Neurobenifical Effect of Anised In Rats Exposed to Mercury

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Received: 27/12/2021
Revised: 26/04/2022
Accepted: 16/05/2022

Abstract

Purpose: Mercury is one of the metallic trace elements. This metal is harmful to health. However, Green anis is a plant widely used in the medical field for its various therapeutic virtues. The aim of this study is to evaluate the effect of subchonic exposure to mercury chloride (HgCl2) on the neurobehavioral status of young wistar rats as well as the neurocorrective effect of essential oil of green anis.

Methods: Extraction of essential oil by hydrodisillation. Exposure of developing rats to a dose of (100mg/L). Then, a group of the intoxicated animals underwent therapy with one dose (0.25 ml/kg) for 21 days intraperitoneally. In addition, the realization of a behavior test battery namely; the forced swimming test to assess the state of despair of the pups, the open field to assess the locomotor activity as well as the exploratory capacities followed by a histopathological study of the brain. Data were analyzed by two-way analysis of variance (ANOVA).

Results: The results of this study demonstrate that exposure to mercury induced significant decreases in body weight ($p < 0.001$) and brain weight ($p < 0.05$), respectively, increases the level of depression ($p < 0.001$), locomotor hypoactivity ($p < 0.01$) compared to control rats. Histological study revealed Purkinje cell degeneration, vascular congestion and lesions. Administration of Pimpinella anisum oil reduced levels of depression ($p < 0.01$), corrected locomotor hypoactivity ($p < 0.05$), and reduced mercury damage.

Conclusion: According to this study, exposure to mercury disrupted the neurobehavioral state of young rats by affecting certain brain structures. These disturbances can be regulated by the essential oil of anis.

Keywords: depression, locomotor activity, brain histology, mercury chloride, Green anis.

Introduction

Mercury (Hg) is a heavy metal highly preponderant in environment. It's used in industrial, medical, agricultural and other applications (Abarikwu et al., 2017) . Exposure to this metal occurs through different pathways and forms (Chan et al., 2017). It can be found in the environment in three different forms: elemental, organic and inorganic (Teixeira et al., 2018). Mercury can damage a wide variety of tissues and organs. It can cause several toxic effects on different systems such as: the central nervous system, the immune system, the kidneys and liver (Hazelhoff et al., 2018). Once in the brain, all of these forms are transformed into inorganic Hg (I-Hg), where they accumulate and bioaccumulate over long periods of time (Chan et al., 2017).

However, herbal medicine plays an important role in recent treatment strategies for various diseases, according to World Health Organization estimates (Doa'a Anwar, 2017). Anise (Pimpinella anisum L.) is one of the oldest known medicinal plants belonging to the Apiaceae family. It is a 30-50 cm high, white-flowered, aromatic annual herb (Amer et al., 2019; Bekara et al., 2016). Pimpinella species have been used as analgesics, anti-inflammatories, appetizers, hypnotics, expectorants, antibacterial and hepatoprotective agents and to increase milk secretion (Cengiz et al., 2008).

The purpose of this study is to evaluate the neurotoxic effect of mercury chloride in young rats exposed during gestation and lactation, and to assess the neuroprotective effect of P. anisum essential oil based on a battery of neurobehavioural tests such as the depression, anxiety and open field test, plus histopathological study of the cerebral cortex.

Materials and methods

1. Plant material and preparation of the essential oil:

The dry and ripe seeds of Pimpinella anisum L were purchased from local herb market in Saida (Algeria) and were identified and authenticated by an expert taxonomist. A voucher specimen was deposited at the herbarium of the department of Biology at Saida University (Algeria). We used 100 g of seeds of Pimpinella anisum. L powder that were processed by steam distillation, over a period of four hours, in an all-glass apparatus, to obtain essential oil with 1.383% yield.

1.1. Determination of the chemical composition of the EOA by CPG/SM:

The analytic study of the AEO was realized on the quality laboratory AFAK Oran with VARIAN CHROMPACK -CP 3900 Gas Chromatography by injecting 0.2 µl of the extract. The vector gas: the helium (He) with a debit of 0.3 ml/min and the column: VF5 capillary column with the dimensions (30 cm X 0.25mm) and 0.25mm of interior diameter. The stationary phase: Nature: 5% phényl-polysinoxane and 95% de methyl. Thickness : 0.25 µm.The computation of the injection's initial column temperature is 70ºC during 2,50 min, then it is elevated with a rate of 15ºC/min at 255ºC during 20 min. The detector: Mass spectrophotometer (Saturne 20200) at 250ºC linked by a computer with an appropriate software and a NIST databank to identify the compounds.

2. Animals and Treatment

Experiments were carried out on Wistar rats (obtained from the Department of Biology, Faculty of Sciences, University of Saida) weighing 210±50 g. The animals were housed with free access to water and food in an animal room, with a 12/12-hour light/dark cycle, at 22 ± 2 °C. They were mated one week after their arrival (three females and one male per cage). After one week of cohabitation with males, females were divided into 02 groups

Group 1: Pregnant females received drinking distilled water.

Group 2: Pregnant females received 100mg/L of $HgCl_2$ in distilled water (Chehimi et al., 2012).

2.1. Experimental Design

At birth, pups issued from intoxicated females, continued to receive HgCl2 in drinking water until weaning, while control pups received only distilled water. In order to test the capacity of anise essential oil (AEO) to attenuate Hg neurotoxicity which induces cognitive deficits, drug therapy was administered, beginning 24 hours after weaning.

At weaning, we got 03 new groups (n = 7) as follow:

Group C: Control rats (issued from control females) received distilled water only.

Group Hg: intoxicated rats with HgCl2 (issued from intoxicated females) that received intoxicated water orally as vehicle solution.

Group Hg-AEO: intoxicated rats (issued from intoxicated females) that received intraperitoneal injection with 0,25ml/kg of body weight AEO daily for 21 days (Asadollahpoor et al., 2017).

All animals were given their injection 30 minutes before the beginning of behaviour tasks. The number of animals that suffered was minimised in accordance with the guidelines of the European Council Directive (86/609/EEC).

3. NEUROBEHAVIORAL STUDY

3.1. Forced Swimming Test

Swimming sessions were conducted by placing the rats in individual glass cylinders (39 cm height × 20 cm diameter) containing water at 22 °C and 30 cm in deep, so that rats could not support themselves by touching the bottom with their paws or tail. Two swimming sessions were conducted: An initial 15 minute pre-test followed by a six minute test, 24 hours later. Following each swimming session, the rats were removed from the cylinders, dried with paper towels and placed into heated cages for 30 minutes, then returned to their home cages. Test sessions were run between 12:00 and 15:00 hours, and videotaped, for later scoring. We recorded the immobility and floating time (Porsolt et al., 1977).

3.2. Open-field Test

General activity was evaluated in the open-field test. The apparatus was constructed of white plywood and measured 72 × 72 cm with 36 cm walls. The lines divided the floor into sixteen 18 × 18 cm squares. A central square (18 cm × 18 cm) was drawn in the middle of the open field. The apparatus was uniformly illuminated with red lights. Each animal was placed individually in the center of the arena and allowed to explore the apparatus for five minutes. A trial was conducted for three consecutive days and the following variables were recorded: Line Crossing, Center Square entries, rearing (count of times that the animal stood on its hind legs), grooming (time, in seconds, used for the animal to groom), and freezing (time, in seconds that the animal remained immobile, often in a crouching posture, with eyes wide open, and irregular respiration), and defecation (number of fecal boli produced) (Dauge et al., 1989).

4. Histological study

Brain samples were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Paraffin sections (5 µm thick) were prepared, routinely stained with hematoxylin and eosin dyes (Suvarna et al., 2013), and then examined microscopically.

5. Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). Data were analysed by the two-way analyses of variance (ANOVAs). When a significant difference was found, the Student-Newman-Keuls post-hoc test was conducted. For all analyses, a difference was considered significant at $p \leq 0.05$.

Results

1.Result of CPG/SM

The chemical analysis of aniseed essential oil revealed 12 constituents which represent 83.55% of the total essential oil.

Table 1. The components identified by CPG/SM

Identified compounds	Time of retention (min)	Concentration ($^0/_0$)
α-pinène	8,11	1,81
Camphène	8,56	0,36
Cis-betaocimène	9,72	0,28
α-phellandréne	10,91	0,22
Limonène	11,68	8,01
3-carène	12,91	0,43
Fenchone	14,22	8,99
α-campholène aldéhyde	16,15	0,47
Ɣ-Elémène	18,1	0,11
Estragole	18,81	7,25
Tans-anéthol	20 ,33	55,44
Cis-Anéthol	21,60	0,18

2. Body and Brain Weight

The weight was evaluated for 15 successive days, the results in table (2) showed that mercury (Hg) decreased significatly the body weight (B.W) (p<0,001), compared with control group. However in the treated group with AEO, we noted a significant increase in the B.W compared (p<0,001) to other groups. Concerning the brain weight (table 2), the results showed that Hg induced a decrease in the brain weight of exposed rats when compared to the controls (p<0,05), whereas in the treated group with AEO, we had observed a significant increase (p<0, 01) compared to intoxicated group.

Table 2. Body weight (g) and brain weight (g) of control (C), Hg-exposed rats during gestation and lactation and Hg-treated rats by anise essential oil (Hg-AEO). Data are mean ± S.E.M. ***p< 0.001 (Hg vs. Control); **p<0.01(Hg vs. Hg-AEO)

Groups	C	Hg	Hg-AEO
Body weight (g)	48,85±3,17	38,28±0,79***	58,42±1,67***
Brain weight (g)	1,62±0,020	1,51±0,014*	1,57±0,008**

3. Neurobehavioral result

3.1. Forced Swimming Test

The results of this test showed a significant increase of immobility time in the exposed animals to mercury when compared to control group (p<0,001) (Figure1). Likewise, we noted a significant (p<0,01) reduction of the immobility time in the treated group with AEO comparatively to other groups.

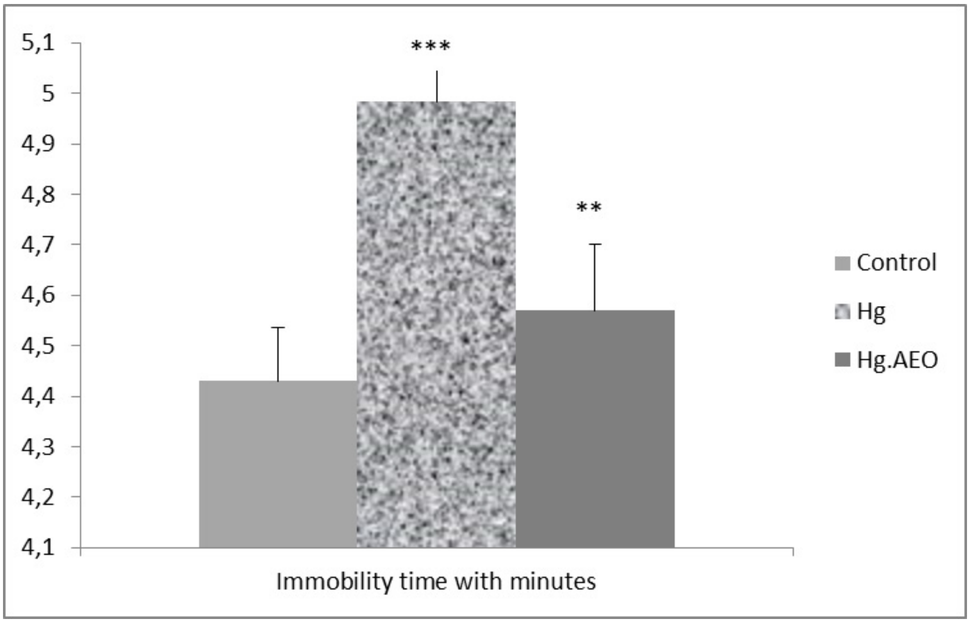

Figure 1. Effect of Hg exposure and anis essential oil during pregnancy and lactation period on depression behavior (FST). Data are mean ± S.E.M. ***p< 0.001 (Hg vs. Control); **p<0.01(Hg vs. Hg-AEO)

3.2. Open field

The statistical analysis in the open-field test, demonstrated that exposure to Hg during pregnancy and lactation increased significantly the latency time (p<0.01) and defecation (p<0.001). On the other hand, there was a significant decrease in line crossing (p<0.01), center square entries (p<0.05), but there was no significant difference in grooming and recovery compared to the control group. However, after the AEO treatment the results relative to this test indicated a significant reduction of latency time (p<0.01), defecation (p<0.001) and a significant rise in the number of tiles crossed (p>0.05) compared to the Hg-vehicle group.

Table 3. parameters of open field test

Parameters	Number of tiles crossed	Number of center visit	Recovery	Grooming	Defecation	latency time (min)
Control	150,85±10,10	4,14±1,83	7,71±2,45	3,57±0,68	1,28±0,6	0
Hg	76± 20,28**	0,28±0,18*	3,85±0,63	3,71±0,35	5,42±0,68***	1,48±0,67**
Hg-AEO	123,57±14,88*	2,14±0,34	4,42±0,29	3,85±0,26	1,14±0,45***	0,13±0,04**

4. Histological result

Histological sections performed at the level of the brain and cerebellum of young rats exposed to $HgCl_2$ during the development period showed that exposure of young rats during gestation and lactation to mercury chloride induced degeneration of purkinje cells, the presence of microglia, lesions, vascular congestions and neuronal cell bodies are in vacuoles. However, animals that have undergone P. anisum EO therapy have a reduced number of Purkinje cells compared to controls treated with essential oil, normal nuclei and absence of necrosis and cell infiltration compared to animals exposed to mercury.

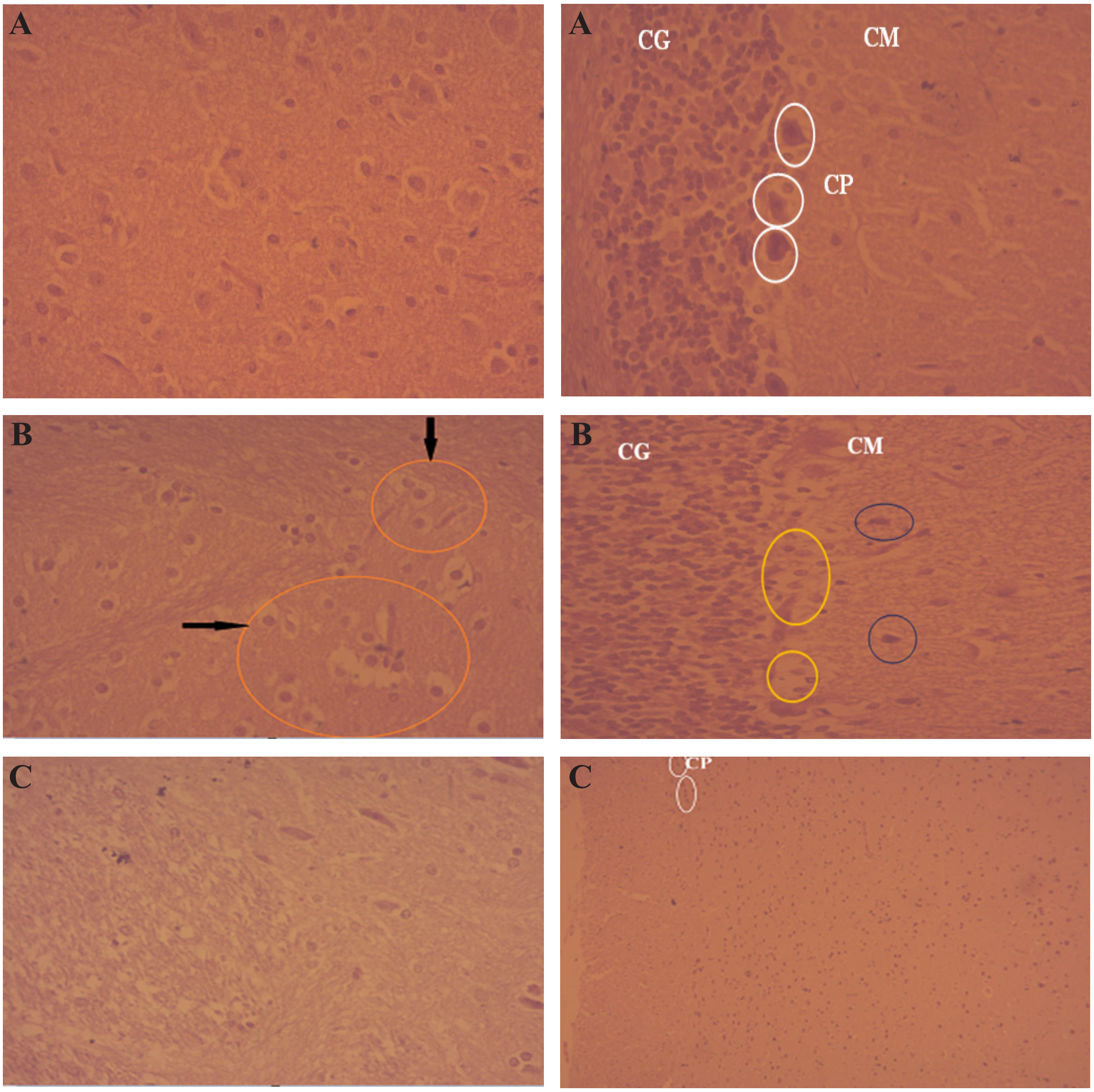

Figure 3. Histological sections at the level of the brain with Gr×40 and Gr×10 with A: control group, B: intoxicated group, C: treated intoxicated group; CG: granular layer, CM: molecular layer; white circle: purkinje cell; orange circle: vacuolation of cellular bodies ; yellow circle: purkinje cell degeneration, blue circle: microglia

Discussion

Exposure to environmental chemicals cause adverse health effects that are determined by the time of exposure and the stage of development, of which fetal and postnatal periods appear to be most vulnerable to the effects of pollutants (Grandergin et al., 2008). Mercury is considered an environmental pollutant linked to a high health risk (Azevedo et al., 2016). Poisoning by this metal induces serious complications, mainly neurological, nephrological and cutaneous (Girault et al., 2008). However, Pimpinella anisum has a variety of properties such as antifungals, antivirals, antimicrobials, antioxidants, muscle relaxants, anticonvulsants and pain relievers. As it has also been used for the management of various neurological diseases (Mosavat et al., 2019).

The results of the variation of weight of the pups from the pregnant females during pregnancy and lactation by the HgCl2 as well as the weight of the brain are significantly reduced.

According to Chehimi et al., 2012, exposure to a high dose of mercury chloride (HgCl2) has shown a decrease in the weight gain of the offspring at birth and during the postnatal period. Moreover , Vimy et al., 1990 explained the decrease in the weight of the offspring by very high levels of Hg at the levels of the pituitary gland of the fetuses knowing that the pituitary gland is an endocrine gland responsible for the secretion of several hormones, including growth hormone.

In addition, the essential oil therapy of pimpinella anisum allowed a significant increase in weight as well as the weight of the brain of the raccoons. The administration of low doses of anis to broilers allows for better feed intake, weight gain and organ weight (Mahmood et al., 2014).

The results of the forced swimming test showed a significant immobility time of the pregnant and lactating addicted rats compared to the control rats. This explained why the animals exposed to HgCl2 presented a depressive state. For thus, their mobility time is reduced.

Following the exposure of a group of workers to inorganic mercury vapor, psychological problems appeared on their behavior, such as: emotional problems, depression, anxiety and social withdrawal (Haut et al., 1999).

Furthermore, subjects professionally exposed to mercury in a chronic manner show depressions in comparison with subjects exposed occasionally or unexposed (Soleo et al., 1990). On the other hand, the treatment of the young poisoned rats with anise oil significantly reduced their immobility time. These results are in agreement with those who have proved that anise oil causes a reduction in the immobility time of animals previously exposed to lead (Kahloula et al., 2013).

In addition, it has been proven that the different extracts (aqueous and ethanolic) of this plant have the antidepressant effect, and this by reducing the animals immobility time in the forced swimming test (Sharmat et al,. 2016).

The results obtained from the open-field test showed a significantly reduced number of tiles crossed and a significantly reduced number of poisoned rats compared to the control rats. Animals exposed to Hg were hyporactive compared to control animals that were hyperactive.

In addition, the latency time and the number of defecations of the poisoned batch were high compared to the control group, which meant that the Hg exposed animals took time to explore a new environment. Several studies have shown that exposure to MeHg can produce behavioural deficits related to locomotor activity and motor performance in adult mice (Dos Santos et al., 2016).

However, following the treatment of rats exposed to mercury with a dose of 0.25 ml / kg of anised, we observed a significant increase in the number of tiles crossed compared to the intoxicated rats and a significant decrease in the latency time as well as in the number of defecations. The essential oil of anise and the aqueous extract may have a corrective effect on the locomotor activity (Kahloula et al., 2013; Bekara et al., 2015).

The central nervous system is considered to be the target organ when humans inhale mercury vapour (Bensefa et al., 2011).

Mercury induced a degeneration of Purkinje cells which may explain the motor hypoactivity observed in the open field test, the presence of microglia which are the defensive lines of the nervous system, the presence of lesions and vascular congestion. All this indicates that mercury can cross the blood-brain barrier and induce inflammation in the brain.

Our results are consistent with those of Kumar et al., 2012 and Pereira et al., 2016. Kumar et al., 2012, revealed that oral exposure to a dose of 1 mg/kg/day HgCl2 in rats for 28 days resulted in: vascular congestion, neuronal degeneration and Purkinje cell necrosis. In addition, Pereira et al., 2016 showed that harmful levels of inorganic mercury can reach the brains of fish after exposure to high concentrations of this metal in water, resulting in significant loss of brain cells (neurons and glial cells) in specific regions (hypothalamus, optic tectum and molecular layer of the cerebellum), decreased cerebellar volume (molecular layer) and altered swimming behaviour of fish. In addition, some authors have reported that inorganic mercury can reach the CNS due to changes in the integrity of the blood-brain barrier (Teixeira et al., 2018). Inorganic mercury enters the brain either through the bloodstream after crossing the blood-brain barrier (Korbaset et al., 2013) or through axonal transport after direct sensory absorption from water (Rouleau et al., 1999; Pereira et al., 2016). Chronic exposure to elemental mercury in animals appears to result in accumulations of elemental mercury in the cerebellum, temporal and frontal lobes (Ostertaget al., 2013). Previous toxicological studies have already indicated that decreases in astrocyte and neuron numbers are generally associated with functional damage observed in behavioural tests following exposure to inorganic mercury (Teixeira et al., 2018).

However, animals exposed to mercury and treated with the essential oil of aniseed show a significant number of Purkinjes cells, normal nuclei and the absence of necrosis compared to mercury poisoned animals. Given the lipophilic nature of the essential oil, it allows it to cross the blood-brain barrier and reach the brain. Our results are consistent with those of Abdul-Hamid et al., 2014, who showed that treatment of rats with P. anisum essential oil could correct the damage caused by exposure to aspartame such as: Purkinje cells had a narrowed contour, irregular perikaryon associated with deep stained cytoplasm and pycnotic nuclei. The treatment with essential oil of P. anisum revealed that the majority of Purkinjes cells appear to have normal architecture with well-defined nuclei and prominent nucleoli.

Conclusion

In conclusion, the results of this study show that exposure of young wistar rats during development to mercury chloride induced depression and hypoactivity followed by tissue damage. In addition, treatment with P. anisum oil has corrected the damage caused by mercury.

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التأثير العصبي للدينونة في الجرذان المعرضة للزئبق

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تاريخ استلام البحث: 2021/12/27
تاريخ تعديل البحث: 2022/04/26
تاريخ قبول البحث: 2022/05/16

المُستَخلَص

الهدف: الزئبق هو أحد العناصر المعدنية النادرة. هذا المعدن ضار بالصحة. ومع ذلك، فإن اليانسون الأخضر هو نبات يستخدم على نطاق واسع في المجال الطبي لفضائله العلاجية المختلفة. الهدف من هذه الدراسة هو تقييم تأثير التعرض لكلوريد الزئبق على الحالة السلوكية العصبية لفئران ويستار الصغيرة وكذلك التأثير التصحيحي للزيت العطري لليانسون الأخضر.

الطريقة: استخلاص الزيت العطري عن طريق التحلل المائي. تعرض الفئران النامية لجرعة (100 مجم/لتر). ثم خضعت مجموعة من الحيوانات المخمرة للعلاج بجرعة واحدة (0.25 مل/كجم) لمدة 21 يومًا داخل الصفاق. بالإضافة إلى ذلك، تحقيق بطارية اختبار السلوك وهي، اختبار السباحة القسري لتقييم حالة اليأس لدى الجرذان، الحقل المفتوح لتقييم النشاط الحركي بالإضافة إلى القدرات الاستكشافية تليها دراسة الأنسجة المرضية للدماغ. تم تحليل البيانات عن طريق تحليل التباين ثنائي الاتجاه (ANOVA).

النتائج: أظهرت نتائج هده الدراسة أن الانخفاض الكبير في وزن الجسم ووزن الدماغ ($P < 0.05, P < 0.001$) التوالي، الناجم عن التعرض للزئبق، يزيد من مستوى الاكتاب، ($P < 0.001$) وينقص النشاط الحركي ($P < 0.05, P < 0.001$) مقارنة مع الفئران الشواهد. كما كشفت الدراسة النسيجية عن تنكس خلايا بركنجي واحتقان الاوعية الدموية والاغات. أدى استخدام زيت الينسون الأخضر إلى تقليل أضرار الزئبق من بينها نقص نسبة الاكتآب ($P < 0.01$) وتصحيح نقص النشاط الحركي ($P < 0.05$).

الاستنتاج: وفقًا لهذه الدراسة، أدى التعرض للزئبق إلى تعطيل الحالة السلوكية العصبية للفئران الصغيرة من خلال التأثير على بعض هياكل الدماغ. يمكن تنظيم هذه الاضطرابات بواسطة زيت اليانسون الأساسي.

الكلمات المفتاحية: الاكتئاب، النشاط الحركي، أنسجة المخ، كلوريد الزئبق، اليانسون الأخضر.