ORIGINAL ARTICLE

Neuro beneficial effects of *Pimpinella anisum* against lead exposure

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Background: The essential oil of *Pimpinella anisum* has been widely used in traditional medicine to treat a variety of diseases, including some neurological disorders. **Aims:** This study was aimed to test, *in vivo*, the possible anxiolytic and antidepressant effects, of the essential oil of *Pimpinella anisum* against chronic lead acetate (0.2%) intoxication during the gestation and lactation period, in Wistar rat pups. **Settings and Design:** Wistar rat pups were exposed to lead via their dams' drinking water from postnatal day (PND) 1 to (PND) 21. After weaning, the lead-exposed rats received injections of essential oil of *Pimpinella anisum* (0.5 ml/kg) for 15 days. The level of anxiety, depression and locomotor activity were studied. **Materials and Methods:** The behaviours evaluated were: Locomotor activity (open-field test), anxiety (dark and light compartment and elevated plus maze tests), and depression (forced swimming test). **Statistical Analysis:** The data were analysed by two-way analyses of variance (ANOVAs). When a significant difference was found, the Student-Newman-Keuls post-hoc test was conducted. For all analyses, the difference was considered to be significant at $P \leq 0.05$. **Results:** The results of the present study demonstrate that developmental lead exposure induces, on the one hand, impairments of body ($P < 0.001$) and brain weight ($P < 0.05$), respectively, and on the other hand, increases the level of anxiety ($P < 0.001$), depression ($P < 0.001$) and locomotor hyperactivity ($P < 0.001$), compared to control rats. Administration of the essential oil of *Pimpinella anisum* entrains reduction in the level of anxiety ($P < 0.001$), depression ($P < 0.001$) and correct locomotor hyperactivity ($P < 0.001$) in rats exposed to lead beforehand. **Conclusion:** In conclusion, our results demonstrate that developmental lead exposure induces significant perturbation of emotional reactivity that can be improved by treatment with the essential oil of *Pimpinella anisum*. Further evaluation of the use of anise oil in the treatment of neurological disorders is suggested.

Key words: Anxiety, depression, lead acetate, locomotor activity, *Pimpinella anisum*

INTRODUCTION

Lead (Pb) is still widely distributed in the environment, in ambient air, in a number of food stuffs, in drinking water and finally in dusts.[1] The consequences of chronic exposure to low levels of lead in childhood have been a matter for extensive research during recent years. Exposure to low levels of lead during early development has been implicated in long-lasting behavioural abnormalities and cognitive deficits in children and experimental animals.[2-5] The learning impairment and other behavioural disturbances like anxiety and depression that affect children and laboratory animals, suggests that the hippocampus may be one region that is adversely affected during early life. Recent developments suggest that gestation through lactation is a sensitive period, when exposure to low levels of Pb cause long-lasting cognitive deficits.[6-9]

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The direct neurotoxic actions of Pb include: Apoptosis; excitotoxicity; influences on the neurotransmitter storage and release process, cerebrovascular endothelial cells and astroglia; and proliferation, differentiation and synaptogenesis in the developing brain.[10-13] Recent findings demonstrate that early lead exposure disrupts expression and phosphorylation of the cAMP-responsible element binding protein (CREB), a transcription factor directly related to the neuronal plasticity in the hippocampus of juvenile rats.[14]

The physiological and pharmacological studies indicate that the serotoninergic and glutamatergic neurotransmitter systems play critical roles in the regulation of cognitive processes and locomotor activity. Although the neurobiological bases dealing with how Pb impairs the neurobehavioural function remain unclear, evidence suggests that disruptions of these neurotransmitter systems may be involved in Pb-related neurobehavioural dysfunction. Lead-induced effects on the glutamatergic system include: Changes in the synthesis, turnover and reuptake of glutamate, and changes in the number of N-Methyl-D-aspartate (NMDA) receptors.[15]

Essential oils made out of natural aromatic molecules are endowed with so many physiological and

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pharmacological properties that they find applications in almost every field of medicine, not only curatively, but also from the preventative medicine point of view.[16]

Aromatherapy can provide useful complementary medical service both in healthcare settings and in private practice, for example, in dementia and depression. There are also many indications for the useful and successful application of essential oils, such as in cases of stress and sleep disorders.[16]

The essential oil of *Pimpinella anisum* (anise oil) is used today as an ingredient in cough medicine and is reported to have diuretic and diaphoretic properties.[17] *Pimpinella anisum* has been also used as a carminative, antiseptic, antispasmodic, expectorant, stimulant and stomachic medicament. In addition, only a few studies point to the possible effects of *Pimpinella anisum* on neuronal activity. The plant's and especially its fruit's essential oil have been used in the treatment of some neurological diseases, including seizures and epilepsy; it also has a protective effect against the development of cerebrovascular diseases.[18]

The aim of the present study is to examine the probable anxiolytic and antidepressant effects of anise oil and to attenuate the Pb neurotoxicity-induced cognitive deficits, *in vivo*, on rats exposed to lead during gestation and lactation.

MATERIALS AND METHODS

Plant Material and Preparation of Aqueous Suspension

Seeds of anise '*Pimpinella anisum L'* (family, Apiaceae) were purchased from local herb shops in Saida and were identified by an expert taxonomist. The sample was preserved, and a voucher specimen coded P-04061976 was deposited at the herbarium of the Department of Biology, Sciences Faculty, Saida University, Algeria, for future reference. We used 50 g of anise seeds that were processed by steam distillation, over a period of four hours, in an all-glass apparatus, to obtain essential oil with 3.6% yield.

Animals and Treatment

Experiments were carried out on Wistar rats (obtained from Charles River) weighing 200-50 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). On pregnancy day 0 the dams were divided into three groups: One group received 0.2% lead acetate in drinking distilled water during gestation and lactation (Pb), the second group received lead acetate and anise essential oil (AEO), whereas, rats in the control group received distilled water without lead acetate (C), as described previously. At birth, the Pb pups continued to receive lead acetate during lactation until postnatal day (PND) 21 and the rats were weaned on this day. Immediately after the behavioural evaluation, the rats were decapitated and blood samples were taken for glucose blood determination. The number of suffering animals were minimised in accordance with the guidelines of the European Council Directive (86/609/EEC).

Experimental Design: Chronic Administration Prior to Assessment

Animals were exposed to lead during gestation and lactation (Pb) and the controls animals received distilled water. In order to test the ability of anise essential oil (AEO) to attenuate Pb neurotoxicity-induced cognitive deficits, drug therapy was administered, beginning 24 hours after weaning. Randomly chosen animals of each group were injected (i.p.) with 0.5 ml/kg of body weight AEO (n = 8; n = 8, respectively) or distilled water (vehicle) (n = 9). All animals were injected once daily on days 1-15 after weaning. During PND 32-6 all subjects were given their injection 30 minutes prior to beginning the behaviour tasks.

Dark and Light Compartments

In this test, we allowed the animals to investigate an arena formed by two compartments: One with light the other one dark. Rats generally loathed places with light. Hence, the more the animal was not anxious, the more its exploration would be reduced in the dark compartment. We estimated the residence time between both compartments, the one with light and the other dark, to be three minutes of exploration of the environment by the rat. The experiment lasted approximately 30 minutes.[19]

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 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 24 hours later by a six minute test. Following each swimming session, the rats were removed from the cylinders, dried with paper towels and placed into heated cages for 30 minutes, and 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.[20]

Open-field Test

The 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).[21]

Elevated Plus Maze

The anxiety was evaluated in the elevated plus maze test. The elevated plus maze apparatus consisted of two open arms, 50 × 10 cm (length × width) and two closed arms, 50 × 10 × 50 cm (length × width × height) with an open roof arranged such that the two arms of each type were opposite each other. The maze was elevated from the floor.

For the test, each animal was placed in the center of the maze, facing one of the closed arms, the number of entries into the two arms, the time spent in the open and the closed arms were registered for five minutes.[22]

Biochemical Assay

The dosage of glucose is made on the serum after separation of the total blood. The blood glucose is estimated by using the organic kit (kit Bio systems).

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

Administration of lead acetate showed a significant decrease ($P < 0.001$) in the body weight of exposed rats compared to the Pb-AEO and control groups, respectively.

However, there was no significant difference ($P > 0.05$) in body weight between the Pb-OAS and control groups [Table 1]. The brain weight also decreased significantly ($P < 0.05$) in the Pb-treated rats compared to the Pb-AEO and control groups, respectively [Table 1].

With regard to the anxiety-like behaviour, the Pb ingested by mother rats during gestation and lactation entrain an increase of residence time in the compartments with light compared to control rats ($P < 0.001$). On the other hand we estimated a significant diminution in the residence time in the compartments with light for Pb-AEO ($P < 0.001$) [Figure 1]. Furthermore, the test of plus maze revealed that Pb administration reduced the time spent exploring the open arms ($P < 0.05$), the time in the open arms ($P < 0.05$) and percentage of entries into the open arms ($P < 0.05$). However, there was no significant difference in all the parameters of this test between Pb-AEO and control rats [Figure 2].

Table 1: Body weight (g) and brain weight (g) of control (C) and lead-exposed rats during gestation and lactation and Pb-treated rats by anise essential oil (Pb-AEO)

Groups (g)	Control	Pb	Pb-AEO
Body weight	75,90±0,27	55,78±0,26***	74,65±0,29
Brain weight	1,73±0,03	1,52±0,03*	1,70±0,03

Data are mean±S.E.M. ***P<0.001, *P<0.05, (Pb vs. Control) P<0.001, (Pb vs. Pb-AEO) P<0.001, (Pb vs. Control) P<0.05, (Pb vs. Pb-AEO) P<0.05, AEO – Anise essential oil

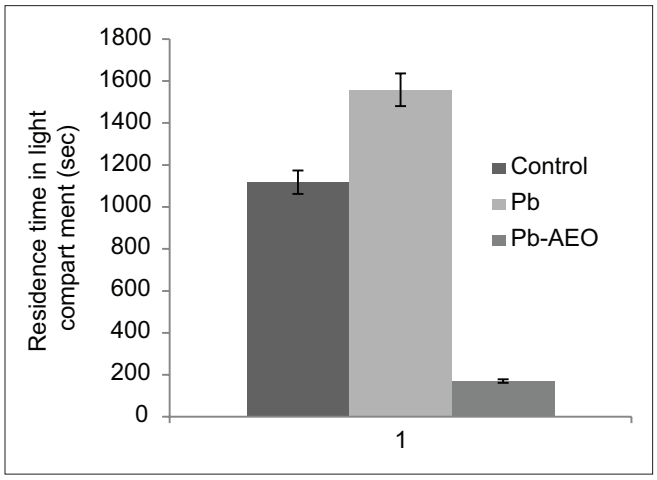

Figure 1: Effect of Pb exposure and anis essential oil during pregnancy and lactation period on anxiety behaviour (dark and light compartment). Data are mean ± S.E.M. ***P < 0.001

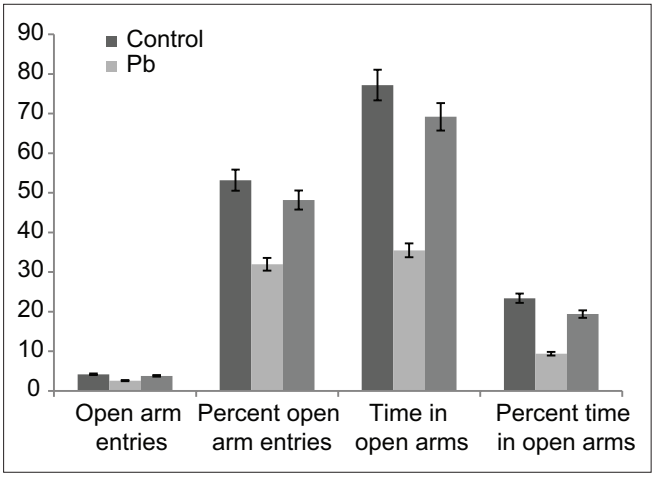

Figure 2: Effect of Pb exposure and anis essential oil during pregnancy and lactation period on anxiety behaviour (plus maze test). Data are mean ± S.E.M. *P < 0.05. (Pb vs. Control); P < 0.001 (Pb vs. Pb-AEO); P < 0.05

In addition, we registered a significant increase of immobility time in Pb-treated animals, in the forced swimming test (FST), compared to the controls ($P < 0.001$) [Figure 3]. Likewise, we noted a significant reduction ($P < 0.001$) of immobility time in the Pb-AEO group compared to the Pb group [Figure 3].

The results showed that females exposed to 0.2% of Pb during pregnancy and lactation allowed to record a significant increase of the glycaemia rate, with a mean of 1.58 ± 0.07 compared to control rats, 0.96 ± 0.01, [Figure 4]. On the other hand, no significant difference was observed in terms of blood glucose concentration in Pb-poisoned rats that were treated with AOE, compared to the control rats ($P > 0.05$) [Figure 4].

The Student's *t* test, in an open-field test, demonstrated that exposure to Pb during pregnancy and lactation increased the latency time ($P < 0.001$), line crossing ($P < 0.001$), center square entries ($P < 0.001$), rearing ($P < 0.001$), grooming ($P < 0.001$) and defecation ($P < 0.01$) compared to the control group [Table 2]. However, after the AEO treatment the results relative to this test indicated a significant reduction of all parameters (measuring latency time ($P < 0.001$), line crossing ($P < 0.01$), rearing ($P < 0.001$)) excepting the center square entries ($P > 0.05$), grooming ($P > 0.05$) and defecation ($P > 0.05$) compared to the Pb-vehicle group [Table 2].

DISCUSSION

This study was carried out to provide, on the one hand, a comprehensive assessment of the effects of Pb exposure during pregnancy and lactation on the behaviour of weaned pups submitted to a range of tasks, and on the other hand, to attenuate the Pb neurotoxicity, which is translated into anxiogene and depression states by using anise essential oil treatment on rats.

Several studies have revealed that Pb exposure produces neurological damage and behavioural disruptions in human beings and in experimental animals.[9,23-27] Prenatal lead exposure is extremely dangerous and its effects on various aspects of brain development, function and behaviour have been examined. Does prenatally transferred lead come from the skeletal lead stores of a woman herself, poisoned as a youngster, or from environmental sources? A recent study indicates deleterious effects on childhood cognitive development, with placental blood lead levels below 10 mg/dl.[28]

The main constituents of anise oil are trans-anethole (89.1%), estragol (3.6%), linalool (1.1%), α-terpineol (0.2%), and cis-anethole (0.2%).[29] All these active agents may be responsible for the effects observed in this study.

The main component of anise oil is anethole (1-(4-Methoxyphenyl)-1-propen).[29] It is largely used

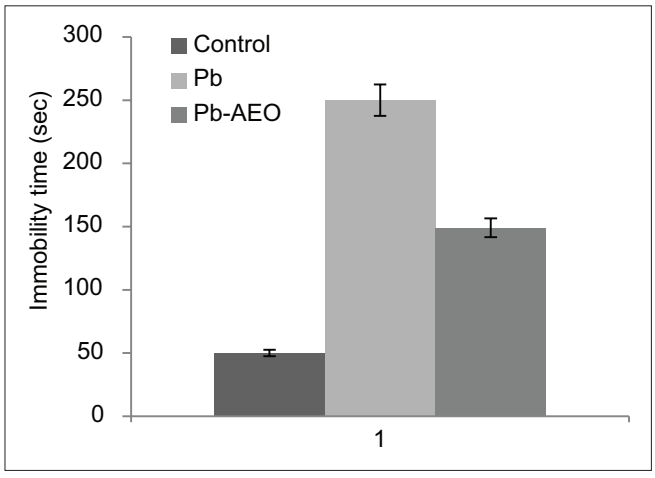

Figure 3: Effect of Pb exposure and anis essential oil during pregnancy and lactation period on depression. Data are mean ± S.E.M. ***$P < 0.001$. (Pb vs Control) $P < 0.001$; (Pb vs Pb-AEO); $P < 0.001$

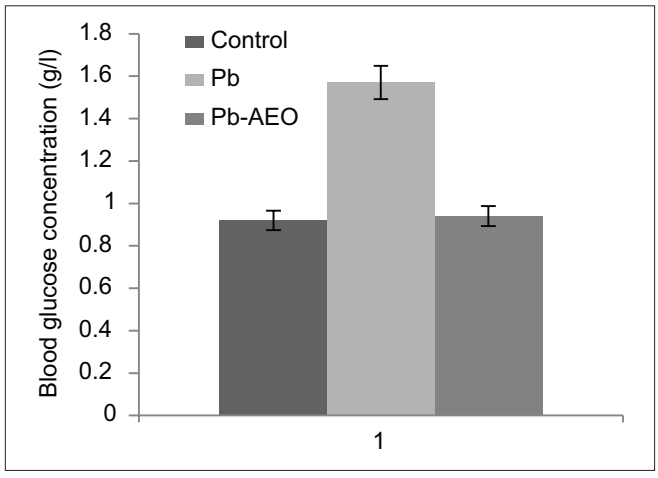

Figure 4: Blood glucose level (g/l) of control (C) and lead exposed during gestation lactation (Pb) and treated rats by anis essential oil (Pb-AEO). Data are mean ± S.E.M. ***$P < 0.001$. (Pb vs. Control); $P < 0.001$ (Pb vs. Pb-AEO); $P < 0.001$

Table 2: Effect of the exposure to Pb and anise essential oil treatment during pregnancy and lactation

	Latency time (second)	Line crossing	Center square entries	Rearing	Grooming	Defecation
Control	306,28±4,21	62,7±2,83	1,42±0,36	9,14±1,73	8,42±0,36	1,71±0,42
Pb	75,85±5,9***	199,28±4,1***	3,57±0,36***	27,42±1,41***	2,85±0,34***	2,42±0,29**
Pb-AEO	174,75±1,73***	86±3,26**	0,71±0,18	14,71±0,47***	2,42±0,20	2,57±0,20

Data are mean±S.E.M. ***$P<0.001$, **$P<0.01$, (Pb vs. Control) $P<0.001$, (Pb vs. Pb-AEO) $P<0.001$, Pb-AEO – Pb-treated rats by anise essential oil

as a substrate for the synthesis of various substances of neuro-pharmaceutical interest, such, as, anticonvulsant and sedative drugs.[30]

The present findings show that Pb exposure during the pregnancy and lactation period causes a loss of both body and brain weight. This shows that Pb induces reduction of food intake, which is due to the action of Pb on certain neuro-centers responsible for the control of hunger in the brain. Even the hyperglycemia reduces the hunger sensation. It has been indicated that the endocrine and biochemical mechanisms underlying the growth suppression, produced mainly by gestational and lactational lead exposure, are related to the decreases in growth hormones and associated factors.[31] Similar observations have been reported by different authors.[32,33] In the same way, a significant decrease in the weight of the brain is a result of lead toxicity. Lead can cause a delay in the neuronal maturation of the cerebellum, possibly as a result of vascular disruption.[34] Reduced weights of various brain areas, including the cerebral cortex, cerebellum, and hippocampus, have also been reported during lead encephalopathy in suckling rats.[35] Some authors have reported a number of alterations in the lead-exposed cerebellum, including a decrease in the molecular layer width, granular cell density, and dendritic arborization, after a decrease in total weight.[36]

However, the results of the present study clearly demonstrate that prenatal exposure to Pb produces a profound effect on anxiety-like behaviour in the dark and light compartments test and plus maze test. Although the mechanism by which Pb alters behaviour in the dark and light compartments is yet to be established, it appears that there is a link with the hippocampal serotonin and dopamine neurons. These systems are involved in the regulation of Corticotropin Releasing Factor (CRF), which plays an important role on the systems implicated in anxiety-like behaviour.

The serotoninergic and glutamatergic systems play a central role in modulation of anxiety and an increase in the 5-HT and glutamate levels in the hippocampus is associated with an anxiogenic effect.[37]

Moreover, during the forced swimming test we observed that the time of immobility in the Pb-exposed group was significantly higher than that in the control group. This could be due to the fact that Pb acted like a depressive element on the dopaminergic and serotonnergic systems in different brain areas, mainly the striatum, hippocampus, and hypothalamo-hypophysaire axis. This result was in good agreement with those who had observed that Pb-treated rats during both pregnancy and lactation had a depressive-like behaviour detected in the forced swimming test.[38] Certain authors explained this as being the effect of Pb on serotoninergic and glutamatergic transmission in the brain and their receptors 5-HT1A and NMDA, respectively, in depression physiopathology.[39,40] It has been shown in reports that hippocampus is an important target of neurotoxic agents, and it accumulates Pb to a greater degree; this accumulation entrains an elevated rate of serotonin, which controls depressive behaviour.[41]

Likewise, our results present that Pb has a chemical stress because it is involved in the rise of glycaemia, probably under some stress hormones. Reports state that stress causes an increase of glycaemia under the action of the corticotropin releasing factor, corticotropine, cortisone in the hypothalamus, hypophysis and surrenal gland, respectively, by activating an enzyme of carbohydrate metabolism.[42]

On the other hand, we observed that Pb-exposed rats and those treated with AEO presented anxiety and depression state less important than poisoned Pb animals, which was due the neuroprotective effects of anise oil. The hippocampal area is characterised by the NMDA receptor predominance, which is highly vulnerable to damage from Pb exposure. The dysfunction of NMDA receptors seems to play a crucial role in the neurobiology of disorders such as Parkinson's disease, Alzheimer's disease, epilepsy, anxiety and depression. The key role of glutamate accumulation and activation of NMDA receptors in the pathophysiology of depression is well established.[43] Pb exposure induces NMDA receptor function changes, induces a wide variety of changes in intracellular signalling synaptic plasticity in the hippocampus and blocks the NMDA ion channel.[15,44,45] In the present study, addition of anise oil presents a neuroprotective effect, probably by enhanced modulation of the NMDA functions, such as, activation of the glycine site of the NMDA receptor.

Lead-exposed rats also show higher locomotor activity in the open-field. These results suggest that Pb induces hyperlocomotor activity in rats, which strengthens the concept that Pb may induce hyperactivity in the experimental animals. Previous investigations in several laboratories have demonstrated that Pb-exposures are associated with hyperactivity in human and experimental animals.[46,47]

In fact, this hyperlocomotor activity is reduced by the effect of anise oil, maybe due to activation of GABA A receptors.[48] It has been shown that anise oil exerts its effect on opioid receptors via activation of GABA A receptors in mice. In addition, it has been revealed that anise oil enhances the activity of the Na^+-K + ATPase.[49,50] Na^+-K + pumps play an important role in the regulation of neuronal excitability

In conclusion, the results from the present study show that anxiety and depression induced by chronic Pb intoxication

during gestation, until weaning, can be corrected by administration of anise essential oil. Understanding the cellular action of this essential oil can help to find a strategy to prevent and treat the effect of Pb intoxication during development.

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