Abstract
Pregnant rats received 0, 2% of lead acetate (Pb) in drinking water. The treatment with the aqueous extract of Pimpinella anisum L (P.A.E.) started after weaning with dose of 750 mg/kg and for 15 successive days. The effect of the extract was evaluated through behavioral tests : open filed (OF) , Forced swimming test (FST) and dark /light test. Beside; Lactate dehydrogenase (LDH), total plasmatic proteins, alkaline phosphatase (ALP), lipid peroxidation (TBARS) and Catalase (CAT) were analyzed. Lead induced a hyperactivity in the open field which was reduced after administration of P.A.E. In the FST, the treatment with plant extract enhanced significantly the mobility time compared with intoxicated group. We had observed that time spent in the dark compartments in dark and light test was reduced after oral administration of P.A.E. Lead caused significant increase in LDH , TBARS and ALP , in contrary this toxic agent decrease the levels of plasmatic proteins and catalase activity in cerebellum and cerebrum. We can conclude that oral treatment with aniseed aqueous extract was effective in reducing the level of some of biochemical parameters and ameliorate behavior of intoxicated rats by lead.

Keywords P.anisum L, Aqueous Extract, Lead Acetate, Neurobehavioral Tests, Cerebellum and Cerebrum

1. Introduction
For many ages , herbs formed the main source of therapeutics that have been used to help in relief from illness, hence herbal treatment is still used but a small percentage of living plant have been phytochemically investigated[1]. Plants extracts are considered nowadays as a potential source for bioactive molecules that can affect positively or negatively on different cellular processes [2].

Pimpinella anisum L (Family: Apiaceae) is an annual herb indigenous to near East and widely cultivated in the Mediterranean. The principal constituents of the fruit are volatile oil, fatty acids, coumarins, flavonoids, glycosides, proteins and carbohydrates [3]. The seeds as well as essential oil have been used as anti-oxidants, antispasmodic, anti-microbial, digestive stimulant, galactogogue [4]. Whereas few studies have investigated the effect of this plant on neurological disorders, however, in Iranian folk medicine, the traditional healers described aniseed as a treatment for epilepsy and convulsion [5].

Lead is the most common neurotoxin agent that is widely distributed in the environment; many studies suggested that the neurotoxin effect of this non-essential element are mediated by interference directly or non-directly with cholinergic and aminergic system [6]. Exposure to this heavy metal in earlier life has been showed to produce impairment in the processes of learning, memorization and motor coordination.

In the presence of all this huge data which confirmed the hazardous effect of lead on nervous system especially in children, this study was conducted to investigate the possible beneficial effect of an oral administration of P. anisum L aqueous extract on lead induced neuro-behavioral damage in young rats.

2. Material and Methods

2.1. Plant Material
The dry and ripe seeds of Pimpinella anisum L were purchased from a local herbs market in Chief Center (Algeria) and were identified by an expert taxonomist. A voucher specimen was deposited in the herbarium of department of Biology, Faculty of Science, Oran University (Algeria).

2.1.1. Aqueous Extract Preparation
The seeds of Pimpinella anisum L were grounded; 100 g of
the powder were immerged in 1 L of distilled water on heat for 15 minutes [7]. The aqueous extract was filtered through Wathman paper N°1, and the filtrate was after that lyophilized (CHRISI, ALPHA 1-2LD, Germany). The yield of extraction was 20, 99%.

2.2. Animals

Females Wistar rats (Rattus norvegicus) weighting 200 ± 30 g were used in this study. All animals were obtained from Department of Biology, Faculty of Science, University Of Oran 1. The animals were housed in standards conditions with free access to food and water (12 h light/dark, T° 22±2°C). All the procedure performed on animals were approved and conducted in accordance with the National Institute of health Guide (Reg. No. 488/160/1999/CPCSEA). After one week of cohabitation with males, females were divided into 02 groups [8]:

Group 1: Pregnant females received drinking water without lead acetate (Pb).

Group 2: Pregnant females received 0, 2% of lead acetate (Pb) in drinking water.

2.2.1. Experimental Design

At birth, pups issued from intoxicated females, continued to receive lead acetate in drinking water until weaning, while control pups received only distilled water. At weaning, we got 03 new groups (n = 8) as follow:

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

Group Pb: intoxicated rats with lead (issued from intoxicated females) that received distilled water orally as vehicle solution.

Group Pb + P.A.E: intoxicated rats (issued from intoxicated females) that received orally P. anisum aqueous extract (P.A.E) at dose of 750 mg/Kg daily for Two weeks [3].

Neurobehavioral Study

Open Field Test (OF)

This test evaluated the general motor activity of rats. The apparatus was constructed of poly- wood and measured 72×72 cm with 36 walls. The lines divided the floor into sixteen 18×18 cm squares. Each animal was placed in the center of the arena and allowed to explore the apparatus for 5 minutes. The following parameters were recorded through the test: Number of squares crossing, Center squares entries, Rearing (Frequency with which the animal stood on their hind legs) Grooming and Defecation (Number of boli produced).

Forced Swimming Test (FST)

The rat was placed in glass cylinder (39 cm height × 20 cm diameter) containing water at 22 °C and 30 cm of deep. Tow swimming session were conducted: an initial 10 minutes pre-test followed 24 hours by six minutes test. After swimming session, each rat was removed from the cylinder, dried and returned to their home cage. The parameters recorded during the test were time of mobility and immobility time.

Dark and Light Test (DLT)

In this test, we allowed animal to discover the arena formed by two compartments: one with light and the other dark. Rat’s generally hated places with light, hence more the animals is no anxious, more its exploration would be reduced in the dark compartment. The parameters recorded in this test are time spent in the dark compartment and time spent in the light compartment.

Sacrifice

At the end of the behavioral tests, rats were killed in the morning after 12 hours of fasting. Sacrifice was done by cervical decapitation after injection in I.P of anesthesia (Chloral solution 3ml/Kg). The brain was removed, rinsed with physiological solution (NaCl 0, 9%) and stored at -80 °C until use. Blood was collected in heparin tubes, and then it was centrifuged 3000 rpm/ 10 minutes. Samples of plasma were conserved for further Biochemical [9].

2.3. Biochemical Analyses

The dosage of Lactate Dehydrogenase (LDH) and total plasmatic proteins was made on the recuperated plasma by using commercial kits (CHRONOLAB).

2.3.1. Preparation of Brain Homogenates

Cerebrum and Cerebellum were separated and cleaned with ice-cold saline solution. Brain parts were homogenized in a proportion of 1:10 (W/V) ice-cold KCL buffer (1, 15%; pH 7, 2). The homogenate was centrifuged at 10.000×g for 10 minutes at 4°C to obtained post –mitochondrial supernatant (PMS) which was used for the quantification of ALP , Lipid peroxidation and Catalase.

2.3.2. Dosage of Alkaline Phosphatase (ALP)

The assessment of alkaline phosphatase (ALP) in the brain was measured by using commercial kits (CHRONOLAB).

2.3.3. Lipid Peroxidation (TBARS)

The level of lipid peroxidation was determined as described by [10]. Briefly , to 0,2 ml of brain homogenate , we add in the following order : 0,2 ml of Sodium lauryl phosphate (8,1%), 1,5 ml of acid acetic (20 %, pH 3,5) and 1,5 ml of aqueous solution of Thiobarbituric acid (0,8%). The volume of the solution was made up to 4 ml by adding distilled water and heated at 95 °C for 60 minutes. After cooling in ice bath, 4 ml of n-Butanol and 1 ml of distilled water were added to the mixture and centrifuged. The organic layer separated and its absorbance was measured at 532 nm. The result was expressed as µmol of MDA/ mg of proteins tissue.
2.3.4. Catalase Activity (CAT)

The catalase activity was measured following the methods of [11]. Briefly, 50 µl of homogenate and 750 µl of phosphate buffer (0, 1 M; pH 7, 5) were incubated into tube, hence after adding 200 µl of H₂O₂ (50mM), the chronometer was set on. After one minute, the reaction was stopped by adding 2 ml of Potassium Dichromate solution (5%). Each tube was heated at 100 °C for 10 minutes. After cooling, the absorbance of the samples was measured at 570 nm. The catalase activity was expressed as mM of H₂O₂ degraded / min/mg of proteins.

2.4. Statistical Analysis

All results were expressed as mean ±S.E.M (Standard of Error). The data analysis was carried out by using statistical software: R. Kruskal Wallis rank test (a non-parametric test) was used to examine the difference between independent groups and the Wilcoxon rank sum test (a non-parametric test) was used to examine the difference between the dependent groups using R software. Value of p < 0.01 and p < 0.05 were taken as the significant level.

3. Results

Body and Brain Weight

The weight was evaluated for 15 successive days, the results in table (1) showed that lead increased the body weight (B.W), in contrary we observed that the control group represented the lower body weight, however in the treated group with P.A.E we noted a significant increase in the B.W compared (p<0.01) to other groups. Concerning the brain weight (table 1), the results showed that lead induced a decrease in the brain weight of exposed rats when compared to the controls (p<0.01), whereas in the treated group with P.A.E, we had observed a significant increase (p<0.01) compared to intoxicated group.

Open Field Test

The open field experiment demonstrated that exposure to lead (Pb) during pregnancy and lactation increased the number of crossed squares (p<0.05) and reduced the grooming (p<0.01) compared to control group (Table 2). Hence, the oral administration of P.A.E. showed a significant reduction in some parameters of the open field test like Number of visit in central squares (p<0.05) and defecation (p<0.01), whereas for the other parameters no significant changes was recorded.

| Table 1. Effect of Pb and P.A.E on body and brain weight of tested rats. |
|-----------------------------|-----------------------------|
|                             | Body weight (g) | Brain weight (g) |
| Group C                     | 32.16 ± 1.68      | 1.61 ± 0.054    |
| Group Pb                    | 37.74 ± 4.80 **   | 1.40 ± 0.099**  |
| Group Pb+ P.A.E.            | 61.19 ± 2.60**    | 1.57 ± 0.072*   |

(*** p<0.01)

| Table 2. Effect of lead (Pb) and oral administration of P.A.E on Open Field parameters. |
|---------------------------------|------------------|------------------|----------------|------------------|------------------|
|                                | Number of crossed squares | Number of visits in central square | Rearing | Grooming | Defecation   |
| Group C                        | 128.2± 29.20      | 7.8 ± 2.57       | 55±15.61 | 12.4 ±1.46 | 1 ± 0.02       |
| Group Pb                       | 158 ±14.13*      | 28.8 ± 12.2 *    | 68 ±11.71| 5.8 ±1.23**| 2.66 ±0.40*    |
| Group Pb+ P.A.E.               | 141.6± 27.48     | 24.4 ± 5.55*     | 88 ± 19.01| 19.8±2.98**| 1.30 ± 0.58**  |

(*) p<0.05; (**) p<0.01.
Forced Swimming Test

The results of this test showed a significant increase of immobility time in the exposed animals to lead (Pb) when compared to control group (p<0.05) (Figure 1). Likewise, we noted a significant (p<0.01) reduction of the immobility time in the treated group with P.A.E comparatively to other groups.

Dark and Light Test

Lead (Pb) ingested by mother rats during gestation and lactation caused a decrease of time spent in the dark compartment of exposed rats when compared to controls rats (p<0.05). Thus, oral treatment with aniseed aqueous extract increased significantly the time spent in the dark compartment comparatively with other groups (p<0.05), figure (2).

LDH and Total Proteins

Exposure to lead induced a significant increase in the LDH levels (1348±317 U/L) in intoxicated Group compared to the control (801, 95±261 U/L) with p<0.01. In treated group with P.A.E., no significant reduction of LDH level was observed, in contrary a little augmentation in the concentration of this enzyme was registered (1500, 27±391 U/L) when compared to the intoxicated group. (Table 3).

As showed in table (03), lead induced a significant decrease in total plasmatic proteins compared to the control group. Whereas, after oral treatment with aniseed aqueous no significant increase in the proteins levels of Pb+ P.A.E group was observed.
increased from 10.79±1.36 µM for Control group to 17.7±2.71 µM for intoxicated group. A significant reduction of lipid peroxidation levels was observed after treatment with 71 µM for intoxicated group. A significant reduction of lipid peroxidation levels was observed after treatment with P.A.E. as compared to intoxicated group (p<0.01).

MDA is a marker of lipid peroxidation, the results showed that the level of MDA in intoxicated group was 23.71±5.94 µM in Cerebrum and 14.65±1.09 µM in Cerebellum, respectively (p<0.05). Hence, Non-Significant elevation of MDA is a marker of lipid peroxidation, the results showed that the level of MDA in intoxicated group was (16,60±1,09* 17,71±2,71**) compared to control group (14,65± 0,69 µM) respectively (p<0.05).

For the catalase activity (Table 4), we can observed that lead poisoning induced depletion in the enzyme activity from 2,82 ± 0,31 mM H2O2 (control rats) to 1,75±0.52 mM H2O2 (Pb group) in Cerebrum, and from 3,27±0.41 mM H2O2 to 1,68±0.05 mM H2O2 in cerebellum as compared to control group respectively (p<0.05). Hence , Non-Significant elevation of catalase was observed after treatment of rats with P.A.E as compared to intoxicated group (Pb), therefore the plant extract increase the level of catalase as : 2,82 ± 0,53 mM H2O2 in cerebrum and 3,27± 0.03 mM H2O2 in Cerebellum respectively (p<0.05).

## 4. Discussion

The primary site of action of lead is nervous system [12]. This heavy metal has shown demonstrable neurological effects [13] and behavioral disorders [14] including deficits in learning, spatial memory and motor skills. Previous data suggested that the neurotoxic effects of lead are mediated through interference with cholinergic and amnergic system [6]. Hence, oxidative stress has been also proposed to be another mechanism involved in the Pb toxicity. Moreover, earlier exposure to lead through placenta and lactation is more dangerous because Blood-Brain –Barrier (BBB) is still immature and it is highly permeable during the first phase of brain development. In fact, several studies have demonstrated that neuronal development start from fetal stage and continue until adolescence [15].

### Alkaline Phosphatase (ALP), Lipid Peroxidation and Catalase Activity

The results of the study (Table 4) showed that lead intoxication caused a significant increase in the ALP Level from 46,29± 14,88 U/L (Control group) to 58,5 ± 22,06 U/L (Pb group) in Cerebrum and from 23,71 ± 5.94 U/L (Control group) to 27,54± 4,75 U/L (Pb group) in Cerebellum. However, we had observed a non-significant decrease in ALP level after treatment with aniseed aqueous extract in both cerebrum and cerebellum respectively as compared to intoxicated group : 57,12± 18,63 U/L (Cerebrum) and 22,79 ± 6,99 U/L (Cerebellum).

| Group   | LDH (U/L) | Proteins (g/dl) |
|---------|-----------|-----------------|
| Group C | 801.95±95 | 71,32±3,90      |
| Group Pb| 1348.21±21** | 58,24±7,21**     |
| Group Pb+ P.A.E | 1500.27±391 | 43,12±4,13     |

(*) p<0.05 (**) p<0.01

| Level of ALP (U/L) | Lipid peroxidation (µM of MDA/Mg of proteins tissue) | Catalase (mM H2O2/min/mg of proteins) |
|--------------------|-----------------------------------------------------|---------------------------------------|
| Cerebrum | Cerebellum | Cerebrum | Cerebellum | Cerebrum | Cerebellum |
| Group C | 46,29±14,88 | 23,71±5,94 | 14,65±0,69 | 10,97±1,36 | 2,82±0,31 | 3,27±0,41 |
| Group Pb | 58,5±22,06* | 27,54±4,75* | 16,60±1,09* | 17,71±2,71** | 1,75±0,52 * | 1,68±0,05* |
| Group Pb+ P.A.E | 57,12±18,63 | 22,79±6,99 | 15,33±1,11* | 8,00±1,37 ** | 2,82 ± 0,53 | 3,27±0,41 |

(*) p<0.05 (**) p<0.01

### Body and BrainWeights

Our results showed that lead exposure during gestation and lactation caused an increase in the body weight of the pups but many others studies have demonstrated that lead caused a decrease in the body weights [8], [16]. In contrary to this data and in accordance with our finding, two studies have previously reported higher body weight in offspring developmentally exposed to low level of lead [17], [18]. Refereeing to [18] and its team have proposed a possible compensatory response to high toxic insult , refereeing to [17] suggested a number of mechanisms , such as lead (Pb) induced polymorphisms in Vitamin D receptors , interference with endocrine signaling pathways and altered hypothalamic – pituitary –adrenal axis (HPA) to explain high weight gain . In agreement to this data [19] have studied the effect of low level chronic exposure to lead on physical development of Children in Boston and concluded that early Pb exposure can lead to obesity that persist in adulthood. Besides, lead caused a significant decrease in the weight of Cerebrum and Cerebellum; this effect could result of high vulnerability of cerebral cortex towards various toxic.

Treatement with *Pimpinella anisum* L aqueous extract (P.A.E) at 750 mg/kg for 15 days increased significantly the body weight. This effect may be attributed to the bio-actives compoundes of aniseed such as Anethole, Eugenol, Anisaldehyde, Estragol and Methylchavicol, which have a
particular stimulant effect on digestive system, cause aniseed oil and its main compound in *Pimpinella anisum L.* affected pathogen micro-organism in the digestive system and showed increasing effect on weight gain and feed conversion. It was reported that anise oil affected positively the digestibility of nutrient [20]. Indeed, aniseed is rich in nutritive compounds which can have a positive effect on body weight gain like: proteins 18%, Fatty oil 8-23%, essential oil 2-7%, sugars 3-5% and crude fiber 12-15% [21].

**Open Field (OF)**

The current finding showed that exposure to lead during gestation and lactation affected the locomotor activity in the Open Filed test. Further, lead caused a significant hyperactivity, which was in accordance with lot of studies [8], [22]. Many suggestions have been proposed to explain this effect, some of this relied the change in the open field behavior to the alteration of dopaminergic, serotonergic and cholinergic systems [14]. Hence, lead caused a direct inhibition of acetylcholine during brain development. Moreover, alterations caused by this toxic element on different neurotransmitters in prefrontal cortex are involved in the hyperactivity recorded during open field test. The cholinergic system is responsible for neurobehavioral manifestations during lead poisoning which result most often by a locomotive and cognitive dysfunctions in animals. This effect was clearly reflected by the hyperactivity recorded in the Open Field test.

In another hand, lead caused anxiety, which was translated by the high number of visits in central squares; this can be considered as a good measurement for anxiety behavior cause rodents seems to prefer the corners and avoids the center of the apparatus. In addition, the high number of rearing recorded in this test was considered also as indicator for anxiety [25]. Lead caused an anxiety like behavior, this effect may have a link with the hippocampal serotonin and dopamine neurons. These systems are involved in the regulation of Corticotropin releasing factor (CRF) which play a key role in anxiety behavior. Hence, lead caused a direct inhibition of acetylcholine during brain development. Moreover, alterations caused by this toxic element on different neurotransmitters in prefrontal cortex are involved in the hyperactivity recorded during open field test.

**Forced Swimming Test (FST) and Dark /Light Test (DLT)**

In FST, immobility time was higher in exposed rats to lead compared with the controls. This result is in accordance with the study of [37]. The decrease in serum total proteins was attributed to both hepatic and renal damage induced by this toxic agent, hence this effect may be due also to the binding of lead ions to plasmatic proteins, where its caused alteration in high number of enzymes and can also disturb proteins synthesis in hepatocytes. Moreover, the decreasing in the proteins levels may be explained by the decrease of hepatic DNA and RNA induced by lead intoxication or due to diminution of utilization of free amino acids for proteins synthesis [38]. Pb exposure caused an increase in the LDH levels, which was in agreement with the studies of [37], [22]. This effect may be produced by the damage of red blood cells (RBC) membrane, hence this augmentation may be attributed also to the alterations of redox status, and however accumulation of free radicals can act as stimulant of LDH release.

Alkaline phosphatase play an important role in transmission and development of brain, hence the function of this enzyme are more active in certain part of brain such as...
primary air and frontal [39]. Our results indicated that lead exposure during development induced a significant increase of ALP levels in both cerebrum and cerebellum which was in agreement with others studies[37], [22]. The increase in PAL values may be attributed to the damage of Kidney , liver and bone following lead exposure . Moreover, it has been reported that the leakage of ALP activity was associated to the deficit in mineralization [9].

The most probable mechanism for the lead toxicity was through inducing oxidative stress in different cells whereas many studies have reported that Pimpinella anisum exhibited a potential antioxidant activity [40], [4]. In fact , several studies have confirmed that P. anisum L possess a significant reducing power which can serve as an indicator of its potential antioxidant propriety. The compounds that are responsible of antioxidant activity in aniseed: Caffeic acid, Camphene, Chlorogenic acid, Rutin and Stigmasterol. Some of them reduce radicals by donating hydrogen atoms [40].

**Lipid Peroxidation and Catalase Activity**

The most widely test used for the oxidative stress is the measurement of MDA, a product of lipid peroxidation by the Thiobarbituric Acid Reacting Substance Assay. Ours results showed that lipid peroxidation level was increased in both Cerebrum and Cerebellum after an exposure to lead acetate during gestation and lactation which was in accordance with the study of[41]. The high levels of MDA recorded in brain of intoxicated rats may be explained by alteration of lipid membranes attributed to lead which consequently caused an alteration in membranes integrity , permeability and functions[42]; these data correlate with the sensitivity of brain to lead intoxication during development until Blood-Brain –Barrier (BBB) is fully functional[43]. It has been reported that lead is a potential agent for inducing oxidative stress by production of ROS. The concentration of ROS in the organism is controlled by several defense mechanisms, which involved antioxidant and detoxifying enzymes [44]. Therefore, the neurotoxic effect of lead may be due in part to the disruption of pro-oxidant and anti-oxidant balance. Ours results showed that treatment with P. anisum aqueous extract decreased the level of lipid peroxidation in brain of intoxicated rats, which agree with the finding of [40]. This effect could be attributed to the Phyto-chemicals radicals scavenging present in the plant. Hence, [45] through their study on P. anisum extract confirmed that polyphenols are efficient in preventing lipid peroxidation. Polyphenols represent a group of bioactive compounds that occur in almost plants. Several studies have reported that polyphenols presents in the seeds of P. anisum L donate electron and react with free radical to convert them to more stable products . Thus, allow to make equilibrium again between pro-oxidant/antioxidant balances, which reduced the lipid peroxidation.

Catalase activity was significantly decreased after lead exposure during gestation and lactation, which was in agreement with [46]. This diminution of enzyme levels in both cerebrum and cerebellum can be attributed to the reduction of iron absorption or to the inhibition of Hem biosynthesis. Moreover, the decrease of catalase activity could be the result of decrease in substrate levels [47] or to the reduced synthesis of the enzyme, it’s self as a result of high intra – Cellular concentration of lead [22]. The oral administration of aniseed aqueous extract enhanced the activity of catalase in both cerebrum and cerebellum. It has been suggested that bio- actives constituents of aniseed are responsible of this effect, because they play a role of radical scavenging by donating hydrogen atoms or by acting as chain breaking agents in lipid peroxidation. According to[4]: P. anisum aqueous extract exhibited a potential anti-oxidant activity which was attributed to various mechanisms such as: prevention of chain interaction , binding of transition metal ion catalysts , decomposition of peroxides , prevention of continued hydrogen abstraction , reductive capacity and radicals scavenging [48], [49]. Moreover, this activity is attributed to the presence of some bioactive compounds such as : Caffeic acid , Camphene, Chlorogenic acid, Rutin and Stigmasterol which reduced radicals (like H2O2) by donating hydrogen atom thus leading to the improvement of catalase activity exhausted after exposure to lead acetate.

**5. Conclusions**

From this data, we can conclude that P. anisum L aqueous extract may have a possible beneficial effect against neurological disorders caused by lead intoxication especially during development. This effect could be due to the anti-oxidant potential of aniseeds, which was related to their content of bio-actives molecules such as Flavonoids and phenolic or due to the presence of Anethole, which is structurally related to Catecholamine, Adrenaline, Noradrenaline and Dopamine. Further, more studies have to be conducted to know the compound(s) is (are) responsible for the neuro-beneficial effect of aniseed and which mechanism (s) is (are) involved.

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**Declaration of Interest**

No financial or personal conflict, were recorded between the authors of this document during the realization of this work.
Abbreviation

P.A.E: *Pimpinella anisum* aqueous extract, Pb: Lead, OF: Open Filed; FST: Forced swimming test; EO: Essential oil, ALP: Alkaline phosphatase, TBARS: Thiobarbituric assay, CAT: Catalase, LDH: Lactate dehydrogenase, NMDA: N-methyl-D-aspartate,

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