

EFFECT OF *PIMPINELLA ANISUM L.* ON HISTOLOGICAL AND BIOCHEMICAL DAMAGE IN CEREBRUM AND CEREBELLUM OF YOUNG RATS INTOXICATED BY LEAD ACETATE.

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Abstract

Lead is non-essential element for human body; hence, earlier exposure to this metal induced deleterious effects especially on brain of newborn and child. *Pimpinella anisum L.*, is an annual herb that has been investigated for its neuroprotective effect. The aim of this study is to determine either oral administration of *Pimpinella anisum L.*, extract can correct the damages caused by lead exposure. Rats were exposed to 0.2% of lead in drinking water during gestation, lactation and weaning. Treatment with *Pimpinella anisum L.*, aqueous extract was at 03 different doses: 250 mg/kg, 500 mg/kg and 750 mg/kg for 15 days. The results showed that lead induced a significant increase in the level of phosphatase acide ($p < 0.05$) and a non-significant increase of hydroperoxides concentration in both cerebrum and cerebellum. Histological damages were observed in the brain of intoxicated rats, which were more remarkable in cerebrum than cerebellum. Oral administration of *Pimpinella anisum L.*, aqueous extract had decreased non-significantly the level of biochemical parameters with no changes in the tissular architecture of cerebrum and cerebellum when compared to the control group. It can be conclude that *Pimpinella anisum L.*, can possess a beneficial effect on neurotoxicity induced by lead exposure.

Key words: *Pimpinella anisum L.*, Lead, Cerebrum, Cerebellum, Phosphatase acide and Hydroperoxydes.

Introduction

Pimpinella anisum L., (Aniseed) is an annual herb that belong to the family of Apiaceae and it is largely cultivated all around the world especially in warm region [1]. This plant has been used since the past as condiment for food preparation [2] and as treatment for many diseases such as: dyspnea, bloating, common cold, colic and for gastro intestinal disorders [3]. Moreover, aniseed has been used also for its anti-inflammatory, appetizing, hypotonic and Hepatoprotective activities [4]. This same plant has been investigated for its beneficial effect on nervous system [5,6], whereas in traditional medicine *Pimpinella anisum L* is largely recommended for neurological disorders such as : depression and epilepsy [7].

Lead is very known to induce neurological impairment especially in earlier life exposure and consequently disturbs the whole brain by interference directly or non-directly with the aminergic and cholinergic system [8]. By crossing the Blood Brain Barrier (BBB), lead can easily damage cellular processes, neurons development and arrangement of tissues in both cerebrum and cerebellum [9].

The deleterious effects of lead on animal and human brain with their different part is well documented, but until now, no specific treatment was effective in removing or correcting the damage induced by earlier exposure to this heavy metal. For all this reason, researches are moving towards find some new natural sources of healing with lower side effects and costs.

This study was conducted in the aim of determine the possible beneficial effect of an oral administration of *Pimpinella anisum L.*, aqueous extract on histopathological and biochemical damages induced by lead on the cerebrum and cerebellum of young rats intoxicated through uterine life.

Material and Methods:

Plant Material

Dry and ripe seeds were purchased from a local herbal market in Chlef region (Algeria) then they were grounded to obtain a powder. Preparation of aqueous extract was done by immersing 100 g of powder in 1L of distilled water, the mixture was boiled for 15 minutes after that it was filtered and lyophilized [10]. The extraction yield was 20, 99%.

Animal study:

Rats of 21 days (n=40) were used to conduct this

study; they were housed in standard cages with free access to food and water. 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**).

At weaning rats were divided 05 groups as follow: [11, 12].

Group C: Control rats that received distilled water as vehicle solution.

Group Pb: Intoxicated rats with 0.2% of lead acetate in drinking water for 42 days (issued from intoxicated females).

Group Pb+ P.A.E 250: Intoxicated rats with 0.2% of lead acetate in drinking water for 42 days (issued from intoxicated females). They received 250 mg/kg of *Pimpinella anisum L.*, (P.A.E) for aqueous extract for 15 day by oral gavage.

Group Pb+ P.A.E. 500: Intoxicated rats with 0.2% of lead acetate in drinking water for 42 days (issued from intoxicated females). They received 500 mg/kg of *Pimpinella anisum L.*, (P.A.E) for aqueous extract for 15 day by oral gavage.

Group Pb+ P.A.E. 750: Intoxicated rats with 0.2% of lead acetate in drinking water for 42 days (issued from intoxicated females). They received 750 mg/kg of *Pimpinella anisum L.*, (P.A.E) for aqueous extract for 15 day by oral gavage.

Sacrifice and Biochemical analysis

After achievement of experimental protocol, fasted rats were sacrificed in the morning. Hence, brain was rinsed *in situ* with ice saline solution (0, 9%) before being divided into two part: cerebrum and cerebellum. Cerebrum and cerebellum were homogenized in ice phosphate buffer solution, the supernatant were used to assess the phosphatase activity (Commercial kits, CHRONOLAB), and hydroperoxides levels by colorimetric method [13].

Histopathological study

Samples taken from the brain (cerebrum and cerebellum) of the studied rats in different groups were fixed in formalin (10%). After that, they were washed under tap water then introduced in bath containing serial dilutions of graduated alcohol (methyl, ethyl and absolute ethyl) which are used for dehydration. Samples were cleared in xylene and embedded in liquid paraffin at 56°C. Next, sections of 4 µm of thickness were cut, deparaffinized and stained with Hematoxylin /Eosin stains for histopathological examination under light microscope.

Statistical analysis

All results were expressed as mean \pm S.E.M (Standard of Error). The data analysis was carried out by using statistical software: R (2010). Kruskal Wallis rank test and the Wilcoxon rank sum test were used to examine the level of significance between groups. Value of $p < 0.01$ and $p < 0.05$ were taken as significant.

Results

Biochemical parameters

The results of the biochemical analysis are represented in the table 01. We noted that lead exposure caused a significant increase in the Phosphatase acide level in cerebrum and cerebellum when compared to the control group. Hence, the oral treatment with aniseed aqueous extract at dose of 250mg/kg and 750 mg/kg has reduced non-significantly the PAC level in cerebrum and cerebellum respectively. The concentration of hydroperoxides was non-significantly increased in Pb group when compared with Control group in both cerebrum and cerebellum. Whereas the administration of *Pimpinella anisum L.*, aqueous extract for 15 days and with three doses decreased non-significantly the concentration of hydroperoxides in cerebrum and cerebellum respectively

Histopathological study

Cerebrum

The brain histology of control rats showed well-developed neurons, no vascular damage or hemorrhages were observed (Figure 01). whereas the brain of intoxicated rats with 0.2% of lead revealed some changes in the structure such as inflammatory reactions and cells were bigger in size with large vascular spaces around them (Figure 02). The intoxicated groups with lead (0.2%) and then were treated by aniseed aqueous extract for 15 days at 250 mg/kg, 500 mg/kg and 750 mg/kg respectively, showed some improvement in the general structure of cells but vacuolization still persist.(Figure 03, 04 and 05 respectively).

Cerebellum

Light microscope examination of cerebellum sections of control rats, stained with Hematoxylin-Eosin showed a normal structure with no particularity and without lesion or damage (Figure 06). Whereas in intoxicated group with lead (0.2%), histology of cerebellum was pale with the presence of eosinophil with no notable lesion (Figure 07). Treatment of intoxicated rats with aqueous

extract of *Pimpinella anisum L.*, at the three doses (250 mg/kg, 500 mg/kg and 750 mg/kg) showed a similar structure of those observed in control group with no inflammatory processes or frankly damage (Figure 08, 09 and 10 respectively).

Discussion

Exposure to lead at developmental stage has induced an elevation in the concentration of phosphatase acid (PAC) in brain-studied regions (cerebrum and cerebellum); our findings were in accordance with previous studies [14, 15, 16]. This effect is attributed to the destruction of cellular membranes, which leads to the augmentation of permeability and/or cells necrosis. Moreover, we recorded an increase in the level of hydroperoxides in both cerebrum and cerebellum after lead intoxication. This result was in agreement with the finding of [17, 18]. This augmentation in hydroperoxides level is attributed to the generation of reactive oxygen species by lead [19]. In fact, exposure to lead acetate can affect cytoplasmic and mitochondrial membranes, which contribute to the liberation of hydroperoxides in circulation [20].

The non-significant effect of *Pimpinella anisum L.*, aqueous extract on PAC and hydroperoxides levels may be attributed to its antioxidant proprieties [21]. In our previous work, we had demonstrated that *Pimpinella anisum L.*, reduced the lipid peroxidation and enhanced catalase activity [22]. The possible suggested mechanism is that aniseed extract contain bioactive compounds, which act as a chelating and scavenging agent of free radicals and reactive oxygen species. The developmental stage of different brain regions determine the extent of lead impairment. In rats, neurogenesis of some areas of brain is completed or nearly completed before day fifteen. While the cerebellum is under development until postnatal day 20 [8]. It has been reported that lead considered to be a neurodevelopmental teratogen and caused neurobehavioral deficits. This complication could be attributed to the immaturity of the Blood Brain Barrier (BBB) [23]. Morphological changes in cerebellar tissue are supported by previous neuropathology finding which showed changes in the cerebral cortex following lead exposure [24, 25]. The histology of Brain (cerebrum and cerebellum) was altered after lead exposure during gestation and lactation which was in agreement with the study of [26]. This effect may be related to the fact that ions of lead (Pb) bond with sulfhydryl (SH) group in the membranes and damage them through lipid peroxidation. Moreover, heavy metals such as lead labialize lysosomal

membranes, inhibit protein synthesis, affects structures and synthesis of RNA and DNA, beside they disturb structure and function of mitochondrial membrane [27]. These mechanisms are involved in the neurotoxicity and histopathological impairment caused after lead exposure.

Administration of *Pimpinella anisum L.*, aqueous extract after lead exposure and for duration of 15 days was effective in improving the histopathological impairments caused by intoxication of lead. This effect can be attributed to the antioxidant proprieties of the studied plant, further many studies have reported the antioxidant potential of *Pimpinella anisum L.*, [28].

Conclusion

From this study, we can conclude that *Pimpinella anisum L.*, aqueous extract may have a beneficial effect on biochemical and histological damages induced by earlier exposure to lead, and hence this effect is mainly attributed to its antioxidant activity and its bioactive compounds.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

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Table 1. Effect of lead (Pb) and *Pimpinella anisum* L aqueous extract (P.A.E) on the levels of biochemical parameters in cerebrum and cerebellum.

| | Level of Phosphatase acide (U/L) | | Concentration of hydroperoxides (nmol/l) | |
|----------------------------|----------------------------------|-------------|--|------------|
| | Cerebrum | Cerebellum | Cerebrum | Cerebellum |
| Group C | 25.75±4.41 | 27.21±4.15 | 0,61±0,01 | 0,78±0,37 |
| Group Pb | 28.76±4.45* | 32.12±3.92* | 1,14±0,28 | 0,86±0,21 |
| Group Pb+ P.A.E 250 | 29.34±6.33 | 38.93±7.08 | 0,71±0,26 | 0,72±0,37 |
| Group Pb+ P.A.E 500 | 48.05±6.4 | 52.92±0.63 | 0,69± 0,14 | 1,01± 0,05 |
| Group +P.A.E 750 | 42.19±3.27 | 31.49±1.81 | 0,82±0,07 | 0,93±0,93 |

(*)<0.05.

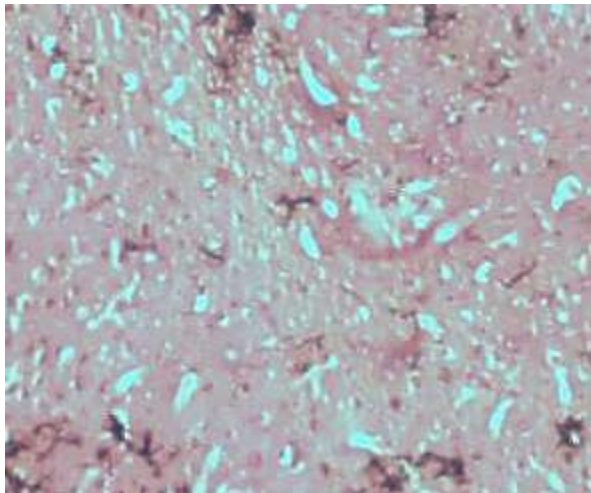


Figure 1. Brain section of control rats (group C) stained by hematoxylin eosin observed under light microscope (40×10)

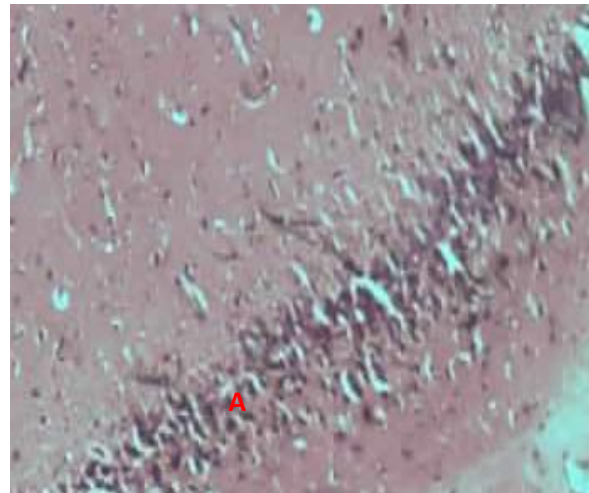


Figure 2. Brain section of intoxicated rats (group Pb) stained by hematoxylin eosin observed under light microscope (40×10). (A) Pyramidal cells with big size

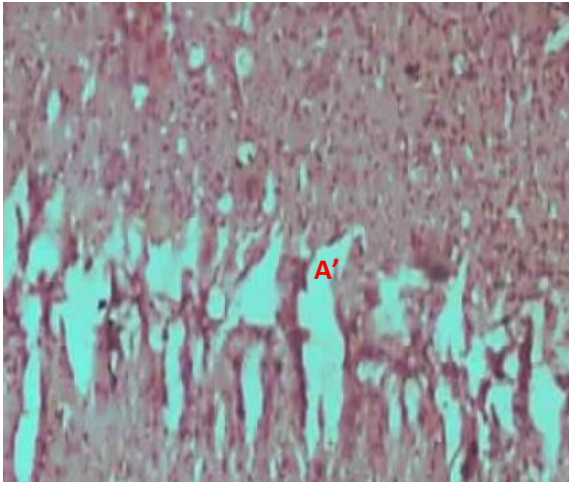


Figure 3. Brain section of intoxicated rats with Pb and treated by *P. anisum L* aqueous extract for 15 days (group Pb +250), stained by hematoxylin eosin observed under light microscope (40×10).

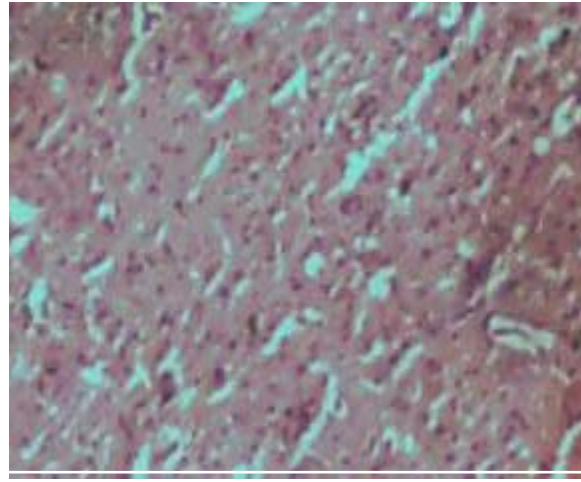


Figure 4. Brain section of intoxicated rats with Pb and treated by *P. anisum L* aqueous extract for 15 days (group Pb+ P.A.E. 500), stained by hematoxylin eosin observed under light microscope (40×10).

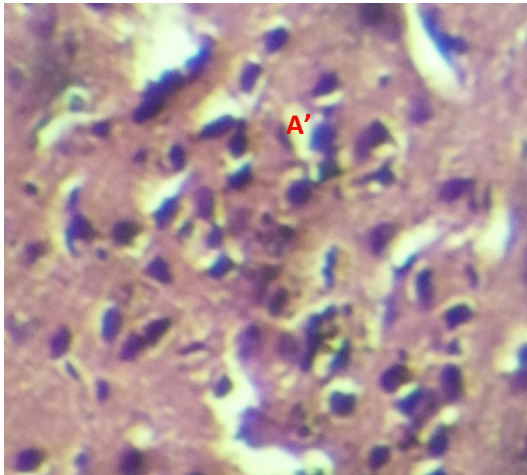


Figure 5. Brain section of intoxicated rats with Pb and treated by *P. anisum L* aqueous extract for 15 days (group Pb+ 750), stained by hematoxylin eosin observed under light microscope (40×10). **A'**: vacuolization cells.

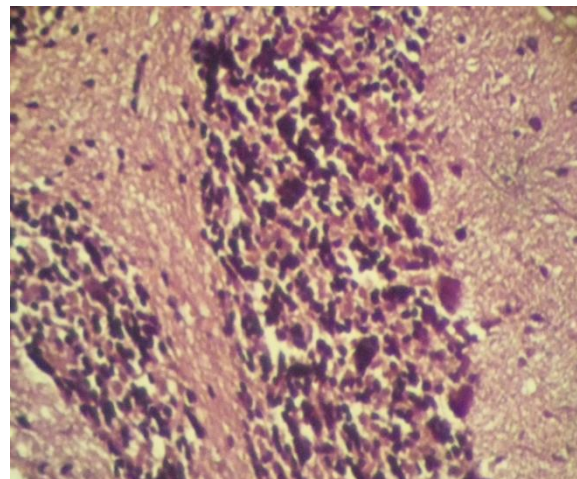


Figure 6. section of cerebellum of control rats (Group C) stained by hematoxylin eosin and observed under light microscope (40×10).

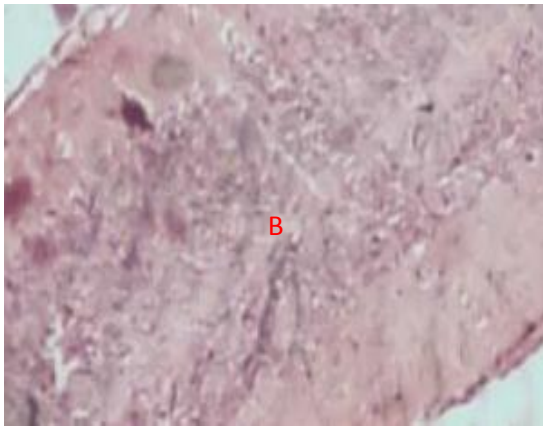


Figure 7. section of cerebellum of intoxicated rats (Group Pb) stained by hematoxylin eosin and observed under light microscope (40×10). **(B)** Molecular layer in cerebellum, which is pale

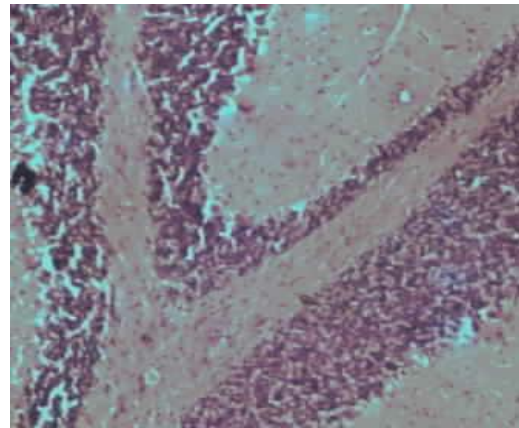


Figure 8. section of cerebellum of intoxicated rats (Group Pb+ 500) and treated with *P.anisum L* aqueous extract for 15 days. Stained by hematoxylin eosin and observed under light microscope (40×10).

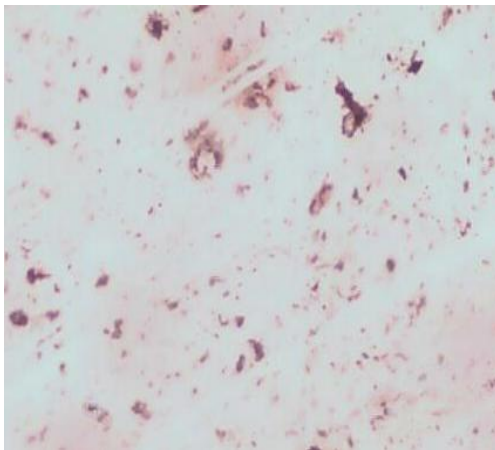


Figure 9. section of cerebellum of intoxicated rats (Group Pb+ P.A.E 500) and treated with *P.anisum L* aqueous extract for 15 days. Stained by hematoxylin eosin and observed under light microscope (40×10).

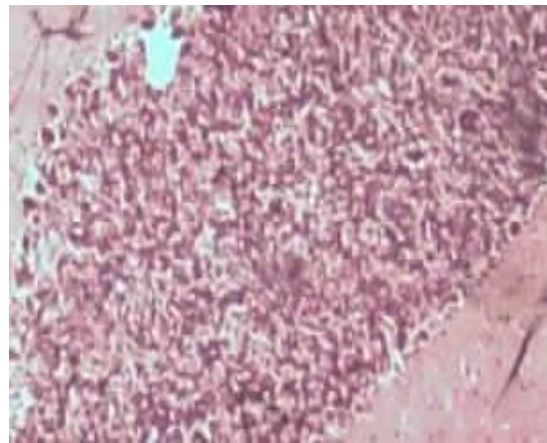


Figure 10. Fig. 10: section of cerebellum of intoxicated rats (Group Pb+ P.A.E 750) and treated with *P.anisum L* aqueous extract for 15 days. Stained by hematoxylin eosin and observed under light microscope (40×10).