A Comparative Study between the Role of Stem Cells and Flax Seeds Oil Against Neurotoxicity Induced by Lead Acetate in Brain Tissue of Male Albino Rats: Histological Study

Original Article

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ABSTRACT

Introduction: Lead is an industrial pollutant and has hazardous effects on the brain. Lead induces oxidative stress, inhibition of antioxidant activity, and damage to neurons. Mesenchymal stem cells (MSCs) are important cells that have ability to renew and convert into other cell types. MSCs release antioxidant and anti-inflammatory agents. Flax seeds oil (FSO) is a vital source of essential nutrients for the brain. Also, FSO is used as a natural antioxidant and a source of omega3.

Aim of the Work: The present study was conducted to evaluate the ameliorative role of stem cells and flax seeds oil on the brains of lead-intoxicated rats and restitution of damaged brain tissues.

Material and Methods: The current experiment used 40 male Albino rats that weight about 250–280 g, and were equally divided into four groups (10 rats/group) as follows: Group 1, rats were used as normal. Group 2, rats intoxicated with lead acetate (100 mg/kg) intraperitoneally. Group3, rats treated with mesenchymal stem cells (1×10^6 cells/rat intravenously injection). Group 4, rats administrated orally with flax seeds oil (1 ml/kg). At the end of the experiment, we collected blood and brain tissues for biochemical examinations and histopathology.

Results: Elevation of blood lead levels, reduction in neurotransmitters such as serotonin, norepinephrine, dopamine, and antioxidant activities (CAT and SOD) in brain tissue also an increase in MDA, cognitive defects, necrosis, congestion in the blood vessels, vaculation in brain tissue of the lead intoxication group, but treatment of stem cells showed marked improvement compared to flax seeds oil which revealed moderate improvement in brain tissue in all parameters.

Conclusion: The experiment revealed effectiveness of MSCs and FSO against the toxic effect of lead on brain tissue and ability of MSCs and FSO to repair damaged brain tissue. MSCs treatment was more effective than FSO treatment.

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Key Words: Brain, flax seeds oil, lead, mesenchymal stem cells, oxidative stress.

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INTRODUCTION

The nervous system is essential for practically all mammalian processes, including cognitive activity, movement, perception, and homeostasis[1]. Lead (Pb) is a ubiquitous metal that was one of the first metals to be discovered by humans. Unique features of lead, such as softness, low melting point, ductility, high malleability, and corrosion resistance, lead is widespread use in various industries, such as paint, autos, plastics, ceramics, etc. This has brought about a multifold increase in the presence of free lead in the inanimate environment and biological systems[2].

In terms of the number of persons who have been exposed to lead and the general public's health can be estimated, lead is one of the greatest environmental medicine concerns. Environmental contamination caused by lead includes industrial lead production and metal recycling. Lead poisoning often called colica pictonium or plumbism is a condition caused by high levels of heavy metal lead in the body. Pain, paranesthesia, muscle weakness, gastrointestinal symptoms, and weight loss are the major clinical manifestations of subacute lead poisoning. Lead is transmitted to humans and animals via the food chain.Lead toxicity is determined by the chemical form given to the animal, the method of administration, and the duration and frequency of administration[3].

The brain is the organ that is most vulnerable to lead poisoning. Lead has a significant impact on synapse formation in a child's developing cerebral cortex. Lead also inhibits the production of neurochemicals, such as neurotransmitters, and the structuring of ion channels. The myelin layer of neurons is also lost as a result of lead poisoning, inhibits neuronal development, and interferes with neurotransmission[4,5].

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Mesenchymal stem cells resemble an adhering population of fibroblasts within the stroma of bone marrow. In addition to neuroectodermal-derived lineages, chondrocytes, Adipocytes, myoblasts, and osteoblasts are mesoderm-derived lineages that cells can differentiate into many cells[6,7].

MSCs can be generated from a variety of sources, including placental tissue, amniotic fluid, cord blood, adipose tissue, and tooth pulp. Nonetheless, bone marrow aspiration remains the preferred source of MSCs in the majority of laboratories[8].

In the medical world, stem cell therapy is gaining popularity as a treatment for neurological diseases. Stem cells can divide and develop in a variety of ways due to their ability to self-renew[11]. Stem cells are also capable of differentiating into Types of specialized cells. These characteristics make stem cells a potential therapy option for Alzheimer's disease by promoting the growth and repair of damaged brain structures[9-10].

Several scientific research suggests that flax seed oil can help avoid a variety of debilitating diseases (cancer, mental disorders, cardiovascular diseases, and diabetes)[11].

Using flax seeds for oil extraction offers several health advantages, as it reduces the risk of chronic illnesses. Because its edible oil is high in omega-3 fatty acid Alpha-Linolenic Acid, lignans, proteins, vitamins, minerals, etc., it is considered a superfood, flax seed oil has achieved immense popularity. As a result of its omega-3 fatty acid concentration, it is used to minimize the risk of a variety of cardiovascular disorders. Flaxseed offers potential health advantages in addition to its nutritional value[14]. Numerous research has been conducted to enhance its oxidative stability, which is essential for the manufacture of food items[12].

By evaluating neurotransmitters, antioxidant activities, oxidative stress indicators, and histological abnormalities, this study intended to demonstrate the ameliorative role of bone marrow-mesenchymal stem cells and flax seeds oil against the toxicity of lead in the brain tissues of male Albino rats.

MATERIAL AND METHODS

Experimental design

Forty adult Wistar (white male Albino) rats aged between 2 and 3 months old and weighing about 250–280 g were included in this study. Rats were divided into 4 groups (each group has ten rats) as follows: Group (1) (control group): Rats received dist. Water.

Group (2) (lead intoxication): rats were intraperitoneally injected with lead acetate dissolved in distilled water at 100 mg/kg b.w.t for seven days[13].

Group (3) (lead+ MSCs): rats were intraperitoneally injected with lead acetate (100 mg/kg b.w.t) for seven days and after 24 hours, a single dosage of mesenchymal stem cells was given (1×10^6 cells/rat intravenously injection)[14] then left for 31 days.

Group (4) (lead + FSO): rats were intraperitoneally injected with lead acetate (100 mg/kg b.w.t for seven days) and after 24 hours, rats were administered orally with flax seeds oil (1 ml/kg b.w.t for 30 days)[15] then left one day.

Group (5): There were five Wistar rats used as the source for extraction MSCs only.

Rats were placed at the animal house of South Valley University's Faculty of Science in Qena, Egypt, and kept at room temperature (23±2oC), with a 12-hour light/dark cycle, and given a balanced commercial diet. For drinking, tap water was provided ad libitum.

At the end of the study, all rats were sacrificed by using a suitable dose of ethyl ether. And the blood was collected from retro-orbital in clean EDTA tubes to determine the lead level in the blood. After dissection, we extracted brain tissues, and one part put them on ice immediately, and then transferred in liquid nitrogen to our facility, where they were instantly frozen at -80°C until biochemical examination, then the other part was fixed in a fixative (natural buffer fermol 10%) for 24 hours. Then it was kept in 70% ethyl alcohol for histological examination.

Preparation, isolation, and culture of bone marrow-derived mesenchymal stem cells

The bone marrow of five Wistar male rats aged 2-3 months old was extracted from their femurs. The removed bone marrows were incubated for cell culture in Dulbecco's modified Eagle's medium-filled 25 cm^2 flasks (DMEM, In*vitro*gen, Carlsbad, CA). Incubations of 15 minutes at 37 °C would be carried out in a water bath while the flasks were shaken at 120 r/min for ten and fifteen minutes later, respectively. The flasks were forcefully stirred for 10 seconds, and then the contents were filtered through a nylon screen with a pore size of 250 m in order to capture any residual undetected tissue. The suspension of cells was centrifuged at about 300 g for three minutes. After achieving a homogenous cell suspension, the suspended cells were centrifuged at 1200 rpm for seven minutes, and 3 ml of cultured media was added to the cell pellets and filtered. Cells were incubated in 25 cm^2 flasks with 5 ml of DMEM at 37 °C in a humidified 5% CO air. Every two days, the culture media was changed. The confluence of the cells reached roughly 90 percent. The mesenchyme group was segregated based on its ability to stick to the flask's bottom, and MSCs were seen using an inverted microscope. This method was according to Huang *et al*[16] (Figure 1).

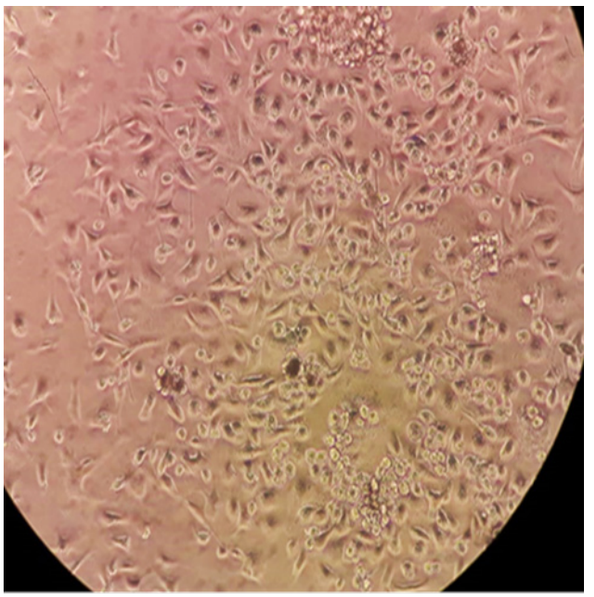

Fig. 1: Bone-marrow derived mesenchymal stem cells

Identification and characterization of bone- marrow mesenchymal stem cells by Using Flow Cytometer CD90, CD73, CD271, and CD45:

The immunophenotyping of bone marrow mesenchymal stem cells was performed with antibodies against rat antigens CD73, CD271, CD34, and CD90, and their isotope controls Bayati *et al*[17].

Chemical and Reagent

El-Gomhouria Company for Chemicals and Laboratory Supplies, located in Assiut, Egypt, provided the lead acetate.

EL Captin Company was the source of flax seed commercial oil (FSO) (Al Obour City, Cairo, Egypt).

Biochemical assay

Measurement of lead levels in the blood: lead levels in the blood were analyzed by using the lead ELISA kit (Molecular probes Tm In*vitro*gen detection technologies, CAT. NO. M36353). Following the manufacturer's instructions. The absorbance of each well was measured at a wavelength of 495/520 nm using a microplate reader.

Determination of neurotransmitters (serotonin, norepinephrine, and dopamine)

The concentration of norepinephrine in brain tissue was analyzed by using the rat norepinephrine ELISA kit (my BioSource. San Diego, California, United States, CAT. No. MBS775077. The serotonin concentration in brain tissue was analyzed by using the rat serotonin ELISA kit (my BioSource. San Diego, California, United States, CAT. No. MBS9362408. The dopamine concentration in brain tissue was analyzed by using the rat dopamine ELISA kit (Eagle biosciences, INC., United States. CAT. NO. DOU39-K01(1× 96 wells). Following the manufacturer's instructions. At a wavelength of 450 nm, a microplate reader was used to measure the absorbance of each well.

Determination of Superoxide dismutase (SOD)

The colorimetric technique was used to determine the SOD described by Nishikimi *et al*., 1972[18].

Determination of Catalase (CAT)

CAT was determined using the colorimetric technique outlined by Aebi, 1984[19].

Determination of Malondialdehyde (MDA)

The measurement of MDA was done using a technique described by Ohkawa *et al*., 1979[20].

Assessment of cognitive function by T-maze

The T-maze is a horizontally positioned T-shaped elevated or enclosed device. Animals begin at the base of the T and are permitted to select one of the target arms at the opposite end of the stem. According to Deacon and Rawlins 2006[21], we performed rewarded alternation with a T-maze, which is a simple maze being used in animal cognitive tests. We give 5 trials for each rat to choose the correct goal arms. Alteration score %= (number of correct choices/numbers of total trials) ×100

Histopathological examination

For histological studies, samples of brain tissue were fixed in neutral buffered formalin of 10% concentration with a pH of 7.2, dehydrated was done in a series of alcohols in an ascending manner, cleaned inside cedarwood oil, and embedded inside paraffin wax. After preparing paraffin slices with a thickness of 5 micrometers, the following dyes were applied: hematoxylin and eosin Harris stain[22].

The degree of severity of the detected histopathological changes in both cerebral and cerebellar tissues was expressed using a four-point scoring system (ordinal approach) as follows: typical histological architecture damage levels (-), mild (+), moderate (++), and severe (+++). The following pathological injuries on 5 slides from five different rats in each group were graded: Destruction and degeneration of neurons. The number of damaged slides and the number of affected areas on a single slide was used to determine the severity of various pathological lesions in diverse groups of rats. For various pathological damage, the individual score for each animal was obtained, and the mean score for each group was then calculated[23,24].

Statistical analysis

Means± Standard Deviation of means (Mean ± S.D) was used to express the degree of results variability. A one-way ANOVA analysis of variance was used to assess the data statistically (the Prism pad computer software, USA) then the Newman-keuls T-test, and to assess for treatment differences, the least significant difference (L.S.D.) was utilized. When the *P-value* of the results is < (0.001), they are considered statistically significant.

RESULTS

Identification and characterization of mesenchymal stem cells by using a flow cytometer

A flow cytometer was used to evaluate the expression of MSC surface markers in order to confirm their identification and purity. Antibodies were used to stain MSCs (cell suspension) specific for CD90 FITC, CD73 and CD271 for MSCs, and CD34 for hematopoietic cells.

CD 34 (Figure 2) was consistently negative in MSCs, although CD90, CD73, and CD271 (Fig.2) were all positive.

Clinical observations

We observed a change in the behavior of rats intoxicated with lead acetate when compared to normal rats. Rats intoxicated with lead were inactive, slow movement, tired, had hair loss, increased heart pulsations through the chest wall, and tended to be isolated and didn't interact with each other. Also, there was aggressive behavior noticed in the rats as they hurt each other, so we separated them into separate cages. Although we separated them into separate cages, we observed that rats hurt themselves. For example, one hurt his tail and another one hurt his eye (Figure 3).

Biochemical results

Lead levels in the blood: lead levels in groups (2), (3), and (4) indicated higher than normal group. However, lead levels in rats from groups (3) and (4) were recorded lower than in the lead intoxication group (Table1, Figure 4).

Neurotransmitters concentration in brain tissue

Serotonin, norepinephrine, and dopamine concentration revealed a significant decrease at (P<0.001) in all groups when compared with the normal group. While group 3 and group 4 showed a significantly increase at (P<0.001) in serotonin, norepinephrine, and dopamine concentration in comparison to the lead intoxication group (Table 2, Figures 5,6,7).

CAT and SOD activities in brain tissue

CAT and SOD activities in rats in groups (2), (3), and (4) showed a significantly reduction at (P<0.001) compared with normal rats. Also, groups (3) and (4) indicated a significantly elevation at (P<0.001) in CAT and SOD activities when compared with the lead intoxication group (Table 3, Figures 8,9).

Oxidative stress biomarker (MDA) in brain tissue

MAD levels of all groups showed a significantly increase at (P<0.001) as compared with the normal group. In addition, group (3) and group (4) indicated a significantly decrease at (P<0.001) in MAD levels when compared with the lead intoxication group (Table 4, Figure 10).

Assessment of cognitive function by T maze

All groups showed a significantly decrease at (p<0.001) in the number of correct choices of trials for a reward. This means a reduction of cognitive ability, short memory, and learning when compared with normal. Also, group 3, and group 4 induced a significantly increase at (p<0.001) in the number of correct choices of trials for a reward. This means an elevation of cognitive ability, short memory, and learning in comparison with rats intoxicated with lead (Table 5, Figure 11).

Histological results

In the control group (group 1), the brain tissues showed normal histological architecture cerebral tissues showed normal neurons (Figure 12.a). While cerebellum revealed centrally granular cells surrounded by molecular and purkinje cells layers. (Figure 13a).

Lead intoxication group (group 2): The brain cerebrum lesions characterized by vascular and neuronal changes were observed in most of the animals in this group. Cerebral vacuoles around the neurons with pyknotic nucleus with congested blood vessels and dilated Virchow robin spaces. In addition to, numerous microglia cells aggregation were seen around the blood vessels, (Figures 12 b,c,d). While the cerebellum revealed disruption and degenerative changes in granule cell layer neurons with pyknotic nuclei and degeneration with vacuolation in the molecular cell layer which is represented by a dark nucleus with and vacculation around nucleus (Figure 13b).

Group (3): The cerebrum of rats in this group exhibited mild necrosis appear in some cells of neurons which expressed by decreased in necrosed cells number, which appeared dark in color with a pyknotic nucleus and shrinkage of the cytoplasm, besides mild cerebral vacculation (Figure 12e). Cerebellum showed necrosed neurons with vacculation with normal arranged molecular layer and hypocellular granular layer (Figure 13c).

Group (4): The cerebrum of rats still showed large number of necrosed neurons and congestion in the blood vessels with dilated Virchow-Robin spaces in the cerebrum and is considered better than group (2) (Figure 12f). Cerebellum revealed a vacuolated and loosely arranged molecular layer and hypocellular granular layer (Figure 13d).

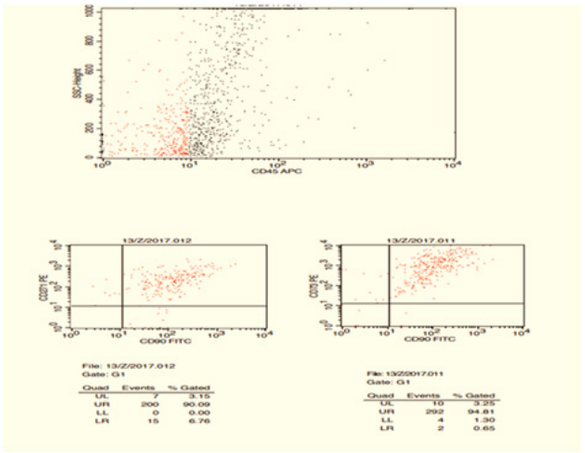

Fig. 2: diagram of flow cytometry dot plot of MSCs isolated from bone marrow, (a) shows that cells are negative for cd45 ABC (b) CD90 FITC is represented on X-axis and CD73 PE and Cd 271 PE are represented on Y-axis cells are positive for Cd90, Cd73, and CD271.

Fig. 3: The violence, seen in eye and tail injuries.

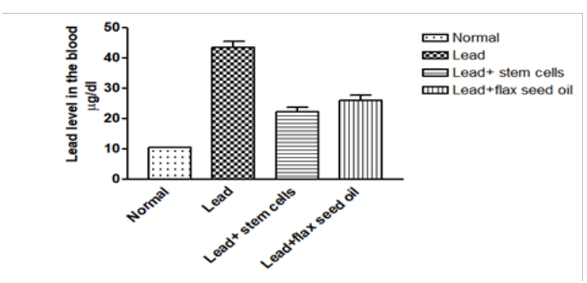

Fig. 4: Effect of mesenchymal stem cells and oral administration of flax seeds oil on the lead level in the blood of rats intoxicated with lead acerate.

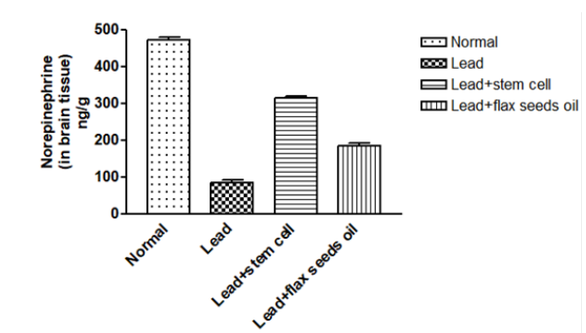

Fig. 5: Effect of mesenchymal stem cells and oral administration of flax seeds oil on norepinephrine in brain tissue of rats intoxicated with lead acetate.

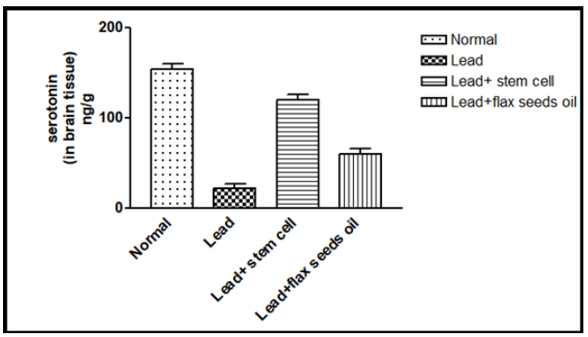

Fig. 6: Effect of mesenchymal stem cells and oral administration of flax seeds oil on serotonin in brain tissue of rats intoxicated with lead acetate.

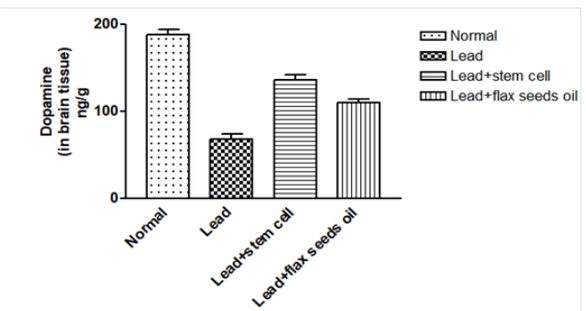

Fig. 7: Effect of mesenchymal stem cells and oral administration of flax seeds oil on dopamine in brain tissue of rats intoxicated with lead acetate.

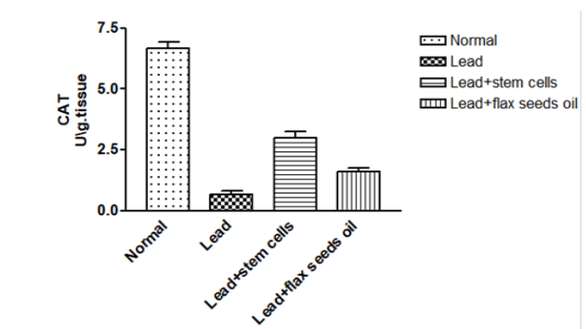

Fig. 8: Effect of mesenchymal stem cells and oral administration of flax seeds oil on catalase (CAT) in brain tissue of rats intoxicated with lead acetate.

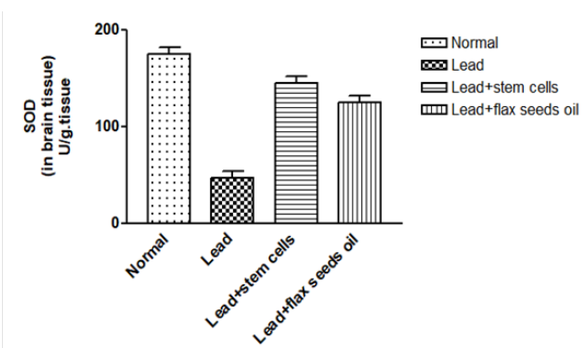

Fig. 9: Effect of mesenchymal stem cells and oral administration of flax seeds oil on superoxide dismutase (SOD) in brain tissue of rats intoxicated with lead acetate.

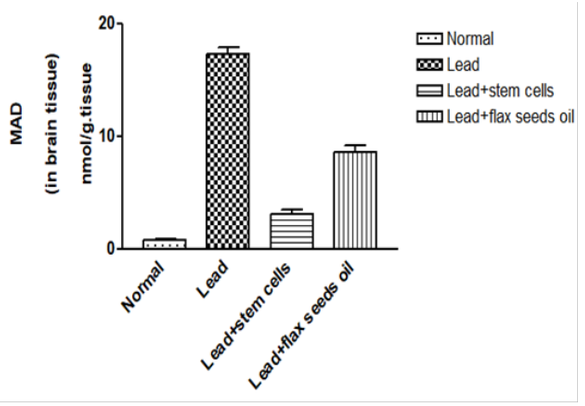

Fig. 10: Effect of mesenchymal stem cells and oral administration of flax seeds oil on Malondialdehyde (MDA) in brain tissue of male Albino rats intoxicated with lead acetate.

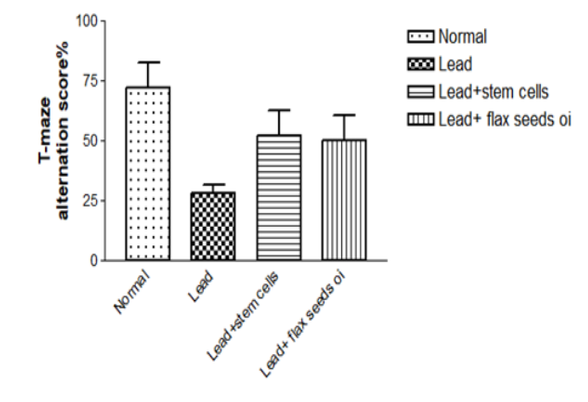

Fig. 11: Effect of stem cells and oral administration of flax seeds oil on cognitive ability by using T-maze test alternation score % of rats intoxicated with lead acetate.

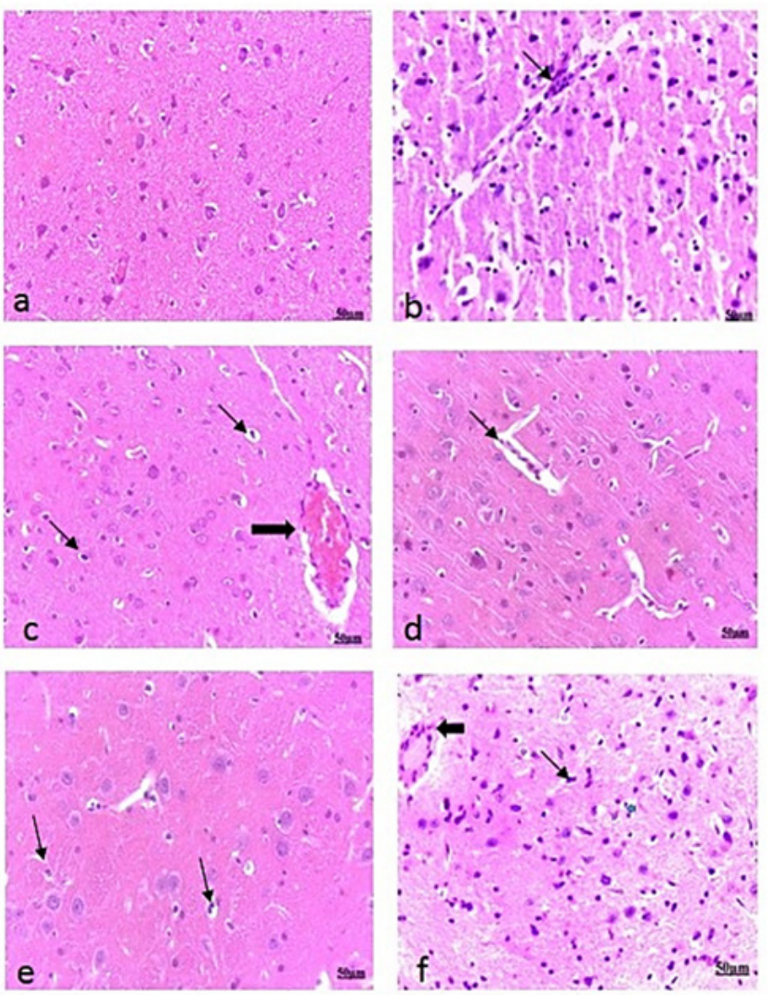

Fig. 12: Photomicrographs of H&E (bar= 50 µm) brain cerebrum sections of adult male rats of groups 1,2,3,4. (a) control group(1) showing normal brain morphology of the neurons. (b, c &d) group 2 showing numerous microglia cells were seen around the blood vessels (arrow), (c) cerebral vacuoles around the neurons with pyknotic nucleus (thin arrows) with congested blood vessels and dilated Virchow robin spaces (thick arrow). (d) Besides, vacculation around blood vessels (arrow). (e) group (3) showing few necrosed neurons which, appeared dark in color with pyknotic nucleus and shrinkage in cytoplasm, (arrows). (f) group 4 showing moderate numbers of necrosed neurons which, appeared dark in color with pyknotic nucleus and shrinkage in cytoplasm (thin arrow) and congestion in the blood vessels (thick arrow).

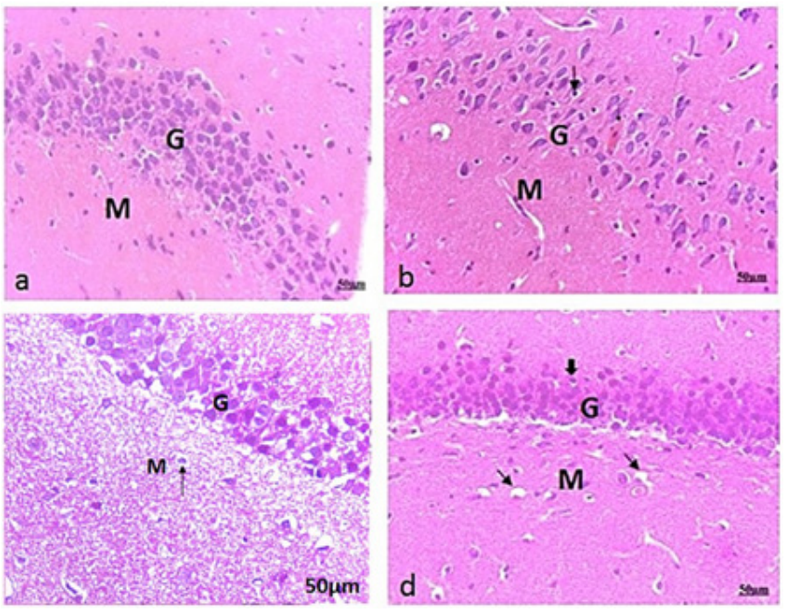

Fig. 13: Photomicrographs of H&E (bar= 50 µm) brain cerebellum sections of adult male rats of groups 1,2,3,4. (a) control group(1) rats showing normal architecture of cerebellum with granular cells surrounded by molecular and purkinje cells layers. (b) group 2 showing disruption and degenerative changes in granule cell layer neurons with pyknotic nuclei (G) and degeneration with vacuolation in the molecular cell layer (M) (arrows). (c) group 3 showing few cerebellum necrosed neurons and vacuolated cytoplasm cells. (d) group 4 showing a vacuolated and loosely arranged molecular layer and hypocellular granular layer (arrows).

Table 1: Effect of mesenchymal stem cells and oral administration of flax seeds oil on the lead level in the blood of rats intoxicated with lead acetate

Groups	Lead level in blood (µg/dl)
	Mean ± S.D.
Normal	10.40 ± 0.09
Intoxicated with lead	$43.40 \pm 1.9^{+++a}$
Intoxicatedwith lead+stem cells	$22.20 \pm 1.5^{+++a---b}$
Intoxicated with lead+flax seeds oil	$25.90 \pm 1.8^{+++a---b}$

Table 2: Effect of mesenchymal stem cells and oral administration of flax seeds oil on norepinephrine, serotonin and dopamine in brain tissue of rats intoxicated with lead acetate

Groups	norepinephrine (in brain tissue) (pg/g)	Serotonin (in brain tissue) (ng/mg)	dopamine (in brain tissue) (ng/mg)
	Mean ± S.D.	Mean ±S.D.	Mean ± S.D.
Normal	187.2 ± 6.68	154.1±5.20	470.9 ±7.22
Intoxicated with lead	$67.2 \pm 6.89^{---a}$	$21.5 \pm 5.34^{---a}$	$84.9 \pm 6.70^{---a}$
Intoxicatedwith lead+stem cells	$135.7 \pm 5.95^{---a+++b}$	$119.4 \pm 6.43^{---a+++b}$	$314.9 \pm 4.43^{---a+++b}$
Intoxicated with lead+flax seeds oil	$110.1 \pm 3.51^{---a+++b}$	$59.40 \pm 5.91^{---a+++b}$	$184.4 \pm 6.63^{---a+++b}$

Table 3: Effect of mesenchymal stem cells and oral administration of lax seeds oil on CAT and SOD in brain tissue of rats intoxicated with lead acetate

Groups	CAT (U/g. tissue)	SOD (U/g. tissue)
	Mean ± S.D.	Mean ± S.D.
Normal	174.7 ± 7.29	6.65 ± 0.29
Intoxicated with lead	$46.6 \pm 7.35^{---a}$	$0.67 \pm 0.15^{---a}$
Intoxicated with lead+stem cells	$144.3 \pm 7.48^{---a+++b}$	$2.99 \pm 0.26^{---a+++b}$
Intoxicated with lead+flax seeds oil	$124.9 \pm 6.28^{---a+++b}$	$1.59 \pm 0.15^{---a+++b}$

Table 4: Effect of mesenchymal stem cells and oral administration of flax seeds oil on Malondialdehyde (MDA) in brain tissue of male Albino rats intoxicated with lead acetate

Groups	MDA (in brain tissue) (n mol/g. tissue)
	Mean ± S.D.
Normal	0.77 ± 0.12
Intoxicated with lead	$17.28 \pm 0.62^{+++a}$
Intoxicatedwith lead+stem cells	$3.04 \pm 0.47^{+++a---b}$
Intoxicated with lead+flax seeds oil	$8.53 \pm 0.60^{+++a---b}$

Table 5: Effect of stem cells and oral administration of flax seeds oil on cognitive ability by using T-maze test alternation score % of rats intoxicated with lead acetate

Groups	T-maze test (alternation score %)
	Mean ± S.D.
Normal	72 ± 10.33
Intoxicated with lead	$28 \pm 10.33^{---a}$
Intoxicatedwith lead+stem cells	$52 \pm 10.33^{---a+++b}$
Intoxicated with lead+flax seeds oil	$50 \pm 10.54^{---a+++b}$

Table 6: Histopathological scores of albino rats of control, lead acetate, mesenchymal stem cells and oral administration of flax seeds oil classified according to severity of lesions into absent, (-), mild (+), moderate (++), and severe (+++)

Lesions	Normal	Lead	Stem cells	Flax seeds oil
Necrosis in neurons	-	+++	+	++
Cerebral edema	-	+++	+	++
Congested blood vessels	-	+++	-	++
Gliosis	-	+++	+	+

DISCUSSION

The central nervous system is the major target of lead poisoning, and the developing brain appears to be particularly vulnerable to lead neurotoxicity[4]. Lead (Pb) -induced neurotoxicity has been linked to oxidative stress as a probable mechanism. Damage caused by lead-induced oxidative stress could result from[1] Pb inhibiting 5-aminolevulinic acid (ALA) dehydratase, resulting in an accumulation of ALA, which could be a source of free radicals;[2] Pb's direct engagement with biological membranes causes lipid peroxidation;[3] mitochondrial function is impaired by a rise in intracellular calcium levels, and[4] Pb causes a reduction in glutathione and free radical scavenging enzymes. The great affinity of Pb for metal cofactors or sulfhydryl groups in these molecules and enzymes is largely responsible for this[25].

The present study showed that the lead-intoxicated rats showed an elevation in lead levels in the blood which is consistent with previous research that has shown a significant blood lead accumulation[26,27]. Possible reasons for this observation might be the affinity of the lead to react with protein transporters[26]. Previous research showed that red blood cells have a strong affinity for lead and often contain the majority of the lead in the bloodstream, which agrees with our observation[28].

The current study showed a pronounced decrease in the concentration of lead in the blood of rats treated with mesenchymal stem cells or flax seeds oil in comparison to rats treated with lead acetate without any treatment. This improvement may be attributed to mesenchymal stem cells and flax seeds oil which have chelating properties that are similar to those of EDTA[29,30].

Elevation of lead blood levels is associated with an elevated oxidative reaction, which might be responsible for lead-caused toxic effects[31].

The current results showed that lead acetate induces an increase in MDA in brain tissue by increasing free radical production. Free radicals caused oxidative stress by producing lipid peroxidation (oxidation of lipids) such as MDA. So the presence of a huge percentage of phospholipids containing polyunsaturated fatty acids makes the brain particularly vulnerable to peroxidation[32,33]. These results are consistent with Galal *et al*[32] and Gazwi *et al*.,[34] who reported that the reduction of phospholipids was also noticeable in the brain as a result of lead accumulation and increased levels of lipid peroxidation.

In this study, the rats treated with mesenchymal stem cells (MCSs) after being intoxicated with lead acetate, showed a decrease in MDA in brain tissue. This result is in agreement with Ali *et al*.,[35] who reported that treatment of MCSs decreases MDA in the midbrain and frontal cortex of rats intoxicated with sodium nitrite. MSCs decreased oxidative stress indicators in brain tissue by secreting a vast array of cytokines, chemokines, and growth factors that are possibly implicated in tissue repair and this mechanism may describe how MCSs decrease MDA in the brain tissue[36].

Also Castorina *et al*.,[37] documented the ameliorating effect of mesenchymal stem cells on oxidative stress in neurodegenerative diseases. This previous study showed that MSCs decreased the levels of lipid peroxidation and oxidative stress indicators by activation of free-radical trapping or the enzymatic antioxidant system prompted by cytokine activation, as a result, brain injury is avoided, and the clinical prognosis is improved[38].

The current investigation indicated that the rats treated with flax seeds oil showed a reduction in MAD in brain tissue. The present results are in agreement with Ismail *et al*.,[39] who suggested that flax seeds oil decrease MAD in the brain of γ-irradiated. Badawy *et al*.,[40] reported that treatment of flax seeds oil decrease MDA in brain tissue in diabetic rats.

Flax seeds oil induces a reduction in MAD by reducing oxidative stress, this may be due to flax seed oil's free radical scavenging activity, which protects against pathological changes caused by OH- and O-2. In possible 2 ways that suggest the usage of omega-3 polyunsaturated fatty acids

in flax seed oil may help to mitigate this oxidative damage. First, omega-3 may raise catalase levels in cytoplasm and the peroxisome, resulting in greater protection against free oxygen radicals. Second, supplemental omega-3 could be replaced with fatty acids components of membranes that have been damaged by oxygen free radicals[41].

Radical-scavenging enzymes like CAT and SOD are the first-line defense of cells against oxidative stress[42]. In the present research, lead induces a decrease in antioxidant CAT and SOD activities in brain tissue. These results are in accordance with Galal *et al*.,[32] and Hatice *et al*.,[43]. This result may be attributed to elevated MDA which may be a reason for the reduction of antioxidant activity. Lead lowers the antioxidant capacity of cells, particularly thiol-containing antioxidants and enzymes[44]. Also, Lead inhibits the activity of antioxidant enzymes in the tissues through the production of reactive oxygen species (ROS)[45].

As well, the results of this study showed that the treatment by mesenchymal stem cells induced elevation in antioxidant CAT and SOD activities in brain tissue after intoxication with lead acetate. In this study discussed above, MSCs reduced MDA, which may be a reason to restore antioxidant activity. The current study is in agreement with Calió *et al*.,[46] who suggested that mesenchymal stem cells suppress oxidative stress in the stroke model. Also, mesenchymal stem cells improved antioxidant enzyme activities in Conscious State Patients[47]. Elzayat *et al*.,[48] reported that treatment of stem cells induces improvement in SOD and CAT in the brain tissue of rats intoxicated with AlCl3.

Also, The present study showed that the treatment by flax seeds oil in lead intoxication brain tissue improved antioxidant CAT and SOD activities. This result is in accordance with Ismail *et al*.,[39] who stated that rats irradiated and intoxicated with carbon tetrachloride then treated with flax seeds oil showed enhancement in CAT and SOD activities in brain tissue. Modulation of the enzymatic antioxidant defenses of the brain tissues was largely responsible for the ameliorative effects of flax seeds oil. Several findings reported that flax seeds oil inhibited cellular ROS generation. Flax seed oil antioxidants prevented cell damage induced by hydrogen peroxide[49]. The flax seed oil has two antioxidant systems: one is a water-soluble component, which accounts for the majority of the system, and the other is a phenolic molecule[50,51]. Those may be the reasons for flax seeds oil improved antioxidant CAT and SOD activities.

By histopathological examination in the present study, brain cerebrum lesions characterized by vascular and neuronal alterations, Cerebral edema exhibited with dilated Virchow robin spaces, microglia cells around degenerated neurons, congestion of meningeal blood vessels, and necrosis in the neurons of the cerebrum was seen in the lead-intoxicated group. These changes are in accordance with Sidhu and Nehru,[52] and Saleh and Meligy,[53] who stated that degeneration of neurons was found in parts of the cerebrum and the normal arrangement of the cellular layer of the cerebellum was disrupted after lead intoxication. Galal *et al*.,[32] reported that the histopathological alterations occurred in the brain exposed to acute lead such as astrogliosis, edema, and gliosis which are in agreement with this study's results.

These pathologic changes in the cerebral cortex and the cerebrum may be linked to oxidative stress, particularly an imbalance between reactive oxygen species production and antioxidant systems' ability to deactivate them. Most of these reactive oxygen mediators destroy cellular organelles and cause the production of another hazardous molecule[54]. The present findings revealed that Pb-exposed rats had cerebral edema, which might be associated with a disruption in blood-brain barrier function, resulting in a change in blood dynamics and fluid escape into the central nervous tissue, and these findings are consistent with Al-Mzaien *et al*.,[55]. Also, Kahle *et al*.,[56] demonstrated that Ischemia-related edema is caused by activation of the brain's Na+, K+, and Cl- co-transporter system, which promotes the production of edema and swelling of endothelial tissue.

The current results indicated that lead acetate produced gliosis. the formation of gliosis is one of the processes through which lead exerts its detrimental effects on the central nervous system, relating to the damage of neurons[57].

Additionally, in the present work, treatment by mesenchymal stem cells after lead intoxication showed marked improvement in the histopathology of brain tissue which indicated as a reduction in the numbers of degenerative neurons and enhance the repair of neurons to nearly similar to normal. Kamal *et al*.,[58] documented that mesenchymal stem cells improved the brain tissue damage of Alzheimer's model rats.

According to Ali *et al*.,[35] stem cells improve brain tissue similarly to normal tissue. Stem cells protect themselves against infection and secrete five growth factors, allowing mesenchymal stem cells to specialize and repair damaged tissues. Mesenchymal stem cells may be attributed to cell repair in several tissue types, including the brain[59]. Mesenchymal stem cells have the potential to replace lost cells by developing into functional brain tissue, modify the immune system to avoid further neurodegeneration, and provide trophic support for disordered nervous systems[60].

Also, in the present study, treatment by flax seeds oil after lead intoxication revealed moderate improvement in the histopathology of brain tissue which indicated as reduction in the degenerative neurons compared to lead intoxication group but not nearly similar to normal. This finding is in agreement with Singh *et al*.,[61] who investigated that omega-3 ameliorated pathologic alteration in the cerebellum and cerebral of rats intoxicated with lead. These results may be due to flax seeds oil containing omega-3 fatty acids. Omega-3 fatty acids can help to

minimize the damage caused by oxidative stress through docosanoids, which are produced by Docosahexaenoic acid and function as neuroprotectants[61,62]. Also, as known the supplementation of Omega-3 fatty acids reduces microglial activation and inflammatory response[63].

From the study results, it was shown that the mesenchymal stem cells showed a reduction in the numbers of degenerative neurons and enhance the repair of neurons to nearly similar to normal. Flaxseed oil also showed an improvement in reducing the degenerative neurons, as it did not reach a degree nearly similar to normal cells, but this improvement for flaxseed oil is less compared to the effect of stem cells. The difference between the effect of MSCs and FSO is due to MSCs can differentiate and replace damaged cells, in addition to MSCs secret bioactive agents such as antioxidants, and anti-inflammatory and anti-apoptotic agents. So MSCs is more superior to FSO which has natural antioxidant and anti-inflammatory properties.

In the present study, rats intoxicated with lead acetate showed a reduction in norepinephrine, serotonin, and dopamine in brain tissue. These results are in agreement with Abdel Moneim,[64]. As well, Lead induces depression in rats by reducing norepinephrine and serotonin concentrations in serum and brain tissue[65].

Elevation of lead levels in the blood induced an increase in monoamine oxidase A, which caused a reduction in serotonin, norepinephrine, and dopamine[66]. Lead may penetrate the intracellular environment of neurons. Moreover, lead competes with calcium for common binding sites and is transported into calcium transport channels in the nervous system, where it is required for neurotransmitter release and regulation[67]. This may be the reason for the decrease in serotonin, norepinephrine, and dopamine.

The current study indicated an improvement in norepinephrine, serotonin, and dopamine in the brain tissues of rats treated with mesenchymal stem cells after lead acetate intoxication. These results are in accordance with Ali *et al*.,[35] who recorded that treatment of mesenchymal stem cells induces improvement in norepinephrine, serotonin, and dopamine in the frontal cortex and midbrain of rats intoxicated with Sodium nitrite. Interestingly, mesenchymal stem cells migrate to inflammation and differentiate into a neuron in various parts of the brain, such as the cerebral cortex and hippocampus[68]. This might be the cause of improvement of norepinephrine, serotonin, and dopamine.

In addition, treatment by flax seeds oil after intoxication with lead acetate revealed an elevation in norepinephrine, serotonin, and dopamine in brain tissues. these results are in agreement with Abdel Moneim,[64]. This improvement may be attributed to flax seeds oil which has a free radical scavenging activity, and this could exert a beneficial effect against pathological alterations caused by the presence of O- 2 and OH-. A reduction in brain MDA was shown to have a significantly beneficial correlation with improved neurotransmitter levels[40]. Lipoic acid in flax seeds oil induce increased serotonin, and this improvement in serotonin levels led to improvement in other neurotransmitters. The current study observation of behavior indicated alterations in the behaviors of the lead intoxicated group. This alteration may be the result of depression due to reducing norepinephrine and serotonin[65].

The present results showed that lead acetate induces a reduction in cognitive ability. Lead-induced impaired cognitive function on short- and long-term memory, according to Alves Oliveira *et al*.,[69]. Lead causes cognitive deficits associated with ROS formation, peroxidation of lipids, and lowering in antioxidant enzymes, resulting in neuronal deficits[25,70].

In this study, the treatment by mesenchymal stem cells after lead intoxication showed improvement in cognitive performance, learning, and memory deficits. Choi *et al*.,[70] stated that stem cells induce improvement in cognitive functions, this may be due to the antioxidant properties of stem cells by inhibition of ROS and lipid peroxidation formation. Mesenchymal stem cells ameliorated cognitive defects in Alzheimer's[71].

Also, flax seeds oil recovered cognitive deficits induced by lead acetate. The current data is in accordance with Noroozi *et al*.,[72] who suggested that flax seeds oil prevent learning and memory deficits caused by lead. This may be due to flax seeds oil inducing inhabitation ROS and lipid peroxidation generation, also, reducing oxidative stress. Also, alpha-linoleic acid in flax seeds oil enhances neurogenesis, notably in the hippocampus's dentate gyrus. Flax seeds oil improved behavior defects associated with poor cognitive function[73].

CONCLUSION

Recently, many problems linked to brain function, such as behavioral or poor memory and learning difficulties, particularly in children. These problems are due to environmental pollution with harmful substances such as heavy metals. This study was conducted to show the use of stem cells and flax seeds oil has the ability to mitigate the damage to the brain tissue caused by lead, whose toxic effects are similar to the harmful effects of other environmental pollutants. Both stem cells or flaxseed oil showed a repair and an improvement in the brain tissue which was reflected in the performance of its functions. In the future, it is possible to consider the use of stem cells or flax seeds oil as one of the therapeutic agents used to treat brain damage caused by environmental pollutants, especially lead. Mesenchymal stem cells showed marked improvement in damaged brain tissues which is nearly similar to normal tissues compared to flax seeds oil which showed moderate enhancement in damaged brain tissues.

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AUTHORS CONTRIBUTIONS

Prof. Abdel Rahim and Dr. Rana planned the protocol and manuscript. Dr. Zainab read the histological results. Mariam carries out the project in practice, analyzed the results, and wrote the draft manuscript.

ETHICS STATEMENT

All of the rats' experiments were conducted in compliance with the National Institute of Health Council's guidelines for animal care and use. The ethical research committee of the faculty of veterinary medicine at the South Valley University of Qena-Egypt reviewed the number (No. 39/12.062022). I confirm the experiment of the manuscript is stated in agreement with Animal Research Reporting of In *Vivo* Experiments guidelines. All rats were clinically examined before being used in the experiment.

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Abbreviation

Pb: Lead, **MSC:** Mesenchymal stem cells, **FSO:** Flax seeds oil, **CAT:** Catalase, **SOD:** Superoxide dismutase, **MDA:** Malonaldehyde, **EDTA:** Ethylenediaminetetraacetic acid, **DMEM:** Dulbecco's Modified Eagle Medium, **L.S.D.:** least significant difference, **ALA:** 5-aminolevulinic acid, **ROS:** Reactive oxygen species.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور التحسيني للخلايا الجذعية وزيت بذور الكتان ضد السمية العصبية التي يسببها أسيتات الرصاص في انسجه المخ لذكور الجرذان البيضاء

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المقدمه: يعتبر الرصاص من الملوثات الصناعيه واسعه الانتشار التي لها اثار خطيره علي انسجه المخ .الرصاص يؤدي الي تلف الخلايا العصبيه وتثبيط عمل مضادات الاكسده وحدوث الاجهاد التاكسدي. تتميز الخلايا الجذعيه المتوسطيه بقدرتها علي التجديد والتميز الي انواع مختلفه من الخلايا وايضا قدرتها علي اطلاق مواد ذات خصائص مضاده للاكسده والتهابات. يعتبر زيت بذور الكتان مصدرا حيويا للمغذيات الاساسيه لانسجه المخ وايضا مصدرا طبيعيا كمضادا للاكسده وايضا مصدر للاوميجا٣.

الهدف من البحث: هدف الدراسه الحاليه تقييم التاثير السام لماده خلات الرصاص علي انسجه المخ والدور التحسيني للخلايا الجذعيه المتوسطيه وزيت بذور الكتان ضد سميه الرصاص وايضا امكانيه استخدامهم لعلاج انسجه المخ التالفه.

مواد وطرق البحث: تم استخدام اربعون من ذكور الجرذان البيضاء البالغه والتي قسمت الي اربع مجموعات متساويه. المجموعه الاولي: مجموعه طبيعيه تم اعطاءها ماء مقطر فقط.المجموعه الثانيه: تم حقنها بخلات الرصاص بالغشاء البريتوني بجرعه ١٠٠ مجم/كيلوجرام لمده سبع ايام.المجموعه الثالثه: تم حقنها بخلات الرصاص وعلاجها بالخلايا الجذعيه المتوسطيه مليون خليه لكل فأرلمره واحده.المجموعه الرابعه:تم حقنها بخلات الرصاص وعلاجها بزيت بذور الكتان ١ملي/كيلو جرام لمده شهر.وفي نهايه التجربه تم تجميع عينات الدم وانسجه المخ وذلك لاجراء الفحوصات البيوكيميائيه والهستوباثولوجيا.

نتائج البحث: اظهرت المجموعه المسممه بالرصاص ارتفاعا في مستوي الرصاص في الدم مع تركيز انخفاض النواقل العصبيه كالسرتونين والدوبامين والإبنيفرن وايضا مضادات الاكسده والتي تشمل الكاتليز وديسموتاز فوق أكسيد وزياده المالونالدهيد في انسجه المخ وايضا بعد الفحص الباثولوجي تبينا احتقان في الاوعيه الدمويه وتلف في الخلايا العصبيه في انسجه المخ وايضا مشاكل في الذاكره والسلوك. اما المجموعات المعالجه بالخلايا الجذعيه المتوسطيه وزيت بذور الكتان اظهرت تحسنا واضحا في تركيز النواقل العصبيه ومضادات الاكسده وانسجه المخ وتحسنا في الذاكره والسلوك والتي استدلالنا عليها بالمتاهه.

الاستنتاج: أشارت الدراسة إلى كفاءة العلاج بالخلايا الجذعيه المتوسطيه وزيت بذور الكتان للتخفيف من التأثير السام لماده الرصاص على أنسجة المخ وامكانيتهم علي اصلاح الانسجه التالفه.وكانت كفاءه الخلايا الجذعيه المتوسطيه في العلاج افضل من زيت بذور الكتان.