

Comparative Study between Mesenchymal Stem Cells and Flax Seeds Oil against Toxicity of Lead Acetate on the Spinal Cord Tissue of Male Albino Rats

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ABSTRACT

Background: Lead is a common heavy metal that persists in the environment and has many toxic effects on the central nervous system, especially the spinal cord. **Objective:** The current study investigates the effect of mesenchymal stem cells (MSCs) and flax seeds oil (FSO) on the spinal cord tissue against lead acetate toxicity in male albino rats.

Materials and Methods: Forty adult male albino rats were divided equally into four groups. The first group served as control rats. The second group received lead acetate (100 mg/kg) intraperitoneally daily for seven days. Group 3 after lead acetate intoxication then treated with a single dose of MSCs (1×10^6 cells/rat intravenously injection). In the 4th group 3 after lead acetate intoxication then rats were treated orally with FSO (1 ml/kg) for thirty days. At the end of the experiment, spinal cord tissues were collected to determine the lead level in the spinal cord, histopathology, immunohistochemistry for cleavage caspase3, and estimate DNA damage by comet assay.

Results: Our results revealed significantly increased lead concentration in spinal cord tissue in group 2. In addition to, upregulation of cleavage caspase 3 and elevation of DNA damage in the spinal cord tissue and histopathological alterations in spinal cord tissues. Nevertheless, the treatment of MSCs and FSO groups recorded a decline in lead levels in the spinal cord tissue and downregulation of cleaved caspase 3 and DNA damage and histopathological improvement.

Conclusion: Our investigation showed that MSCs are more effective than FSO against lead acetate induced toxicity.

Keywords: Lead acetate toxicity, Spinal cord, Mesenchymal stem cells, Flax seeds oil.

INTRODUCTION

The World Health Organization (WHO) has issued lead as one of ten chemical groups that are harmful to human health. Lead is a common environmental and industrial pollutant. Industrial emissions, soil, car exhaust gases, and contaminated foods are considered the most important sources of lead exposure⁽¹⁾. Lead is a multi-organ toxin that has been linked to malignancies, hepatic, renal, reproductive, and central nervous system failure. Also, lead exposure induces anemia and immunotoxicity. Lead concentrations in the blood as low as 5 mg/dL were originally believed to be safe⁽²⁾. Many heavy metals, including lead, were known to cause excessive generation of reactive oxygen species (ROS), which increases lipid peroxidation and decreases antioxidant activities.

MSCs therapy is a novel treatment for neurodegenerative diseases of the central nervous system. It has the potential to solve major pathophysiology due to its ability to reduce inflammation, reconstruct the blood-brain barrier, and induce neural regenerations by secreting neurotrophic factors such as basic fibroblast growth factor, brain-derived neurotrophic factor, endothelial growth factor, and vascular endothelial growth factor⁽³⁾. Using of flax seeds oil can help in avoiding of many chronic diseases such as diabetes, mental disorders, and cardiovascular diseases this is because of its high content of polyunsaturated fatty acids (omega-3 omega-6), lignans, high-quality proteins, and fiber. The significant antioxidant properties of these lignans of flax seeds oil are due to their high levels of a free radical scavenger. The antioxidant and anti-inflammatory activities of flax

seeds oil enhance the antioxidant levels and suppress the inflammatory responses and regulatory elements in the nervous system⁽⁴⁾.

MATERIALS AND METHODS

1. Chemical:

Lead acetate was purchased from El-Gomhouria Company for Chemicals and Laboratory Supplies, located in Assiut, Egypt. Flax seed commercial oil was purchased from EL Captin Company (Al Obour City, Cairo, Egypt). Kit of comet assay was purchased from RnDSsystems, 19 Barton Lane -Abingdon Science Park-Abingdon, OX14 3NB- United Kingdom, CAT. NO. TA800. Polyclonal IgG anti-cleaved Caspase 3 antibody purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

2. Animals:

Forty adult male Albino rats aged 2 and 3 months old and weighing about (250–280 g) were purchased from South Valley University's Faculty of Science in Qena, Egypt, as lab animals and were housed in standard conditions and fed on a normal diet and water ad libitum. Rats were divided into 4 groups (each group has ten rats) as follows: **Group (1) (control):** Rats received dist. Water. **Group (2):** rats were injected intraperitoneally with lead acetate (100 mg/kg b.w.t for seven days) and then, kept for 30 days later. **Group (3):** rats were intraperitoneally injected with lead acetate (100 mg/kg b.w.t for seven days) after that by 24 hours, rats received a single dosage of mesenchymal stem cells (1×10^6 cells/rat intravenously) then left for 30 days. **Group (4):** 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).

At the end of the study, all rats were sacrificed by using a suitable dose of ethyl ether. After dissection, we extracted spinal cord tissues, and one part was put on ice immediately, and then transferred in liquid nitrogen, where they were instantly frozen at -80°C until DNA comet assay and lead levels examination. The other part was fixed in natural formalin 10% for 24 hours. Then it was kept in 70% ethyl alcohol for histopathology and immunohistochemistry.

Ethics statement:

All the experimental procedures were carried out according to the principles and guidelines of the Ethics committee of the faculty of veterinary medicine at the South Valley University of Qena-Egypt conformed to the Guide for the care and use of Laboratory Animals, Published by US National Institutes of Health (NIH Publication No. 39/12.062022).

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

The bone marrow of male rats was extracted from their femurs. The removed bone marrows were incubated for cell culture in Dulbecco's modified Eagle's medium-filled 25 cm² flasks (DMEM, Invitrogen, 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 cm² flasks with 5 ml of DMEM at 37°C in a humidified 5% CO₂ air. Every two days, the culture media was changed. The confluence of the cells reached roughly 80~90 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 (Fig. 1).



Figure (1): Bone-marrow mesenchymal stem cells during incubation showing a relatively homogenous cell culture reached among 80~90% confluence (x200).

3. Identification and characterization of bone-marrow mesenchymal stem cells by Using Flow Cytometer CD90, CD31, and CD45:

The immunophenotyping of bone marrow mesenchymal stem cells was performed with antibodies against rat antigens CD90, CD31, CD34, CD45 and their isotope controls. Flow cytometry dot plot of MSCs isolated from rat bone marrow shows that cells are negative for CD 34 FITC.

4. Biochemical analysis:

4.1. Measurement of lead levels in the spinal cord tissue

Half grams of spinal cord tissues were digested in a mixture of sulfuric, nitric, and perchloric acid buffer. The tissue was applied to a hot plate for complete digestion for 15 minutes. The precipitated lead carbonate was dissolved in nitric acid and the lead is measured with atomic absorption spectrometry (Thermo AA S4) (GFS-97).

5. Estimation of DNA damage by comet assay:

100 mg of crushed spinal cord tissues were put in 1ml ice-cold PBS including 20 mM EDTA/ 10% DMSO then mixed for 5min and filtered. 100 μl of cell suspension was completely mixed with 600 μl of low melting agarose, then spread 100 μl of the mixture on pre-coated agarose slides. After solidifying at 4°C , the slides were placed in the cold lysing solution for 1 hour at 4°C . The slides were removed and put in a horizontal electrophoresis container for 20 minutes, in which they were filled with freshly made electrophoretic buffer. The slides were gently rinsed in 0.4 M Tris-HCl solution after electrophoresis after that, stained by ethidium bromide. A fluorescent microscope was used to investigate the DNA movement patterns of 100 cells, and photos were recorded using a camera. The Comet analysis software of R&D Systems was used to determine the qualitative and quantitative amount of DNA damage in the cells by measuring tail length, tail moment, % DNA tail, olive tail, comet length head length, and %DNA head.

6. Immunohistochemical investigation of anti-cleaved -caspase-3

For Immunohistochemistry, small pieces of the spinal cord were fixed in 10% neutral buffered formalin pH 7.2. The paraffin-embedded tissues were deparaffinized and rehydrated in a series of ethanol solutions (100% to 70%) ethanol, embedded in paraffin wax and Paraffin sections of 5 micrometers in thickness. They were then incubated with antibodies against cleaved caspase 3 (1:10), after which the sections were washed and stained with 3, 3'- diaminobenzidine (DAB) for 2-3 min before being counterstained with hematoxylin for 2-5 min.

7. Histopathological examination:

Specimens from spinal cord tissue were fixed in 10% neutral buffered formalin pH 7.2, dehydrated in ascending series of alcohols, cleared in xylene, and embedded in paraffin wax. Paraffin sections of 5

micrometers in thickness were prepared and then stained with Harris hematoxylin and eosin stain.

8. Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). The variability degree of results was expressed as Means± Standard Deviation of means (Mean±S.D). The data were statistically analyzed by one-way ANOVA analysis of variance (Newman–Keuls T-tests) by using prism computer program 3 and the least significant difference (L.S.D) was used to test the difference between treatments. Results were considered statistically significant when P < (0.05).

RESULTS

1. Lead levels in the spinal cord tissue:

Lead levels in groups (2), (3), and (4) revealed higher concentrations than in normal rats. On the other hand, lead tissue concentration in groups (3) and (4) were decreased significantly in comparison to the lead intoxication group (Table 1, Fig. 2).

Table (1): Effect of a single dose of mesenchymal stem cells and oral administration of flax seeds oil on lead levels in spinal cord tissues of male Albino rats intoxicated with lead acetate:

Groups	Lead level in spinal cord tissue (µg/g)
	Mean ± S.D.
Normal	0.24 ± 0.01
Intoxicated with lead	1.65 ^{+++a} ± 0.05
Intoxicated with lead+stem cells	0.34 ^{+++a ---b} ± 0.02
Intoxicated with lead+flax seeds oil	0.38 ^{+++a ---b} ± 0.01

Results are expressed as mean ± S.D. of 10 rats for each group.

+++a = significant increase compared with normal at p<0.001.

---b = significant decreased compared with intoxicated with lead group at p<0.001.

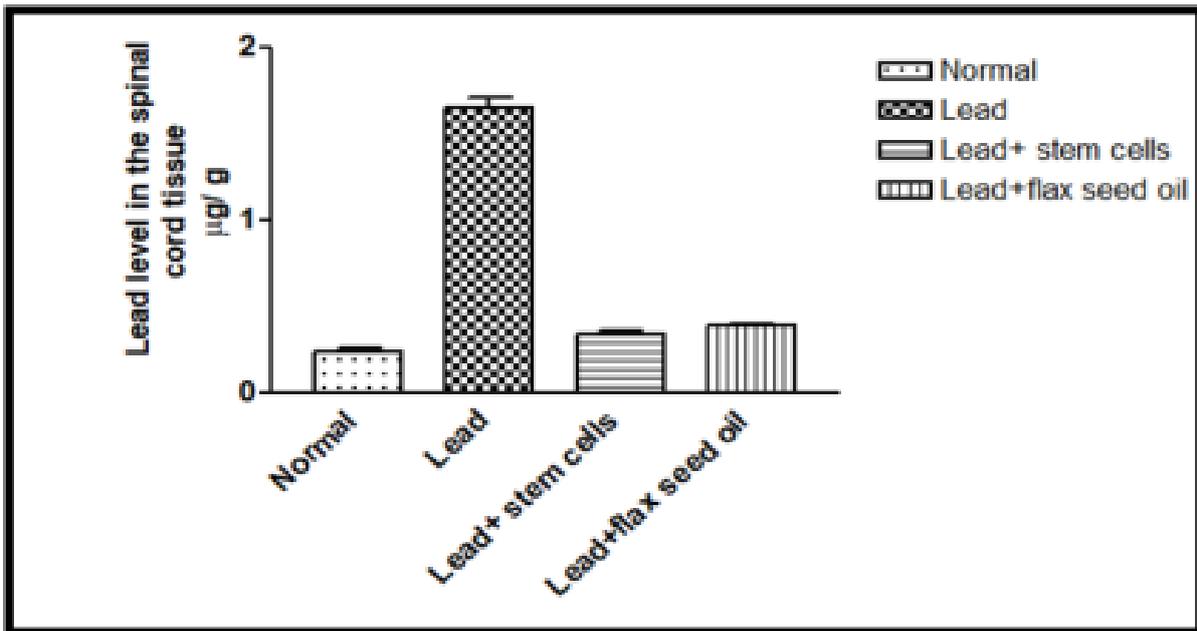


Figure (2): Effect of a single dose of mesenchymal stem cells and oral administration of flax seeds oil on lead levels in spinal cord tissue (µg/g) of male Albino rats intoxicated with lead acetate.

2. DNA damage examination using comet assay:

The exposure to lead acetate induced a significant marked elevation of DNA damage in the spinal cord tissue as evidenced by a significant increase in the mean values of tail length, tail moment, % DNA tail, olive tail, comet length, and decrease in the mean values of head length and %DNA head when compared with the normal group. Conversely, treatment with MSCs and FSO indicated significantly reduced DNA damage in the spinal cord tissues as showed by a significant decrease in the mean values of tail length, tail moment, % DNA tail, olive tail, comet length, and an increase in the mean values of head length and %DNA head (Tables 2 and 3, Figs. 3,4&5).

Table (2): Effect of a single dose of mesenchymal stem cells and oral administration of flax seeds oil on tail length, tail moment, % DNA tail, olive tail and comet length in spinal cord tissue of male Albino rats intoxicated with lead acetate:

Groups	Tail length (µm) (in spinal cord tissue)	Tail moment (µm) (in spinal cord tissue)	DNA tail% (%) (in spinal cord tissue)	Olive tail (µm) (in spinal cord tissue)	Comet length (µm) (in spinal cord tissue)
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Normal	6.57± 0.51	0.8 ± 0.1	4 ± 1	0.35± 0.03	26.33 ± 1.53
Intoxicated with lead	11.73 ^{+++a} ± 0.68	1.8 ^{+++a} ± 0.1	11 ^{+++a} ± 1	0.58 ^{+++a} ± 0.01	35.33 ^{+++a} ± 1.52
Intoxicated with lead+stem cells	9.77 ^{+++a - -b} ± 0.32	1.2 ^{+++a - -b} ± 0.1	7.33 ^{+++a - -b} ± 0.6	0.40 ^{+a - -b} ± 0.02	30.67 ^{+a - -b} ± 2.08
Intoxicated with lead+flax seeds oil	10.03 ^{+++a - -b} ± 0.15	1.3 ^{+++a - -b} ± 0.1	8 ^{+++a - -b} ± 1	0.46 ^{+++a - -b} ± 0.02	31.00 ^{+a - -b} ± 2.08

Results are expressed as mean ± S.D. of 10 animals for each group.

+a = significant increased compared with normal at p<0.05.

+++a = significant increased compared with normal at p<0.001.

++a = significant increased compared with normal at p<0.01.

-b = significant decreased compared with intoxicated with the lead group at p<0.05.

- -b = significant decreased compared with intoxicated with the lead group at p<0.01.

- - -b = significant decreased compared with intoxicated with the lead group at p<0.001.

Table (3): Effect of a single dose of mesenchymal stem cells and oral administration of flax seeds oil on head length and %DNA head in spinal cord tissue male Albino rats intoxicated with lead acetate:

Groups	Head length (µm) (in spinal cord tissue)	%DNA head (%) (in spinal cord tissue)
	Mean ± S.D.	Mean ± S.D.
Normal	22.67 ± 1.15	96± 1
Intoxicated with lead	15 ^{- - -a} ± 1	89 ^{- - -a} ± 1
Intoxicated with lead+stem cells	18.67 ^{- - -a ++b} ± 0.58	92.67 ^{- - -a ++b} ± 0.57
Intoxicated with lead+flax seeds oil	17 ^{- - -a +b} ± 1	92 ^{- - -a +b} ± 1

Results are expressed as mean ± S.D. of 10 animals for each group.

- - -a = significant decreased compared with normal at p<0.001.

- -a = significant decreased compared with normal at p<0.01.

+b = significantly increased compared with intoxicated with the lead group at p<0.05.

++b = significantly increased compared with intoxicated with the lead group at p<0.01.

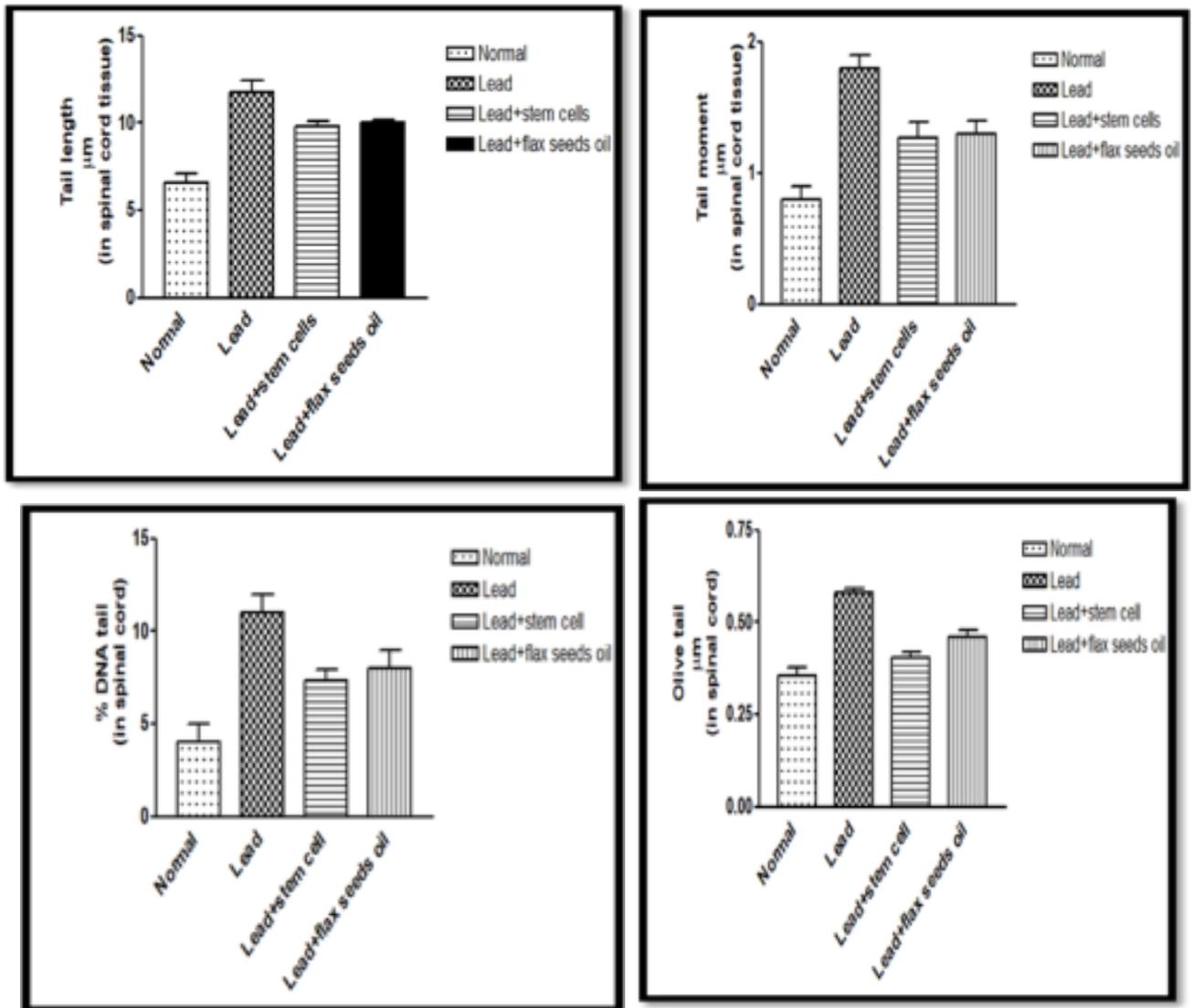


Figure (3): Effect of single dose of mesenchymal stem cells and oral administration of flax seeds oil on tail length, tail moment, % DNA tail and olive in spinal cord tissue of male Albino rats intoxicated with lead acetate.

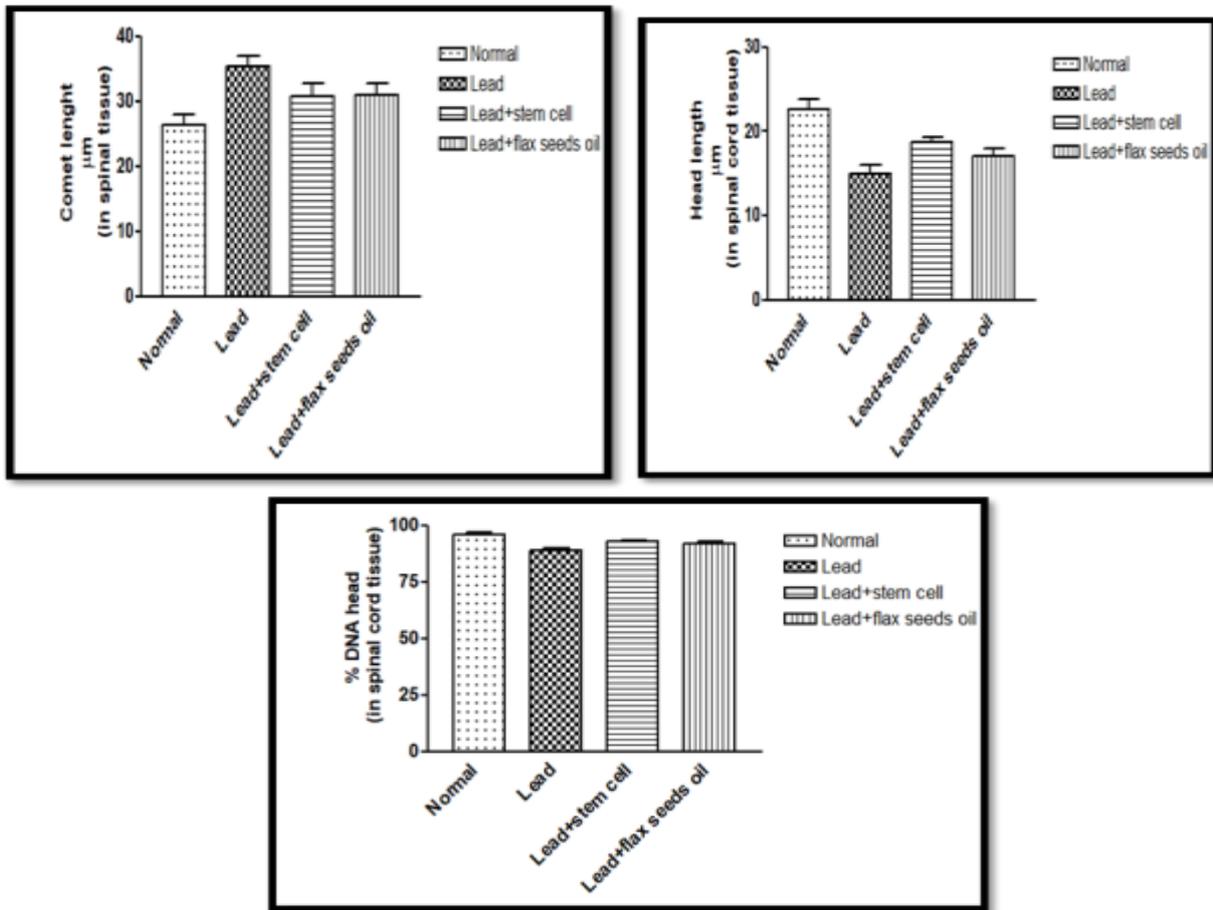


Figure (4): Effect of a single dose of mesenchymal stem cells and oral administration of flax seeds oil comet length, head length and %DNA head in spinal cord tissue of male Albino rats intoxicated with lead acetate.

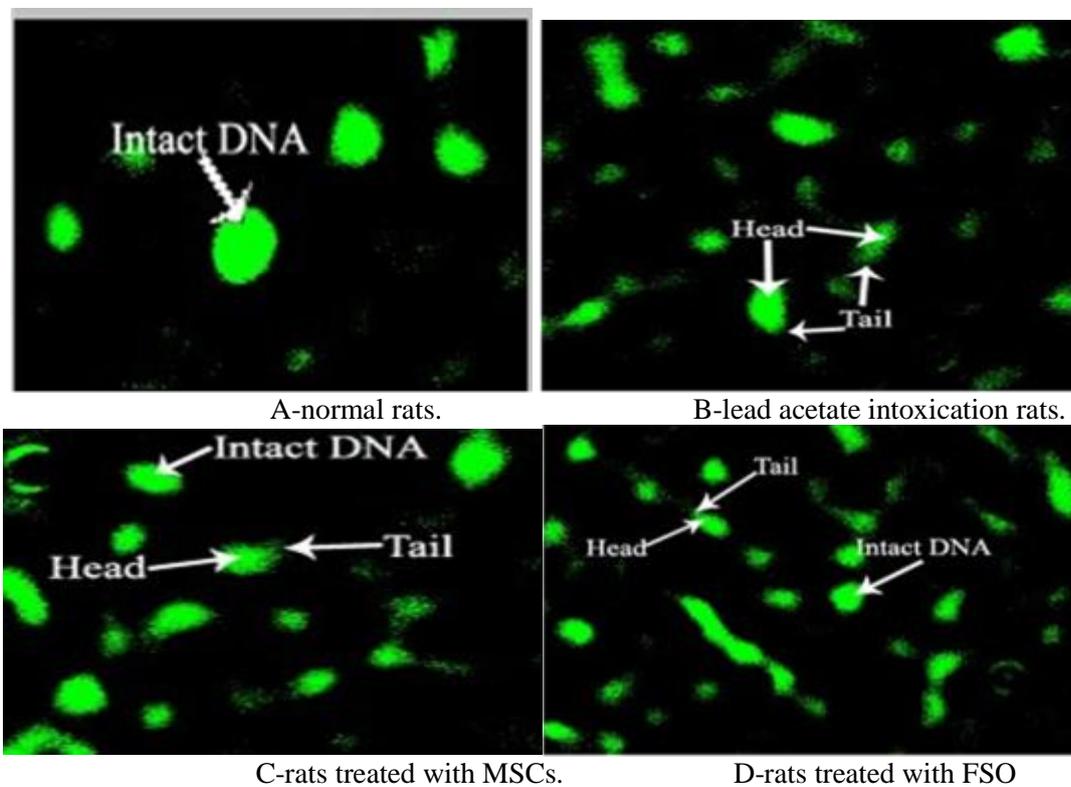


Figure (5): Fluorescent microphotograph of spinal cord of rats stained by ethidium bromide. Comet figures showing A: normal spinal cord with intact DNA, B: spinal cord of lead acetate intoxication with a high degree of damaged DNA, C: spinal cord of treatment of MSCs with a low degree of damaged DNA and intact DNA, D: spinal cord of treatment of FSO with a low degree of damaged DNA. (Intact DNA is shown as a complete circle and damaged DNA has a head and tail like a comet).

3. Immunohistochemistry examination:

Immunohistochemistry for the apoptosis effector cleaved caspase 3 showed that lead acetate extensively elevated apoptotic activity (cleaved caspase-3 expression) in the spinal cord tissue when compared with the normal group. On the other hand, cleaved caspase-3 expression was reduced by both MSCs and FSO when compared to the lead intoxication group. MSCs were more effective than FSO at inhibiting lead-induced apoptosis in the spinal cord (**Fig. 6**).

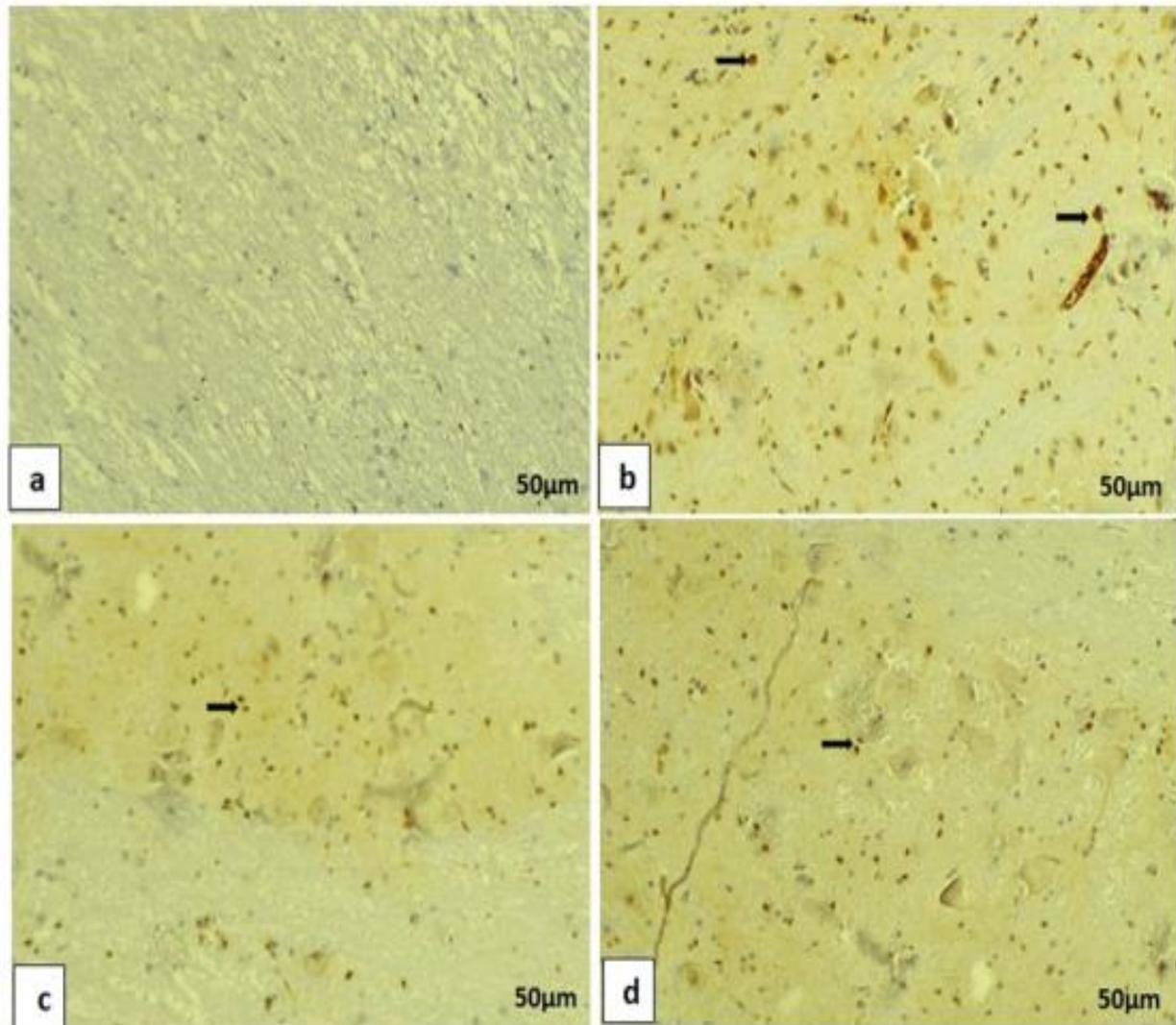


Figure (6): photomicrographs of spinal cord sections of rats stained with apoptosis effector cleaved caspase 3 (barr=50mµ). (a) control group showing mild positive reaction cleaved caspase-3 expression, (b) showing positive reaction as elevated apoptotic activity of cleaved caspase 3 in group 2, (c & d) showing positive reaction as a decreased cleaved caspase-3 expression both MSCs and FSO.

4. Pathological examination:

Group (1) (Normal rats)

The spinal cord of normal rats showed normal histological structure (**Fig. 7a**). In group 2 the spinal cord tissues of rats injected with lead acetate were characterized by increased depressed and degenerated astrocytes with increased area of cavitation besides, a decreasing number of normal neurons also there was necrosis in the tissue of the spinal cord. These changes were observed in most of the spinal cord tissues of rats in this group (**Fig. 7b**). Meanwhile rats in group 3 exhibited an increased number of normal neurons with mild degenerated astrocytes and few cavitation areas (**Fig. 7c**). Moreover, group 4 showed moderate degenerated astrocytes and moderate cavitation areas (**Fig. 7d**). **Table (4)** showed histopathological 212 scores of the spinal cord of normal, lead acetate, mesenchymal stem cells and oral administration of flax seeds oil classified according to the severity of lesions.

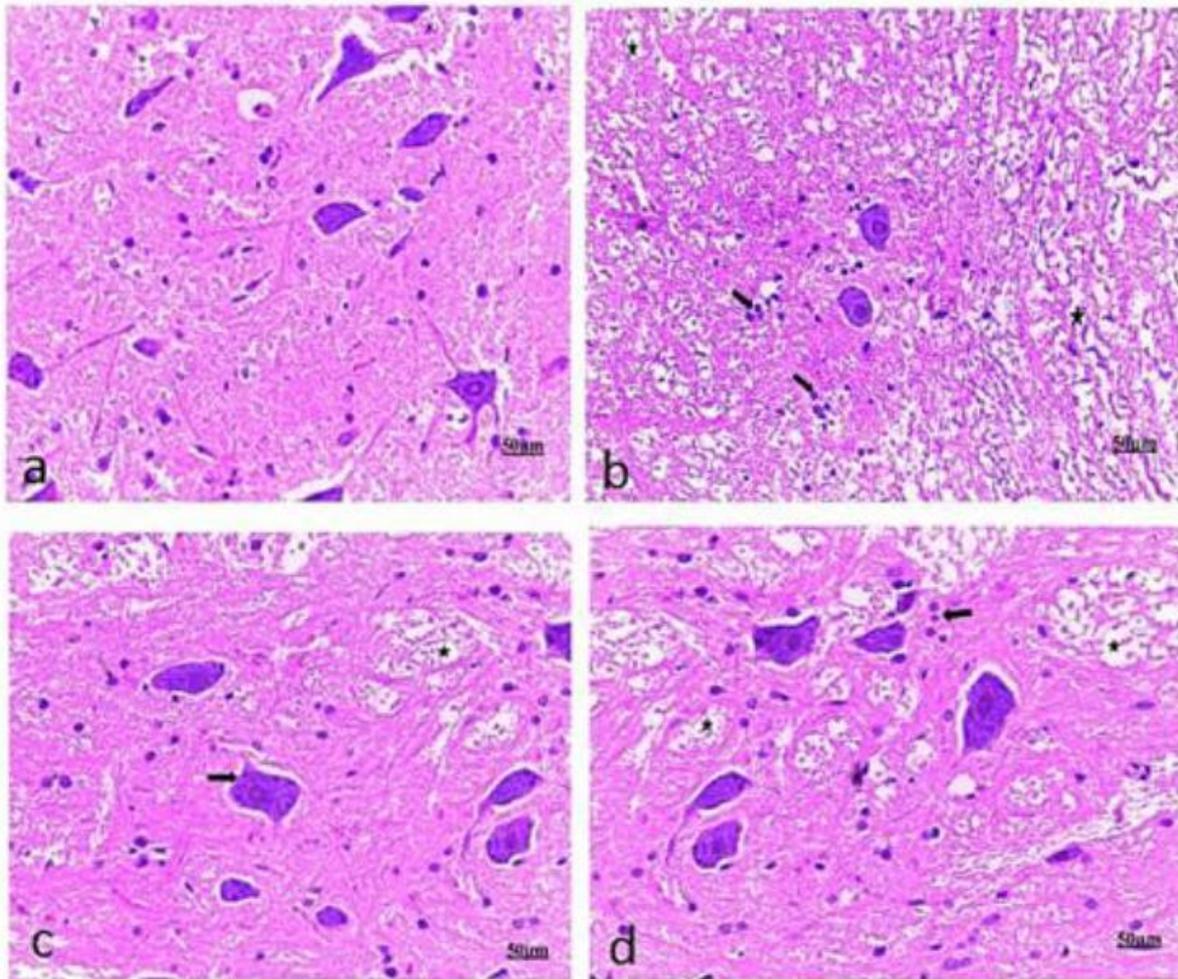


Figure (7): Photomicrographs of spinal cord sections of rats stained with H&E; barr=50µm. (a) control group showing normal architecture of the spinal cord. (b) Showing increased depressed and degenerated astrocytes (thick arrow) with increased area of cavitation (stars) and a decreased number of normal neurons in the lead intoxication group. (c) Rats treated with mesenchymal stem cells showing an increased number of normal neurons (thick arrow) with mild degenerated astrocytes and few cavitation areas (star). (d) Rats treated with flax seeds oil revealing an increased number of normal neurons (thick arrow) with mild degenerated astrocytes and few cavitation areas (star).

Table (4): Histopathological scores of spinal cord of normal, lead acetate, mesenchymal stem cells and oral

administration of flax seeds oil classified according to the severity of lesions into absent, (-, mild (+), moderate (++) , and severe (+++).

Lesions	Control	Lead	Stem cells	Flax seeds oil
Necrosis in neurons	-	+++	+	++
Vacuolation	-	+++	+	++
Congested blood vessels	-	++	-	-
Gliosis	-	+++	+	++

DISCUSSION

Oxidative stress plays an essential role in the pathogenesis of lead and caused the pathogenesis and toxicity of the associated disease. Lead neurotoxicity is caused by different ways of cellular, intracellular, and molecular mechanisms. According to previous reports, lead causes oxidative stress by increasing the generation of reactive oxygen species (ROS), and reducing antioxidants, which play a critical role in reducing lead toxicity. Free radicals can damage cell membranes by lipid peroxidation, activating inflammatory signaling cascades⁽⁵⁾.

The present study showed that lead level was elevated in the spinal cord tissues of group 2. As blood circulates through the soft tissues such as the brain, and spinal cord, lead is precipitated and bio-accumulated⁽⁶⁾. Increased lead concentration in spinal cord tissue may be attributed to the blood-brain barrier alteration in the involved portion of the nervous system, which could permit lead acetate penetrate from the blood into the spinal cord⁽⁷⁾.

The current study showed a pronounced decrease in the concentration of lead in the spinal cord of rats treated with MSCs or FSO. This improvement may be attributed to MSCs and FOS is possible to chelating properties of lead⁽⁸⁻⁹⁾. The phenolic lignans of FSO and other phytoestrogens have antioxidant activity⁽⁴⁾. Also, MSCs release many chemicals that exhibit antioxidant and anti-inflammatory properties⁽¹⁰⁾. These antioxidant and anti-inflammatory properties may be the cause of its own the chelating properties of lead. **Ismail et al.**⁽⁴⁾ suggested that FSO regulates the trace elements in the central nervous system of γ -irradiated and CCl4.

Our results revealed that lead acetate causes increasing DNA damage in the spinal cord tissues as demonstrated by a significant increase in the mean values of tail length, tail moment, % DNA tail, olive tail, comet length, and reduction in the mean values of head length and %DNA head. These results are in accordance with **Arif et al.**⁽¹¹⁾ who reported that lead caused DNA damage which was expressed by a significant increase in the mean values of tail length, tail moment, % DNA tail, olive tail, and comet length in erythrocytes of rats.

Also, the possible mechanism of lead acetate genotoxicity is either because of its reaction with DNA

or by the production of ROS which induced DNA damage as the higher level of NO formed through lead acetate intoxication inhibits cellular respiration and triggers apoptosis causing DNA damage⁽¹²⁻¹³⁾.

The results of the study indicated that treatment with MSCs recorded significantly reduced DNA damage in the spinal cord tissues as showed by a significant decrease in the mean values of tail length, tail moment, % DNA tail, olive tail, comet length and increase in the mean values of head length and %DNA head. **Hamza et al.**⁽¹⁴⁾ stated that diabetic rats treated with MSCs recorded a decrease in DNA damage in the pancreas which was estimated by the comet assay method, this is consistent with our findings. This improvement may be due to the antioxidant and anti-inflammatory activity effects of MSCs, which lead to a decrease in free radicals and an increase in antioxidant enzymes⁽¹⁵⁾.

Dagci et al.⁽¹⁶⁾ used embryonic neural stem cells to treat spinal and brain injury, in which treatment of stem cells showed reduced the DNA damage as signified by low Comet assay parameters, such as the % DNA in the tail, tail moment, and tail length.

Our study revealed that treatment with FSO recorded significantly reduced DNA damage in the spinal cord tissues as indicated by a significant decrease in the mean values of tail length, tail moment, % DNA tail, olive tail, comet length, and increase in the mean values of head length and %DNA head. Also, the ameliorative effect of FSO on lead acetate which causes DNA fragmentation in the brain tissue of rats was stated by **Abdel-Moneim et al.**⁽¹²⁾, this is in harmony with our results. **El Makawy et al.**⁽¹⁷⁾ reported that flax seeds oil treatment induces a decrease in DNA damage as expressed by elevation in tail length, tail DNA %, and tail moment in testes and liver of rats intoxicated with bisphenol-A. The amelioration provided by FSO may be attributed to its intrinsic antioxidant and free radical scavenging properties associated with its constituent bioactive components such as omega-3 and lignans⁽¹⁸⁾.

The present study showed an increase in cleaved caspase 3 expression in spinal cord tissue of rats intoxicated with lead acetate. Our findings are in agreement with **Nasr et al.**⁽¹⁹⁾ who stated that increased cleaved caspase3 levels in testis tissues due to lead-induced formed ROS and inhibited antioxidant system. Lead causes an elevation in enzymatic activities of the caspase family in the liver, kidney, and central nervous system tissues are referred to as an apoptotic effect of lead due to lead stimulated extracellular signal-regulated kinase dephosphorylation and caspase cascade are the most effective pathways directly related to apoptotic signals⁽²⁰⁾.

Also, the present study showed a decrease in cleaved caspase 3 expression in spinal cord tissue of rats after treatment with MSCs. our investigations are in accordance with **Dasari et al.**⁽²¹⁾ who stated that MSCs reduced cleaved caspase3 in the spinal cord tissue after

injury. Similarly, **Zhang et al.**⁽²²⁾ suggested that the treatment of MSCs causes a decline in caspase 3 activity in rats with cerebral ischemia.

In addition, the present study recorded a decrease in cleaved caspase 3 expression in spinal cord tissue of rats treated with FSO. These results are in agreement with **Diab et al.**⁽²³⁾ who stated that FSO reduced apoptotic markers including caspase9 in liver tissue of rats intoxicated with cadmium chloride. These results suggest that flax seeds oil has antioxidant, anti-inflammatory, and anti-apoptotic properties. Also, **Abdel Moneim**⁽²⁴⁾ documented that FSO induces a decline in BAX expression in brain tissues of rats injected with lead due to FSO inhibiting MDA and NO production and restoring antioxidant enzymes activity.

A recent study showed that the spinal cord of rats intoxicated with lead acetate was characterized by increasing depressed and degenerated astrocytes with increased area of cavitation besides, a decreased number of normal neurons also there was necrosis in the tissue of the spinal cord. These changes are in agreement with **Al-Khafaf et al.**⁽²⁵⁾ who documented that spinal cord tissue of rats intoxicated with lead revealed histological changes described by degenerative alterations and necrotic lesions, neurons losing their dendrites, pyknosis of the nucleus, and infiltration of microglial cells. Degeneration of neurons suggested two mechanisms, the production of gliosis is one of the mechanisms by which lead causes its adverse effects on the central nervous system. In the central nervous system, astrocytes are numerous cells that support neurons, contribute to the formation and function of synapses, thin synapses by phagocytosis, and perform a variety of homeostatic processes⁽²⁶⁾. Another mechanism is calcium ions control a wide range of biological functions in a healthy CNS, including neurogenesis, differentiation, and synaptic activity. Lead may have inhibited calcium's regulating action on neuronal cell integrity and suppressed various intracellular biological functions⁽²⁷⁻²⁸⁾.

Lead causes neuropathological and metabolic changes in the central nervous system, resulting in serious damage. All of these abnormalities were related to a positive caspase-3 activation in most neurons with varying degrees of astrogliosis⁽²⁹⁾. On the other hand, the current study explained that spinal cord tissues of rats treated with MSCs exhibited an increased number of normal neurons with mild degenerated astrocytes and few cavitation areas. Our data are in accordance with **Kim et al.**⁽³⁰⁾ suggested that the treatment of MSCs reduced damage to spinal cord injury. This ameliorative effect of MSCs on the injured spinal cord because MSC produces neuroprotective growth factors and decreases inflammation in the damaged spinal cord tissue.⁽³¹⁾

Moreover, in our experiment treatment of FSO recorded a moderate improvement in histopathology of spinal cord tissue as showed by moderate necrosis and degenerated astrocytes and moderate cavitation areas. **Gholaminejhad et al.**⁽³²⁾ reported that flax seeds

improve the damage of spinal cord tissue after injury. This improvement is due to suppressed oxidative stress and enhancing the antioxidant system by flax seeds⁽⁴⁾.

CONCLUSION

Collectively, this study supports the application of MSCs and FSO for combating oxidative stress to spinal cord tissues induced by lead acetate reflected by improvement of histopathological alterations, reduction of DNA damage, and down-regulating apoptotic markers, thereby preserving spinal cord function. In addition, MSCs may also be an effective therapeutic agent against spinal cord injury.

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

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