THERAPEUTIC EFFECTS OF CALCIUM DISODIUM EDETATE WITH ALPHA-LIPOIC ACID AND CHIA SEEDS AGAINST LEAD-CADMIUM-INDUCED NEUROTOXICITY IN RATS

By

Abd El-Ghany, M. A

Department of Home Economics,
Faculty of Specific Education,
Mansoura University, Egypt

Ghozy, S. F.

Department of Home Economics,
Faculty of Specific Education,
Mansoura University, Egypt

Sultan, Y. M.

Department of Home Economics,
Faculty of Specific Education,
Mansoura University, Egypt

Research Journal Specific Education
Faculty of Specific Education
Mansoura University

ISSUE NO. 73 JANUARY , 2023

Therapeutic effects of calcium disodium edetate with alpha-lipoic acid and chia seeds against lead-cadmium-induced neurotoxicity in rats

*Abd El-Ghany, M. A** *Ghozy, S. F.**

*Sultan, Y. M.**

Abstract:

The present study was designed to investigate the effects of mixture Sodium calcium edetate (CaNa2EDTA) and Alpha lipoic acid (ALA) with chia seeds powder (ChS.P) against lead and cadmium-induced Neurotoxicity in rats. Twenty-four healthy Albino male rats, weighing 158 ± 3 g were subdivided randomly into four groups (6 each), one served as negative control group (-ve) and lead-cadmium induced neurotoxicity rats, that classified into untreated neurotoxicity group (+ve) and two treated groups with CaNa2EDTA and ALA combined and ChS.P. The experiment lasted for 60 days after induction of neurotoxicity. Food intake amount and rat's weight were recorded to obtain nutritional parameters. Blood samples were collected to assays the levels of Lactic Dehydrogenase (LDH), C-reactive protein level (CRP), Cyclooxygenase-2 (COX-2), Dopamine (DA), Serotonin (ST), Acetyl cholinesterase (AChE), some serum heavy metals, lipid peroxidation, antioxidant enzymes activity, some kidney and liver functions. Also, the histopathological examination changes in brain tissue. The study results treated with CaNa2EDTA and ALA combined and ChS.P showed a significant improvement in serum levels of LDH, CRP, COX-2, DA, ST, AChE some heavy metals, Lipid peroxidation, Antioxidant enzymes activity, and some kidney and liver function, when compared to untreated neurotoxicity group (+ve).

It can be recommend that the consumption of Alpha-lipoic acid and chia seed in diets have ant-neurotoxicity and antioxidants effects and that

* Department of Computer Teacher Preparation, Faculty of Specific Education, Damietta University

play significant safer role from calcium disodium edetate in treatment of lead-cadmium toxicity and inhibit of complications.

Keywords: Salvia hispanica L, Neurotoxicity, Thioctic acid, Sodium calcium edetate, Cadmium, Lead and Rats.

INTRODUCTION:

Lead (Pb) and Cadmium (Cd) are ecological prevalent neurotoxic heavy metals. They are also known to induce diversified organ damage, cause degenerative bone disease, and destroyed the central nervous system even at the lowest levels of exposure **(Diaconu *et al.*, 2020 and Alengebawy *et al.*, 2021)**.

Sodium calcium edetate or edetate calcium disodium (CaNa2EDTA) is an EDTA derivative salt with two sodium atoms and one calcium atom **(Barton, 2014)**. CaNa2EDTA can be useful for treated of specific neurodegenerative disorders by chelating metal ions to prevent inflammatory changes from taking place, such as short-term and long-term poisoning of Pb, and Cd, and remove excess iron from the body. **(Mostafalou *et al*, 2015)**.

Alpha-lipoic acid (ALA) and also known as Lipoic acid, α-lipoic acid, and thioctic acid is an antioxidant naturally occurring compound that's made in the body to protect against damage to the body's cells. It is also available as a pharmaceutical drug, as an antioxidant nutritional supplement, The Alpha-lipoic acid it can be form complex compounds with metal ions such as iron, cadmium, lead, zinc, nickel, cobalt, and manganese to prevent the free radical-induced tissue damage or inactivation of the antioxidant enzymes in the body **(Yuan *et al.*, 2021)**.

Chia seed are the edible part of the *Salvia hispanica L.* and have protruded as plant-based nutraceuticals and have gained nutritionist's attention due to their balanced nutritional composition of vitamins (A, B, K, E and D), minerals (iron, Zinc, potassium, calcium, magnesium, and phosphorus), proteins, polyunsaturated fatty acids (omega-3), antioxidants, and fiber**,**. Moreover, antioxidant analysis of chia seeds revealed the presence of more than 27 elements, including flavonoids, phenolic acids

"such as caffeic acid, cinnamic acid, rosmarinic acid, quercetin, and myricetin", proanthocyanidin-related phenolics, and procyanidin dimers **(Abdel-Aty *et al.*, 2021)**. The Chia seeds possess many important functional properties such as solubility, water-holding capacity, viscosity, oil holding capacity, chelator, emulsion stability, foam enhancer and foaming stability "which prove its potential to be used as a thickening agent", gel-forming agent, emulsifying agent, rehydrating agent, clarifying agent and as suspension formers in the formulation of food products at commercial level or home **(Knez Hrnčič *et al.*, 2019)**. Chia seed consumption has growing over the years, given its health benefits related to chronic diseases such as cardiovascular diseases, obesity, cancer, anemia treatment, analgesic, antidepressant, vision and immunity improver, diabetes and neurodegenerative diseases such as Alzheimer's disease **(Din *et al.*, 2021 and Motyka *et al.*, 2022)**. Therefore, this study was carried out to evaluate the possible modifying effects of Alpha lipoic acid and CaNa2EDTA combined and chia seeds powder on against Cd- and Pb-induced Neurotoxicity in male rats.

MATERIALS AND METHODS

A-Materials:

Chia seeds (*Salvia Hispanica L.*) was purchased from the products selling unit at the National Research Center, Dokki, Egypt. Lead Acetate [Pb (CH3COO)2.3H2O] 99% pure and Cadmium Chloride (CdCl2.H2O) 98% pure were purchased from El-Gomhouria Company for trading Drugs, Chemicals, Mansoura city branch, Dakahlia Governorate, Egypt. Thioctic acid drugs were purchased from a pharmacy at Mansoura city. Ethylenediaminetetraacetic acid calcium disodium salt (CaNa2EDTA) was purchased commercially from Sigma-Aldrich Corporation (St. Louis, Missouri, USA). Basal diet was prepared according to **NRC (1995)**. Twenty-four healthy adult Albino male rats of Sprague–Dawley strain weighing 158 ± 3 g were purchased from the laboratory animal farm of Veterinary Medicine at Zagazig University in Egypt.

B-Methods:

Chia seeds powder:

Seeds were thoroughly checked to remove any impurities and then ground into a powder. The whole seed powder was saved until used in diet preparation in well-closed, opaque glass jars in the refrigerator to prevent lipid oxidation. The diet was supplemented with 5% chia seeds powder by substituting some of the basic diet components.

Induction of Neurotoxicity (NT):

Cadmium chloride ($CdCl_2$) and Lead Acetate Pb (CH_3COOH_2) are used for experimental induction of neurotoxicity in rodents. $CdCl_2$ and Pb were prepared as a mixture of a toxic solution by dissolving the following doses in 1 ml distilled water at a dose of Cd (5 mg/kg b.w.) according to **Mohammed *et al.*, (2014)**, combined with the dose of Pb (30 mg/kg b.w.) according to **Saleh & Meligy (2018)**. The toxic solution was freshly prepared daily and administered orally gavage in a volume of 1 mL/kg b.w. once daily for 30 days before treatment.

Alpha lipoic acid solution:

Thioctic tablets, manufactured by EVA pharma for pharmaceuticals and Medical Appliances, as a pack of contained 30 film-coated tablets. Each tablet contains 300 mg of alpha lipoic acid. The tablets were crushed and suspended in distilled water, and administrated orally gavage as a freshly prepared daily solution at a therapeutic once daily dose of (54 mg/kg b.w.) for 30 days after toxicity according to **El-Sayed *et al., (2016).***

$CaNa_2EDTA$ solution:

$CaNa_2EDTA$ solution was made fresh daily by dissolving $CaNa_2EDTA$ in sterile saline to a concentration of 50 mg/mL, then was given as intraperetinoel injection in a therapeutic once daily dose of (50 mg/kg) for 5 consecutive days depending on the body weight of each rat according to **Sánchez-Fructuoso *et al.*, (2002)**.

Experimental Animals Protocol:

Rats were kept under surveillance for seven days for adaptation and fed on basal diet. 6 rats served as negative control group and 18 rats were orally gavage received a mixture of a toxic solution of (Cd 5 mg/kg b.w.) according to **Mohammed *et al.*, (2014)**, combined with the dose of Pb (30 mg/kg b.w.) according to **Saleh & Meligy (2018)** for 30 days to induce Neurotoxicity which classified into untreated group (positive control) and 2 treated rat groups that treated with combined of CaNa2EDTA (50 mg/kg b.w. i.p) a once-daily dose for 5 consecutive days according to **Sánchez-Fructuoso *et al.*, (2002)**, along with Thioctic acid tablets (ALA) administrated by oral gavage as a freshly prepared daily solution at a once-daily dose of (54 mg/kg b.w.) according to **El-Sayed *et al.*, (2016)** for 30 days, and group treated with Chia seeds powder (+ChS.P): treated with 5% chia seeds powder by substituting some of the basic diet components, for 30 days. Food and water was provided *ad-libtum*. Food intake was recorded daily and body weight of rats was measured once weekly, until the end of experimental period (60 days). All the biological experimental procedures were applied according to Internationally Ethical Guidelines for the care and use of laboratory animals. And permission for the experiment was obtained from the Research Ethics Committee at the Faculty of Specific Education, Mansoura University.

Chemical composition of chia seeds samples:

Moisture, protein, fat, ash and fiber contents in dry weight (D.w) were determined according to the methods of the **AOAC (2005**). Total carbohydrates and Nitrogen-free extract (NFE) were calculated by difference as following:

Total carbohydrates% = 100 - (moisture % + protein % + fat % + ash %).

Nitrogen-free extract% = 100 - (moisture% +protein% +fat % +ash %+ fiber %).

Energy was expressed in kilocalories per 100g according to **Watt & Merrill (1963)**, using the following formula:

Energy (kcal.100g) = (g of protein x 4) + (g of fat x 9) + (g of carbohydrate x 4).

Nutritional Parameters:

The amount of food intake was recorded daily, while rat's weight was measured once a week to identify body weight gain. Body weight gain, feed efficiency ratio (FER), and protein efficiency ratio (PER) calculate according to **Chapman *et al*., (1959)**.

Biological analyzes:

At the end of experimentation, all animal were anesthetized by di-ethyl ether and blood samples were collected from the inner canthus of the rat's eye using heparinized capillary tubes, and then the serum was obtained after centrifugation at 3000 rpm for 10 minutes. The serum biochemical analysis includes the following:

Lactic Dehydrogenase (LDH),).C-reactive protein level (CRP), Cyclooxygenase-2 (COX-2).were measured depending on the method of **(Vassault, 1983; Vaishnavi, 1996 and Van Weemen & Schuurs, 1971, respectively)**. Dopamine (DA) level was assessed by enzyme-linked immunosorbent assay using the manufacturer's protocol of a Mouse/Rat Dopamine ELISA Assay Kit (No. DOU39-K01; Eagle Biosciences, Inc., Boston, USA). Serotonin (ST) levels were assessed by using the manufacturer's protocol of a Rat Serotonin ELISA Kit (No. LS-F27987; LifeSpan Biosciences, Inc., Seattle, Washington, USA). Acetyl cholinesterase (AChE) levels were assessed by using the manufacturer's protocol of a Rat Acetylcholinesterase ELISA Kit Principles (No. DEIASL417; Creative Diagnostics Co., Shirley, New York, USA). Serum Heavy metals: Zinc (Zn), Cadmium (Cd) and Lead (Pb) were quantified using method according to **(Kelishadi *et al*., 2018 and Manton *et al*., 2001, respectively)**.

Serum Lipid peroxidation (Malondialdhyde "MDA") and Antioxidant enzymes parameters superoxide dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH). Glutathione Peroxidase (GPx) were determined according to the method described by **(Eze *et al*., 2008;**

Paoletti & Macali, 1990; Sinha, 1972; Rice-Evans & Miller, 1994 and Paglia & Valentine, 1967, respectively). Some serum kidney and liver function parameters Creatinine , Urea, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) , Alkaline phosphatase (ALP) and Total Bilirubin (T.BiL) were determined according to **(Henry *et al.*, 1974; Rock *et al*., 1987; Belfield & Goldberg, 1971; Orlowski & Meister, 1963 and Young *et al.*, 1975, respectively)**.

Histopathological examination of the brain tissue:

The brain tissue was stained with hematoxylin and eosin (H&E) for histopathological examination according to **Bancroft *et al.*, (1996).**

Statistical data analysis:

Collected data were subjected to analysis according to SPSS program according to **Abu-Bader (2011)**.

RESULTS AND DISCUSSION:

Chemical composition of chia seeds samples:

The data in table (1) showed chemical composition of the content in percent of moisture, protein, lipid, ash, total carbohydrate (T. Carb), fiber, Nitrogen-free extract (NFE) and energy of chia seeds. It noticed that the chemical composition of chia seeds are as the following: moisture content is (7.63 ± 0.11 %), protein content is (22.86 ± 0.11 %), lipid content is (23.87 ± 0.05 %), ash content of is (4.76 ± 0.05 %), T. Carb content is (40.88 ± 0.22 %), fiber content is (21.11 ± 0.03 %), and NFE content is (19.76 ± 0.24 %).

A review showed that the chemical composition of chia seeds contains (16:26%) protein, (31:34%) fat, (37:45%) total carbohydrates, and (23-35%) total dietary fiber **(Marcinek & Krejpcio, 2017)**. The chemical compositions of some different seeds are a good source of essential nutritional compounds associated with health promotion and disease prevention **(Senila *et al.*, 2021).**

Table (1): Chemical composition (%) of raw Chia seeds:

Component (%)	Values
Moisture	**7.63 ± 0.11**
Protein	**22.86 ± 0.11**
Fat	**23.87 ± 0.05**
Carbohydrates	**40.88 ± 0.22**
Ash	**4.76 ± 0.05**
Fibers	**21.11 ± 0.03**
NFE	**19.76 ± 0.24**
Energy (kcal.100g)	**469.79 ± 3.06**

Each value is average repetition three times, whereas illustrating mean ± SD

Nutritional indicators of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder at the end of study:

The statistical data in table 2 showed rat's body weight gain, body weight gain percent, food intake, feed efficiency ratio (FER), protein intake, and protein efficiency ratio (PER). The untreated neurotoxicity rats group (+ve) showed a significant decrease in body weight gain, body weight gain percent, food intake, FER, protein intake, and PER, when compared with the control group (-ve). The neurotoxicity rat groups treated with the combination of (+CaNa2EDTA & ALA) and chia seeds powder showed significant decrease in body weight gain, body weight gain percent, food intake, FER, protein intake, and PER, when compared with the control group (-ve). While, CaNa2EDTA & ALA and chia seeds powder treated groups marked a significant improvement when compared with the untreated neurotoxicity group (+ve).

Rat's chronic administration of Pb and Cd is associated with lessening body weight, anorexia, nausea, and vomiting associated with muscle wasting and oxidative stress which typically follow continuous exposure **(Fiati Kenston *et al.*, 2018 and Lopotych *et al.*, 2020)**. Also, treated with chia seeds achieved significant improvement in the weight

gained and different nourishment effects when compared to the positive control group **(Alamri, 2019)**.

Table (2): Nutritional indicators of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

groups \ Parameters		Weight Gain (g)	Weight Gain %	Food Intake (g)	feed efficiency ratio (FER)	Protein Intake (g)	Protein efficiency ratio (PER)
untreated	Control (-ve)	79.83 ±2.32[a]	50.43 ±1.82[a]	18.08 ±0.34[a]	0.073 ±0.003[a]	3.62 ±0.07[a]	0.37 ±0.02[a]
	Control (+ve)	34.17 ±2.86[c]	21.61 ±1.83[c]	11.99 ±0.34[c]	0.047 ±0.004[c]	2.40 ±0.07[c]	0.24 ±0.02[c]
treated	+CaNa2EDTA & ALA	55.33 ±1.21[b]	35.06 ±0.52[b]	15.33 ±0.34[b]	0.060 ±0.002[b]	3.07 ±0.07[b]	0.30 ±0.01[b]
	+ChS.P	54.67 ±3.08[b]	34.67 ±1.84[b]	15.02 ±0.34[b]	0.061 ±0.003[b]	3.00 ±0.07[b]	0.30 ±0.01[b]

The results were illustrating as mean ± SD values in each column having different combinations of superscript letters (a, b, c, d...).

Biological analyzes:

1- Serum Lactic Dehydrogenase (LDH), C- reactive protein (CRP), and Cyclooxygenase-2 (COX-2) levels of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

The statistical data in table 3 showed a significant increase in LDH, CRP, and COX-2 levels in the untreated neurotoxicity rats group (+ve) when compared with the control group (-ve). While the neurotoxicity group treated with the combination of (+CaNa2EDTA & ALA) showed a significant increase in LDH, and COX-2 levels, while CRP showed non-significant differences when compared with the control group (-ve). Also, the neurotoxicity group treated with (ChS.P) showed a significant increase in LDH, CRP, and COX-2 levels when compared with the control group (-ve). Moreover, treated groups with combination of CaNa2EDTA & ALA

and chia seeds powder marked a significant improvement in the levels of LDH, CRP, and COX-2, when compared with the untreated neurotoxicity group (+ve).

The exposure to toxic Pb and Cd compounds, where their studies showed a significant increase in the levels of serum LDH, CRP, and COX-2 after Pb and Cd administrated **(Rafiei-Asl *et al.*, 2019)**.

The protective potential of Alfa-lipoic acid as strong antioxidants and heavy metal chelators against cadmium-induced, results showed significantly decreased activity of LDH, CRP, and COX-2 when administrated ALA supplements which defend against Cd toxicity **(Markiewicz-Górka *et al.*, 2019)**. Also, administrated the protein hydrolysate and peptide fractions isolated from chia seeds have anti-inflammatory properties, especially Cyclooxygenases enzymes like COX-2 which stimulate the chemotactic process and the entry of cells to the inflammation site **(Chan-Zapata *et al.*, 2019 and Rabail *et al.*, 2021)**.

Table (3): Serum Lactic Dehydrogenase (LDH), C- reactive protein (CRP), and Cyclooxygenase-2 (COX-2) levels of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

groups \ Parameters		LDH (U/L)	CRP (mg/mL)	COX-2 (pg/mL)
untreated	Control (-ve)	1377.83 ±12.95^{d}	2.14 ±0.17^{c}	33.85 ±4.15^{d}
	Control (+ve)	4195.67 ±12.83^{a}	5.55 ±0.62^{a}	79.90 ±2.07^{a}
treated	+CaNa2EDTA & ALA	1551.67 ±12.55^{c}	2.29 ±0.25^{c}	43.95 ±7.14^{c}
	+ChS.P	2624.33 ±13.06^{b}	3.29 ±0.28^{b}	58.98 ±3.00^{b}

The results were illustrating as mean ± SD values in each column having different combinations of superscript letters (a, b, c, d...).

2- Serum Dopamine (DA), Serotonin (ST), and Acetylcholinesterase (AChE) levels of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

The statistical data in table 4 showed a significant increase in of DA and AChE levels, and a significant decrease in ST level in the untreated neurotoxicity rats group (+ve) when compared with the control group (-ve). While, the neurotoxicity groups treated with the combination of (+CaNa2EDTA & ALA) and (ChS.P) showed a significant increase in DA and AChE levels, While, ST level showed a significant decrease when compared with the control group (-ve). Moreover, neurotoxicity groups treated with the combination of (+CaNa2EDTA & ALA) and (ChS.P) marked a significant improvement in the levels of DA, ST and AChE when compared with the untreated neurotoxicity group (+ve).

Pb and Cd increased the AChE activity in brain which affects cholinergic neurotransmission efficacy in the synapsis, and decreases the response and stimulation of acetylcholine receptors causing cognitive dysfunction. Also, Pb and Cd increase the dopamine and glutamate levels, and decrease serotonin concentration, which causes impairment of catecholaminergic and serotoninergic transmission **(Al-Kahtani, 2019)**. Chia seed contains Leucine (a branched-chain amino acid) that is used as an energy source and helps reduce muscle protein breakdown, modulates the uptake of brain neurotransmitter precursors, and releases encephalins, which inhibits pain signals entering the nervous system **(Melo-Ruíz *et al.*, 2016)**.

Table (4): Serum Dopamine (DA), Serotonin (ST), and Acetylcholinesterase (AChE) levels of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

groups \ Parameters		DA (ng/ml)	ST (ng/ml)	AChE (pg/ml)
untreated	Control (-ve)	11.55 ±0.51[d]	184.72 ±4.81[a]	25.98 ±2.80[d]
	Control (+ve)	23.22 ±0.41[a]	136.50 ±7.05[d]	127.43 ±11.10[a]
treated	+CaNa2EDTA & ALA	13.88 ±0.60[c]	170.17 ±6.45[b]	39.67 ±7.68[c]
	+ChS.P	18.27 ±0.37[b]	148.85 ±3.31[c]	74.88 ± 5.78[b]

The results were illustrating as mean ± SD values in each column having different combinations of superscript letters (a, b, c, d...).

3- Serum heavy metals: Lead (Pb), Cadmium (Cd), and Zinc (Zn) levels of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

The statistical data in table 5 showed a significant increase in Pb, and Cd levels, while Zn level showed a significant decrease in the untreated neurotoxicity group (+ve) when compared with the control group (-ve). While the neurotoxicity group treated with the combined of CaNa2EDTA & ALA showed a significant decrease in Pb and Zn levels, while Cd level showed non-significant differences when compared with the control group (-ve). The neurotoxicity rats group treated with (+ChS.P) showed a significant increase in Pb and Cd levels, while Zn level showed a significant decrease when compared with the control group (-ve). Also, the (+ChS.P) treated group marked a significant decrease in Pb and Cd levels when compared with the untreated neurotoxicity group (+ve).

Pb and Cd are toxic metals humans are exposed to because they can be accumulated in human organisms, especially the brain, which causes neurotoxicity and poses a major threat to public health. Zinc (Zn) may be beneficial in low-dose heavy metal mixture-induced neurotoxicity in rats by its anti-inflammatory and antioxidant mechanisms **(Anyanwu *et al.*, 2020)**. CaNa2EDTA chelates binds Pb and Cd strongly, eliminating them in the urine. During this process, CaNa2EDTA causes greater losses of many essential minerals. Also, the levels of minerals didn't have a big significantly change and were within the reference ratios after heavy metals administration **(Shaban *et al.*, 2021)**.

Chia seeds is a great source of minerals that are considered inorganic nutrients essential for the maintenance of life's physicochemical processes **(Prathyusha *et al.*, 2019 and Senila *et al.*, 2021)**. Also, ALA intervenes in the pathomechanisms of neurodegeneration and its disturbing neuronal energy metabolism and shows a positive effect on dementia sufferers **(AlMomen & Blaurock-Busch, 2022)**.

Table (5): Serum heavy metals: Lead (Pb), Cadmium (Cd), and Zinc (Zn) levels of neurotoxicity rats treated by calcium disodium edetate with alpha-lipoic acid and chia seeds powder:

groups \ Parameters		Pb (µg/l)	Cd (ng/ml)	Zn (nmol/ml)
untreated	Control (-ve)	2.27 ±0.13^{c}	0.78 ±0.21^{c}	2.67 ±0.19^{a}
	Control (+ve)	6.51 ±0.39^{a}	3.45 ±0.08^{a}	2.15 ±0.9^{b}
treated	+CaNa2EDTA & ALA	1.80 ±0.11^{d}	0.72 ±0.04^{c}	1.03 ±0.21^{d}
	+ChS.P	4.99 ±0.07^{b}	2.81 ±0.17^{b}	1.72 ±0.11^{c}

The results were illustrating as mean ± SD values in each column having different combinations of superscript letters (a, b, c, d...).

4- Serum free radical (Malondialdhyde "MDA") concentration levels and antioxidant enzymes Catalase (CAT), Reduced Glutathione (GSH), Glutathione Peroxidase (GPX) and superoxide dismutase (SOD) levels of the experimental rat groups:

The statistical data in table 6 showed a significant increase in MDA levels, and showed a significant decrease in CAT, GSH, GPX and SOD levels in the untreated neurotoxicity rats group (+ve) when compared with the control group (-ve). While, the neurotoxicity groups treated with a combined of CaNa2EDTA & ALA and chia seeds powder (+ChS.P) showed a significant increase in MDA levels, and showed a significant decrease in the levels of CAT, GSH, GPX and SOD when compared with the control group (-ve). Also, the CaNa2EDTA & ALA and (ChS.P) treated groups marked a significant improvement in CAT, GSH, GPX and SOD levels when compared with the untreated neurotoxicity group (+ve).

The ubiquity of heavy metals such as lead and cadmium may adversely affect the balance between antioxidants enzymes and free radicals scavenger in the human body. Therefore, heavy metals have been linked to

many health problems including neurotoxicity, infertility, cancer, osteoporosis, and human organ failure **(Abd El-Ghany *et al.*, 2017 and Abdulidha *et al.*, 2020)**. In addition, when the formation of ROS has exceeded antioxidant levels, the free radicals will attack macromolecules, especially, proteins, DNA, polysaccharides, and cell membranes that consist of polyunsaturated fatty acids, leading to an increase in MDA levels and a decrease in antioxidant enzymes activity, which causes progressive damage of cellular structures eventually **(Halliwell, 2011 and Poljsak *et al.*, 2013)**.

Alpha-lipoic acid has an amphiphilic property which allows it to easily cross the blood-brain barrier and cell membranes and also helps to activate other antioxidants such as ubiquinone (coenzyme Q10) vitamin C, and vitamin E. Also, Alpha-lipoic acid has a promising natural and soft chelating agent **(AlMomen & Blaurock-Busch, 2022)**. Also, rats fed on chia seeds showed a significant decrease in MDA and a significant increase in the antioxidant indices including SOD, CAT, GPX and GSH activities. Seeds antioxidant activity is due to its contents of bioactive components such as phenolic and flavonoids, because it's scavenging effect on the free radicals **(Marcinek & Krejpcio, 2017 and El-Feky *et al.*, 2022)**.

Table (6): Serum Malondialdhyde (MDA) and antioxidant enzymes Catalase (CAT), Reduced Glutathione (GSH), Glutathione Peroxidase (GPX) and superoxide dismutase (SOD) levels of the experimental rat groups:

Parameters / groups		MDA nmol/ml	CAT (U/L)	GSH (µmol/L)	GPX (mU/ml)	SOD (U/L)
untreated	Control (-ve)	6.07 ±0.20^{d}	0.95 ±0.01^{a}	2.06 ±0.08^{a}	96.12 ±2.92^{a}	42.49 ±0.90^{a}
	Control (+ve)	21.18 ±3.23^{a}	0.23 ±0.02^{d}	0.69 ±0.03^{d}	25.57 ±8.46^{d}	8.11 ±4.19^{d}
treated	+CaNa2EDTA & ALA	9.93 ±0.72^{c}	0.79 ±0.01^{b}	1.57 ±0.12^{b}	72.36 ±5.78^{b}	38.35 ±0.93^{b}
	+ChS.P	14.02 ±1.19^{b}	0.65 ±0.01^{c}	1.18 ±0.06^{c}	56.27 ±3.09^{c}	23.77 ±5.93^{c}

The results were illustrate as mean ± SD values in each column having different combinations of superscript letters (a, b, c, d...).

5- Some serum Kidney and Liver function: Creatinine, Urea, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma-glutamyl Transferase (GGT) and Total Bilirubin (T.BiL) levels of the experimental rat groups:

The data in table (7) showed significant increase in Creatinine, Urea, ALT, AST, ALP, GGT and T.BiL levels in the untreated neurotoxicity group (+ve) when compared with the control group (-ve). While the neurotoxicity group treated with the combined of CaNa2EDTA & ALA showed a significant increase in Urea, ALT and AST Levels when compared with the control group (-ve). While, the neurotoxicity rats group treated with chia seeds powder (+ChS.P) showed significant increase in Creatinine, Urea, ALT, AST, ALP, GGT and T.BiL levels when compared with the control group (-ve). Moreover, neurotoxicity treated groups with the combined of CaNa2EDTA & ALA and ChS.P marked a significant improvement in Creatinine, Urea, ALT, AST, ALP, GGT and T.BiL levels when compared with the untreated neurotoxicity group (+ve).

The prolonged exposure to heavy metals, mostly Pb and Cd impairs kidney functions through the induction of oxidative stress, which appears to significantly increase urea and creatinine levels **(Bhattacharjee *et al.*, 2016 and Rana *et al.*, 2018)**. Pb and Cd cause the increase in liver enzymatic activities especially AST and ALT, the release of intracellular enzymes into the bloodstream, irritability, free radical production, raised the cellular metabolic rate, and liver damage **(Zou *et al.*, 2020 and Hassan *et al.*, 2022)**.

CaNa2EDTA effectively slowness the progression of chronic kidney disease in patients with body Pb burden, as shown by increased levels of creatinine clearance and glomerular filtration. While, Alpha-lipoic acid has a protective effect on Cd-induced oxidative stress, due to its potent antioxidant and metal chelator activity that can repair oxidative damaged and exert antioxidant cellular activities **(Luo *et al.*, 2016)**. The

administration of Alpha-lipoic acid significantly decreased the activity of serum AST, ALT, LDH, and GGTP, which counteracts organ damage caused by cadmium **(Markiewicz-Górka *et al.*, 2019)**.

The administration of chia seed in diet may decrease liver enzymatic activities and slightly improve the health state of liver disease **(Ali *et al.*, 2019 and Fernández-Martínez *et al.*, 2019)**. Also, chia seeds had shown significant improvement in Creatinine and Urea levels, due to the high content of dietary fiber, and high antioxidant activity in the seeds **(Khafagy & Samir, 2022)**.

Table (7): Some serum Kidney and Liver function: Creatinine, Urea, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma-glutamyl Transferase (GGT) and Total Bilirubin (T.BiL) levels of the experimental rat groups:

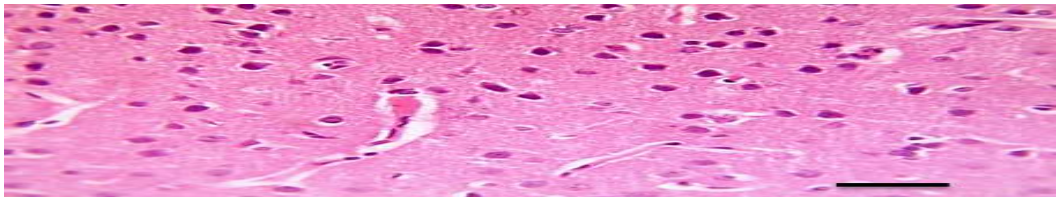
groups \ Parameters		Creatinine (mg/dl)	Urea (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	T.BiL (mg/dl)
untreated	Control (-ve)	0.53 ±0.02^{d}	25.65 ±4.62^{d}	34.67 ±5.20^{d}	140.50 ± 9.20^{d}	86.00 ±10.99^{c}	3.29 ± 0.46^{d}	0.31 ±0.09^{c}
	Control (+ve)	1.57 ±0.12^{a}	101.17 ±9.56^{a}	83.33 ±3.56^{a}	419.33 ±8.82^{a}	344.00 ±11.20^{a}	19.28 ±3.28^{a}	1.56 ±0.20^{a}
treated	+CaNa2EDTA & ALA	0.59 ±0.02^{c}	37.33 ±3.51^{c}	44.17 ±6.27^{c}	170.17 ±7.28^{c}	95.33 ±8.29^{c}	4.30 ±0.54^{c}	0.41 ±0.14^{c}
	+ChS.P	0.84 ±0.03^{b}	60.50 ±5.87^{b}	63.50 ±7.09^{b}	304.33 ±8.29^{b}	207.83 ±9.35^{b}	11.68 ±2.04^{b}	0.64 ±0.12^{b}

The results were illustrate as mean ± SD values in each column having different combinations of superscript letters (a, b, c, d...).

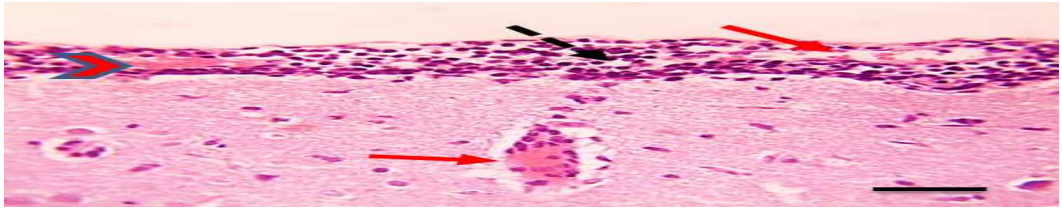
Histopathological analysis of Brain specimens:

The microscopic pictures from the Brain cortical sections appear as the following: the control group (-ve) and +CaNa2EDTA & ALA treated group showing normal neurons and neuropil in pictures (1&3) respectively. While, the untreated neurotoxicity rats group (+ve) showing leukocytic cell

infiltration, congestion and hemorrhage in meninges, congested blood vessels, focal area of necrosis infiltrated by leukocytic cells, and vacuolation in the neuropil in picture (2). The neurotoxicity rat groups treated with chia seeds powder (+ChS.P) showing mildly congested blood vessels in pictures (4).



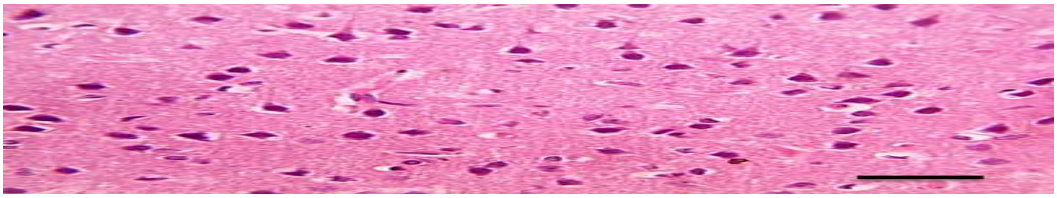
Pic (1): Brain cortical sections for negative control group (-ve) showing normal neurons and neuropil (H&E-stained, X: 400 bar 50).



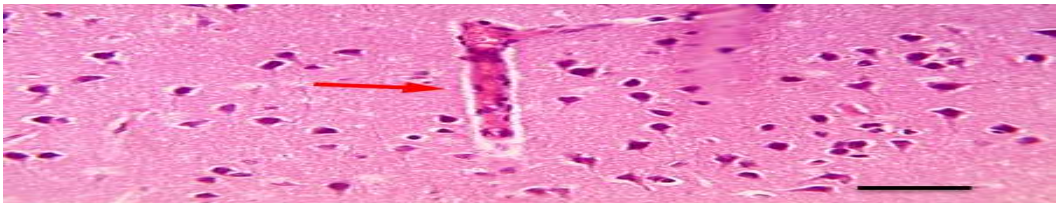
Pic (2): Brain cortical sections for untreated neurotoxicity group (+ve) showing leukocytic cells infiltration (dashed black arrows), congestion (red arrow) and hemorrhage (red arrowhead) in meninges, congested blood vessels (red arrows), focal area of necrosis (black arrows) infiltrated by leukocytic cells (blue arrows), and vacuolation (arrowheads) in the neuropil (H&E-stained, X: 400 bar 50).

The ability of Pb to replace calcium ions allows its passage through the blood-brain barrier and accumulation in the brain using calcium-ATPase pumps, which might play a significant role in the development of neurodegenerative diseases **(Shaban *et al.*, 2021 and Enogieru & Egbon, 2022)**. The use of nutrients combination from medicinal plants may reduce the toxic effect of heavy metals Cd and Pb mediated oxidative damage in the liver, brain, kidney, thyroid gland and testes evident by histological sections **(Abd El-Ghany *et al.*, 2019 and Abdulidha *et al.*, 2020)**. Moreover, the deleterious effect of the drugs and chemicals on the

histological state and functions of the brain can be prevented and treated through the administration of healthy medicinal plants such as nuts, chia seeds, flax seeds, and moringa seeds **(Henrich, 2020 and Ansari & Fatima, 2021)**.



Pic (3): Brain cortical sections for treated group by CaNa2EDTA &ALA showing normal neurons and neuropil (H&E-stained, X: 400 bar 50).



Pic (4): Brain cortical sections for treated group by ChS.P showing mildly congested blood vessels (red arrows) (H&E-stained, X: 400 bar 50).

CONCLUSION:

In conclusion, we recommend including Alpha-lipoic acid and chia seed in diets for those who exposed to lead and cadmium poisoning because they have the ability to reduce the neurotoxic effects of lead and cadmium, on the nervous system.

REFERENCES:

- **Abd El-Ghany M. A.; Ghozy S. F. and Sultan Y. M. (2019):** Efficacy of curcuma extract compared with glucosamine sulfate for treatment of osteoarthritis in rats World journal of pharmacy and pharmaceutical sciences 8(11), 61-76.
- **Abd El-Ghany, M. A.; El-Mehiry, H.; Nagib, R. and El-Sayed, H. M. (2017):** Effect of orexin drug and some minerals on rats exposed to obesity and alzheimer disease. World journal of pharmacy and pharmaceutical sciences 6

(1), 21-37.

- **Abdel-Aty, A. M.; Elsayed, A. M.; Salah, H. A.; Bassuiny, R. I. and Mohamed, S. A. (2021):** Egyptian chia seeds (*Salvia hispanica L.*) during germination: Upgrading of phenolic profile, antioxidant, antibacterial properties and relevant enzymes activities. Food Science and Biotechnology, 30(5), 723–734.
- **Abdulidha, N. A.; Jaccob, A. A. and AL-Moziel, M. S. G. (2020):** Protective effects of Co-Q10, Ginkgo biloba, and l-carnitine on brain, kidney, liver, and endocrine system against sub-acute heavy metals toxicity in male rats. Toxicol. Environ. Health Sci. **12**, 331–341.
- **Abu-Bader, S. H. (2011):** Using Statistical Methods in Social Science Research: With a Complete SPSS Guide (2nd ed.) Oxford University Press.
- **Alamri, E. (2019):** The Influence of Two Types of Chia Seed on Some Physiological Parameters in Diabetic Rats. International Journal of Pharmaceutical Research & Allied Sciences, 8(3), 131-136.
- **Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R. and Wang, M.Q. (2021):** Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics, 9(3), 42-74.
- **Ali, B.; Yaha, A. E. and Nehad, R. (2019):** Biological Studies on the Effects of Chia Seeds (*Saliva hispanica L.*) On the Healthy Status of Rats with Liver Cirrhosis. Journal of Research in the Fields of Specific Education. 6(18), 3-16.
- **Al-Kahtani, M. (2019):** Effect of both selenium and biosynthesized nanoselenium particles on cadmium-induced neurotoxicity in albino rats. Human &Amp; Experimental Toxicology, 39(2), 159–172.
- **AlMomen, A., and Blaurock-Busch, E. (2022):** Alpha-Lipoic Acid (ALA), fatty acid and promising chelating agent for neurological ailments.World, 3(01)009-014.
- **Ansari, S. and Fatima, S. (2021):** Prevention of liver problems from adverse chemical agents by healthy nutrition and Unani dietotherapy. In Phytochemistry, the Military and Health. 257-273.
- **Anyanwu, B. O.; Orish, C. N.; Ezejiofor, A. N.; Nwaogazie, I. L. and Orisakwe, O. E. (2020):** Neuroprotective effect of Costus afer on low dose heavy metal mixture (lead, cadmium and mercury) induced neurotoxicity via

antioxidant, anti-inflammatory activities. Toxicology Reports. 7, 1032-1038.

- **AOAC (2005):** Official method of Analysis of Association of Officiating Analytical Chemists. 18 th Edition. Washington, D.C.
- **Bancroft, J. D.; Suvarna, K. S. and Layton, C. (1996):** Bancroft's Theory and Practice of Histological Techniques E-Book (8^{th} Ed.) Elsevier health sciences, P. 139- 157.
- **Barton, C. (2014):** EDTA (Ethylenediaminetetraacetic Acid). In: P. Wexler, (Ed.), Encyclopedia of Toxicology (3rd ed.), Elsevier Inc. pp. 310-311.
- **Belfield, A. and Goldberg, D. M. (1971):** Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme, 12(5), 561–573.
- **Bhattacharjee, T.; Bhattacharjee, S. and Choudhuri, D. (2016):** Hepatotoxic and nephrotoxic effects of chronic low dose exposure to a mixture of heavy metals-lead, cadmium and arsenic. International Journal of Pharmaceutical, Chemical & Biological Sciences, 6(1). 39–47.
- **Chan-Zapata, I.; Arana-Argáez, V. E.; Torres-Romero, J. C. and Segura-Campos, M. R. (2019):** Anti-inflammatory effects of the protein hydrolysate and peptide fractions isolated from *Salvia hispanica L.* seeds. Food and Agricultural Immunology, 30(1), 786–803.
- **Chapman, D. G.; Castillo, R. and Campbell, J. A. (1959):** Evaluation of protein in foods: 1. A method for the determination of protein efficiency ratios. Canadian Journal of Biochemistry and Physiology, 37(5), 679–686.
- **Diaconu, M.; Pavel, L.V.; Hlihor, R.M.; Rosca, M.; Fertu, D.I.; Lenz, M.; Corvini, P.X. and Gavrilescu, M. (2020):** Characterization of heavy metal toxicity in some plants and microorganisms-A preliminary approach for environmental bioremediation. New biotechnology, 56, 130–139.
- **Din, Z. U.; Alam, M.; Ullah, H.; Shi, D.; Xu, B.; Li, H. and Xiao, C. (2021):** Nutritional, phytochemical and therapeutic potential of chia seed (*Salvia hispanica L.*). A mini-review. Food Hydrocolloids for Health, 1, 100010.
- **El-Feky, A. M.; Elbatanony, M. M.; Aboul Naser, A. F.; Younis, E. A. and Hamed, M. A. (2022):** Salvia hispanica L. seeds extract alleviate encephalopathy in streptozotocin-induced diabetes in rats: role of oxidative stress, neurotransmitters, DNA and histological indices. Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals,

27(5), 427–440.

- **El-Sayed, G. R.; Hasanee, J. A. and Ahmed, M. I. (2016):** Determination of cadmium and lead residues in tissues and administration protective treatment. Bulletin of Faculty of Science, Zagazig University, 2016(2016), 91–101.
- **Enogieru, A. and Egbon, F. (2022):** Neurobehavioural and Histological Alterations in Lead Acetate-Exposed Rats Pretreated with Aqueous Leaf Extract of Vernonia amygdalina. African Journal of Biomedical Research, 25(2), 205–213.
- **Eze, J. I.; Anene, B. M. and Chukwu, C. C. (2008):** Determination of serum and organ malondialdehyde (MDA) concentration, a lipid peroxidation index, in Trypanosoma brucei-infected rats. Comp Clin Pathol 17, 67–72.
- **Fernández-Martínez, E.; Lira-Islas, I. G.; Cariño-Cortés, R.; Soria-Jasso, L. E.; Pérez-Hernández, E. and Pérez-Hernández, N. (2019):** Dietary chia seeds (*Salvia hispanica*) improve acute dyslipidemia and steatohepatitis in rats. Journal of food biochemistry, 43(9), e12986-13002.
- **Fiati Kenston, S.; Su, H.; Li, Z.; Kong, L.; Wang, Y.; Song, X.; Gu, Y.; Barber, T.; Aldinger, J.; Hua, Q.; Li, Z.; Ding, M.; Zhao, j. and Lin, X. (2018):** The systemic toxicity of heavy metal mixtures in rats. Toxicology Research, 7(3), 396-407.
- **Halliwell, B. (2011):** Free radicals and antioxidants–quo vadis?. Trends in pharmacological sciences, 32(3), 125-130.
- **Hassan, M. A.; Mahmoud, Y. K.; Elnabtiti, A.; El-Hawy, A. S.; El-Bassiony, M. F. and Abdelrazek, H. (2022):** Evaluation of Cadmium or Lead Exposure with Nannochloropsis oculata Mitigation on Productive Performance, Biochemical, and Oxidative Stress Biomarkers in Barki Rams. Biological trace element research.
- **Henrich, C. (2020):** The Role of Nutrition in Integrative Oncology. In: Noland, D., Drisko, J., Wagner, L. (eds) Integrative and Functional Medical Nutrition Therapy. Humana, Cham. https://doi.org/10.1007/978-3-030-30730-1_26.
- **Henry, R. J.; Cannon, D. C. and Winkelman, W. (1974):** Clinical Chemistry Principles and Techniques, 11th ed. Happer and Row Publishers. p. 1629.
- **Kelishadi, R.; Pourmirzaiee, M.; Chehrazi, S. and Heidari-Beni, M. (2018):** Serum Zinc Level and Eating Behaviors in Children Receiving Zinc

Supplements without Physician Prescription. Advanced Biomedical Research, 7(1), 120. https://doi.org/10.4103/abr.abr_77_18.

- **Khafagy, M. M. and Samir, B. M. (2022):** Remedial Effects of Moringa Leaves and Chia Seeds on Diabetic White Rats. Home Economics Journal.15 (15) 739-753.
- **Knez Hrnčič, M.; Ivanovski, M.; Cör, D. and Knez, Ž. (2019):** Chia Seeds (*Salvia hispanica L.*): An Overview-Phytochemical Profile,Isolation Methods, and Application. Molecules (Basel, Switzerland), 25(1), 11-38.
- **Lopotych, N.; Panas, N.; Datsko, T. and Slobodian, S. (2020):** Influence of heavy metals on hematologic parameters, body weight gain and organ weight in rats. Ukrainian Journal of Ecology, 10(1), 175-179.
- **Luo, T.; Liu, G.; Long, M.; Yang, J.; Song, R.; Wang, Y.; Yuan, Y.; Bian, J.; Liu, X., Gu, J.; Zou, H. and Liu, Z. (2016):** Treatment of cadmium-induced renal oxidative damage in rats by administration of alpha-lipoic acid. Environmental Science and Pollution Research, 24(2), 1832–1844.
- **Manton, W.; Rothenberg, S. and Manalo, M. (2001):** The Lead Content of Blood Serum. Environmental Research, 86(3), 263–273.
- **Marcinek, K. and Krejpcio, Z. (2017):** Chia seeds (Salvia hispanica): health promoting properties and therapeutic applications - a review. Roczniki Państwowego Zakładu Higieny, 68(2):123-129.
- **Markiewicz-Górka, I.; Pawlas, K.; Jaremków, A.; Januszewska, L.; Pawłowski, P. and Pawlas, N. (2019):** Alleviating Effect of α-Lipoic Acid and Magnesium on Cadmium-Induced Inflammatory Processes, Oxidative Stress and Bone Metabolism Disorders in Wistar Rats. International journal of environmental research and public health, 16(22), 4483-4507.
- **Melo-Ruíz, V.; Schettino-Bermúdez, B.; Rodríguez-Diego, J.; Díaz-García, R.; Calvo-Carrillo, C. and Gazga-Urioste, C. (2016):** Chia seeds (Salvia hispanica L) wild plant rich in nutrients. Journal of Life Sciences, 10(5), 221-227.
- **Mohammed, E.; Hashem, K. and Rheim, M. (2014):** Biochemical study on the impact of Nigella sativa and virgin olive oils on cadmium-induced nephrotoxicity and neurotoxicity in rats. Journal of Investigational Biochemistry, 3(1), 1-8.

- **Mostafalou, S.; Baeeri, M.; Bahadar, H.; Soltany-Rezaee-Rad, M.; Gholami, M. and Abdollahi, M. (2015):** Molecular mechanisms involved in lead induced disruption of hepatic and pancreatic glucose metabolism. Environmental Toxicology and Pharmacology, 39(1), 16–26.
- **Motyka, S.; Koc, K.; Ekiert, H.; Blicharska, E.; Czarnek, K. and Szopa, A. (2022):** The Current State of Knowledge on *Salvia hispanica* and *Salviae hispanicae semen* (Chia Seeds). Molecules, 27(4), 1207-1226.
- **NRC, (1995):** National Research Conical, Nutrition Requirements of Laboratory Animals. Forth Revised Edition, Institute for Laboratory Animal Research. National Institute of Health. Academic Press. Washington DC, USA.
- **Orlowski, M. and Meister, A. (1963):** Gamma-glutamyl-p-nitroanilide: a new convenient substrate for determination and study of l- and d-gamma-glutamyltranspeptidase activities. Biochimica et biophysica acta, 73, 679–681.
- **Paglia, D. E. and Valentine, W. N. (1967):** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine, 70(1), 158–169.
- **Paoletti, F. and Mocali, A. (1990):** Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. Methods in enzymology, 186, 209–220.
- **Poljsak, B.; Šuput, D. and Milisav, I. (2013):** Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. Oxidative medicine and cellular longevity vol. 2013, 11 pages.
- **Prathyusha, P.; Kumari, B. A.; Suneetha, W. J. and Srujana, M. N. S. (2019):** Chia seeds for nutritional security. Journal of Pharmacognosy and Phytochemistry, 8(3), 2702-2707.
- **Rabail, R.; Khan, M. R.; Mehwish, H. M.; Rajoka, M.; Lorenzo, J. M.; Kieliszek, M.; Khalid, A. R.; Shabbir, M. A. and Aadil, R. M. (2021):** An overview of chia seed (*Salvia hispanica L.*) bioactive peptides' derivation and utilization as an emerging nutraceutical food. Frontiers in bioscience (Landmark edition), 26(9), 643–654.
- **Rafiei-Asl, S.; Khadjeh, G.; Jalali, S. M.; Jamshidian, J. and Rezaie, A. (2019):** Investigating the protective effects of bromelain against inflammatory marker alterations induced by cadmium pulmonary intoxication in rat. Iranian

Veterinary Journal, 16(2), 75-88.

- **Rana, M. N.; Tangpong, J. and Rahman, M. M. (2018):** Toxicodynamics of lead, cadmium, mercury and arsenic-induced kidney toxicity and treatment strategy: a mini review. Toxicology reports, 5, 704-713.
- **Reitman, S. and Frankel, S. (1957):** A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28(1), 56–63
- **Rice-Evans, C. and Miller, N. J. (1994):** Total antioxidant status in plasma and body fluids. Methods in enzymology, 234, 279–293
- **Rock, R. C.; Walker, W. G. and Jennings, C. D. (1987):** Nitrogen metabolites and renal function. In: Tietz NW. Fundementals of clinical chemistry, 3 rd Ed: 669-704. Philadelphia: WB Saunders.
- **Saleh, S. and Meligy, F. (2018):** Study on Toxic Effects of Lead Acetate on Cerebellar Cortical Tissue of Adult Albino Rats and the Role of Vitamin E as a Protective Agent. Ain Shams Journal of Forensic Medicine and Clinical Toxicology, 31(2), 110–118.
- **Sánchez-Fructuoso, A. I.; Blanco, J.; Cano, M.; Ortega, L.; Arroyo, M.; Fernández, C.;Prats, D.and Barrientos, A.(2002):**Experimental lead nephropathy: treatment with calcium disodium ethylenediaminetetraacetate. American journal of kidney diseases: the official journal of the National Kidney Foundation, 40(1), 59–67.
- **Senila, L.; Neag, E.; Cadar, O.; Kovacs, M. H.; Becze, A. and Senila, M. (2021):** Chemical, Nutritional and Antioxidant Characteristics of Different Food Seeds. Appl. Sci. 10(5), 1589-1604.
- **Shaban, N. Z.; El-Kader, A.; Sara, E.; Mogahed, F. A.; El-Kersh, M. A. and Habashy, N. H. (2021):** Synergistic protective effect of Beta vulgaris with meso-2, 3-dimercaptosuccinic acid against lead-induced neurotoxicity in male rats. Scientific Reports, 11(1), 1-18.
- **Sinha, A. K. (1972):** Colorimetric assay of catalase. Analytical biochemistry, 47(2), 389–394.
- **Vaishnavi, C. (1996):** C-reactive protein in bacterial infections. In: Immunology and Infectious Diseases. 3(6): 139-144.
- **Van Weemen, B. K. and Schuurs, A. H. (1971):** Immunoassay using antigen-

enzyme conjugates. FEBS Lett 15: 232-236.

- **Vassault, A. (1983):** Lactate dehydrogenase: UV-method with pyruvate and NADH. In: Methods of enzymatic analysis, 3rd edition, Elsevier, New York, NY, pp 118–25.
- **Watt, B. and Merrill, A. L. (1963):** Composition of foods: raw, processed, prepared. Washington, DC: Consumer and Food Economics Research Division / Agricultural Research Service, 198 (Agriculture Handbook, 8).
- **Young, D. S.; Pestaner L. C. and Gibberman V. (1975):** Effects of drugs on clinical laboratory tests. Clin Chem 21(5): 1D-432D.
- **Yuan, Y.; Zhao, S. W.; Wen, S. Q.; Zhu, Q. P.; Wang, L.; Zou, H.; Gu, J. H.; Liu, X. Z.; Bian, J. C. and Liu, Z. P. (2021):** Alpha-Lipoic Acid Attenuates Cadmium- and Lead-Induced Neurotoxicity by Inhibiting Both Endoplasmic-Reticulum Stress and Activation of Fas/FasL and Mitochondrial Apoptotic Pathways in Rat Cerebral Cortex. Neurotoxicity Research, 39(4), 1103–1115.
- **Zou, H.; Sun, J.; Wu, B.; Yuan, Y.; Gu, J.; Bian, J.; Liu, X. and Liu, Z. (2020):** Effects of Cadmium and/or Lead on Autophagy and Liver Injury in Rats. Biological Trace Element Research, 198(1), 206–215.

التأثيرات العلاجية لإديتات الكالسيوم ثنائي الصوديوم مع حمض ألفا ليبويك وبذور الشيا ضد السمية العصبية التي يسببها الرصاص والكادميوم في الفئران

عبد الغني محمود عبد الغني – شيماء فتحي عبد الغني غزي – ياسمينا محمد ربيع سلطان*

الملخص العربي:

صممت الدراسة الحالية لدراسة تأثير خليط ايديتات كالسيوم ثنائي الصوديوم (CaNa2EDTA) مع حمض ألفا ليبويك (ALA) معاً و مسحوق بذور الشيا (ChS.P) ضد السمية العصبية التي يسببها الكادميوم والرصاص اجريت الدراسة علي أربعة وعشرون من ذكور الفئران الألبينو البالغة، متوسط وزنها (١٥٨ ± ٣ جم) تم تقسيمها عشوائيًا إلى أربع مجموعات (ستة بكل مجموعة)، المجموعة الاولي الضابطة السالبة و ثلاث مجموعات تم اصابتهم بالسمية العصبية، وتم اعادة تقسيمهم إلى المجموعة الثانية الضابطة الموجبة غير معالجة والمجموعة الثالثة تم معالجتها بخليط ايديتات كالسيوم ثنائي الصوديوم مع حمض ألفا ليبويك والمجموعة الرابعة تم معالجتها بمسحوق بذور الشيا. واستمرت التجربة لمدة ٦٠ يومًا. تم تسجيل كمية الطعام المتناولة يوميا ووزن الفئران اسبوعيا وفى نهاية التجربة تم جمع عينات الدم لفحص مستويات إنزيم نازع لهيدروجين اللاكتات (LDH)، ومستوى البروتين التفاعلي (CRP) و انزيمات الأكسدة الحلقية- ٢ (COX-2)، الدوبامين (DA) ، السيروتونين (ST) ، أسيتيل كولينستراز (AChE) ، بعض المعادن الثقيلة في الدم، بيروكسيد الدهون، نشاط إنزيمات مضادات الأكسدة، بعض مؤشرات وظائف الكلى و الكبد. كذلك، تم فحص التغيرات النسيجية في أنسجة المخ. أظهرت نتائج الدراسة تحسنًا معنوي في مستويات المصل من LDH، CRP، COX-2، DA، ST، AChE، بعض المعادن الثقيلة، بيروكسيد الدهون، نشاط الإنزيمات المضادات للأكسدة، وبعض وظائف الكلى والكبد في المجموعات المعالجة بخليط CaNa2EDTA مع ALA و ChS.P، عند مقارنتها بمجموعة الضابطة الموجبة الغير معالجة (+ve).

وتوصي الدراسة بضرورة استهلاك حمض ألفا ليبويك وبذور الشيا في الوجبات الغذائية لتأثيراتهم المضادة للسمية العصبية والمضادات لأكسدة والتي تلعب دورًا مهم و أكثر أمانً من مركبات إديتات الكالسيوم في علاج سمية الرصاص والكادميوم وكما تعمل عي تثبيط المضاعفات.

الكلمات المفتاحية: سالفيا هيسبانيكا إل - السمية العصبية - حمض الثيوكتيك - ايديتات كالسيوم ثنائي الصوديوم - الكادميوم - الرصاص - فئران.

* قسم الاقتصاد المنزلي ، كلية التربية النوعية ، جامعة المنصورة ، جمهورية مصر العربية