Role of pumpkin and sunflower seeds in mitigating neurotoxicity induced by some heavy metals in rats

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Research Journal Specific Education
Faculty of Specific Education
Mansoura University

ISSUE NO. 92 MAY , 2025

Therapeutic effects of dried persimmon consumption with diet against Triton X-induced hyperlipidemia in male rats

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Abstract

The current study was aimed to investigate the therapeutic effects of pumpkin (PS.P) and sunflower (SFS.P) seeds powder against lead and cadmium-induced neurotoxicity in rats. Twenty-four male Albino rats (weight: 158 ± 3 g) were randomly allocated into four groups (6 each). The first group served as a negative control group fed only a basal diet. while 18 rats were orally gavaged with a toxic mixture of Cd (5 mg/kg b.w.) and Pb (30 mg/kg b.w.) for 30 days to induce neurotoxicity and redivided into a positive control group fed on basal diet and two treated groups with 5% from basal diet PS.P and SFS.P. The experimental duration was 60 days. Food intake and body weight were measured to assess nutritional parameters. Blood samples were collected to assays the levels of C- reactive protein (CRP), Lactic Dehydrogenase (LDH), Cyclooxygenase-2 (COX-2), Acetyl cholinesterase (AChE), Serotonin (ST), Dopamine (DA), some Lipids profile parameters, lipid peroxidation, antioxidant enzymes activity, some liver and kidney functions .The results demonstrated that groups treated with PS.P and SFS.P exhibited significant improvements in serum levels of CRP, LDH, COX-2, AChE, ST, DA, some lipids profile parameters, lipid peroxidation, antioxidant enzymes activity, and some liver and kidney functions, when compared to positive control group It can be recommended that the incorporating pumpkin and sunflower seeds into the diet due to their anti-neurotoxicity and antioxidant properties, which offer a safer therapeutic approach against lead-cadmium toxicity and help mitigate its complications especially for those exposed to lead and cadmium poisoning.

Keywords: pumpkin and sunflower seeds, Neurotoxicity, Lead, Cadmium and Rats.

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INTRODUCTION:

Lead (Pb) and cadmium (Cd) exert neurotoxic effects by disrupting nervous system function and increase oxidative stress, mitochondrial impairment, and neurotransmitter interference. These metals accumulate in neural tissue, triggering neuronal apoptosis, neuroinflammation, and cognitive dysfunction, posing great risks to nervous system development and function **(Sanders *et al.,* 2009; Branca *et al.,* 2018 and Alengebawy *et al.,* 2021)** Pb/Cd disrupt dopaminergic signaling linked to ADHD, while epigenetic mechanisms including DNA methylation, histone modifications perpetuate cognitive deficits and neurodegeneration risks across generations **(Bouchard *et al.,* 2010; Neal *et al.,* 2011; Anderson *et al.,* 2012 and Sanders *et al.,* 2015)**.

Pumpkin (*Cucurbita pepo L.*), is an annual herbaceous and angiosperm plant belonging to the *Cucurbitaceae* family **(Caili *et al.,* 2006)**. Pumpkin seeds contain numerous bioactive compounds including vitamin E, α/β-carotene, cryptoxanthins, vanillic, ferulic, cucurbitosides, and squalene. These components demonstrate therapeutic potential, particularly in oncology, through their antioxidant and anti-proliferative activities. The diverse phytochemical profile also includes γ-aminobutyric acid, violaxanthin and luteoxanthin, their high flavonoid and cucurbitacin content, exhibit anti-proliferative effects in colon, lung, and breast cancer cell lines **(Ratnam *et al.,* 2020)**. Pumpkin seeds are rich in essential minerals such as sodium, manganese, calcium and zinc which regulate blood pressure, fluid balance, enhance cellular nutrient transport and neuromuscular function. Zinc supports macronutrient metabolism, vitamin A mobilization, and nucleic acid synthesis. (**Elinge *et al.,* 2012 and Syed *et al.,* 2019)**. Pumpkin seeds contain a good amount of tryptophan which lead to a form of the neurotransmitter (serotonin), thus aiding in depression **(George & Nazni, 2012 and Lachance & Ramsey, 2018)**.

Sunflower is the fruits of *Helianthus annuus L.* and is one of the world's essential oil-producing seeds **(Seifi & Alimardani, 2010)**. Sunflower seeds store sulfur-rich proteins essential for human metabolism, supporting insulin production, tissue development, and antioxidant functions. Their amino acid profile is dominated by glutamic acid (26.91%), aspartic acid (10.50%), and arginine (9.75%), with notable cysteine (3.47%) and methionine (6.18%) content **(Guo *et al.,* 2017)**. Also, provide significant vitamin E (α-tocopherol), folate, niacin, and essential minerals (Ca, Mg, Se, Zn) **(Adeleke & Babalola, 2020 and Khurana & Singh, 2020)**. Sunflower seeds exhibit strong antioxidant activity from phenolic compounds (chlorogenic acid 214.3 mg/100 g) **(Filho & Egea, 2021)**. Additionally, its contains β-sitosterol, which promotes neurite outgrowth and had a therapeutic potential for Alzheimer's disease **(Koga *et al.,* 2020)**. Accordingly, this study aimed to assess the possible effects of pumpkin and sunflower seeds powder in mitigating neurotoxicity induced by exposure Pb and Cd in male rats.

MATERIALS AND METHODS

A-Materials:

Pumpkin and sunflower seeds were obtained from the products selling unit at the National Research Center, Dokki, Egypt. All kits for biochemical analysis were purchased from Kamiya Biomedical Company, Cairo, Egypt. Cadmium chloride (CdCl) and lead acetate (Pb CHCOO) were purchased from El-Gomhoria Company for chemicals, El-Mansoura city, Egypt. The basal diet raw materials were purchased from Al-Gomhouria Company and veterinary pharmacy according to **NRC (1995)**. Twenty-four healthy adult Albino male rats of Sprague–Dawley strain (weight: 158 ± 3 g) were obtained from the laboratory animal farm of Veterinary Medicine at Zagazig University in Egypt.

B-Methods:

Preparation of seeds powder:

Seeds of Pumpkin, and Sunflower seeds were inspected for impurities, then ground into fine powder using a laboratory mill. Whole seed powder was saved in light-protected glass in the refrigerator to prevent lipid oxidation until used in diet preparation. Experimental diets were prepared by replacing 5% of the basal diet components with seed powder, water ad libitum, nutritional treatment continued for 30 days after neurotoxicity.

Induction of Neurotoxicity (NT):

Cadmium chloride (CdCl) and lead acetate (Pb CHCOO) were employed to induce experimental neurotoxicity in rodents. A standardized toxic solution was prepared by dissolving $CdCl_2$ (5 mg/kg body weight) and lead acetate (30 mg/kg body weight) in 1 mL distilled water, following established protocols **(Mohammed *et al.,* 2014; Saleh & Meligy, 2018)**. The freshly prepared solution was administered via oral gavage (1 mL/kg body weight) once daily for 30 days prior to nutritional treatment initiation.

Experimental Animals Protocol:

Twenty-four rats were kept under surveillance for seven days to adapt and were fed a basal diet. Six rats served as a negative control group, while 18 rats were orally gavaged with a toxic mixture of Cd (5 mg/kg b.w.) and Pb (30 mg/kg b.w.) for 30 days to induce neurotoxicity. These rats were then divided into a positive control group (untreated) and two treatment groups: one received 5% pumpkin seed powder (+PS.P) and the other 5% sunflower seed powder (+SFS.P), by substituting some of the basal diet components for 30 days. Food and water were provided *ad-libtum*. Food intake was recorded daily, and the body weight of the rats was measured weekly until the end of the experimental period (60 days). All biological experimental procedures were conducted in accordance with internationally accepted ethical guidelines for the care and use of laboratory animals. Approval for the experiment was obtained from the Research Ethics Committee at the Faculty of Specific Education, Mansoura University.

Chemical composition of seeds samples:

Moisture, fat, protein, fiber and ash 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 the rats were weighed once a week to assess body weight gain. Body weight gain, feed efficiency ratio (FER), and protein efficiency ratio (PER) were calculated according to the method described by **Chapman *et al.*, (1959)**.

Biological analyzes:

At the experimental endpoint, rats were anesthetized using diethyl ether. Blood samples were collected via the medial canthus of the eye using heparinized capillary tubes. Serum was subsequently isolated by centrifugation at 3,000 rpm for 10 min. The serum biochemical analysis includes the following: C- reactive protein (CRP), Lactic Dehydrogenase (LDH) and Cyclooxygenase-2 (COX-2) were measured depending on method of (**Vaishnavi, 1996; Vassault, 1983 and Van Weemen & Schuurs, 1971,respectively**). An acetyl cholinesterase (AChE) level was assessed by using the manufacturer's protocol of a Rat Acetylcholinesterase ELISA Kit Principles (No. DEIASL417; Creative Diagnostics Co., Shirley, New York, USA). Serotonin (ST) level was assessed by using the manufacturer's protocol of a Rat Serotonin ELISA Kit (No. LS-F27987; LifeSpan Biosciences, Inc., Seattle, Washington, USA). 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). Lipid profile parameters were evaluation using kits bought from Diamond Biodiagnostic (Egypt) and Reactivos Spinreact Company (Spain), as the following: Total Lipids (T. Lipid), Triglyceride (TG), Total Cholesterol (T.CH), High-Density Lipoprotein cholesterol (HDLc), concentration were determined according to **(Frings *et al.,* 1972; Fossati & Prencipe 1982; Deeg & Ziegenhorn 1983 and Burstein *et al.,* 1970, respectively)**. Low-Density

Lipoprotein cholesterol (LDLc) was measured according to (**Friedewald *et al.*, 1972)**, using the formula: LDLc = T.CH - (TG /5) – HDLc. Very Low-Density Lipoprotein cholesterol (VLDLc) was measured according to (**Ross, 1992)** using the formula: VLDLc = TG / 5.

Serum Lipid peroxidation (Malondialdhyde "MDA") and Antioxidant enzymes: Catalase (CAT), superoxide dismutase (SOD), Reduced Glutathione (GSH). Glutathione and Peroxidase (GPx) were determined according to the method described by **(Paoletti & Macali, 1990; Eze *et al.,* 2008; Sinha, 1972; Rice-Evans & Miller, 1994 and Paglia & Valentine, 1967, respectively)**. Some serum kidney and liver functions: Urea, Creatinine, Total Bilirubin (T.BiL), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were determined according to **(Rock *et al.*, 1987; Henry *et al.*, 1974; Young *et al.,* 1975; Belfield & Goldberg, 1971 and Orlowski & Meister, 1963, respectively)**.

Statistical data analysis:

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

RESULTS AND DISCUSSION:

Chemical composition of 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 pumpkin and sunflower seeds. The results showed that sunflower seeds had higher percentages of moisture (6.75%), protein (23.84%), ash (3.93%), total carbohydrates (41.76%), and nitrogen-free extract (29.85%) compared to pumpkin seeds. In contrast, pumpkin seeds exhibited significantly higher fat (35.68%) and fiber (18.42%) content than sunflower seeds. Additionally, the energy value of pumpkin seeds (550.91 kcal/100 g) exceeded that of sunflower seeds (475.83 kcal/100 g), indicating a greater caloric contribution.

This finding is nearly similar to a study showed the chemical composition of whole pumpkin seeds contains as the following: 6.70%

moisture, 28:50% protein, 35.43% oil, 2.26% dietary fiber, 4.50% Ash and 29.31% NFE **abd-elnoor (2019)**. Another study showed that the chemical composition of sunflower seeds contains 6.16% moisture, 23.73% protein, 32.50% lipids, 36.52% carbohydrates 12.64% fiber and 3.31% Ash **Petraru *et al.,* (2021)**.

Table (1): Chemical composition of pumpkin and sunflower seeds:

Seeds / Component	Pumpkin seeds	Sunflower seeds
Moisture %	4.12 ± 0.07^{b}	6.75 ± 0.10^{a}
Protein %	18.38 ± 0.07^{b}	23.84 ± 0.11^{a}
Fat %	35.68 ± 0.03^{a}	23.71 ± 0.04^{b}
Ash %	2.75 ± 0.04^{b}	3.93 ± 0.04^{a}
T. Carb %	39.06 ± 0.07^{b}	41.76 ± 0.09^{a}
Fiber %	18.42 ± 0.04^{a}	11.92 ± 0.05^{b}
NFE %	20.64 ± 0.11^{b}	29.85 ± 0.14^{a}
Energy (kcal.100g)	550.91 ± 0.25^{a}	475.83 ± 0.40^{b}

Each analysis was performed triplicate. Results were expressed as Mean ± SD in each row having different letters (a, b, c, d..) are significantly at P>0.05.

Nutritional indicators of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Data in table 2 showed: the untreated neurotoxicity rats group (+ve) had a significant decrease in body weight gain, body weight gain %, food intake, feed efficiency ratio (FER), protein intake, and protein efficiency ratio (PER), when compared with control group (-ve). While, the neurotoxicity rat groups treated with pumpkin seed powder (+PS.P) and sunflower seed powder (+SFS.P) in neurotoxic rats led to a significant improvement in final body weight, body weight gain, body weight gain percentage, food intake, FER, protein intake, and PER, compared to the untreated neurotoxicity group (+ve). Despite being significantly lower than the negative control group (-ve), these parameters were notably higher than in untreated rats. 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)**. These results are roughly consistent with studies showed that the body weight of the rats in untreated groups were significantly decreased, while the groups treated with seeds powder achieved significant improvement in the weight gained and different Nutritional effects **(Abd El-Ghany *et al.,* 2010; Sun *et al.,* 2017 and Al-Awar *et al.,* 2020)**

Table (2): Nutritional indicators of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower 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[d]	21.61 ±1.83[d]	11.99 ±0.34[c]	0.047 ±0.004[d]	2.40 ±0.07[c]	0.24 ±0.02[d]
treated	+PS.P	50.67 ±3.39[c]	32.06 ±2.48[c]	14.79 ±0.34[b]	0.057 ±0.004[c]	2.96 ±0.07[b]	0.29 ±0.02[c]
	+SFS.P	57.00 ±2.53[b]	36.13 ±1.82[b]	14.53 ±0.34[b]	0.066 ±0.004[b]	2.91 ±0.07[b]	0.33 ±0.02[b]

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

Biological analyzes:

1- Serum C- reactive protein (CRP), Lactic Dehydrogenase (LDH), Cyclooxygenase-2 (COX-2) of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Data in table 3 showed: the untreated neurotoxicity rats group (+ve) had a significant increase in CRP, LDH and COX-2 when compared with the control group (-ve) neurotoxicity rat groups treated with pumpkin seed powder (+PS.P) and sunflower seed powder (+SFS.P) showed a significant increase in the levels of CRP, LDH and COX-2 compared to the negative control group (-ve). However, when compared with the untreated neurotoxicity group (+ve), both PS.P and SFS.P treatments led to significant improvements (i.e., reductions) in CRP, LDH and COX-2 levels, indicating a partial protective effect against neuroinflammation. Pumpkin and sunflower seeds possess several biological activities which can be characterized as antimicrobial, anti-inflammatory, cytotoxic, antiviral, antitumor activities and reducing the toxic effects of heavy metals (**Júnior *et al.,* 2016; Shaban & Sahu 2017 and Elhamalawy, 2018)**. Pumpkin seeds are rich in bioactive compounds such as phytosterols, tocopherols and phenolic compounds, which have been shown to modulate inflammatory pathways by inhibiting pro-inflammatory cytokines and enzymes like COX-2 and nitric oxide synthase **(Al-Okbi *et al.,* 2014)**. Sunflower seeds have a high content of vitamin E, selenium and phenolic acids. These compounds scavenge free radicals and downregulate inflammatory mediators, reducing the expression of CRP, TNF-α, and COX-2 **González-Castejón *et al.,* 2012)**.

Table (3): Serum C- reactive protein (CRP), Lactic Dehydrogenase (LDH), Cyclooxygenase-2 (COX-2) levels of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

groups	Parameters	CRP (mg/mL)	LDH (U/L)	COX-2 (pg/mL)
untreated	Control (-ve)	2.14 ±0.17^{c}	1377.83 ±12.95^{d}	33.85 ±4.15^{c}
	Control (+ve)	5.55 ±0.62^{a}	4195.67 ±12.83^{a}	79.90 ±2.07^{a}
treated	+PS.P	3.51 ±0.35^{b}	3164.17 ±17.80^{c}	55.50 ±5.35^{b}
	+SFS.P	3.87 ±0.31^{b}	3337.33 ±8.12^{b}	59.57 ±4.57^{b}

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

2- Serum Acetyl cholinesterase (AChE), Serotonin (ST) and Dopamine (DA) levels of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Data in table 4 showed that treatment with pumpkin seed powder (+PS.P) and sunflower seed powder (+SFS.P) resulted in a significant increase in DA and AChE levels, along with a decrease in ST, when compared to the negative control group (-ve). However, both treatments showed significant improvement in all three neurotransmitter markers (DA, ST, and AChE) when compared to the untreated neurotoxicity group (+ve). Lead (Pb) and cadmium (Cd) is associated with dysregulation of neurotransmitters. They increase AChE activity, impairing cholinergic transmission and leading to cognitive dysfunction. Additionally, Pb and Cd elevate dopamine and glutamate levels while reducing serotonin, disrupting catecholaminergic and serotonergic pathways (**Zhang *et al.,* 2009 and Al-Kahtani 2019**). Pumpkin seeds are rich in L-tryptophan and choline. L-tryptophan is a precursor to serotonin, which supports various neuropsychological functions, while choline contributes to brain development and serves as a precursor to acetylcholine, essential for cholinergic neurotransmission **(Dutta *et al.,* 2020)**. Sunflower seeds have been shown to elevate levels of dopamine, serotonin, norepinephrine, acetylcholine, and brain-derived neurotrophic factor (BDNF) in mice. These effects are associated with reduced inflammation and oxidative stress, along with improved aromatic amino acid metabolism and correction of abnormal metabolite levels **(Lu *et al.,* 2021)**.

Table (4): Serum Acetyl cholinesterase (AChE), Serotonin (ST) and Dopamine (DA) levels of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

groups \ Parameters		AChE (pg/ml)	ST (ng/ml)	DA (ng/ml)
untreated	Control (-ve)	25.98 ±2.80[d]	184.72 ±4.81[a]	11.55 ±0.51[d]
	Control (+ve)	127.43 ±11.10[a]	136.50 ±7.05[d]	23.22 ±0.41[a]
treated	+PS.P	75.12 ±6.52[b]	145.63 ±5.43[d]	18.80 ±0.47[b]
	+SFS.P	76.83 ±9.51[b]	144.97 ±7.56[d]	19.07 ±0.39[b]

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

3- Serum Total Lipids (T. Lipid), Total Cholesterol (T.CH), Triglyceride (TG), High-Density Lipoprotein cholesterol (HDLc), Low-Density Lipoprotein cholesterol (LDLc) and Very Low-Density Lipoprotein cholesterol (VLDLc) of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Data in table 5 showed serum lipids profile parameters including Total Lipids (T. Lipid), Triglyceride (TG), Total Cholesterol (T.CH), High-Density Lipoprotein cholesterol (HDLc), Low-Density Lipoprotein cholesterol (LDLc) and Very Low-Density Lipoprotein cholesterol (VLDLc) levels in control group (-ve) and neurotoxicity (+ve) rat groups which feed on the basal diet and treated with pumpkin seed powder (+PS.P) and sunflower seed powder (+SFS.P). Treatment with PS.P and SFS.P resulted in a significant increase in T. Lipid, T.CH, TG, LDLc, and VLDLc, and a significant decrease in HDLc when compared with the normal control group (-ve). However, when compared with the untreated neurotoxicity group (+ve), both PS.P and SFS.P treatments showed notable improvements across all lipid profile markers.

The findings of this study closely align with previous research who find that PSP and SFS are rich in linoleic acid (omega-6) and oleic acid (omega-9), which enhance HDLc synthesis and promote LDLc receptor activity, improving cholesterol clearance **(Ryan *et al.,* 2007 and Alagawany *et al.,* 2021)**. Also, β-Sitosterol in pumpkin seeds blocks intestinal cholesterol absorption, lowering TC and LDLc. While, Soluble fiber in PSP binds bile acids, forcing the liver to use circulating cholesterol for bile synthesis, reducing serum TC. **(El-Adawy & Taha, 2001, Phillips *et al.,* 2005 and Abd El-Ghany *et al.,* 2019)**. Vitamin E and phenolic compounds in sunflower seeds reduce oxidative stress, preventing LDLc oxidation **(González-Pérez, 2021)**.

Table (5): Serum Total Lipids (T. Lipid), Total Cholesterol (T.CH), Triglyceride (TG), High-Density Lipoprotein cholesterol (HDLc), Low-Density Lipoprotein cholesterol (LDLc) and Very Low-Density Lipoprotein cholesterol (VLDLc) of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Groups	Parameters	T. Lipid (mg/dl)	T.CH (mg/dl)	TG (mg/dl)	HDLc (mg/dl)	LDLc (mg/dl)	VLDLc (mg/dl)
untreated	Control (-ve)	294.00 ±4.33[d]	53.83 ±5.74[c]	57.83 ±4.75[c]	40.00 ±3.82[a]	4.27 ±0.87[c]	11.57 ±0.95[c]
	Control (+ve)	607.00 ±15.18[a]	114.50 ±7.39[a]	126.67 ±9.27[a]	23.33 ±1.63[c]	65.83 ±5.48[a]	25.33 ±1.85[a]
treated	+PS.P	399.67 ±11.27[c]	84.33 ±2.16[bc]	89.17 ±3.79[b]	30.17 ±5.60[b]	36.33 ±3.55[b]	17.83 ±0.56[b]
	+SFS.P	433.83 ±9.22[b]	88.83 ±5.40[b]	94.83 ±3.31[b]	30.83 ±1.33[b]	39.03 ±5.31[b]	18.97 ±0.66[b]

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at $P>0.05$.

4- Serum Malondialdhyde (MDA) and antioxidant enzymes Catalase (CAT), superoxide dismutase (SOD), Reduced Glutathione (GSH) and Glutathione Peroxidase (GPX) levels of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Data in table 6 showed that neurotoxicity group (+ve) rats had a significantly increased in MDA and decreased in antioxidants enzymes levels (CAT, GSH, GPX, SOD) when compared with the control group, (-ve). While, Pumpkin (+PS.P) and sunflower (+SFS.P) seed treatments significantly reduced MDA and improved antioxidant enzymes levels (CAT, GSH, GPX, SOD) compared to neurotoxicity group (+ve), though not to normal levels. These findings demonstrate both seeds' partial neuroprotective effects against oxidative stress. Studies have shown that pumpkin seeds exhibit antioxidant properties by reducing MDA and enhancing antioxidant enzymes (GSH, SOD) in neurotoxicity models, consistent with our observed improvements due to its content of carotenoids, Zinc, and vitamin E. **(Zdunić *et al.,* 2016; Alagawany *et al.,* 2021 and Niazi *et al* 2022)**. Similarly, sunflower seeds contain tocopherols and phenolic compounds that mitigate oxidative stress by lowering MDA and elevating GPX/CAT activities **(Ryan et al., 2007; Saad *et al.,* 2019 and González-Pérez, 2021)**. These mechanisms align with our results, where both seeds partially restored oxidative balance in neurotoxic conditions.

Table (6): Serum Malondialdhyde (MDA) and antioxidant enzymes Catalase (CAT), superoxide dismutase (SOD), Reduced Glutathione (GSH) and Glutathione Peroxidase (GPX) levels of control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Parameters / groups		MDA nmol/ml	CAT (U/L)	SOD (U/L)	GSH (µmol/L)	GPX (mU/ml)
untreated	Control (-ve)	6.07 ±0.20[c]	0.95 ±0.01[a]	42.49 ±0.90[a]	2.06 ±0.08[a]	96.12 ±2.92[a]
	Control (+ve)	21.18 ±3.23[a]	0.23 ±0.02[c]	8.11 ±4.19[c]	0.69 ±0.03[c]	25.57 ±8.46[c]
treated	+PS.P	14.08 ± 0.19[b]	0.64 ±0.02[b]	22.38 ±1.30[b]	1.14 ±0.02[b]	54.02 ±5.14[b]
	+SFS.P	14.36 ± 0.31[b]	0.62 ±0.06[b]	21.94 ±1.60[b]	1.08 ±0.05[b]	51.22 ±6.46[b]

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

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 control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

Data in table 7 showed that neurotoxicity rats group (+ve) significantly increased kidney (creatinine, urea) and liver (ALT, AST, ALP, GGT, T.BiL) markers in rats compared to control group (-ve). Treatment with pumpkin and sunflower seed powders significantly reduced these elevated parameters when compared with the untreated neurotoxicity group (+ve), though levels remained higher than control group (-ve). These results demonstrate the seeds' partial protective effects against neurotoxicity-induced kidney and liver damage, suggesting their potential therapeutic value, likely due to their antioxidant properties. Pb and Cd exposure elevates liver enzymes (AST, ALT) by inducing oxidative stress, cellular damage, and metabolic disruption, leading to hepatocyte necrosis and enzyme leakage. This heavy metal toxicity further exacerbates tissue injury through free radical production, lipid peroxidation, and antioxidant depletion, causing liver/kidney damage **(Sellaoui *et al.,* 2016; Bhattacharjee *et al.,* 2016; Zou *et al.,* 2020 and Hassan *et al.,* 2022)**. Several studies corroborate the nephroprotective and hepatoprotective effects of pumpkin and sunflower seeds observed in our study. Pumpkin seed has been shown to significantly reduce creatinine and urea levels in toxin-induced renal damage models, attributed to its antioxidant phytochemicals like tocopherols and carotenoids **(Zdunić *et al.,* 2016 and Mansour *et al.,* 2019)**. Similarly, sunflower seeds demonstrate hepatoprotective effects by lowering elevated liver enzymes, likely due to their high vitamin E and phenolic content **(Naziroğlu *et al.,* 2014, González-Pérez, 2021 and Abd El-Ghany *et al.,* 2023)**. These findings collectively support our results showing both seeds' ability to mitigate neurotoxicity-induced organ damage through antioxidant mechanisms.

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 control group (-ve) and neurotoxicity (+ve) rat groups treated with pumpkin and sunflower seeds powder:

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[c]	140.50 ± 9.20[d]	86.00 ±10.99[d]	3.29 ± 0.46[c]	0.31 ±0.09[d]
	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	+PS.P	0.91 ±0.05[c]	65.50 ±4.07[c]	68.50 ±4.55[b]	319.33 ±5.46[c]	250.00 ±3.58[c]	13.95 ±3.61[b]	0.67 ±0.04[c]
	+SFS.P	1.01 ±0.06[b]	84.17 ±8.56[b]	71.52 ±7.97[b]	336.33 ±6.50[b]	272.00 ±6.99[b]	16.10 ±2.51[a]	0.75 ±0.06[b]

Each value is represented as mean ± SD. Mean values in each column having different letter (a, b, c, d..) are significantly at P>0.05.

CONCLUSION:

We recommend including pumpkin and sunflower seeds in the diet as a safe therapeutic strategy against lead-cadmium toxicity. Their demonstrated anti-neurotoxic and antioxidant properties may help counteract complications of heavy metal exposure, particularly for individuals at risk of Pb/Cd poisoning.

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دور بذور اليقطين ودوار الشمس في تخفيف السمية العصبية الناتجة عن بعض المعادن الثقيلة في الفئران

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الملخص العربي

هدفت الدراسة الحالية إلى دراسة التأثيرات العلاجية لمسحوق بذور اليقطين ودوار الشمس ضد السمية العصبية الناتجة عن التسمم بالرصاص والكادميوم في الفئران. تم تقسيم أربعة وعشرين من ذكور الفئران من فصيلة ألبينو (الوزن: ١٥٨ ± ٣ جم) عشوائيًا على أربع مجموعات (٦ لكل مجموعة). عملت المجموعة الأولى كمجموعة ضابطة سالبة تتغذى على النظام الغذائي القياسي فقط. بينما تم اعطاء ١٨ من الفئران عن طريق الفم مزيج سام من الكادميوم (٥ ملجم / كجم من وزن الجسم والرصاص ٣٠ ملجم / كجم من وزن الجسم) لمدة ٣٠ يومًا للإصابة بالسمية العصبية ثم أعيد تقسيمهم إلى المجموعة الضابطة الموجبة والتي تغذت على نظام غذائي قياسي ومجموعتين تم علاجهم بمسحوق بذور اليقطين ودوار الشمس بنسبة ٥٪ من النظام الغذائي القياسي واستمرت الدراسة لمدة ٦٠ يومًا. حيث تم تقدير كل من المتناول الطعام ووزن الجسم لتقييم المعايير الغذائية. وتم جمع عينات الدم لتحليل مستويات البروتين التفاعلي- سي (CRP)، وديهيدروجيناز اللاكتيك (LDH)، وسيكلوأكسجيناز- ٢ (COX-2)، وأسيتيل كولينستراز (AChE)، والسيروتونين (ST)، والدوبامين (DA)، وبعض مستويات دهون الدم والمانولدهيد ونشاط إنزيمات مضادات الأكسدة، وبعض وظائف الكبد والكلى. وأظهرت النتائج أن المجموعات التي عولجت بمسحوق بذور اليقطين ودوار الشم تحسنًا معنوي في مستويات CRP، وLDH، وCOX-2، وAChE، وST، وDA، وبعض مستويات دهون الدم، وبيروكسيد الدهون، ونشاط إنزيمات مضادات الأكسدة، وبعض وظائف الكبد والكلى، بالمقارنة بالمجموعة الضابطة الموجبة. وتوصي الدراسة بضرورة ادراج بذور اليقطين وعباد الشمس في النظام الغذائي نظرًا لخصائصهما المضادة للسمية العصبية ومضادات الأكسدة بحيث يكون نظام علاجي أكثر أمانًا ضد سمية الرصاص والكادميوم ويساعد في تخفيف مضاعفاتها وخاصة لأولئك المعرضين للتسمم بالرصاص والكادميوم.

الكلمات المفتاحية: بذور اليقطين ودوار الشمس - السمية العصبية - الكادميوم - الرصاص - الفئران

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