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The Neuroprotective effects of Safflower Seeds (*Carthamus tinctorius*) against Lead Acetate - Induced neurotoxicity in Rats

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Safflower Seeds (*Carthamus tinctorius* L. seed (CTS)) have utilized in coloring and flavoring in food industries. The present study aims to evaluate the saver impacts of various CTS levels against neurotoxicity in rats induced by lead (Pb). Thirty-five male rats were classified into five classes each contains 7 rats. Class (I) is the control class and was fed on the basal diet. Class (II) Pb class was administrated oral gavage of lead acetate (30 mg/kg/BW) for eight weeks. Classes (III, IV, and V) were supplemented with different levels of CTS powder formulated with the basal diet (5, 7.5, and 10%, respectively) and combined with Pb intoxication. After eight weeks, serum and brains were collected from rats to measure dopamine, serotonin, antioxidant parameters, and proinflammatory cytokines. Compared to the Pb-intoxicated rats, CTS supplementation ameliorated the Pb-linked elevation in body weight gain, brain weight, and dyslipidemia. Also, it increases the neurotransmitters levels (serotonin and dopamine content) in rats and enhance the antioxidant status of the brain (superoxide dismutase and glutathione functions) while reducing peroxidation of lipid. Moreover, CTS reduced Pro-inflammatory cytokines (tumor necrotic (TNF-α) and acetylcholine esterase (AChE) activity). These outcomes were established by restoring the brain architecture in histological examination. The results of our investigation showed that phenolic-rich CTS supplementation was beneficial in lowering Pb-induced brain inflammation, neuronal damage, and oxidative stress. Therefore, CTS might have a neuromodulatory role against Pb toxicity.

Keywords: Lead Acetate, *Carthamus tinctorius* L. seed, brain, Neuroprotective, Toxicity

INTRODUCTION

Lead (Pb) is a poisonous heavy metal that is exist in several different types of habitats around the world with no favourable effects on biological systems. Pb exposure has long been a worry on a worldwide scale, particularly for young infants (Ye et al. 2015). Because Pb poisoning can influence the body's hematological, hepatocellular, cardiac, reproductive, digestive, and nervous systems, it is recognized that it affects how the body functions (Debnath et al. 2019). Pb poisoning results in both immediate and long-term impairment to the central nervous system (CNS), in addition to a raised incidence of mental retardation, learning disabilities, and peripheral nervous system problems. As a result, Pb has been correlated to a various health issues, including losing weight, irritability, digestive issues, vomiting, constipation, and muscle pain. It has also been linked to changes in speech, nerve conduction, and hearing. In addition, exposure to Pb can result in anemia, convulsions, nephropathy, paralysis, or even death (Jan et al. 2015). According to reports, Pb toxicity results in neurological damage that serves as a foundation for a number of illnesses, including Alzheimer's and Parkinson's diseases (Lamidi and Akefe, 2017). Pb can harm the cerebellum, hippocampus, and prefrontal cortex by substituting calcium ions, which allows it to pass the blood-brain barrier (Ahmed et al. 2013). Pb disruption of the brain's blood-brain barrier allows albumin to enter the tissues, which raises intracranial pressure and produces edema and encephalopathy (Flora et al. 2012). Neurotransmitters such as the dopaminergic, cholinergic, and glutaminergic systems are impacted by Pb exposure in the brain (Lyn, 2006). Acetyl cholinesterase (AChE) dysfunction in the conduction of cholinergic nerve cells is among the hallmarks of Pb toxicity in the brain (Jankowska-Kulawy et al. 2008). The control of cognitive processes depends on the central cholinergic system. As a result, to treat cognitive impairments, AChE

suppressors and cholinergic receptor agonists are utilized to regulate levels of endogenous acetylcholine (Lane *et al.* 2006). Additionally boosting pb neurotoxicity, pro-inflammatory actions in the brain. Pb is believed to promote tumour necrosis factor-α (TNF-α) and interleukin 6 (IL-6) gene expression and secretion, which increases

inflammation in the brain (Lane et al. 2006). Parkinson's, Alzheimer's, and multiple sclerosis disease pathology have all been linked to inflammatory processes (Chibowska et al. 2016). Furthermore, apoptotic neurodegeneration is a known side effect of Pb poisoning (Dribben and Creeley, 2011). According to reports from earlier studies, lead exposure alters the glutathione system (Lopes et al. 2016), causing oxidative stress and inducing neuronal cell death (Mousa et al. 2015; Mujaibel and Kilarkaje, 2015).

One of the many various methods for treating illnesses with plants is traditional herbal medicine. Safflower (*Carthamus tinctorius*) is a commonly utilized plant in the food industry for flavour and colouring (Soraya *et al.* 2017). *C. tinctorius* seeds (CTS) have lately demonstrated bioactive properties against oxidation, adipogenesis, and inflammation (Kim et al. 2013; Yu et al. 2013 and Hwang et al. 2016). Serotonin has been linked to improved cognition and has been confirmed to be formed by CTS. The earliest serotonin derivatives to be discovered were feruloylserotonin and 4-coumaroyl serotonin, which had pharmacological characteristics in nervous system disorders (Smith et al. 2017). Current observations have looked into how CTS protects against oxidative damage brought on by prolonged alcohol use (Choi et al. 2018) and alleviates memory loss brought on by scopolamine (Kim et al. 2019). The ability of CTS's in vitro antioxidant activities to counteract Pb-induced toxicity against inflammation, apoptosis, oxidative stress, and acetyl cholinesterase action is still unknown Therefore, the current research was performed to assess the protective impact of feeding on *C. tinctorius* seeds against lead acetate- induced brain damage in male rats.

MATERIALS AND METHODS

Plant:

Safflower seeds (*Carthamus tinctorius* L.) were obtained from herbs market in Cairo, Egypt.

Chemicals:

Lead acetate [(C2H3O2)2Pb•3H2O, Pb], formalin, diethyl either, basal diet, Casein, cellulose, vitamins and minerals were supplied from Sigma Company, Cairo, Egypt.

Kits:

for biochemical analysis of serum were acquired for chemicals from Gama Trade Company, Cairo, Egypt.

Preparation of safflower seeds

The safflower seeds were carefully washed to eliminate debris, dust, and other contaminants, then safflower seeds were washed with distilled water and were milled into powder by using sunrise to dry, were stored until use in dark-stoppered glass bottles in a cool, dry area in accordance to **Russo and Tyler, (2001)**, who stated that in order to prevent the oxidation of their contents, all plants are kept in a cold, dry, and dark environment.

Chemical composition

According to the **A.O.A.C. (2012)** established procedures, the contents of protein, moisture, ash, fat, and crude fibre were measured. By using the difference, total carbohydrate was determined.

Determination of total flavonoid and total phenolic

Total flavonoid content (TFC) was estimated utilizing aluminum chloride colorimetric approach in accordance to Tohidi et al. (2017). The flavonoid content was determined using the quercetin calibration curve and represented by milligrams of quercetin equivalent per gram of the plant's dry weight (mg Q/g DW). The Folin-Ciocalteu procedure was utilized to assess the total phenolic content (TPC) of all the samples in accordance to Lin and Harnly, (2010). Via the calibration plot of gallic acid, TPC was determined by mg of gallic acid equivalent per gramme of dry weight of the plant (mg GAE/g DW).

Antioxidant activity assay by DPPH

Hatemnia et al. (2014) approach was used to test the effectiveness of every genotype's extracts in trapping DPPH free radicals at a wavelength of 515 nm.

Induction of oxidative brain damage in rats

Pb acetate solution was freshly prepared by dissolving in deionize distilled water at dose of (30 mg/kg body weight) of 1% solution and applied to rats three times/week by gavage tube for 8 weeks (Elgawish and Abdelrazek, 2014).

Experimental design

From the animal house of the National Research Center in Giza, Egypt, 35 adult male Sprague-Dawley rats weighing 180 ± 5 g were taken. They were kept in an air-conditioned room (26 ± 1°C) with a 12-hour light/12-hour dark cycle. Before the experiment began, water and food were supplied to the animals for one week to allow for adaption. The basic diet was established Based to **Reeves et al.(1993)**. Following acclimation, rats were divided randomly between the two following classes: Class (I) (n=7) was the health control and simply received a baseline diet (negative class). Class (II) Lead class (n=28) was given lead acetate at a concentration of (30 mg/kg/BW) of 1% solution for 8 weeks by gavage tube, and were partitioned into four classes. The first class was supplied only the basal diet and represented lead-intoxicated rats (positive class), and the other classes (IV,V,VI) were fed the formed basal diet with supplements of 5, 7.5, and 10% safflower seeds powder, respectively.

Animals were serially anaesthetized with diethyl ether after a 12-hour fasting at the completion of the experiment's eight-week run. Rats were killed, and their internal organs were removed. The posterior vena cava

was used to collect blood samples into dry, clean centrifuge tubes, which were then allowed to clot at room temperature before being spun for 10 minutes at 3000 rpm to separate the serum -20°C was used to freeze serum samples for biochemical examination.

Preparation of brain tissue homogenate

Every animal's entire brain was quickly and carefully dissected, weighed, and split in half sagitally. For histopathological analysis, the first half was submerged in neutral buffered formalin 10%. The second half was homogenised right away to create a 10% (w/v) homogenate in a phosphate buffer-containing ice-cold medium (pH 7.4). In a cooling centrifuge set at 4°C, the homogenate was spun at 1800 x g for 10 min. Supernatant (10%) was isolated, and the neurochemical analysis was performed after keeping it at -80°C **(Suleman et al. 2022)**.

Biological Evaluations

The amounts of food consumed and/or wasted, were recorded every day while total feed intake (FI) was calculated. In addition, body weight (BW) of rats was recorded weekly. Body weight gain percentage (BWG %) and feed efficiency ratio (FER) were calculated according to Champman et al. (1959) using the next equation:

$$\mathbf{BWG}\% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\mathbf{FER} = \frac{\text{weight Gain (g)}}{\text{Feed intake (g)}}$$

Biochemical analysis:

Determination of serotonin (5-HT) and dopamine (DA) were carried out as reported by Hussein *et al.* (2016) utilizing high performance liquid chromatography (HPLC) system. The Ach and AChE activity were assessed in accordance to Knedel and Boottger, (1967); Ellman *et al.* (1961). Serum lipid profile involving total cholesterol (TC), triglycerides (TG) and cholesterol contents of high density lipoprotein (HDL) were evaluated in accordance to Allain *et al.* (1974); Fossati and Principe,(1982); Albers *et al.*(1983), respectively. Calculations of very low-density lipoprotein cholesterol (VLDL) and low density lipoprotein cholesterol (LDL) by the equation of Fruchart, (1982). LDL-c = TC- [HDL-c + (TG/5)] VLDL-c = TG/5.

For assessing lipid peroxidation, plasma level of Malondialdehyde (MDA) was estimated in the supernatant of rat brain following the approach of Draper and Hadley, (1990). The activity of superoxide dismutase (SOD) was assessed according to Spitz and Oberley, (1989). Beutler et al. (1963) calculated the amounts of antioxidant indicators such as reduced glutathione (GSH). Serum tumor necrotic factor- α (TNF-α) was determined α (TNF-α) was determined according to Kandir and Keskin, (2016).

Histopathology analysis

Alcohol was used to dry the tissues of the brain before they were embedded in paraffin and fixed in 10% formalin. After that, tissue slices (5 µm thick) were made, stained with Congo red and Hematoxylin and Eosin, and then examined under a light microscope (Suleman et al. 2022).

Statistical analysis

Data were presented as mean ± standard deviation. Utilizing tests for normality (SPSS version 25), the distribution of the data will be confirmed to be normal. One-way analysis of variance will be utilized to assess statistical significance (ANOVA). Statistical significance is determined by the probability of $p \leq 0.05$.

ETHICS APPROVAL

The experimental protocol was approved by Research Ethics Committee (REC), Faculty of Nursing; Port Said University. Approval code number NUR (6/11/2022) (19). All experiments were carried out in accordance with the guidelines by the committee for the purpose of control and supervision of experiments on animals.

RESULTS

The gross chemical composition of safflower seeds revealed a moisture 3.8%, a fat 28.7%, a protein 14.75%, a carbohydrate 48.3%, a fiber 2.6%, and Ash 1.85% as shown in Table (1).

Table 1: Gross chemical composition of safflower seeds (g per 100 g)

Moisture	Fat	Protein	Carb.	Fiber	Ash
3.8	28.7	14.75	48.3	2.6	1.85

The total bioactive compounds of phenols, flavonoid content, and antioxidant activity of safflower seeds were reported in Table (2). It contains bioactive compounds, including total phenols and total flavonoids, and antioxidant activity being (125.53 mg GAE/g, 3.14 mg QUE/g, and 69.3%), respectively.

In addition, polyphenolic compounds that were identified in the ethanolic extract of safflower seeds on HPLC analysis were reported in Table (3). It contains about 0.51mg of gallic acid, 1.45 mg of Chlorogenic acid, 6.26 mg of Caffeic acid, 6.05 mg of P-Comaric acid, 11.63 mg of rutin, 7.58 mg of ferulic acid, 3.41mg of quercetin and 6.69mg of apigenin.

Table 2: Total phenols, flavonoid content and antioxidant activity of safflower seeds.

Parameters / Sample	Safflower seeds
Total phenols (mg GAE /g)	125.53
Total flavonoids (mg CE/ 100g)	3.14

Antioxidant activity (DPPH, %)	69.3

GAE, Gallic acid equivalent; CE, Catchin equivalent. DW: dry weight

There was no marked difference between rats of various classes in their feed intake and initial body weight, however, there was an apparent elevation in final body weight and body weight gain of the lead-intoxicated rats in comparison to control rats (ve-). Safflower-treated rats at the dosage of 7.5% and 10% displayed a substantial decrease in their body weights and body weight gains in comparison to the control class (ve+). There was no marked difference between Safflower-treated rats at the dose of 7.5 % and 10% neither in their final body weights nor in their body weight gains. Concerning the feed efficiency ratio, there was a marked elevation in FER of the lead-intoxicated rats in comparison to control (ve-). However, safflower-treated rats at the dose of 10% exhibited a marked decrease in their FER in comparison to the control (ve+). It's important to note that there was no apparent difference between the rats treated with safflower at doses of 7.5% and 10% in terms of their FER.

Regarding the brain relative weight, there was an apparent elevation in the brain relative weight of the lead-intoxicated rats in comparison to control (ve-). However, safflower-treated rats at the dose of 7.5 % and 10% exhibited a marked decrease in their brain relative weights compared to the lead-intoxicated rats. It is worth mentioning that there was no apparent difference in the brain relative weight of safflower-treated rats at the dose of 7.5 % and 10%. Also, there was no apparent difference between control rats (ve-) and safflower-treated rats at the dose of 7.5 % Table (4).

As presented in Table (5), there was a marked reduction in the serotonin and dopamine levels in the brain homogenate of lead-intoxicated rats in comparison to the control class. Nevertheless, there was a marked dose-based raise in the serotonin and dopamine levels in the brain homogenate of safflower-treated rats at a dose of 5%, 7.5 %, and 10 % compared to lead- intoxicated rats.

As depicted in Table (6), there was a marked diminish in the serum ACh linked with an apparent raise in the AChE levels of lead-intoxicated rats in comparison to the control class. However, there was a marked dose-based elevation in the serum ACh in the safflower-treated rats of different doses compared to lead-intoxicated rats. Moreover, the serum AChE levels were reduced markedly in the safflower-treated rats of different doses compared to lead-intoxicated rats. No apparent difference was obvious in the serum AChE levels between the safflower-treated rats of different doses (5%, 7.5%, and 10 %).

Data presented in Table (7) revealed that there was a substantial rise in the TG, TC, VLDL-c, and LDL-c levels associated with a marked reduction in the HDL-c levels in the serum of lead-intoxicated rats comparing it to the control class. However, there was a marked dose-dependent reduction in the levels of TG, TC, VLDL-c, and LDL-c associated with a marked elevation in the HDL-c serum levels of safflower-treated rats at a dose of 5%, 7.5 %, and 10 % compared to lead intoxicated rats. It is worth mentioning that there was no apparent difference in the HDL-c levels between safflower-treated classes at a dose of 5%, 7.5 %, and 10%. Also, there was no marked difference in the TG and VLDL levels between safflower-treated classes at a dose of 7.5 and 10 %.

As presented in Table (8), there was a marked elevation in the MDA and TNF-α levels associated with a marked diminish in the GSH and SOD levels in the brain homogenate of lead-intoxicated rats in comparison to the control class. However, there was a marked dose-dependent diminish in the MDA and TNF-α levels associated with a marked elevation in the SOD and GSH levels in the brain homogenate of safflower-treated rats at a dose of (5, 7.5, and 10) % compared to lead intoxicated rats. It is worth mentioning that administration of safflower at a dose of 10 % restored nearly the normal value of SOD in comparison the control class. Also, there was no marked difference in the GSH and SOD levels between safflower-treated classes at a dose of 5% and 7.5%.

Histopathological investigation

Light microscopic examination of the hippocampus of control rats demonstrated the normal histological structure; normal pyramidal neurons with normal vesicular nuclei (Photo1 A). On the other hand, section from Pb-intoxicated rats revealed severe histopathological damage characterized by pyknosis, atrophy, necrosis and shrunken of pyramidal neurons with the flame-shaped

Table 3: Polyphenolic compounds identified in ethanolic extract of seed safflower on HPLC analysis mg/100 g

Sample	Gallic acid	Chlorogenic acid	Caffeic acid	*P*- Comaric acid	Rutin	Ferulic acid	Quercetin	Apigenin
Safflower seeds	0.51	1.45	6.26	6.05	11.63	7.58	3.41	6.69

Table 4: Effect of different levels safflower seeds powder on body weights gain (BWG%), (FI), (FER) and brain weight of brain toxicity rats.

Parameters Sample	IBW	FBW	BWG%	FI	FER	Brain relative weight

Control (ve-)	180.66±4.33[a]	201.20±2.27[b]	11.37±2.81[b]	19.00	0.036±0.09[b]	0.58±0.01[b]
Control (ve+) Pb	178.83±3.24[a]	212.56±2.69[a]	18.19±1.83[a]	21.00	0.054±0.05[a]	0.70±0.02[a]
Safflower seeds (5%)	183.83±1.89[a]	201.16±3.63[b]	9.43±1.65[c]	19.50	0.031±0.05[c]	0.60±0.02[b]
Safflower seeds (7.5%)	179.83±2.04[a]	189.50±2.46[c]	5.38±1.09[d]	18.40	0.018±0.03[d]	0.53±0.01[c]
Safflower seeds (10%)	184.50±1.25[a]	187.16±0.44[c]	1.44±0.59[e]	18.00	0.005±0.02[e]	0.50±0.01[c]

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P ≤ 0.05.

Table 5: Effect of different levels safflower seeds powder on serum dopamine and serotonin of brain toxicity rats.

Parameters / Sample	Dopamine (Pg/ml)	Serotonin (ng/ml)
Control (ve-)	98.56±3.26[a]	217.58±1.97[a]
Control (ve+) Pb	60.99±1.01[e]	150.42±1.57[e]
Safflower seeds (5%)	71.00±1.21[d]	169.30±1.90[d]
Safflower seeds (7.5%)	76.05±2.05[c]	180.65±1.35[c]
Safflower seeds (10%)	84.60±1.51[b]	193.54±1.16[b]

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P ≤ 0.05.

Table 6: Effect of different levels safflower seeds powder on (Ach) and (AChE) of brain toxicity rats.

Parameters / Sample	Acetylcholine (ACh) (mmol/mg)	Acetylcholine esterase (AChE) (unit/mg)
Control (ve-)	5.72±0.24[a]	0.47±0.03[d]
Control (ve+) Pb	0.83±0.07[d]	0.89±0.04[a]
Safflower seeds (5%)	1.57±0.12[c]	0.67±0.05[b]
Safflower seeds (7.5%)	2.18±0.16[bc]	0.68±0.01[b]
Safflower seeds (10%)	2.65±0.12[b]	0.56±0.01[c]

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P ≤ 0.05.

Table 7: Effect of different levels safflower seeds powder on lipid profile of brain toxicity rats.

Parameters / Sample	TC	TG	HDL-c	VLDL-c	LDL-c
	(mg/dl)				
Control (ve-)	112.45±3.36[e]	95.84±1.43[d]	64.72±1.21[a]	19.16±0.28[d]	28.56±4.29[e]
Control (ve+) Pb	238.43±2.52[a]	164.36±2.00[a]	38.63±0.87[c]	32.87±0.40[a]	166.92±2.75[a]
Safflower seeds (5%)	214.72±2.86[b]	143.26±1.96[b]	50.35±2.33[b]	28.65±0.39[b]	135.71±3.01[b]
Safflower seeds (7.5%)	203.30±5.93[c]	127.24±0.75[c]	51.00±2.00[b]	25.44±0.15[c]	126.85±7.78[c]
Safflower seeds (10%)	183.11±3.47[d]	124.95±1.73[c]	51.09±0.49[b]	24.99±0.34[c]	107.82±3.87[d]

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P ≤ 0.05.

Table 8: Effect of different levels safflower seeds powder on oxidative enzymes (MDA,SOD, GSH) and inflammation markers (TNF-α) concentration of brain toxicity rats.

Parameters / Sample	MDA (µmol/ml)	SOD(µ/dl)	GSH (mg/dl)	TNF-α (Pg/ml)
Control (ve-)	87.17 ± 2.94[e]	89.34±2.24[a]	54.33 ± 1.29[a]	98.44±5.93[e]
Control (ve+) Pb	173.20 ± 4.02[a]	51.43±1.34[d]	14.03 ± 3.33[e]	405.05±8.08[a]
Safflower seeds (5%)	132.52 ± 2.03[b]	73.71±2.92[c]	26.23 ± 1.10[d]	313.11±8.11[b]
Safflower seeds (7.5%)	122.85 ± 2.85[c]	69.32±2.34[c]	29.10 ± 3.10[c]	262.83±2.47[c]
Safflower seeds (10%)	115.76 ± 2.34[d]	82.63±1.03[b]	40.65 ± 2.28[b]	203.64±8.65[d]

Mean values are expressed as means ± SD.
Means with different superscript letters in the column are significantly different at P ≤ 0.05.

appearance of neurofibrillary tangles and proliferation of glia cells (Photo1 B). Meanwhile, the hippocampus of rats from group A Safflower seeds (5%) showed moderate to mild histological changes. The examined section revealed necrosis of some pyramidal neurons (back arrow) with the flame-shaped appearance of neurofibrillary tangles

(Photo1 C). Furthermore, a marked enhanced picture was apparent in the hippocampus of rats from group B Safflower seeds (7.5%), examined parts showed no histopathological changes except necrosis of sporadic pyramidal in some sections (Photo1 D&D1). Likewise, some examined sections from group C Safflower seeds (10%) exhibited no histopathological alterations (Photo 1 E), whereas other sections revealed either necrosis of sporadic neurons (Photo1 E1) or necrosis of some pyramidal neurons (back arrow) with the flame-shaped appearance of neurofibrillary tangles (Photo1 E2).

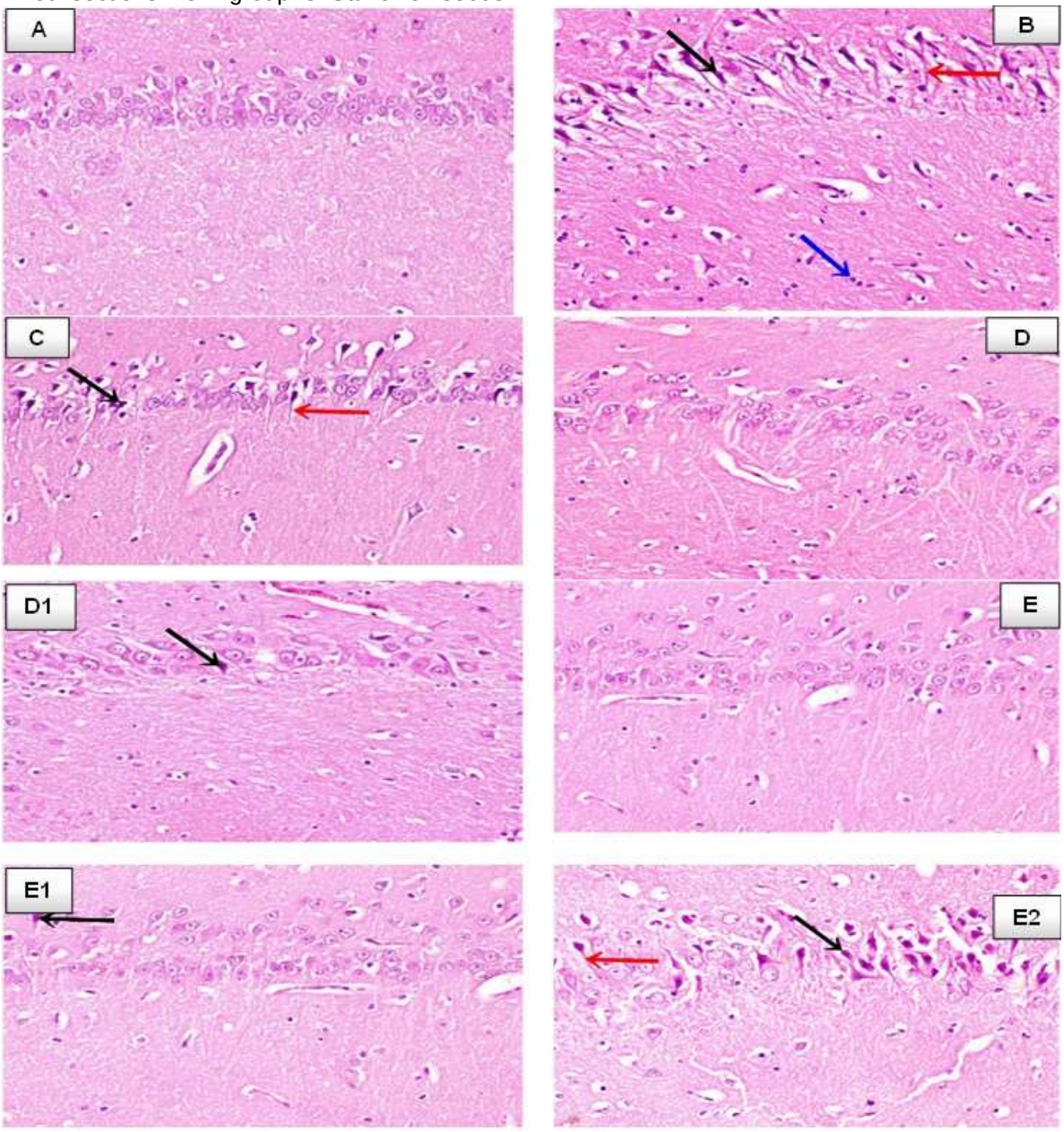

Photo (1): Photomicrographs of rat hippocampus sections H&E **X** 400. **Black arrows** indicate atrophy, pyknosis, shrunken and necrosis of pyramidal neurons, **red arrows** indicate appearance of flame shaped neurofibrillary tangles and **blue arrows** indicate proliferation of glia cells. **A**=Control; **B**= lead acetate; **C**= group A Safflower seeds (5%); **D**= group B Safflower seeds (7.5%); **E**= group C Safflower seeds (10%).

DISCUSSION

It is widely established that Pb-induced neurotoxicity is achieved by cholinesterase activity impairment, ROS production, and pro-inflammatory cytokines. Lead results in oxidative stress, which damages membranes by disrupting the equilibrium between the formation and destruction of reactive oxygen species (ROS) in cellular

components and tissues (Suleman et al. 2022). Due to its capacity to displace calcium ions, Pb has been described like a hazardous heavy metal, which may pass via the blood-brain barrier and produce a number of neurological impairments (Singh *et al.* 2019). Due to this, the goal of the current investigation was to ascertain how CTS affected male rats' neurotoxicity caused by Pb acetate.

According to the current findings, proteins, lipids, and carbs are the primary elements of CTS. These findings support the findings of Bozan and Temelli (2008), who estimated that the overall carbohydrate and fibre content of the seed to be 52%. Various cultivars have varying amounts of fibre in their seeds; the amount is larger in cultivars with thicker walls (about 34%) and lower in those with thinner walls (around 11%) (Hall, 2016). Hamrouni-Sellami et al. (2007) showed that protein content is between 14.9 and 17%. Also, Mariod et al. (2012) showed that raw safflower seed moisture content was 5.3, Fat 34.1.The protein content was 13.2 (g per 100 g) in raw samples. The carbohydrate and fiber contents 43.5 in safflower seeds, 2.6, Ash 1.3.

Also, in our study, the total phenolic content of CTS is 125.53 (mg GAE/100g, flavonoid (3.14 mg CE/100g), antioxidant activity (69.3%). These results are agreed with Hall, (2016) found that the phenolic and flavonoid contents of CSE were 126.0± 2.4 mg (GAE)/g and 62.2 ±1.9 mg (QE)/g, respectively. Similarly Nimrouzi et al. (2020), who demonstrated that phenolic components and antioxidant activity were present in moderate amounts in the extract of CTS.

These findings suggested that CTS might be a useful source of bioactive substances with food and naturally antioxidant characteristics. Caffeic acid, chlorogenic acid, gallic acid, rutin, p-comaric acid, quercetin, apigenin, and ferulic acid were the phenolic substances reported in our work in Table (3). These outcomes are agreed with Hiramatsu et al. (2009); Zhang et al. (2021) within these six polyphenols, they discovered that quercetin, cymaroside, and luteolin were present in significantly larger concentrations than the other substances. Yu et al. (2013) discovered that CTS is a significant source of dietary fibre, proteins, and linoleic acid. Additionally, it includes a number of polyphenols that are now commonly used as antioxidants, including kaempferol. and serotonin. Kaempferol and serotonin in CTS, in specific, have been found to protect against memory loss. In a similar way, Kempuraj et al. (2021); Yeh et al. (2021) stated that eating plants rich in polyphenols or flavonoids may lower the risk of disorders brought on by neuroinflammation.

Exposure of rats to Pb acetate at a dose of 30 mg/kg led to marked elevation in final body weight and body weight gain of the lead-intoxicated rats in comparison to control (ve-). These outcomes were in agreement with Al-Qahtani et al. (2022), who showed that exposure of rats to lead acetate leads to elevation in body weight. While, CTS supplementation exerted an obvious positive impact on the body weight of rats, which can be attributed to steroids, fatty acids, phenolic compounds, and flavonoids (Nimrouzi et al. 2020). The outcomes of this research agree with Elsherif et al. (2022) noticed that the best BWG was fed on (10%safflower seeds) when compared to (+ve) group. In addition the mean values of FI and FER of CTS-treated groups were lower than that of lead-intoxicated rats group. Zahran, (2022) showed that CTS extract at the dose of 100 mg/ kg exhibited a marked decrease in their FER in comparison to the control group. Moreover, Pb intoxication resulted in an increased brain weight index in comparison to the control group. This outcome was on the same line with Rezq et al. (2018); Zahran, (2022);He et al. (2022), who showed that brain weight for Pb- intoxicated group was higher than control group, meanwhile all classes were lower values when compared with Pb-intoxicated group. Also, Kim et al. (2019) suggests that the CTS extract may attenuate atrophy brain in the scopolamine-induced Alzheimer's disease (AD) mice.

Serotonin (5-HT) and dopamine (DA) (catecholamines) are neurotransmitters, which are produced in different zones of the body, including the abdomen, brain, and back. Impairment of the serotonin and dopamine system has been established in various nervous system disorders (Nam et al. 2018). Here, Pb acetate treatment causes a significantly lower level of DA and 5-HT in the brain in comparison to normal control rats. The current results matched those that were attained by Waggas, (2012); Rezq et al. (2018), who demonstrated that delivery of Pb acetate intraperitoneally at a dose of 100 mg/kg BW/day led to a marked decrease in 5-HT and DA content in every examined brain area study (hypothalamus and hippocampus) compared to that of the control rats. As opposed to that, the CTS administration at different levels positively affects the improvement of 5-HT and DA concentrations in the brain, in comparison to Pb intoxicated rats. These findings were on the same line with Li et al. (2020), who found that CSE from safflower was found to inhibit the decreases and restores in levels of serotonin (5-HT) and dopamine (DA) induced by chronic unpredictable mild stress. These effects could be because of the bioactive compounds of CTS, such antioxidants have been shown in ability on promote healthy neurological functions. These findings were on the same line with Hugo et al. (2016); Mehram et al. (2021).

The acetylcholine esterase (AchE) enzyme decreases brain neurotransmitters by breaking down acetylcholine (ACh) into acetate and choline (Rana et al. 2018). The current outcomes revealed that Pb-intoxicated rats displayed an increased AChE activity in comparison to control rats. This outcome is in accordance with Suleman et al. (2022). However, the administration of CTS significantly reduced AchE levels. This finding is on the same line with, Choi et al. (2018), who showed that giving rats with alcohol-induced cognitive deficits safflower seed extract (100 and 200 mg kg−1 day−1) for 30 days decreased AChE function in the brain. Similarly, Kim et

al.(2019); He et al. (2020) clarified that giving the safflower seed extract to the animals reduced the AChE action in the brain, demonstrating that the safflower seed extract lessens cholinergic impairment and enhances memory.

Also, our data revealed that CTS application resulted in a marked decrease in lipid profile levels but resulted in a marked raise in levels of HDL in the rats. These outcomes was on the same line with Nimrouzi et al. (2020) they demonstrated that treating metabolic syndrome-affected rats with safflower seed extract in a dose-based strategy might reduce serum TG and TC levels and enhance the lipid profile. Du et al. (2021); Zahran, (2022) indicated that CTS extract diminished serum TG, TC, VLDL, and LDL and raised HDL level. A similar finding was consistent with Elsherif et al. (2022), who revealed that powdered safflower seed lowered triglycerides, cholesterol, VLDL, and LDL and elevated HDL-c in male rats. Furthermore, Choi et al. (2018); Park et al. (2018) clarified that the safflower seed extract had been shown to have potent antioxidant activities and to limit the oxidation of LDL, hence avoiding the formation of atherosclerotic plaques. Safflower seeds' serotonin derivatives, flavones, polyphenols, and lignans have been primarily ascribed for these impacts.

The brain is especially vulnerable to lipid peroxidation and oxidative damage due to its high oxygen requirements and high lipid content. SOD and CAT, two antioxidant enzymes, are considered to be the initial line of defense for cells against oxidative harm from heavy metals. Superoxide radicals are affected by SOD and reduced to O_2 and H_2O (Rana et al. 2018). Pb has been shown to cause oxidative damage to membrane lipids by increasing peroxidation (Bhatti et al. 2018). The identified rise in serum lipid peroxidation (MDA) levels and decline in serum total antioxidants support the antioxidant depletion caused by Pb acetate (Jackie et al. 2011), which may be the primary factor contributing to the neurotoxicity brought on by Pb exposure (Elgawish and Abdelrazek, 2014). Our findings are in accordance with Khedr and Talkan, (2022), who showed a decrease of brain SOD and GSH actions in the Pb intoxicated class while MDA was increased markedly in comparison to control rats. As contrast to that, CTS supplementation can improve antioxidant defense by elevating the activity of the antioxidant enzyme and lowering peroxidation of lipid as indicated by serum levels of MDA. The present findings were in accordance with that suggested by Kim et al. (2019); de Souza et al. (2022) demonstrated that the treatment of safflower seed extract shields animals given scopolamine from oxidative stress by reducing the formation of ROS and rising the activity of antioxidant enzymes, especially SOD and CAT. Similarly, Nimrouzi et al. (2020), reported that safflower seed extract could reduce oxidative damage in rats undergoing high fructose drinking by increasing TNF-α and MDA levels and decreasing SOD and GSH peroxidase activities. Safflower seed is thought to have a positive impact on memory improvement through its powerful antioxidant action, which comes from its direct scavenging activity of free radicals, which prevents oxidative stress, as well as its anti-inflammatory characteristics (Tayebeh et al. 2021).

TNF- α has a vital role in regulating cascade of cytokine (Griffin, 2013). In the current study, Pb exposure results in an elevated pro-inflammatory cytokines level like TNF-α. This finding agrees with Liu et al. (2015) and Softic et al. (2016), who stated that endotoxemia, which amplifies the release of inflammatory cytokines like TNF-α, may be the cause of the rising TNF-α levels in the Pb-intoxicated class. Additionally, there is a strong connection between oxidative stress and elevated TNF-α levels (Lambertz et al. 2017). However, CTS administration lowered TNF-α level. This finding is on the same line with Zhang et al. (2019); Nimrouzi et al. (2020),they claimed that safflower yellow (SY) therapy dramatically reduced the levels of IL-6, IL-1β, TNF-α and hence reduced the inflammatory response. Furthermore, according to Park et al. (2018), tocopherols and serotonin compounds from safflower seeds can reduce the formation of lipid peroxidation and inflammatory cytokines like TNF-α.

These changes were further confirmed by the histological examination of the brain hippocampus. A bilateral portion of the brain in the temporal lobe called the hippocampus is critical for learning and memory (Kubo et al. 2017). Hippocampus of Pb intoxicated rats displayed pyknosis, atrophy, and necrosis and shrunken of pyramidal neurons with the flame-shaped appearance of neurofibrillary tangles and proliferation of glial cells, and these outcomes are in accordance with Liu et al. (2015), showed that mice hippocampus micro gliosis and astrogliosis after exposure to Pb were significant. Also, Li et al. (2019) indicated that two months of consuming water treated with Pb caused significant pathological alterations in the model class hippocampus and cerebral cortex. Lastly, Suleman et al. (2022) stated that exposure to Pb led to substantial cellular damage in the hippocampus region, which also showed up as vacuolization and edema. Yet, CTS supplementation reduced Pb-induced hippocampus damage. These amendments were more detectable in rats treated with higher levels of CTS. The phenolic compounds in the CTS, including gallic acid, rutin, caffeic acids, ferulic acid, and p-coumaric may account for the noticed protective impact of CTS in this research. These compounds have been demonstrated in experimental models to have antioxidant and anti-inflammatory actions (Tayebeh et al. 2021) as well as anticholinesterase activity (Mangmool et al. 2021**)**.

CONCLUSION

This study revealed that safflower seeds supplementation with different levels has an essential role in preventing the neurotoxic impacts of Pb acetate via different mechanisms, including diminishing Pb-induced brain oxidative stress, inflammation, neuronal damage, and acetyl cholinesterase action due to their high content of

phytochemicals like phenolic compound and flavonoids, which make them powerful antioxidants. Therefore, CTS may be an effective herbal medication for treating Pb-induced neurotoxicity and its subsequent detrimental effects.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

In addition to writing the manuscript, SHN also designed and carried out the experiments. Animal care, flow cytometry research, tissue collection, and data analysis were all carried out by SHN. The manuscript was reviewed by RA and SHN. The final version was read by all authors and got their approval.

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