

Protective Effect of Grape Seeds Powder Against Lead Acetate-induced Brain Toxicity in Male Rats

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Abstract: It is well known that lead (Pb) toxicity induces neurological damage with several disorders like behavioral problems; nerve damage; mental retardation; Parkinson's disease and Alzheimer's disease. Here, we investigated the protective effect of different levels of grape seeds against lead acetate-induced brain toxicity in male rats. Induction of brain toxicity was achieved with intraperitoneally injection of lead acetate (100 mg/kg b.w/day) for two weeks. Body weights gain; relative body weight gain; brain weights and its relative weight to body weight were recorded. Brain tissue concentrations of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) as well as the serum levels of AST, ALT Gamma-Glutamyl-transferase (GGT), alkaline phosphatase (ALP), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) activities, malondialdehyde (MDA) and total antioxidant activity were determined in lead acetate-induced brain toxicity rats and their control. Histopathological examinations of brain tissues were studied. The injection of lead acetate induced significantly reduction in body weight gain and relative body weight gain, concentration of NE, DA and 5-HT in brain tissues, serum level of GSH-Px, SOD, CAT, malondialdehyde and total antioxidant, while increase brain weight and its relative increase of its weight to body weight were compared to that of normal rats. However, the improvements in all of the above parameters were showed in lead brain intoxication rats treated with grape seeds (powder) compared with that of untreated rats. Histopathological examination of brain tissues of untreated positive rats showed focal areas of hemorrhages, leucocytic cell infiltration, focal proliferation of glia cells associated with gliosis and focal areas of atrophied neurons. Brain sections of treated rats with grape seeds at level of 5% have degenerated glia cells while treated rats with 10% have large focal hemorrhagic areas and the higher level of grape seeds have congested blood vessel in some sections and no histopathological changes was shown in other sections of rats. In conclusion, Intake of grape seeds exhibited protective effect against lead (Pb) toxicity, neurological damage and oxidative damage. Therefore, intake of whole grapes with its seeds or grape seeds in food supplement may be beneficial to prevent or protect against lead toxicity.

Key words: Grape Seeds powder • Lead Acetate • Antioxidant Enzymes • Liver Functions

INTRODUCTION

Lead is a poisonous metal, which occurs in both organic as tetraethyl lead and inorganic as lead acetate and lead chloride forms in the environment [1]. It is present in the environment water, soil, dust and paints [2]

and is poisonous to animals and humans throughout the world [3]. Lead-exposure occurs mainly through the respiratory and gastrointestinal systems. Lead which is ingested and absorbed is stored in soft tissues and bone and accumulated in the liver and transported to the kidney, while a small quantity is excreted in urine and the

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reminder accumulates in various body tissues and organs, affecting many biological activities at the molecular, cellular and intercellular levels, which may result in morphological alterations that can remain even after lead levels have fallen [4]. Lead toxicity is related to its accumulation in various tissues and organs and interference with several physiological processes [5]. Consequently, it constitutes a significant public health problem, despite efforts to reduce its level in the ecosystem [6]. Neurological damage induced by lead toxicity is a known condition that has a base for several disorders like behavioral problems; nerve damage; mental retardation; Parkinson's disease and Alzheimer's disease [7].

A plant material in the human diet contains a large number of natural compounds, which have beneficial effects in protecting the body against the development of neurotoxicity. One of these plants with constituents reputed to possess neuron protective properties was grape [8]. Grape is one of the world's largest fruit crops. Grape seeds produced in large quantities by juice industry are increasingly used to obtain functional food ingredients [9]. Grape seed is a better source of antioxidative constituents of grape byproducts. Its extract is a commonly available dietary supplement taken for the antioxidant activity that's attributed to its proanthocyanidin content [10]. It contains several active components including polyphenols, flavonoids, anthocyanins, proanthocyanidins and procyanidines [11]. The antioxidant activity of grape seed is closely associated with activity against various cancer types, cardiovascular diseases and several dermal disorders [12]. It also improves hepatic ischemia-reperfusion injury and reduces the size of the infarct in cardiac ischemia in the rat [13]. Several studies have indicated that grape seed extract inhibit enzyme systems that are responsible for the production of free radicals [14]. Therefore, the present study was undertaken to assess the protective effect of feeding on grape seeds against lead acetate-induced brain toxicity in male rats.

MATERIALS AND METHODS

Materials

Grape Seeds: Grape seeds were purchased from a local market of Spices, Grains and Oils, Holy Makkah, KSA.

Rats and Diet: Seventy five male Sprague-Dawley rats weighing 200 ± 5 g were obtained from the Laboratory Animal Colony, Medicine College, Umm Al Qura University, KSA. Basal diet constituents were purchased

from Baghafar Company for Pharmaceutical and Chemical, Jedda, KSA.

Kits and Chemicals: Lead acetate $[(C_2H_3O_2)_2Pb \cdot 3H_2O, Pb]$, formalin, diethyl ether and kits for biochemical analysis of serum were purchased from Baghafar Company for Pharmaceuticals and Chemicals, Jedda, KSA.

Methods

Preparation of Grape Seeds Powder: Dried grape seeds were cleaned from foreign materials and washed with tap water to remove possible potential dust. Afterwards, it was dried by cotton cloth to remove the excess liquid prior to drying. Then dried grape seeds were ground using grinder mill and sieves were used to obtain a powder particle size of less than 0.2 mm.

Preparation of Basal Diet: The basal diet (AIN-93M) was formulated according to Reeves *et al.* [15] to meet recommended nutrients levels for rats.

Experimental Design: A total of 75 male Sprague-Dawley rats weighing 200 ± 5 g were used in the experiment. All animals were allowed free access to tap water and fed on the standard basal diet and kept under normal health laboratory conditions and adapted for one week. After one week, rats were randomly divided into five groups of 15 rats each. The first group, rats were injected intraperitoneally (i.p.) with saline solution, feed on the basal diet and represented as the health control animals (negative group). The second, third, fourth and fifth groups were injected intraperitoneally (i.p.) with lead acetate (100 mg/kg b.w/day). The second group was represented as lead-intoxication rats (positive group) and the other groups (3, 4 and 5) were feed on the formulated basal diet with 5, 10 and 15% of grape seeds powder, respectively.

The biological value of the different diets was assessed by the determination of its effect on body weight gain (BWG) at the end of the experimental period using the following formula:

$$BWG = \text{Final Body Weight} - \text{Initial Body Weight}$$

At the end of the experimental period (6 weeks), animals were fasted for 12-hr, except of water and then rats sacrificed. Blood samples were collected from the posterior vena cava into dry clean centrifuge tubes and left at room temperature to clot and then centrifuged for 10 minutes at 3000 rpm for serum separation. Serum samples were frozen at -30°C for biochemical analysis.

The whole brain of each animal was rapidly carefully dissected, weighed and sagittally divided into two halves. The first half was immersed in neutral buffered formalin 10% for histopathology examination. The 2nd half was immediately homogenized to give 10% (w/v) homogenate in ice-cold medium containing phosphate buffer (pH 7.4). The homogenate was centrifuged at 1800xg for 10 min in cooling centrifuge at 4°C. The supernatant (10%) was separated and kept at -80°C until being assayed for the neurochemical analysis.

Neurochemical Analysis: Norepinephrine (NE), dopamine (DA) and serotonin (5-HT) were estimated according to the method of Chang [16] and modified by Ciarlone [17]. The fluorescence was measured in Jenway 6200 fluorometer.

Biochemical Analysis: The biochemical indices were determined in rat serum using a UV/Vis spectrophotometer (Humastar 200, automatic biochemistry analyzer, Wiesbaden Germany). The levels of rat serum bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were done using described methods of Bergmeyer *et al.*, [18]. Serum level of ALP was determined by the method of kits (Diamond Co, Hannover, Germany) as described by Roy [19]. Gamma glutamyl transferase (GGT) was determined according to described method by kits (Diamond Co, Hannover, Germany) as described by Young [20].

Oxidative Stress Parameters

Malondialdehyde (MDA): Malondialdehyde was assayed quantitatively in serum using the MDA assay kit (by a spectrophotometric method, ABCAM, UK) according to manufacturer instructions as described by Draper and Hadley [21]. The MDA in the sample reacts with thiobarbituric acid (TBA) to generate a MDA-TBA adduct. The MDA-TBA adduct is quantified colorimetrically (OD = 532 nm). This assay detects MDA levels as low as 1 nmol/well colorimetrically. The results were expressed in nmol/ml.

Glutathione Peroxidase (GPX) Activity glutathione peroxidase enzyme was assayed quantitatively in serum using the GPX assay kit (by a spectrophotometric method, BioAssay Systems, USA) according to the method of Hissin and Hiff [22].

Superoxide Dismutase (SOD): Serum activity of superoxide dismutase was assayed quantitatively in serum using the SOD assay kit (by a spectrophotometric

method BioAssay Systems, USA) as described by Kakkor *et al.* [23]. In the assay, superoxide (O₂⁻) is provided by xanthine oxidase (XO) catalyzed reaction. O₂⁻ reacts with a WST-1 dye to form a colored product. SOD scavenges the O₂⁻ thus less O₂⁻ is available for the chromogenic reaction. The color intensity (OD440nm) is used to determine the SOD activity in a sample. The results were expressed in U SOD/ml.

Catalase Assay (CAT): Serum activity of catalase was assayed quantitatively using the catalase assay kit (by a spectrophotometric method, Bio-Assay Systems, USA) according to method of Sinha [24], measuring catalase degradation of H₂O₂ was using a redox dye. The change in color intensity at 570nm is directly proportional to the catalase activity in the sample. The procedure involves adding a Substrate to sample, incubation for 30 min, followed by a Detection Reagent and reading the optical density. The results were expressed in U Catalase/Liter. *Unit definition:* one unit is the amount of catalase that decomposes 1 µmole of H₂O₂ per min at pH 7.0 and room temperature.

Totals Antioxidant Capacity (TAC): Total Antioxidant Capacity was assayed quantitatively in serum using the catalase assay kit (by a spectrophotometric method, BioAssay Systems, USA) as described by Woodford and Whitehead [25]. In the assay, Cu₂⁺ is reduced by antioxidant to Cu⁺. The resulting Cu⁺ specifically forms a colored complex with dye reagent. The color intensity at 570nm is proportional to TAC in the sample. The results were expressed in µM Trolox Equivalents.

Histopathological Examination: The other halve brain of each animals were carefully washed in an isotonic solution, blotted on a filter paper and then putted in 10% normal formalin. Paraffin section of 6 µm thickness was cut and stained with hematoxylin (HX) and eosin (E) for the histological studies in order to follow up the destruction in the tissues and cells of brain as described by Carleton [26].

Statistical Analysis: The results were expressed as mean ± standard division for 15 rats in each group. Differences between groups were assessed using computerized statistical package of social sciences (SPSS) program (SPSS. 20 software version) with one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. P < 0.05 values were considered to be statistically significant.

RESULTS

The intraperitoneal injection with lead acetate (100 mg/kg b.w/day) for two weeks significantly ($p < 0.05$) reduced body weight gain and increased brain weight and its relative weight to body weight compared to that of the normal control rats. Feeding lead-intoxicated rats on formulated diet with different levels of grape seeds powder significantly ameliorant the lower in body weight and lower brain weight and it's relative to body weight compared with that of the positive control rats feed on basal diet only.

The concentrations of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in brain tissues of lead acetate-induced brain toxicity rats are shown in Table 2. Results indicated that positive rats had significant decrease in the concentration of NE, DA and 5-HT in brain tissues compared to that of normal rats. The lower concentration of NE, DA and 5-HT in brain tissues in lead acetate-induced brain toxicity rats tended towards the significantly increase in all feeding rats on supplemented diet with the different level of grape seeds compared to that of positive rats.

As shown in Table 3, intoxicated rats with lead acetate at a dose of 100 mg/kg b.w/day had significant increase in serum activity of aspartate aminotransferase (AST), Alanine aminotransferase (ALT), gamma-Glutamyl transferase (GGT) and alkaline phosphatase (ALP) enzymes and level of TBr compared with that of the normal rats. Feeding lead-intoxicated rats on supplemented diet with the different levels (5, 10 and 15%) of grape seeds powder significantly decreased serum activity levels of AST, ALT, GGT and ALP enzymes and level of TBr compared to that of positive rats feed on basal diet only.

Intraperitoneal injection of lead acetate to rats caused a significant elevation in serum level of lipid peroxide as indicated by malondialdehyde (MDA) and lowering the serum activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) enzymes and total antioxidant capacity (TAC) compared with that of negative control rats as shown in Table 4. In contrast, feeding lead-intoxicated rats on formulated diet with different levels of grape seeds powder had significant decreased in serum level of MDA and increased in serum activity of GSH-Px, SOD, CAT enzymes and level of TAC compared with that of the positive control rats feed on basal diet only.

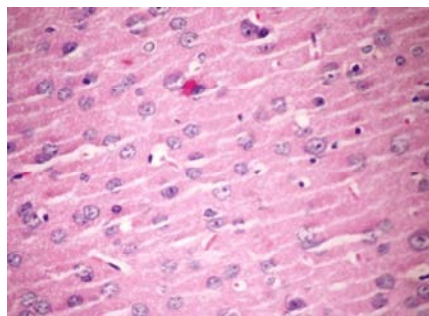


Fig. 1: Brain section of normal rats showing apparently healthy glia cells (H&E X 400)

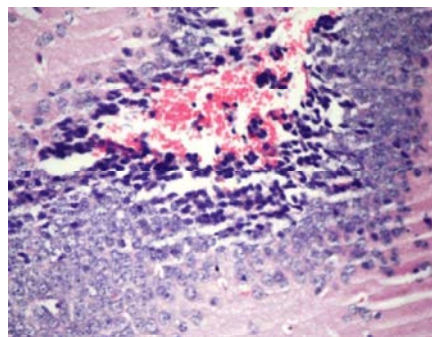


Fig. 2: Brain section of positive rats showing focal area of hemorrhages with leucocytic cell infiltration (H&E X 400)

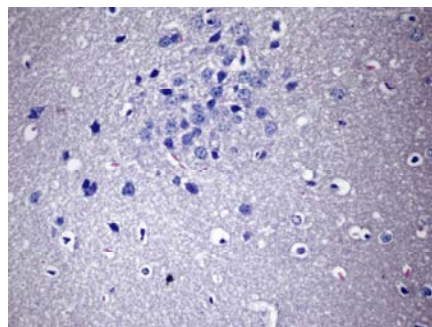


Fig. 3: Brain section of positive rats showing focal proliferation of glia cells, gliosis. (H&E X 400)

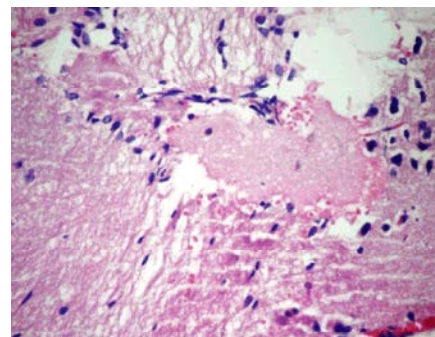


Fig. 4: Brain section of positive rats showing focal area of atrophied neurons (H&E X 400)

Table 1: Effect of feeding different levels of grape seeds powder on body weights gain and brain weight and its weight to body weight in lead acetate-induced brain toxicity rats and their control

Parameters	Treated rats with grape seeds at levels of				
	Normal rats	Positive rats	5%	10%	15%
Initial body weight (g)	246.50±3.37	247.50±3.54	246.50±4.12	246.00±3.16	246.00±3.94
Final body weight (g)	297.80±2.66 ^a	275.30±1.34 ^c	285.70±1.57 ^d	291.60±1.65 ^c	295.40±4.03 ^b
Body weight gain (g)	51.50±2.07 ^a	27.80±3.05 ^d	39.20±3.88 ^c	45.60±3.75 ^b	49.40±3.06 ^a
Brain weight (g)	1.45±0.04 ^c	1.65±0.06 ^a	1.61±0.05 ^{ab}	1.55±0.06 ^{bc}	1.54±0.12 ^c
Relative of brain weight to body weight (%)	0.50±0.01 ^d	0.60±0.02 ^a	0.56±0.02 ^b	0.53±0.02 ^c	0.52±0.03 ^{cd}

-Different superscript letters in the same row denotes significant differences at p<0.05

- SD: Standard Division

Table 2: Effect of feeding grape seeds powder on the concentrations of NE, DA and 5-HT in brain tissues in lead acetate-induced brain toxicity rats and their control

Parameters	Treated rats with grape seeds at levels of				
	Normal rats	Positive rats	5%	10%	15%
NE (ng/ g tissue)	1568.50±1.35 ^a	881.50±1.18 ^c	1005.50±0.97 ^d	1357.00±0.82 ^c	1562.40±0.70 ^b
DA (ng/ g tissue)	1999.30±0.82 ^a	984.20±0.79 ^c	1357.50±0.53 ^d	1785.90±0.57 ^c	1995.10±0.74 ^b
5-HT (ng/ g tissue)	1799.30±0.95 ^a	650.40±0.84 ^c	1007.70±0.82 ^d	1577.30±0.67 ^c	1788.90±0.99 ^b

-Different superscript letters in the same row denotes significant differences at p<0.05

- SD: Standard Division

Table 3: Serum activities of AST, ALT, GGT and ALP enzymes and levels of TBr in lead acetate-induced brain toxicity rats and their control

Parameters	Treated rats with grape seeds at lev				
	Normal rats	Positive rats	5%	10%	15%
AST (U/L)	53.26±0.88 ^c	92.20±0.97 ^a	79.04±0.43 ^b	65.87±1.40 ^c	54.88±1.45 ^d
ALT (U/L)	67.11±.58 ^c	104.06±0.99 ^a	92.89±1.99 ^b	80.17±1.29 ^c	68.36±1.34 ^d
GGT (U/L)	19.35±.61 ^d	74.43±.73 ^a	56.93±0.68 ^b	38.83±.57 ^c	19.87±0.62 ^d
ALP (U/L)	37.49±0.32 ^d	70.64±0.33 ^a	61.53±0.34 ^b	54.98±.14 ^c	54.98±0.28 ^d
TBr (mg/dL)	0.36±0.01 ^d	3.10±0.03 ^a	2.68±0.03 ^b	1.25±0.02 ^c	1.52±0.01 ^c

-Different superscript letters in the same row denotes significant differences at p<0.05

- SD: Standard Division

Table 4: Effect of feeding grape seeds on serum concentration of MDA, activity of GSH-Px, SOD and CAT enzymes and level of TAC in lead intoxicated rats and their control

Parameters	Treated rats with grape seeds at levels of				
	Normal rats	Positive rats	5%	10%	15%
MDA (nmol/l)	34.94±0.14 ^c	72.48±0.38 ^a	60.89±0.40 ^b	49.55±0.43 ^c	35.52±0.33 ^d
GSH-Px (μg/ml)	166.75±0.55 ^a	39.99±0.45 ^c	76.93±0.60 ^d	114.19±0.44 ^c	155.63±0.95 ^b
SOD (μ/ml)	3.3860±0.22 ^a	2.31±0.07 ^c	2.47±0.11 ^d	2.70±0.06 ^c	2.93±0.03 ^b
CAT (mmol/min)	0.18±0.003 ^a	0.09±.004 ^c	0.11±0.01 ^d	0.13±0.01 ^c	0.16±0.01 ^b
TAC (mmol trolox/liter)	2.83±0.14 ^a	1.59±0.09 ^c	1.77±0.08 ^d	2.13±0.06 ^c	2.66±0.132 ^b

-Different superscript letters in the same row denotes significant differences at p<0.05.

- SD: Standard Division

The histopathological examination of brain sections of normal control rats (negative group) showed normal neurons cells (Fig. 1). Brain sections of lead brain intoxication rats (positive group) have focal area of hemorrhages with leucocytic cell infiltration (Fig. 2) and gliosis in the form of focal proliferation of glia cells (Fig.3) as well as focal area of atrophied neurons (Fig. 4). Histopathological examination of brain sections from lead

brain intoxication rats treated with grape seeds at a level of 5% showed degenerated glia cells as shown in Fig. 5. While, brain sections of treated intoxicated-rats with 10% of grape seeds have a large focal hemorrhagic area as shown in Fig. 6. Brain sections of treated intoxicated-rats with a higher level of grape seeds have congested blood vessel as shown in Fig. 7, meanwhile other sections of brain showed have no histopathological changes (Fig. 8).

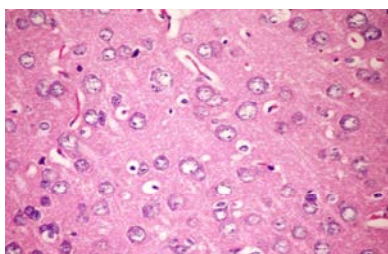


Fig. 5: Brain section of lead brain intoxication rats treated with 5% grape seeds showing degenerated glia cells (H&E X 400)

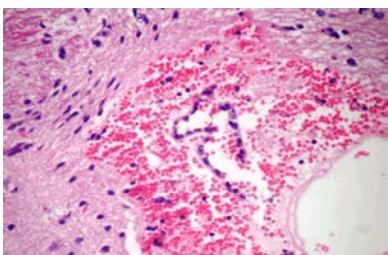


Fig. 6: Brain section of lead brain intoxication rats treated with 10% grape seeds showing large focal hemorrhagic area, (H&E X 400)

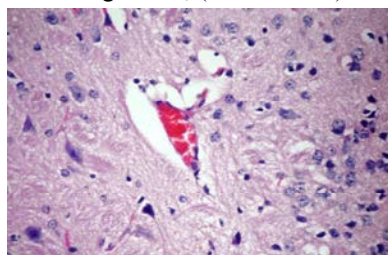


Fig. 7: Brain section of lead brain intoxication rats treated with 15% grape seeds showing congested blood vessel (H&E X 400)

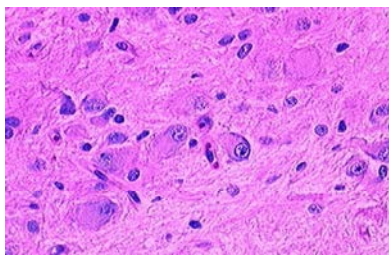


Fig. 8: Brain section of lead brain intoxication rats treated with 15% grape seeds showing no histopathological changes (H&E X 400)

DISCUSSION

The current results showed a significant reduction in body weight (g) and lower in brain weight and its relative weight to body weight in injected rats with lead acetate

(positive group). The present results are in accordance with Xia *et al.* [27] who revealed that exposure of rats to lead acetate causes a decrease in body weight. In addition to, the reduction in body weight was previously observed by Allouche *et al.* [28] and Ibrahim *et al.* [29] who reported that final body weight of intoxicated rats with lead was significantly lower than that of the normal rats. The reduction in body weight may be a result of the direct toxic effect of the lead on the gastrointestinal tract which perhaps results in malabsorption of nutrients or by inhibition of protein synthesis [30]. Also, Hwang and Wang [31] revealed that the lower in body weight may be caused by the effect of toxic lead on zinc status by impairing it in zinc- dependent enzymes which are necessary for many metabolic processes.

The present results showed the ability of grape seeds powder in improving the change in body and brain weights and compared with untreated lead-intoxicated rats (positive group) without any significant changes compared to normal rats. The amendments in body and brain weights induced by administration of grape seeds powder may be related to its effect in prevention of lead toxicity compared to untreated lead-intoxicated rats. However, the deficiency in body weight in lead-intoxicated rats and treated with grape seeds powder compared to that of normal health group may be attributed to the inhibiting effect of grape seeds on both α -amylase and α -glucosidase activity, two key glucosidases required for starch digestion [32]. Whereas, Hasseeb *et al.* [33] reported that grape seed extract can also be used in the treatment of weight loss attributed to metabolic disorders.

From the present result it is clear that intraperitoneally administration of lead acetate induce a significant decrease in NE, DA and 5-HT content in brain tissues, compared to that of the normal control rats. The present results were in accordance with that obtained by Cory-Slechta [34] and Tang *et al.* [35] who showed that intoxication with lead induces a reduction in the metabolites of dopamine in the nigrostriatal and mesolimbic systems. Additionally, Gill *et al.* [36] showed that lead exposure resulted in alterations in concentrations of the brain transmitters, NE and DA. Lead exerts its neurotoxic effects by interfering with Ca^{2+} calmoduline mediated neurotransmitter release that is eventually responsible for behavioral impairment. Waggas [37] revealed that intraperitoneally administration of lead acetate at a dose of 100 mg/kg b.w/day caused a significant decrease in NE, DA and, 5-HT content in all tested brain regions study (cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus)

compared to that of the normal rats. In addition to, Bouton *et al.* [38] showed that lead also disrupts the activity of synaptotagmin, a protein localized in the synaptic terminal that appears to be important for transmitter release. On the other hand, El-Masry *et al.* [39] indicated that lead acetate intoxication induces an oxidative stress situation in rat brain that might be the main mechanism involved in brain neurotoxicity induced by Pb-exposure.

With regard to the effect of grape seeds powder on the concentration of NE, DA and 5-HT in brain tissues, the present results indicated that grape seeds powder have a positive effect in the improvement of NE, DA and 5-HT concentrations in brain tissues, compared to positive rats. The present results agreed with Bagchi *et al.* [40] and Waggas, [37] who mentioned that grape seed extract induce significant reduction in NE, DA and 5-HT in brain tissues of cerebellum, brainstem, striatum, cerebral cortex, hypothalamus and hippocampus, compared to that of intoxicated rats with lead acetate. The effect of grape seed may be related to its antioxidant properties as reported by Yilmaz and Toledo [12] who reported that grape seeds extract contains polyphenols including proanthocyanidins and procyanidins that showed antioxidant and free radical scavengers. Their effects decrease capillary permeability and fragility and scavenge oxidants and free radicals. Other studies indicated that grape seeds extract inhibit enzyme systems that are responsible for the production of free radicals [14]. Many studies have provided evidence that proanthocyanidin has potent radical scavenging ability, antioxidant properties and significant neuroprotective as well as cardiovascular protective effect [41, 42].

The findings of the present study showed that lead acetate caused a significant increase in serum activities of AST, ALT, GGT and ALP enzymes and total bilirubin level. These results were confirmed with histopathological study which revealed multiple focal areas of necrotic and calcified hepatocytes encircled with fibrous connective tissue proliferation in injected rats with lead acetate compared with that of normal control rats. The present results are in agreement with previous studies which revealed that lead has hepatotoxic effect [43] and can cause several changes in the liver structure, where it is conjugated in the liver with glutathione and accumulate in the hepatic tissues, leading to impaired liver functions [44]. Elevation in serum activities of AST and ALT in exposed rats to lead acetate was corroborative with Sivaprasad *et al.* [45] and Abdel-Kader *et al.* [46]. Seddik *et al.* [47] revealed that the elevation in serum

activities of AST and ALT and serum level of bilirubin were accompanied with high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue histogram in treated rats with lead. Ibrahim *et al.* [29] showed significant rise in the serum of AST, ALT and ALP and increased of serum bilirubin level and reduced of serum total protein and albumin levels in lead intoxicated rats as compared to that of control rats. Recently, Azab [48] observed significant increase in serum γ -GT, AST, ALT and ALP activities and showed distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver and necrosis of hepatocytes in lead acetate treated group compared to normal control group.

Regarding to the physiological and histopathological effect of grape seeds on lead-intoxicated rats, the most important result drawn from the current study is the powerful ability of grape seeds in induces significant amelioration in liver functions as well as the improvement in histopathological structure of liver, compared with untreated lead-intoxicated rats (positive group). These amendments were more detectable in treated rats with higher level of grape seeds. These results were in accordance with several results reported that grape seed extract exhibit multi-organ protective properties against drug and chemical-induced toxicity and long-term safety [49] and may be favorable as a therapeutic option in RTX-induced oxidative stress in the rat liver [50]. Grape seed extract is a useful herbal remedy, especially for controlling oxidative damages and is considered as a potent protective agent against hepatotoxicity El-Ashmawy *et al.* [51]. Several other lines of evidence revealed that, grape seed proanthocyanidins exhibit in vivo hepatoprotective and anti-fibrogenic effects against liver injury and act as free radicals scavengers and protective liver damage [52] and significantly reduce serum AST, ALT and ALP activities and bilirubin level and increased serum total protein and albumin levels in dimethylnitrosamine-induced hepatic cirrhosis rats [53]. Grape seeds produce significant hepatoprotective effects by decreasing serum ALT, AST and ALP activities, serum bilirubin and MDA levels and increase albumin level and liver SOD, CAT and GPx activities with amelioration of structural changes induced in liver of diabetic rats [54]. Oral treatment with grape seeds extract significantly ameliorates the indices of hepatotoxicity and lipid peroxidation induced by benzene [55]. The use of high dosage was shown to exert relevant beneficial health effects [56, 57] whereas lower dosages were less effective [58].

The result of the present study clearly showed significant increase in serum MDA level and decrease in serum GSH and total antioxidant levels and activities of GPx, SOD and CAT enzymes in treated rats with lead acetate, compared to normal rats. The significant increased in serum MDA level of lead- intoxicated rats confirmed previous studies [59, 60]. Effect of lead on the activities of SOD and GSH-Px enzymes was previously noticed and in accordance with Sivaprasad *et al.* [61] that showed significant decrease of SOD and GSH-Px activities in lead exposed rats. Also, Abdel-Kader *et al.* [46] observed that rats given lead acetate in drinking water for 4 weeks have significant increase in serum MDA concentrations and decrease in SOD and GSH-Px levels. The observed increase in serum level of lipid peroxidation (MDA), decrease in circulating antioxidant enzymes and decrease of serum total antioxidants level confirm that the lead acetate induced depletion of antioxidants system. The mechanism of lead-induced oxidative stress involves an imbalance between generation and removal of reactive oxygen species (ROS) in tissues and cellular components causing damage to membranes, DNA and proteins [62]. Lead was reported to cause oxidative stress by generating the release of ROS such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipids peroxides [63] and enhance lipid peroxidation and nitric oxide production in serum with concomitant reduction in antioxidant enzymes as GPx, SOD and CAT [64].

The present results showed that grape seeds powder have the ability to improve antioxidant defense through increasing the activity of antioxidant enzyme and total antioxidant and decreased lipid peroxidation as indicated by serum level of MDA. These results confirm the reason for the improvement in liver and brain functions and protect it from damage caused by lead toxicity. The action mechanism of grape seeds may be related to its antioxidant and anti-inflammatory properties [65] and its powerful free radical scavenger [66]. These results are in agreement with Sehirli *et al.* [67] who reported that grape seeds extract could reduce organ injury through its ability to balance the oxidant-antioxidant status and to regulate the release of inflammatory mediators. Grape seeds are rich source of proanthocyanidins, which are mainly composed of dimers, trimers and oligomers of monomeric catechins [68]. Proanthocyanidins are potent natural antioxidants of various polyphenolic components [69]. These compounds possess a broad spectrum of antioxidative properties with greater potency than vitamin E and C, that protects the organs against free radicals and oxidative stress, both *in vitro* and *in vivo* [70]. Many data have shown that the ability of grape seed

proanthocyanidins to improve antioxidant defenses for protecting the main organ function, such as preventing liver injury in the carbon tetrachloride- induced and ischemia/reperfusion-induced [71]. In addition, grape seed proanthocyanidin significantly increase antioxidant enzymes activity such as SOD, GPx and CAT and ameliorate biochemical abnormalities and antioxidant system status in streptozotocin- induced diabetic rats [72]. Thus, grape seeds could prevent toxicity induced of lead by scavenging ROS and/or by induction of cellular antioxidant enzymes. This means that grape seeds could protect against the harmful effect of ROS through its antioxidant activity and by this mean it may be beneficial in protecting the organism against oxidative stress. This is confirmed by findings of Ahmed *et al.* [73] who showed that grape seed extract has antioxidant, anti-inflammatory and antitumor activities and to mediate resistance to free radicals.

CONCLUSION

In conclusion, Grape seed powder (GSPE) has natural antioxidants composed of various polyphenolic compounds generally believed to protect against reactive oxygen species (ROS)-mediated brain degenerative diseases. Intake of grape seeds exhibited protective effect against lead (Pb) toxicity, oxidative damage and neurological damage. Therefore, intake of whole grapes with its seeds or grape seeds in food supplement may be beneficial to prevent or protect against lead toxicity. We recommend eating grapes with their seeds and including the grape seeds in recommended doses in various food products especially in susceptible patients with degenerative neurological disorders. Also more research on Grape seed powder with various degenerative and immunological diseases and more research to explore the various mechanisms of its protective effects

ACKNOWLEDGEMENTS

The authors would like to thank Institute of Scientific Research and Revival of Islamic Heritage at Umm Al-Qura University (project # 43409040) for the financial support.

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