PROTECTIVE EFFECT OF PUMPKIN SEED OIL AGAINST LEAD ACETATE TOXICITY IN MALE MICE.

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Abstract:

The goal of the present work was to investigate the protective effect of pumpkin seed oil (PSO) against lead acetate toxicity in male mice. lead acetate (20 mg/kg b.w.) was administered orally to mice once a day for 30 successive days. Whereas, PSO was administered to the mice orally at 1.5 mL/kg b.w. once a day for 30 successive days concurrently with treatment of lead acetate. The studied parameters were relative organs weights, DNA damage evaluation using comet assay in liver and kidney cells and micronucleus test in bone marrow and biochemical assessment of the liver and kidney function. Results showed that, lead acetate caused DNA damage in tested cells and significant increase in the levels of biochemical measurements. In contrast, PSO administration plus lead acetate effectively alleviated DNA damage in tested cells and improved the biochemical alterations. It can be concluded that PSO may has a protective role against lead acetate toxicity in male mice.

Key words:

Pumpkin seed oil; lead acetate; DNA damage; Micronucleus; Comet assay; Biochemical measurements.

Introduction

In both developing and industrialized countries, environmental and occupational lead pollution is a common problem and may contribute to multi-organ toxicity in man and animals (**El-Tantawy, 2016; Mohammed *et al.*, 2017**).

Lead is a naturally occurring bluish-gray metal found in small amounts in the Earth's crust and can be found in all parts of our environment (**Gupta, 2007**). It is found in our food, water, air and soil. In addition, it is emitted by smelters and boilers that burn used motor oil and is frequently deposited in the soil, where it is taken up by crops (**Chiras, 2009**).

Lead is known as an enzymatic toxicant, neurotoxic, hemato and cardiovascular toxic, nephrotoxic, hepatotoxic, immunotoxic, carcinogenic, teratogenic and mutagenic (**Kiran *et al.*, 2009; Falck *et al.*, 2015**). Lead can readily be absorbed by intestine, lung and less commonly through the skin. Almost 90% of absorbed lead binds to albumin (**Gonick, 2011**). Through endocytosis and/or Erythrophagocytosis, it locates into different tissues and organs including liver and kidney where it exhibits oxidative damage on cells and tissue, and cellular organelles (**Reyes *et al.*, 2013; Kwon *et al.*, 2015**). Kidney is one of targeted site of lead toxicity for being major route of excretion from body and facilitates kidney damage via oxidative stress and lipid peroxidation (**Garçon *et al.*, 2007; El-Nekeety *et al.*, 2009**).

Pumpkin (*Cucurbita pepo*) is a leafy green vegetable; it belongs to the Cucurbitaceae family. Pumpkin seeds are considered as a suitable source of edible oil. Pumpkin seed oil (PSO) has many antioxidants and beneficial nutritional supplements. PSO contains high amounts of vitamin E and tocoferol (**Stevenson *et al.*, 2007**). It also contains considerable amounts of palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1) and linoleic (C 18:2) (**Kulaitiene *et al.*, 2007**). Moreover, it contains phenolic compounds such as tyrosol, vanillic acid, vanillin, ferulic acid, and luteolin (**Andjelkovic *et al.*, 2010**). In addition, it is rich in amino acids, phytosterols, β-carotenes and selenium (**Procida *et al.*, 2012**).

Therefore, the present work aimed to evaluate the deleterious effects of lead acetate on relative organs weights, DNA integrity in liver, kidney and femoral bone marrow; and biochemical measurements of male mice and the protective effects of PSO on these parameters.

Materials and Methods

Chemicals:

Lead acetate was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Pumpkin seed commercial oil (PSO) was purchased from EL Captin Company (Al Obour City, Cairo, Egypt). All other chemicals were of analytical grade and purchased from standard commercial suppliers.

Animals:

Male Swiss albino mice (26 ± 5 g) were purchased from Theodor Bilharz Research Institute, Giza, Egypt, at 10–12 weeks of age. Animals were housed in polypropylene cages (43cm × 30cm × 15cm, five mice per cage) with stainless steel covers in the Animal House of Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University. Animals were kept under controlled temperature (23±4 °C), 50–55% relative humidity and a photoperiod of 12 h light : 12 h dark cycle. Food and water were given *ad libitum*. Animals received human care in compliance with the guidelines of the Animal Care and Use Committee of the National Institutes of Health (NIH publication 86-23 revised 1985). Animals were allowed to adapt to their surrounding environment for 2 weeks prior to the start of the experiments.

Experimental design:

Forty animals were randomly divided into four groups (n = 10) and orally administered for 30 successive days with lead acetate and/or PSO.

Experimental groups were as follows: Control group: animals were orally administered with saline. PSO group: animals were orally administered with PSO at dose of 1.5 mL/kg b.w. **(Ali and Abdelzaher, 2017)**. Lead acetate group: animals were orally administered with lead acetate at dose of 20 mg/kg b.w. **(Aldahmash and El-Nagar, 2016)**. PSO plus lead group: animals were orally administered with 1.5 mL/kg b.w. PSO concurrently with lead acetate (20 mg/kg b.w.).

Relative organs weights:

At the termination of the experiments, internal organs such as liver and kidney were dissected out, trimmed of excess fat and weighted. The organs weight was presented as relative organ weight as follows:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Final body weight (g)}} \times 100$$

Evaluation of DNA damage:

Comet assay in liver and kidney cells:

The comet assay was performed in liver and kidney cells according to **Bandyopadhyaya *et al.* (2008).** Briefly, 50 µL of cell suspension was mixed with 100 µL of 1 % low melting point (LMP) agarose and added to fully frosted slides coated with 80 µL of 1 % normal melting point (NMP) agarose. The cells were then incubated in a lysis solution (2.5 mol L^{-1}NaCl, 100 mmol L^{-1} EDTA, 10 mmol L^{-1}Tris-HCL, 1 % Triton X-100, pH 10) at 4 °C for at least 2 h, at which the slides were placed into an alkaline solution (300 mmol L^{-1}NaOH, 1 mmol L^{-1} EDTA, pH 13) at 4 °C for 20 min so as to allow DNA unwinding, and electrophoresed at 25 V (300 mA) for 20 min. Finally, the slides were neutralized in a 400 mmol L^{-1}Tris buffer (pH 7.5) for 15 min and stained with ethidium bromide (5 µg mL^{-1}). Images of 50 randomly selected nuclei per experimental group were captured using a fluorescence microscope (Eclipse 800, Nikon, Tokyo, Japan) and analyzed with image analysis software (Comet Assay IV, Perceptive Instruments, Suffolk, UK). Scored parameters included tail length, DNA percentage in tail and Olive tail moment (OTM).Tail length is the maximum distance that the damaged DNA migrates from the centre of the cell nucleus. DNA Percentage in tail is the DNA content that migrates from the nucleus into the comet tail. OTM is the product of the tail length and percentage DNA, which gives a more integrated measurement of overall DNA damage in the cell.

Bone marrow micronucleus assay:

The micronucleus (MN) test was carried out in mice femoral bone marrow cells according to **Chauhan *et al.* (2000)**. Numbers of normochromatic, polychromatic erythrocytes and micronuclei were evaluated in control and treated groups. For micronuclei evaluation, 2000 polychromatic erythrocytes were scored per animal. Both normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs) were scored in 500 erythrocytes for determination of the PCEs: NCEs ratio according to the OECD No. 474 guideline of mammalian erythrocyte micronucleus test for chemicals testing (**OECD, 1997**).

Biochemical analysis:

Liver function investigation:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by using kits obtained from Biodiagnostic (Egypt) according to the method of **Reitman and Frankel (1957).** Serum alkaline phosphatase (ALP) was determined by using kits obtained from Biodiagnostic (Egypt) according to the method of **Belfield and Goldberg (1971).**

Kidney function investigation:

Serum creatinine, urea and uric acid were assayed spectrophotometrically using kits provided from Biodiagnostic Co. (Egypt).

Statistical analysis:

Statistical analyses were performed with SPSS 16 software. Experimental data were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range tests was used to determine the significant differences between means. All values expressed as mean ± SD and the significance level was set at $P \leq 0.05$.

Results and discussion

Relative organs weights:

The relative weight of liver and kidney of the male mice treated with lead acetate and/or PSO are summarized in **Table 1**. Oral administration of lead acetate to male mice at a dose of 20 mg/kg b.w. for 30 consecutive days induced a significant loss ($p \leq 0.05$) in the relative weights of liver (3.34±0.04) and kidney (1.56±0.03) as compared to control (3.39±0.05 and 1.62±0.03), respectively. In contrast, oral administration of PSO plus lead acetate produced a significant increase ($P \leq 0.05$) in the relative weights of liver (3.38±0.02) and kidney (1.61±0.02) compared with lead acetate treated male mice.

These results are in coincidence with the findings of **Abdel-Moniem *et al.* (2011)** they found that exposure to lead acetate (20 mg/kg) reduced the body and kidney weight of male rats. **Balubaid (2011)** demonstrated that pumpkin extract ameliorated the highly significant decrease in the infant body weights of mice treated with haloperidol drug. **Hashemi (2013)** reported that pretreatment with PSO increased the weight of testes in sodium valproate's treated rats. **Al-Masri (2015)** revealed that PSO produced a significant protection against lead induced reduction in body weight of male rats. In addition, **Eissa *et al.* (2018)** found that oral administration of PSO for 28 successive days either before, with or after treatment of bisphenol-A significantly elevated the relative weights of liver and testes compared with the bisphenol-A treated male mice.

The protective role of PSO against the adverse effects of lead acetate on relative organs weights could be due to its content of various bioactive compounds such as magnesium, phosphorus, manganese, copper, iron and zinc which are vital in growth (**Alan, 2006**).

Table (1): Relative organs weights of treated male mice with lead acetate and/or pumpkin seed oil (PSO) for 30 consecutive days.

Groups	Relative organs weights (g)	
	Liver	Kidney
Control	3.39±0.05^{a}	1.62±0.03^{a}
PSO (1.5 mL/kg)	3.41±0.02^{a}	1.63±0.02^{a}
Lead acetate (20 mg/kg)	3.34±0.04^{c}	1.56±0.03^{c}
PSO plus lead acetate	3.38±0.02^{ab}	1.61±0.02^{ab}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

DNA damage evaluation:

In the present study, DNA damage was evaluated by comet assay in liver and kidney cells and by micronucleus assay in femoral bone marrow cells. The alkaline comet assay was used as a quantitative and visual method to measure DNA strand breaks that can be applied directly to cells (**Gedik *et al.*, 1998**). DNA damage is an important initial event in carcinogensis. DNA lesions can change in nucleotide sequence, causing mutagenesis and other cellular mechanisms (**Lord and Ashworth 2012**). Results of comet assay showed that the exposure to lead acetate caused an increase in DNA strand breaks leading to greater DNA migration out of the nucleus into the tail of the comet in mice liver and kidney cells.

Comet assay in liver cells:

The comet assay results of liver cells are illustrated in **Table 2**. Lead acetate induced significant increase ($P \leq 0.05$) in the mean values of tailed cells (30.77±0.59), tail length (18.16±0.47), percentage of tail DNA (21.34±0.54) and olive tail moment (3.88±0.17) in liver cells as compared to control (12.86±0.54, 8.22±0.96, 13.32±0.24 and 1.09±0.03), respectively. In contrary, administration of male mice with PSO plus lead acetate diminished significantly ($P \leq 0.05$) the increase in the mean values of comet parameters induced by lead acetate in liver cells.

Table (2): Comet assay parameters in liver cells of treated male mice with lead acetate and/or pumpkin seed oil (PSO) for 30 consecutive days.

Groups	Intact cells (%)	Tailed cells (%)	Tail length (µm)	Tail DNA (%)	Olive tail moment (µm)
Control	87.14±0.54^{b}	12.86±0.54b	8.22±0.96^{c}	13.32±0.24^{c}	1.09±0.03^{c}
PSO (1.5 mL/kg)	88.16±0.26^{a}	11.84±0.26^{c}	8.58±0.25^{c}	12.79±0.14^{c}	1.10±0.02^{c}
Lead acetate (20 mg/kg)	69.23±0.59^{c}	30.77±0.59^{a}	18.16±0.47^{a}	21.34±0.54^{a}	3.88±0.17^{a}
PSO plus lead acetate	86.57±0.55^{b}	13.43±0.55^{b}	9.40±0.41^{b}	14.43±0.33^{b}	1.35±0.09^{b}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

Comet assay in kidney cells:

As shown in **Table 3,** a significant increase ($P \leq 0.05$) in the values of tailed cell percentage (32.83±0.64), tail length (17.45±0.48), tail DNA percentage (19.22±0.66) and olive tail moment (3.35±0.19) was observed in kidney cells of male mice treated with lead acetate compared to control (11.67±0.25, 8.35±0.34, 12.29±0.35 and 1.03±0.04), respectively. On the other hand, administration of male mice with PSO plus lead acetate inhibited significantly ($P \leq 0.05$) the increase in the mean values of tailed

cells percentage (14.85±0.21), tail length (12.31±0.58), DNA tail percentage (14.78±0.51) and olive tail moment (1.81±0.52) in kidney cells compared to those caused by lead acetate.

Micronucleus test:

Micronuclei (MN) are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments of intact whole chromosome lagging behind at the anaphase stage of cell division. They formed due to damaging effects on the spindle, centromere/centromere-associated proteins, or acentric chromosomes due to the clastogenic action and can be recognized in the cytoplasm of immature polychromatic erythrocytes (**Naik and Vijayalaxmi, 2009**).

According to the data in **Table 4**, oral administration of lead acetate significantly increased ($P \leq 0.05$) the mean values of MNPCEs (55.50±5.19) compared to control (9.75±1.71). In contrast, oral administration of PSO plus lead acetate decreased significantly ($P \leq 0.05$) the mean value of MNPCEs (22.25±4.99) in bone marrow cells. In addition, cytotoxicity evaluation of bone marrow erythrocytes showed that lead acetate caused significant reduction ($P \leq 0.05$) in the ratio of PCEs/NCEs (1.32±0.03) as compared to control (2.57±0.08). While, PSO has protection against lead acetate cytotoxicity. This protection was appeared in the elevation of the PCEs/NCEs ratio (2.17±0.07) as compared with those of lead acetate treated group.

Table (3): Comet assay parameters in kidney cells of treated male mice with lead acetate and/or pumpkin seed oil (PSO) for 30 consecutive days.

Groups	Intact cells (%)	Tailed cells (%)	Tail length (µm)	Tail DNA (%)	Olive tail moment (µm)
Control	88.33±0.25[a]	11.67±0.25[c]	8.35±0.34[c]	12.29±0.35[c]	1.03±0.04[c]
PSO (1.5 mL/kg)	88.32±0.38[a]	11.68±0.38[c]	8.82±0.35[c]	12.50±0.29[c]	1.10±0.07[c]
Lead acetate (20 mg/kg)	67.17±0.64[c]	32.83±0.64[a]	17.45±0.48[a]	19.22±0.66[a]	3.35±0.19[a]
PSO plus lead acetate	85.15±0.21[b]	14.85±0.21[b]	12.31±0.58[b]	14.78±0.51[b]	1.81±0.52[b]

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

Our results indicated that the lead acetate oral administration for 30 successive days showed DNA damage activity in tested cells. These results came to a consensus with **Abdel-Moniem *et al.* (2010)** they found that exposure to lead acetate (20 mg/kg) induced DNA fragmentation in male rats. **Azoz and Raafat (2012)** demonstrated that

treatment of rats with lead acetate at a dose of 0.5 g/100 ml drinking water for 2 months caused a significant increases in the percent of multinucleated polychromatic erythrocytes (MNPCEs).

Table (4): Frequencies of micronucleated polychromatic erythrocytes and polychromatic/normochromatic ratio in bone marrow cells of lead acetate and/or pumpkin seed oil (PSO) treated male mice for 30 consecutive days.

Groups	MNPCEs	PCEs	NCEs	PCEs/NCEs ratio
Control	9.75 ± 1.71^{c}	1439.25 ± 13.59^{a}	560.75 ± 13.59^{c}	2.57 ± 0.08^{a}
PSO (1.5 mL/kg)	8.50 ± 1.73^{c}	1460.50 ± 19.12^{a}	539.50 ± 19.12^{c}	2.61 ± 0.13^{a}
Lead acetate (20 mg/kg)	55.50 ± 5.19^{a}	1136.50 ± 13.30^{c}	863.50 ± 13.30^{a}	1.32 ± 0.03^{c}
PSO plus lead acetate	22.25 ± 4.99^{b}	1370.00 ± 14.25^{b}	630.00 ± 14.25^{b}	2.17 ± 0.07^{b}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$). MNPCEs: Micronucleated Polychromatic erythrocytes PCEs: Ploychromatic erythrocitesNCEs: Normochromatic erythrocytes.

The mechanism of lead acetate genotoxicity might work through the induction of oxidative stress. Oxidative DNA damage by free radicals is a common mechanism of lead toxicity (**Valko *et al.*, 2006; Sharma *et al.*, 2014**). Reactive oxygen species (ROS) damage DNA either directly (e.g. via oxidation of bases) or indirectly via interactions with biologically relevant molecules, e.g. via oxidation of fatty acids in cell membranes (**Knasmuller *et al.*, 2008**). Additionally, they destroy also repair enzymes, DNA polymerases and microtubule (**Halliwell, 2007**).

Furthermore, it was shown in *in vitro* studies with cultured mammalian cells that lead binds to zinc-finger proteins including histones and protamines which are involved in the protection of DNA (**Koedrith and Seo, 2011**). In addition, several studies were published which indicate that the metal interferes with DNA-repair mechanisms (**Hartwig *et al.*, 1990; Hartwig, 1994**).

Another relevant mechanism which may lead to DNA damage by lead acetate is an increase of cell proliferation. Increased proliferative lesions were found in experiments with rats and mice, i.e. in the proximal tubular epithelium in the kidneys after i.p. administration of lead acetate (**National Toxicology Program, 2003**). Also in experiments with hepatectomized rats, increased mitogenic activity was observed in the liver tissue when the animals were treated with lead nitrate (**National Toxicology Program, 2003)**. In none of these experiments DNA damage was monitored in parallel in the respective tissues.

Regarding PSO treatment that alleviated the lead acetate induction of DNA damage in tested cells, these results came to a consensus with **Elfiky *et al.* (2012)** they found that oral administration of PSO for ten consecutive days either before or after treatment of azathioprine was effective in the reduction of DNA fragmentation and MNPCEs frequencies, meanwhile, improved the PCEs/NCEs ratio. **Ali and Abdelzaher (2017)** found that treatment with 1.5 mL/kg/day PSO for 5 weeks to sodium nitrite's treated rats significantly alleviated DNA damage and chromosomal abnormalities. In addition, **Abou-Zeid *et al.* (2018)** demonstrated that PSO was effective in reducing the oxidative stress, apoptosis and the percentage of DNA fragmentation in both liver and kidney of emamectin-treated mice. **Eissa *et al.* (2018)** revealed that PSO administration to male mice for 28 successive days either before, with or after treatment of bisphenol-A alleviated significantly the induction of DNA damage in liver, testes and femoral bone marrow cells as a result to bisphenol-A exposure.

Therefore, it might suggest that the protecting effects of PSO against lead acetate genotoxicity could be due to their content of varied bioactive compounds such as vitamin E and phenolic compounds (**Eissa *et al.*, 2018**). Phenolic compounds have antioxidant, anti-mutagenic, anticarcinogenic and anti-inflammatory properties that might be beneficial in protecting the genome stability (**Xie *et al.*, 2013**). In the same manner, polyphenolics ameliorate cell injury and protect oxidant induced DNA from lesion by reducing the free radical mediated oxidative damage (**Urquiaga and Leighton, 2000**). Furthermore, vitamin E has an antioxidant role and can contribute to the cells protection against the free radicals deleterious property (**Santana *et al.*, 2015**).

In addition to the presence of phenolic compounds and vitamin E, selenium in the composition of PSO incorporated into the structure of the glutathione peroxidase enzyme, and directly related to its antioxidant activity (**Al- Zuhair *et al.*, 1997; Fruhwirth and Hermetter, 2007; Zadak *et al.*, 2009**). Moreover, PSO is rich in β-carotene that is a potent free radical quencher, singlet scavenger, and lipid antioxidant (**Fruhwirth and Hermetter, 2007**). Also, the phytosterols in the structure of PSO are reported to have antioxidant effects (**Fruhwirth and Hermetter, 2007; Nyam *et al.*, 2009**).

Biochemical Analysis:

Liver function investigation:

According to data in **Table 5**, a significant increase ($P \leq 0.05$) in the mean values of ALT (43.37±2.61), AST (49.66±2.08) and ALP (128.00±5.61) was observed following the treatment with lead acetate for 30 successive days compared with control (25.33±1.15, 29.67±1.52 and 60.00±2.65), respectively. While, oral administration of PSO plus lead acetate produced a significant amelioration ($P \leq 0.05$) in the levels of ALT (31.00±1.16), AST (34.31±1.43) and ALP (82.33±4.16) compared with lead acetate treated alone.

Table (5): Changes in the levels of ALT, AST and ALP in serum of male mice treated with lead acetate and/or pumpkin seed oil (PSO) for 30 consecutive days.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	25.33±1.15^{c}	29.67±1.52^{c}	60.00±2.65^{c}
PSO (1.5 mL/kg)	24.29±1.52^{c}	28.33±1.56^{c}	54.00±3.00^{d}
Lead acetate (20 mg/kg)	43.37±2.61^{a}	49.66±2.08^{a}	128.00±5.61^{a}
PSO plus lead acetate	31.00±1.16^{b}	34.31±1.43^{b}	82.33±4.16^{b}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

kidney function investigation:

As illustrated in **Table 6**, oral administration of lead acetate caused a significant rise in the levels of creatinine (2.43±0.25), urea (52.00±4.00) and uric acid (7.73±0.42) as compared to control group (0.93±0.15, 21.66±2.31 and 3.66±0.21), respectively. On the other hand, administration of male mice with PSO plus lead acetate diminished significantly ($P \leq 0.05$) the increase in the levels of creatinine (1.42±0.15), urea (31.33±2.52) and uric acid (4.76±0.35) by lead acetate treatment.

Table (6): Changes in the levels of creatinine, urea and uric acid in serum of male mice treated with lead acetate and/or pumpkin seed oil (PSO) for 30 consecutive days.

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
Control	0.93±0.15^{c}	21.66±2.31^{c}	3.66±0.21^{c}
PSO (1.5 mL/kg)	0.83±0.05^{c}	20.62±2.08^{c}	3.36±0.25^{c}
Lead acetate (20 mg/kg)	2.43±0.25^{a}	52.00±4.00^{a}	7.73±0.42^{a}
PSO plus lead acetate	1.42±0.15^{b}	31.33±2.52^{b}	4.76±0.35^{b}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

The biochemical alterations induced by lead acetate are in agreement with the findings of **Suleman *et al.* (2011)** they found that ALT, creatinine and uric acid levels were increased significantly in broiler chickens exposed to lead acetate. **Abdel-Moniem *et al.* (2011)** reported that treatment of rats with lead acetate (20 mg/kg) significantly elevated the level of uric acid, blood nitrogen urea and creatinine as markers of kidney function. **Azoz and Raafat (2012)** revealed that treatment of rats with lead acetate at a dose of 0.5 g/100 ml drinking water for 2 months caused a significant increases in serum AST, ALT and ALP.

Serum levels of liver enzymes such as AST and ALT commonly used as an indicator of liver damage or disease (**Kodai *et al.*, 2007; Campo *et al.*, 2008; Liu *et al.*, 2013**). Of the two, ALT thought to be more specific for hepatic injury because it is present mainly in liver cytosole and in low concentration elsewhere (**Giboney, 2005**). When the liver hepatocytes damaged, these enzymes are released into the blood where the significant increase in AST and ALT activities indicates the damage to the cytosole and mitochondria (**Mathuria and Verma, 2008**). **Gaskill *et al,* (2005**) reported that the high plasma AST and ALT are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue.

The protective role of PSO are in agreement with those obtained by **Abou Seif *et al.* (2014)** they found that the pre-treatment with PSO successfully ameliorated the elevated activities of ALT and ALP by alcohol treatment. Moreover, **Al-Okbi *et al.* (2014)** reported that PSO administration with high fructose diet (HFD) resulted in significant reduction of ALT and AST activity.

The improvement in biochemical alterations alterations afforded by PSO may be attributed to their high content of the polyunsaturated fatty acids (PUFAs). Membrane incorporation of PUFAs may reduce cellular susceptibility to lipid peroxidation and alter membrane fluidity (**Best *et al.*, 2003; Eissa *et al.* 2018)**. Moreover, PSO is also rich in zinc which plays an important role in the structure of proteins and cell membranes and protect against damage (**Bataineh *et al.*, 2002**).

Conclusion:

It can be concluded that lead acetate has the potential to produce genotoxic effects and biochemical alterations in male mice. Accordingly, strict limitations on the use of this compound must be put. PSO appears to have abundant beneficial properties for applications in food. The content of compounds such as polyunsaturated fatty acids, essential amino acids, vitamin E, selenium and polyphenols makes PSO a supply to satisfy essential needs in human diet and health maintenance. Data illustrated that PSO can used as therapeutic agent to attenuate the deleterious effects of lead acetate. Subsequently, it can be categorized as edible oil with a high potential of antioxidant activity.

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التأثيرالوقائي لزيت بذور قرع العسل ضد سمية خلات الرصاص في ذكور الفئران

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أجري هذا العمل لتقييم التأثير الوقائي لزيت بذور قرع العسل ضد سمية خلات الرصاص في ذكور الفئران الصغيرة. تم تجريع الفئران بخلات الرصاص عن طريق الفم بجرعة ٢٠ مجم/كجم من وزن الجسم مرة واحدة يوميا لمدة ٣٠ يوما متتالية. بينما تم تجريع الفئران بزيت بذور قرع العسل (١.٥ مل/كجم من وزن الجسم) عن طريق الفم بالتزامن مع المعاملة بخلات الرصاص. القياسات التي تم دراستها كانت وزن الأعضاء النسبي، تقييم تلف الحامض النووى الديوكسى ريبوزى (دنا) باستخدام تقنية قياس المذنب في خلايا الكبد والكلية واختبار الأنوية الصغيرة في نخاع العظم، التقييم البيوكيميائي لوظائف الكبد والكلية. أوضحت النتائج أن خلات الرصاص أحدثت تلف في الحامض النووي في الخلايا المختبرة، وزيادة معنوية في مستويات القياسات البيوكيميائية. في المقابل أثبتت الدراسة ان تجريع الفئران بزيت بذور قرع العسل بالتزامن مع المعاملة بخلات الرصاص قد خفف من تلف الحامض النووي في الخلايا المختبرة وحسن التغيرات البيوكيميائية. يمكن أن نخلص الي أن زيت بذور قرع العسل قد يكون له دور وقائي ضد سمية خلات الرصاص في ذكور الفئران الصغيرة.

مفاتيح الكلمات:

زيت بذور قرع العسل، خلات الرصاص، تلف الحامض النووي، الأنوية الصغيرة، تقنية قياس المذنب، القياسات البيوكيميائية.