Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-021-15240-3

RESEARCH ARTICLE

Protective effects of olive leaf extract against reproductive toxicity of the lead acetate in rats

Harith Abdulrhman Ahmed[1] · Huda Abdullah Ali[1] · Thulfiqar Fawwaz Mutar[2]

Received: 10 February 2021 / Accepted: 24 June 2021

Abstract

Lead acetate (PbAc) is one of the toxic metals in the environment which causes many effects on different organs of the body. And due to the importance of the olive tree, with its healthy and protective elements against many diseases, the leaf extract of this tree was chosen in our study. Therefore, the aim of this study was to investigate the role of olive leaf (*Olea europea* L.) extract (OLE) against PbAc-induced sperm toxicity, sex hormone changes, oxidative stress, and histopathological changes in rats. Twenty male Wistar rats were divided into four groups (group 1, as control; group 2, OLE; group 3, PbAc; group 4, PbAc+OLE). In the PbAc group, the body weight, testis and epididymis weights, sexual hormones, sperm characteristics, GR, GPx, GST, GSH, SOD, and CAT were significantly decreased, and the sperm abnormality and TBARS level were significant increase when compared with control and OLE groups. Also, numerous damages to testicular tissue were observed in the PbAc group when compared to the control group, while the treatment with OLE in the fourth group led to improvement of sex hormones, semen characteristics, oxidative stress, and testicular tissue damage caused by PbAc. It can be concluded that OLE has a protective and ameliorative effects against PbAc-induced oxidative stress, apoptosis and alterations in testicular tissue, and sperm quality in rats.

Keywords Lead acetate · Environmental toxins · Olive leaf extract · Antioxidants · Reproductive toxicity

Introduction

Lead (Pb) has many applications, and we can be exposed to it through a variety of sources, including factories, car fuel (gasoline), dyes, foods preserved in metal cans, water pipes, building materials, and some cosmetics, and some children's toys (Ashraf et al. 2015; Fouad et al. 2020). In spite of the great efforts to reduce exposure to lead, it still poses a threat and danger to human health, especially for children because they are affected by it more than adults. Lead may enter the body through the skin, mouth, and nose which cause toxic effects to many internal organs, and thus it will lead to acute or chronic toxicity depending on the period of exposure (Meyer et al. 2008; Rerknimitr et al. 2019). Lead acetate (PbAc) (Pb (CH3COO) 2) is a toxic white crystalline chemical compound with a slightly sweet taste, it is soluble in water and glycerin, and poorly soluble in alcohol (Zhang et al. 2013a).

Exposure to large amounts of Pb causes toxicity and damage to many organs inside the body, such as heart, liver, kidneys, brain, and testes, which may increase the death rate, and this explains its severity and toxicity (Patra et al. 2001; Toscano and Guilarte 2005; Zhang et al. 2013b; Abdelhamid et al. 2020). Oxidative stress resulting from exposure to Pb include an imbalance between generation and elimination of reactive oxygen species (ROS) and lipid peroxidation in tissues and cellular components causing damage to membranes, deoxyribonucleic acid (DNA), and proteins (Shan et al. 2009; Patra et al. 2011). The influences on the male reproductive system are one of the critical signs of Pb poisoning, observed that exposure to Pb in industrial cities reduces fertility and reproductive efficiency in men by lowering the count and movement of sperm and an increase deformities (Zhang et al. 2014). Similarly, exposure to high levels of lead induces effects on reproductive system

Responsible Editor: Mohamed M. Abdel-Daim

✉ Thulfiqar Fawwaz Mutar
thulfiqar.fawwaz@uoa.edu.iq

Harith Abdulrhman Ahmed
harithalnajjar81@gmail.com

Huda Abdullah Ali
hudaali85@gmail.com

[1] Environmental Studies, Faculty of Education For Pure Sciences, Al-Anbar University, Ramadi, Iraq

[2] Department of Medical Laboratory Techniques, Al-maarif University College, Al-Anbar, Ramadi, Iraq

Published online: 03 July 2021

activity, defect in sex steroids production, and testicular tissue damage in male rats (Kandeil et al. 2020).

There has been a wide interest worldwide in the medicinal and aromatic plants that provide much beneficial material for the pharmaceutical industries (Mutar et al. 2019; Abd et al. 2020; Almakhatreh et al. 2020; Tousson et al. 2020). The main use of the olive tree *Olea europea* L. for a long period of centuries was based on the oil of its fruits because of its great economic and nutritional importance. However, attention begins in recent decades for the leaves of the olive tree for their high medicinal importance and their use as antioxidants, antibacterial, anti-inflammation, and in the protection against many diseases (Guo et al. 2013; Sofuoglu et al. 2013; Wei et al. 2018; Mao et al. 2019; Abugomaa and Elbadawy 2020).

Many studies have been conducted on olive leaf extract (OLE) due to their antioxidant and anti-inflammatory properties; OLE may help prevent obesity and the associated immune inflammation by reducing weight gain and adipose tissues, improving metabolic functions, and suppressing the induction of inflammatory mediators (Vezza et al. 2019; De Cicco et al. 2020). In the same way, OLE has been correlated with reducing the risk of coronary heart disease to support cardiovascular health as it helps reduce harmful cholesterol levels, maintains blood pressure levels, and has positive effects on atherosclerosis (Lockyer et al. 2012; Varmaghany et al. 2013; Efentakis et al. 2015; Olmez et al. 2015).

Olive leaf extract (OLE) has neuroprotective effects against neurodegenerative diseases and neurotoxicity resulting from oxidative stress, also protection against stroke and brain injury (Castejón et al. 2020; Chiaino et al. 2020). Furthermore, OLE has a protective role against the hepatotoxicity and DNA damage caused by tetrachloride carbon by reducing hepatic damage and fibrosis and improving liver tissues (Taamalli et al. 2020). Moreover, because many health problems are related to diabetes, especially poor fertility in male, olive leaves with their hypoglycemic effect and antioxidant activity have been used to control blood sugar levels; therefore, it will possibly lead to improved testicular damage, stimulate the count and movement of sperm, and regulate hormonal imbalances (Gholami and Zahedi 2019; Soliman et al. 2019). Therefore, the purpose of this study was to investigate the protective role of olive leaf extract (OLE) against lead acetate (PbAc)-induced reproductive toxicity, oxidative stress, sexual hormone imbalance, sperm damage, and histopathological alterations in male rats.

Materials and methods

Tested compounds and doses

Lead acetate (PbAc) was purchased from Sigma Chemical Company (Saint Louis, USA). The doses are based on the previous study by Mokhtari and Zanboori (2011).

Olive leaf extract

Olea europea L. leaves were obtained from the Anbar Governorate, Iraqi. After collecting the leaves, washed well and dried with air, then placed in the shade for 3 days. After that, were dried at 65°C in the air circulating oven overnight until completely dry, then were well crushed and the powder was obtained. Twenty grams of dried powder was put in 500 mL water and mixed in an electric mixer for 10 min and poured in 1-L conical flasks placed in an incubator shaker for 24 h at 37 °C. After extraction, insoluble material was removed using Whatman No. 1 filter paper. The filtrates were then concentrated at 50 °C to yield a dark brown solid extract, with references to the powdered samples; the yields of the OLE were 3.59 g, and extracts were stored in the refrigerator for rat supplementation (Lee-Huang et al. 2003; Al-Attar and Abu Zeid 2013; Wulandari et al. 2016).

Animals and experimental groups

Twenty adult male albino rats with an average weight of 165 ±10g, from 3 to 5 months of age, were obtained from the animal house, College of Veterinary Medicine, University of Baghdad, Iraq. They were assigned to 4 groups and housed in universal galvanized wire cages at room temperature (22–25°C), and 50-–0% humidity, and in a photoperiod of 12h/day and acclimated for 2 weeks before the experiment. Animals were provided with a balanced commercial diet (20% protein, 9% fat, 5% fiber, and 53% starch) and water ad libitum.

Rats were divided into four equal groups (n = 5) and the doses of OLE and PbAc prepared by dissolving in normal saline and given orally by gavages approximately at the same time each morning for 35 consecutive days as follows: group 1, control in which healthy untreated rats; group 2, was orally administered with a dose (150 mg/kg BW/day) of OLE according to Sarbishegi et al. (2018); group 3, was orally administered with a dose (100 mg/kg BW/day) of (PbAc) according to Mokhtari and Zanboori (2011); and group 4, rats were orally administered with PbAc (100 mg/kg BW/day) and then with OLE (150 mg/kg BW/day).

Blood sample collection

At the end of the 30th day of the experimental period, rats of each group were anesthetized with sodium pentobarbital and sacrificed. Blood samples were collected by inferior vena cava from rats into non-heparinized glass tubes for coagulation and serum formation, and blood was sitting for 30 min at room temperature to clot and then centrifuged for 10 min at 4000 rpm; blood serum was separated and kept in a clean stopper vial at −20°C until analysis.

Tissue preparations and sperm morphometric analysis

At the end of the experimental period, the rats were weighed before sacrificed and then anesthetized with sodium pentobarbital (IP) injection. Testes and epididymis were carefully removed, weighed, and washed with saline (0.9%). Testes were minced and homogenized (10% w/v) in ice-cold sucrose buffer (0.25 M) containing in a Potter-Elvehjem type homogenizer and then centrifuged at 10,000 ×g for 20 min at 4°C; and use the supernatant for the analysis of the oxidative stress and antioxidant parameters. Parts of the selected organs were kept in formalin (10%) for histological studies.

Cauda epididymis was carefully cut into small pieces, transferred into Petri dishes containing 5 mL of calcium and magnesium-free Hank's solution at 37°C, and flushed with 3 mL of pre-warmed nutrition medium (M199). The sperm cells were placed for 15 min at 37 °C in the incubator and then the suspension was stirred, and one drop was placed on a warmed microscope slide and examined with an Olympus microscope (Olympus, Tokyo, Japan); at least 10 microscopic fields were observed at 400 × magnification, and the sperm count, abnormality, fast motion, medium motion, slow motion, immotile, and sperm viability were recorded according to the method of Tousson et al. (2018) and Eldaim et al. (2019) according to the sperm morphology criteria.

Body, testis, and epididymis weights

Testes and epididymis were weighed after carefully isolated from male rats and washed by saline solution and removed the connective tissues; the initial body weight (IBW) and final body weight (FBW) were registered, and then body weight gain (BWG) was calculated by the equation = Final weight – Initial weight.

Sexual hormones

Total testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels were measured by using the ADVIA Centaur XP system (two-site sandwich immunoassay using direct chemiluminometric technology; Vidas, France).

Oxidative stress and antioxidant activities

Thiobarbituric acid reactive substances (TBARS) were measured as described by Tappel and Zalkin (1959). One milliliter of testes homogenate was added to 2 mL of 7.5% trichloroacetic acid and mixed. The mixture was centrifuged at 1000 ×g for 10 min. Two milliliters of supernatant was added to 1 mL of 0.7% 2-thiobarbituric acid. After boiling for 10 min, the reactants were cooled and TBARS were measured at 532 ηm. Furthermore, the activities of glutathione reductase (GR), glutathione reduced (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) in the testes homogenates were measured using colorimetric kits (Biodiagnostic, Egypt).

Histopathological examination in testes

Parts of testis tissues were fixed in 10% neutral formalin solution for 2 days and embedded in paraffin wax and cut with a microtome for 5-μ thick sections. The sections were stained with Hematoxylin and Eosin (H & E) stains and photographed on the PC screen using a light microscope (Olympus, Tokyo, Japan) according toTousson et al. (2012, 2018).

Statistical analysis

Results were expressed as means ± SE. Statistical analysis for all experimental groups was compared using a one-way analysis of variance. The significant differences between mean values among the four groups were tested using Duncan's new multiple range test. Values of $p<0.05$ were considered statistically significant.

Results

Initial and final body weights, body weight gain, and organs weights

Table 1 shows that treatment with OLE in the second group (G2) did not cause any changes in rat's body weights or in testis and epididymis weights when compared with the weights of the control group (G1), whereas exposure to PbAc in the third group (G3) caused a significant decrease ($P< 0.05$) in the final body weight (FBW), body weight gain (BWG), testis, and epididymis weights compared to the control group (G1). On the other hand, the presence of OLE in the fourth group (G4) led to the protection of the body weights, and testis and epididymis weights from loss as a result of Pb poisoning.

Changes in sperm characteristics

As shown in Table 2, the control (G1) and OLE (G2) groups exhibit normal characteristics in each of the sperm count, fast motion, medium motion, slow motion, immotile, abnormality, and viability. While the presence of the PbAc in (G3) group causes a significant ($P< 0.05$) decrease in sperm characteristics and significant ($P< 0.05$) increase in sperm abnormality and immotile compared with (G1) and (G2) groups, the sperm characteristics showed amelioration after treatment of OLE with the PbAc (G4) group when compared with PbAc group (G3) (Table 2).

Table 1 The body and organ weights of male rats treated with olive leaf extract (OLE) and lead acetate (PbAc)

Groups	Control	OLE	PbAc	PbAc+OLE
Initial body weight (gm)	165 ±2.317[a]	167±2.423[a]	166 ±2.339[a]	164 ±1.315[a]
Final body weight (gm)	219 ±4.768[a]	217 ±1.803[a]	188 ±1.992[c]	205 ±4.650[b]
Body weight gain (gm/35 days)	54 ±4.05[a]	50 ±3.43[a]	22 ±3.36[c]	42 ±5.39[b]
Testis weight (gm)	4.89 ± 0.34[a]	4.73 ± 0.21[a]	3.32 ± 0.16[c]	3.91 ± 0.13[b]
Epididymis (gm)	1.3±0.17[a]	1.2±0.09[a]	0.6±0.14[c]	0.9±0.13[b]

Data are expressed as mean ± SE of 5 observations; the groups with different superscript letters are significantly different at $p<0.05$

Abbreviations: *OLE*, olive leaf extract; *PbAc*, lead acetate

Changes in sexual hormones

The variations of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in serum of male rats for all the studied groups are represented in Figure 1. The normal levels of the sex hormones were observed in the control (G1) and OLE (G2) groups, while the rat's group treated with PbAc (G3) showed a significant ($p<0.05$) decrease in levels of testosterone, FSH, and LH compared to the (G1) and (G2) groups. In contrast, the treated rats with PbAc+ OLE in the fourth group (G4) showed a significant ($p<0.05$) increase and improvement in sex hormone level when compared to the PbAc group (G3).

Oxidative stress and antioxidant activity

As shown in Table 3, there was a significant ($p<0.05$) increase in TBARS levels, while a significant decrease in GR, GPx, GST, GSH, SOD, and CAT activity was detected in testis tissues in PbAc (G3) as compared with the control (G1) and OLE (G2) groups. In contrast, the treatment of rats with PbAc+OLE (G4) showed ameliorative effects in the activities of oxidative stress and antioxidant parameters when compared to PbAc group (G3).

Olive leaf extract improved testis tissue against PbAc-induced histopathological changes

The histopathological changes of testes sections in male rats for all studied groups are demonstrated in Figure 2A–D. Testes sections in control (G1) and OLE (G2) groups showed the normal structure of the seminiferous tubules, interstitial tissues (Leyding cells), spermatocytes, and spermatogenesis (Figure 2A, B). However, the light microscopic examination of the testis section in the PbAc group (G3) revealed morphological disorganization of the testis structure with atrophy in the seminiferous tubule, degeneration of germinal epithelium, and incomplete spermatogenesis (Figure 2C). The treated rats with OLE +PbAc (G4) showed a regular and normal distribution of the spermatogenic cells in the seminiferous tubules with an increase of sperms. Also, the lumen of the seminiferous tubules was fully packed with sperms due to the role of OLE in alleviating the adverse effect of PbAc (Figure 2D).

Discussion

Lead acetate (PbAc) is known as an environmental toxic mineral that affects many organs in the body and this is related to physiological and morphological changes, as some abnormal

Table 2 Changes in sperm count, fast motion, medium motion, slow motion, immotile, sperm abnormality, and sperm viability in sperms of male albino rats administrated with olive leaf extract (OLE) and lead acetate (PbAc)

Groups	Control	OLE	PbAc	PbAc+OLE
Sperm count (10^6/mL)	135 ± 4.82[b]	143 ± 4.04[a]	117 ± 3.56[d]	130 ± 2.00[c]
Fast motion (%)	38.80 ± 0.66[b]	43.83 ± 0.69[a]	18.50 ± 0.81[d]	32.50 ± 21.38[c]
Medium motion (%)	26.20 ± 0.49[a]	29.85 ± 0.58[a]	19.10 ± 0.93[c]	22.70 ± 21.55[b]
Slow motion (%)	32.80 ± 1.07[b]	36.75 ± 0.99[a]	28.10 ± 0.68[c]	29.20 ± 19.43[c]
Immotile (%)	28.20 ± 1.59[c]	23.87 ± 0.50[d]	35.30 ± 0.54[a]	31.20 ± 21.47[b]
Sperm abnormality (%)	9.30 ± 0.37[c]	8.97 ± 0.14[c]	17.50 ± 0.22[a]	11.20 ± 0.33[b]
Sperm viability (%)	69.30 ± 0.74[b]	73.46 ± 2.13[a]	49.40 ± 0.40[d]	58.50 ± 12.91[c]

Data are expressed as mean ± SE of 5 observations; the groups with different superscript letters are significantly different at $p<0.05$

Abbreviations: *OLE*, olive leaf extract; *PbAc*, lead acetate

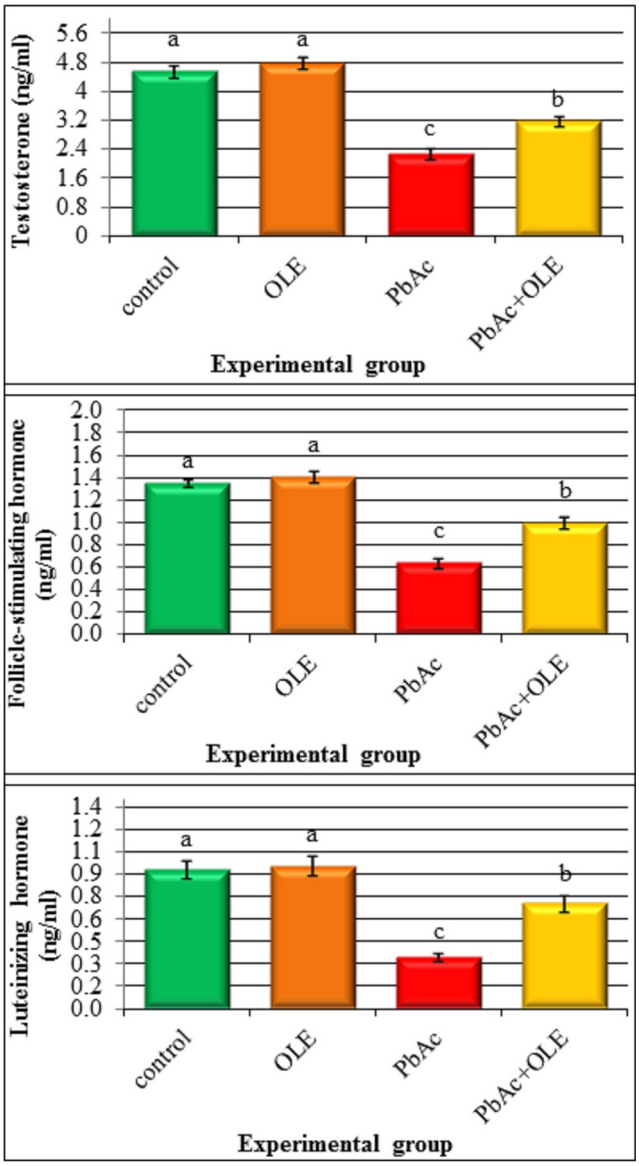

Fig. 1 Mean values ± SE of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in all studied groups. Abbreviations: OLE, olive leaf extract; PbAc, lead acetate

symptoms such as lethargy, loss of appetite, and diarrhea were observed in rats treated with lead acetate, which in turn led to changes in body weight, testicle weight, and epididymis. Our results are in agreement with some studies that indicate that PbAc may impair normal intestinal absorption of food or the occurrence of intestinal cramps and diarrhea associated with lead poisoning (Ibrahim et al. 2012), or it may cause an imbalance in vitamin D and glucose metabolism as well as inhibit some essential enzymes in the production of proteins and nucleic acids, causing a loss in body weights (Rahman et al. 2018). One of the main factors that could explain lead's negative effects is oxidative stress. Lead may stimulate lipid peroxidation and reactive oxygen species in cell membranes that impede growth due to their interference with metabolic processes (Dkhil et al. 2016), and it may also inhibit the activity of antioxidant enzymes and damage nucleic acids in cell membranes that may affect the building process and thus weight loss (Ponce-Canchihuamán et al. 2010).

The current results revealed a decrease of the testis and epididymis weights caused by PbAc in the third group (G3) when compared to control and OLE groups, and this finding is in accordance with Hari Priya and Reddy (2012) who found that PbAc-induced marked effects on the reproductive system include damage and decrease in the testis and epididymis weight. In contrast, these results showed that treated rats with olive leaf (*Olea europea* L.) extract (OLE) in the fourth group (G4) did not cause any side effects such as diarrhea and loss of appetite; also, OLE reduced the inflammatory toxicity and improved the weights of the body and reproductive organs in male rats that have been decreased by the effect of PbAc, which agree with Cazarin et al. (2015), who stated that OLE has the ability to improve infections in the membranes lining the digestive system and reducing colitis, as these infections reduce the absorption of nutrients in the stomach and intestine, which causes a decrease in the weight of rats; in the same context, Žugčić et al. (2019) reported that OLE supports the digestive functions via protection from disorders and diseases in a large way, such as constipation and indigestion, as well as works as an intestinal cleanser and detoxification, and it also speeds up the process of absorption of essential nutrients during the digestive process. This explains the effectiveness and activity of olive leaves against infections or toxins, as well as its important role in the metabolism and maintenance of the weight of rats.

The present study showed many negative effects and imbalances caused by PbAc on the spermatic characteristics and sexual hormones (total testosterone, FSH, and LH) of male rats. So, these results are compatible with the previous study that indicated PbAc lowers the seminal characteristics which include sperm count, motility, and viability as well as induce many changes in the testis tissues such as testicular damage, necrosis of seminiferous tubules, and lack of sperm in male rats (Ileriturk et al. 2020), whereas the rat's administration of olive leaf extract (OLE) with the PbAc (G4) showed a recovery in the seminal characteristics and sex hormones (total testosterone, FSH, and LH) when compared with the PbAc group (G3). However, the recovery in the sperm and hormones is due to the important health benefits that OLE can provide related to healthy sexual function as it has antioxidant activity and a protective role against hormonal changes and impotence caused by diabetes via stimulating blood circulation and a raised efficiency which helps improve blood flow in various parts of the body, including the reproductive organs (Park et al. 2013). Similarly, OLE contains oleuropein, which is one of the antioxidants responsible for many health benefits in the body including activation of sperm and sex hormones. In addition, OLE contains many polyphenols and flavonoids

Table 3 Changes in TBARS, GR, GPx, GST, GSH, SOD, and CAT in all the studied groups

Groups	Control	OLE	PbAc	PbAc+OLE
Thiobarbituric acid reactive substances (μmol/g tissue)	15.89 ±1.15^{c}	14.21 ±1.276^{c}	27.14 ±1.39^{a}	19.24 ±1.52^{b}
Glutathione reductase (IU/g tissue)	22.31 ±0.320^{a}	21.37±0.346^{a}	14.31 ±0.964^{c}	18.81 ±0.363^{b}
Glutathione peroxidase (IU/g tissue)	265.2 ±21.26^{b}	278.3 ±1.057^{a}	124.4 ±4.315^{d}	185.9 ±5.518^{c}
Glutathione-S-transferase (IU/g tissue)	23.48 ±0.671^{a}	25.06 ±0.494^{a}	16.97 ±1.184^{c}	20.28 ±0.797^{b}
Glutathione reduced (mg/g tissue)	73.30 ± 4.841^{b}	78.7 ±3.341^{a}	45.01± 1.519^{d}	62.23 ±0.287^{c}
Superoxide dismutase (U/mg protein)	55.4 ±0.66^{a}	57 ±0.79^{a}	42 ±0.46^{c}	50 ±0.67^{b}
Catalase (U/mg protein)	71 ± 0.26^{a}	69 ± 0.98^{a}	48±0.64^{c}	62 ±0.39^{b}

Data are expressed as mean ± SE of 5 observations; the groups with different superscript letters are significantly different at $p<0.05$

Abbreviations: *OLE*, olive leaf extract; *PbAc*, lead acetate; *TBARS*, thiobarbituric acid reactive substances; *GR*, glutathione reductase; *GPx*, glutathione peroxidase; *GST*, glutathione-S-transferase; *GSH*, glutathione reduced; *SOD*, superoxide dismutase; *CAT*, catalase

that are important to counteract free radicals that cause damage in many parts of the body such as the reproductive system (Irakli et al. 2018). Furthermore, Leskovec et al. (2019) expound that OLE contains many important elements for sperm validity, including phenolic compounds and vitamin E, as there was a positive relationship between treatment with olive leaves rich in these elements and an increase in the sperm count and their activity. Also, Almeer and Abdel Moneim (2018) mentioned that there was a clear improvement in the quality and number of sperms when treated with olive leaves, which in turn also increased the level of testosterone in males, in addition to increasing the level of the luteinizing hormone, which stimulates the cells in the testicle to produce testosterone.

The toxic effects of PbAc at the cellular level may be explained by lipid peroxidation and histological alterations. Also, the changes in the antioxidant defense system and oxidative stress are some of the mechanisms that can act in testicular dysfunction. Therefore, the treated rats with PbAc showed a reduction in the antioxidants activity (GR, GPx, GST, GSH, SOD, and CAT), and the elevation in the levels of TBARS. Also, PbAc caused many histopathological damages to include incomplete spermatogenesis and degeneration of seminiferous tubules in the testicular tissue, mild decrease in spermatogenesis with degeneration of germinal epithelium, and sloughing of germ cells into the tubular lumen. Our findings agree with Ansar et al. (2015) who reported that PbAc has many effects on sperm morphology and can

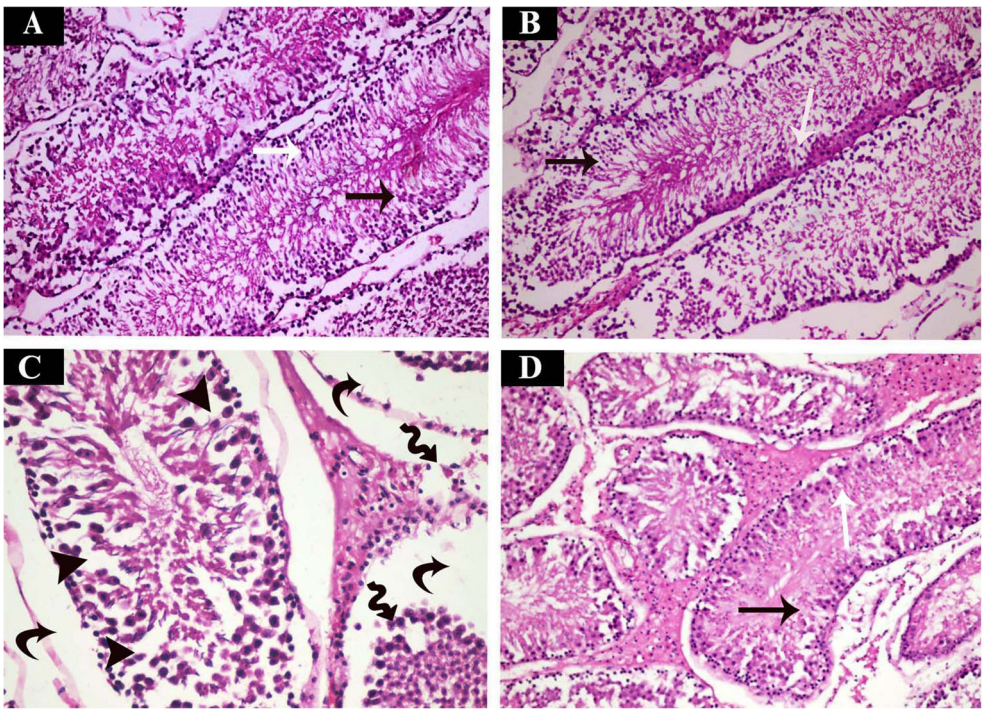

Fig. 2 (A, B) Photomicrographs of rat testes sections in control and olive leaf extract (OLE) groups showed the normal structure of seminiferous tubules, spermatid, sperm formation, and spermatogenesis found of primary (white arrows) and secondary (black arrows) spermatocytes. (C) Testis in the lead acetate (PbAc) group revealed a mild decrease in spermatogenesis with degeneration of the germinal epithelium and reduced Leydig cell concentration (zigzag arrows). Many vacuoles among the spermatogenic cells (arrowheads) and widening of the interstitial space (curved arrows). (D) Testes in the lead acetate (PbAc) and olive leaf extract (OLE) groups showed a regular distribution of the spermatogenic cell layers and normal spaces between the tubules

induce oxidative stress, toxicity, and injury in the testis tissues of male rats. Likewise, Udefa et al. (2020) demonstrated that PbAc increases lipid peroxidation, inflammation, and necrotic in testes that induced possible infertility and impairment of reproductive ability in male rats. Furthermore, Elgawish and Abdelrazek (2014) proposed that PbAc may begin oxidative stress via two pathways which include decreasing the activity of the antioxidant enzymes and promoting reactive oxygen species (ROS) production. Al-Megrin et al. (2020) demonstrated that PbAc induces harmful effects in testis tissue such as testicular destruction, necrosis in the seminiferous tubules, dispersed endothelial cells, and loss of spermatid in male rats.

However, following the treatment of rats with OLE in the fourth group (G4), the results observed that levels of antioxidant enzymes and TBARS were restored to normal values and improved in the damaged testicular tissues, which indicate OLE capacity to regulate the testicular function and lipid peroxidation in male rats affected by PbAc metal. Moreover, OLE contains antioxidants which are molecules that can inhibit free radicals such as ROS that producing in the oxidation process; thus, it can improve the damaged tissues that have been exposed to toxic substances such as PbAc (Al-Quraishy et al. 2017). Additionally, Rostamzadeh et al. (2020) reported that OLE has a potential protective effect via reducing oxidative stress and promote the antioxidant against cyclophosphamide-induced reproductive dysfunction and spermatotoxicity in male mice. In the same context, phenolic compounds in OLE have anti-inflammatory and antioxidant properties and prevent free radicals that lead to depletion of antioxidant enzymes SOD and GPx in the rats with ethanol-treated (López-Miranda et al. 2010; Alirezaei et al. 2012). Furthermore, the olive leaf contains hydroxytyrosol and tyrosol phenolic components which may be contributing to pharmacologic effects that reduce oxidative stress resulting from exposure to toxins (Ashkanani et al. 2020).

Conclusion

The present results indicate that lead acetate causes testicular toxicity, abnormal changes in sperms, sex hormonal imbalance, and antioxidant depletion which might be related to oxidative damage. Our results showed that olive leaves can enhance reproductive fertility and reduce the toxic effects of PbAc on the reproductive system in male rats. Moreover, studies of these promising beneficial effects of OLE might be a great role in promoting clinical strategies to treat patients with PbAc-induced testicular damage.

Author contribution All authors, Harith A. Ahmed, Huda A. Ali, and Thulfiqar F. Mutar, contributed to the study conception, design, material preparation, data collection, analysis, and approved the final manuscript.

Availability of data and materials All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate All rats were handled by the standard guide for the care and use of laboratory animals. The approval of the Institutional Animal Care and Use Committee for the experiment design was performed at the College of Veterinary Medicine, University of Baghdad, Iraq.

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

References

Abd HH, Ahmed HA, Mutar TF (2020) Moringa oleifera leaves extract modulates toxicity, sperms alterations, oxidative stress, and testicular damage induced by tramadol in male rats. Toxicol Res (Camb)

Abdelhamid FM, Mahgoub HA, Ateya AI (2020) Ameliorative effect of curcumin against lead acetate–induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. Environ Sci Pollut Res 1–16

Abugomaa A, Elbadawy M (2020) Olive leaf extract modulates glycerol-induced kidney and liver damage in rats. Environ Sci Pollut Res 1–12

Al-Attar AM, Abu Zeid IM (2013) Effect of tea (Camellia sinensis) and olive (Olea europaea L.) leaves extracts on male mice exposed to diazinon. Biomed Res Int 2013:

Alirezaei M, Kheradmand A, Heydari R, Tanideh N, Neamati S, Rashidipour M (2012) Oleuropein protects against ethanol-induced oxidative stress and modulates sperm quality in the rat testis. Med J Nutrition Metab 5:205–211

Almakhatreh M, Mutar TF, Tousson E (2020) Therapeutic role of rosemary extract against vepesid induced kidney injury. Proliferation and Apoptosis in Male Rats 3:16–25

Almeer RS, Abdel Moneim AE (2018) Evaluation of the protective effect of olive leaf extract on cisplatin-induced testicular damage in rats. Oxid Med Cell Longev 2018

Al-Megrin WA, Alkhuriji AF, Yousef AOS et al (2020) Antagonistic efficacy of luteolin against lead acetate exposure-associated with hepatotoxicity is mediated via antioxidant, anti-inflammatory, and anti-apoptotic activities. Antioxidants 9:10

Al-Quraishy S, Othman MS, Dkhil MA, Moneim AEA (2017) Olive (Olea europaea) leaf methanolic extract prevents HCl/ethanol-induced gastritis in rats by attenuating inflammation and augmenting antioxidant enzyme activities. Biomed Pharmacother 91:338–349

Ansar S, AlGhosoon HT, Hamed S (2015) Evaluation of protective effect of rutin on lead acetate-induced testicular toxicity in Wistar rats. Toxin Rev 34:195–199

Ashkanani M, Farhadi B, Ghanbarzadeh E, Akbari H (2020) Study on the protective effect of hydroalcoholic olive leaf extract (oleuropein) on the testis and sperm parameters in adult male NMRI mice exposed to Mancozeb. Gene Reports 21:100870

Ashraf U, Kanu AS, Mo Z, Hussain S, Anjum SA, Khan I, Abbas RN, Tang X (2015) Lead toxicity in rice: effects, mechanisms, and mitigation strategies—a mini review. Environ Sci Pollut Res 22:18318–18332

Castejón ML, Montoya T, Alarcón-de-la-Lastra C, Sánchez-Hidalgo M (2020) Potential Protective role exerted by secoiridoids from Olea

europaea L. in cancer, cardiovascular, neurodegenerative, aging-related, and immunoinflammatory diseases. Antioxidants 9:149

Cazarin CBB, da Silva JK, Colomeu TC, Batista ÂG, Meletti LMM, Paschoal JAR, Bogusz Junior S, Braga PAC, Reyes FGR, Augusto F, de Meirelles LR, Zollner RL, Maróstica Júnior MR (2015) Intake of Passiflora edulis leaf extract improves antioxidant and anti-inflammatory status in rats with 2, 4, 6-trinitrobenzenesulphonic acid induced colitis. J Funct Foods 17: 575–586

Chiaino E, Micucci M, Cosconati S, Novellino E, Budriesi R, Chiarini A, Frosini M (2020) Olive leaves and hibiscus flowers extracts-based preparation protect brain from oxidative stress-induced injury. Antioxidants 9:806

De Cicco P, Maisto M, Tenore GC, Ianaro A (2020) Olive leaf extract, from Olea europaea L., reduces palmitate-induced inflammation via regulation of murine macrophages polarization. Nutrients 12:3663

Dkhil MA, Moneim AEA, Al-Quraishy S (2016) Indigofera oblongifolia ameliorates lead acetate-induced testicular oxidative damage and apoptosis in a rat model. Biol Trace Elem Res 173:354–361

Efentakis P, Iliodromitis EK, Mikros E, Papachristodoulou A, Dagres N, Skaltsounis AL, Andreadou I (2015) Effects of the olive tree leaf constituents on myocardial oxidative damage and atherosclerosis. Planta Med 81:648–654

Eldaim A, Tousson E, El Sayed IET, Awd WM (2019) Ameliorative effects of Saussurea lappa root aqueous extract against Ethephon-induced reproductive toxicity in male rats. Environ Toxicol 34:150–159

Elgawish RAR, Abdelrazek HMA (2014) Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. Toxicol Rep 1:795–801

Fouad AA, Foda NT, Diab IH, Badr el Dine FMM, Balah MIF (2020) Evaluation of possible molecular toxicity induced by occupational exposure to lead and concomitant effect of smoking. Environ Sci Pollut Res 27:411–423

Gholami R, Zahedi SM (2019) Reproductive behavior and water use efficiency of olive trees (Olea europaea L. cv Konservolia) under deficit irrigation and mulching. Erwerbs-Obstbau 61:331–336

Guo J, Wu W, Sheng M et al (2013) Amygdalin inhibits renal fibrosis in chronic kidney disease. Mol Med Rep 7:1453–1457

Hari Priya P, Reddy PS (2012) Effect of restraint stress on lead-induced male reproductive toxicity in rats. J Exp Zool Part A Ecol Genet Physiol 317:455–465

Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE (2012) Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2:41–46

Ileriturk M, Benzer F, Aksu EH, et al (2020) Chrysin protects against testicular toxicity caused by lead acetate in rats with its antioxidant, anti-inflammatory, and antiapoptotic properties. J Food Biochem e13593

Irakli M, Chatzopoulou P, Ekateriniadou L (2018) Optimization of ultrasound-assisted extraction of phenolic compounds: oleuropein, phenolic acids, phenolic alcohols and flavonoids from olive leaves and evaluation of its antioxidant activities. Ind Crop Prod 124:382–388

Kandeil MA, Abd Eltwab MA, Safwat GM (2020) Antioxidant role of vitamin C in alleviating the reproductive toxicity of lead acetate in male rats. J Adv Vet Res 10:9–12

Lee-Huang S, Zhang L, Huang PL et al (2003) Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochem Biophys Res Commun 307:1029–1037

Leskovec J, Rezar V, Nemec Svete A et al (2019) Antioxidative effects of olive polyphenols compared to vitamin e in piglets fed a diet rich in n-3 pufa. Animals 9:161

Lockyer S, Yaqoob P, Spencer JPE, Rowland I (2012) Olive leaf phenolics and cardiovascular risk reduction: physiological effects and mechanisms of action. Nutr Aging 1:125–140

López-Miranda J, Pérez-Jiménez F, Ros E, de Caterina R, Badimón L, Covas MI, Escrich E, Ordovás JM, Soriguer F, Abiá R, Alarcón de la Lastra C, Battino M, Corella D, Chamorro-Quirós J, Delgado-Lista J, Giugliano D, Esposito K, Estruch R, Fernandez-Real JM, Gaforio JJ, la Vecchia C, Lairon D, López-Segura F, Mata P, Menéndez JA, Muriana FJ, Osada J, Panagiotakos DB, Paniagua JA, Pérez-Martinez P, Perona J, Peinado MA, Pineda-Priego M, Poulsen HE, Quiles JL, Ramírez-Tortosa MC, Ruano J, Serra-Majem L, Solá R, Solanas M, Solfrizzi V, de la Torre-Fornell R, Trichopoulou A, Uceda M, Villalba-Montoro JM, Villar-Ortiz JR, Visioli F, Yiannakouris N (2010) Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008. Nutr Metab Cardiovasc Dis 20:284–294

Mao X, Xia B, Zheng M, Zhou Z (2019) Assessment of the anti-inflammatory, analgesic and sedative effects of oleuropein from Olea europaea L. Cell Mol Biol 65:52–55

Meyer PA, Brown MJ, Falk H (2008) Global approach to reducing lead exposure and poisoning. Mutat Res Rev Mutat Res 659:166–175

Mokhtari M, Zanboori M (2011) The effects of lead acetate on sexual behavior and the level of testosterone in adult male rats. Int J Fertil Steril 5:13

Mutar TF, Salem SB, Tousson E et al (2019) Ameliorative effects of vitamin B17 on the kidney against Ehrlich ascites carcinoma induced renal toxicity in mice. Environ Toxicol 35:1–10. https://doi.org/10.1002/tox.22888

Olmez E, Vural K, Gok S, Ozturk Z, Kayalar H, Ayhan S, Var A (2015) Olive leaf extract improves the atherogenic lipid profile in rats Fed a high cholesterol diet. Phyther Res 29:1652–1657

Park J-H, Jung J-H, Yang J-Y, Kim H-S (2013) Olive leaf down-regulates the oxidative stress and immune dysregulation in streptozotocin-induced diabetic mice. Nutr Res 33:942–951

Patra RC, Swarup D, Dwivedi SK (2001) Antioxidant effects of α tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. Toxicology 162:81–88

Patra RC, Rautray AK, Swarup D (2011) Oxidative stress in lead and cadmium toxicity and its amelioration. Vet Med Int 2011

Ponce-Canchihuamán JC, Pérez-Méndez O, Hernández-Muñoz R, Torres-Durán PV, Juárez-Oropeza MA (2010) Protective effects of Spirulina maxima on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. Lipids Health Dis 9:35

Rahman A, Al-Awadi AA, Khan KM (2018) Lead affects vitamin D metabolism in rats. Nutrients 10:264

Rerknimitr P, Kantikosum K, Chottawornsak N, Tangkijngamvong N, Kerr SJ, Prueksapanich P, Sithisarankul P, Kumtornrut C, Asawanonda P, Sutheparuk S, Panchaprateep R (2019) Chronic occupational exposure to lead leads to significant mucocutaneous changes in lead factory workers. J Eur Acad Dermatology Venereol 33:1993–2000

Rostamzadeh A, Amini-Khoei H, Korani MJM, Rahimi-Madiseh M (2020) Comparison effects of olive leaf extract and oleuropein compounds on male reproductive function in cyclophosphamide exposed mice. Heliyon 6:e03785

Sarbishegi M, Gorgich EAC, Khajavi O et al (2018) The neuroprotective effects of hydro-alcoholic extract of olive (Olea europaea L.) leaf on rotenone-induced Parkinson's disease in rat. Metab Brain Dis 33: 79–88

Shan G, Tang T, Zhang X (2009) The protective effect of ascorbic acid and thiamine supplementation against damage caused by lead in the testes of mice. J Huazhong Univ Sci Technol Medical Sci 29:68–72

Sofuoglu SC, Yayla B, Kavcar P, Ates D, Turgut C, Sofuoglu A (2013) Olive tree, Olea europaea L., leaves as a bioindicator of atmospheric PCB contamination. Environ Sci Pollut Res 20:6178–6183

Soliman GA, Saeedan AS, Abdel-Rahman RF, Ogaly HA, Abd-Elsalam RM, Abdel-Kader MS (2019) Olive leaves extract attenuates type II diabetes mellitus-induced testicular damage in rats: molecular and biochemical study. Saudi Pharm J 27:326–340

Taamalli A, Feriani A, Lozano-Sanchez J, Ghazouani L, el Mufti A, Allagui MS, Segura-Carretero A, Mhamdi R, Arráez-Roman D (2020) Potential hepatoprotective activity of super critical carbon dioxide olive leaf extracts against CCl4-induced liver damage. Foods 9:804

Tappel AL, Zalkin H (1959) Inhibition of lipide peroxidation in mitochondria by vitamin E. Arch Biochem Biophys 80:333–336

Toscano CD, Guilarte TR (2005) Lead neurotoxicity: from exposure to molecular effects. Brain Res Rev 49:529–554

Tousson E, El-Moghazy M, Massoud A, Akel A (2012) Histopathological and immunohistochemical changes in the testes of rabbits after injection with the growth promoter boldenone. Reprod Sci 19:253–259

Tousson E, Bayomy MF, Ahmed AA (2018) Rosemary extract modulates fertility potential, DNA fragmentation, injury, KI67 and P53 alterations induced by etoposide in rat testes. Biomed Pharmacother 98:769–774

Tousson E, Hafez E, Abo Gazia MM, Salem SB, Mutar TF (2020) Hepatic ameliorative role of vitamin B17 against Ehrlich ascites carcinoma–induced liver toxicity. Environ Sci Pollut Res 27: 9236–9246. https://doi.org/10.1007/s11356-019-06528-6

Udefa AL, Amama EA, Archibong EA et al (2020) Antioxidant, anti-inflammatory and anti-apoptotic effects of hydro-ethanolic extract of Cyperus esculentus L.(tigernut) on lead acetate-induced testicular dysfunction in Wistar rats. Biomed Pharmacother 129:110491

Varmaghany S, Rahimi S, Torshizi MK, et al (2013) Effect of olive leaves on ascites incidence, hematological parameters and growth performance in broilers reared under standard and cold temperature conditions. Anim Feed Sci Technol 185:60–69

Vezza T, Rodríguez-Nogales A, Algieri F et al (2019) The metabolic and vascular protective effects of olive (Olea europaea L.) leaf extract in diet-induced obesity in mice are related to the amelioration of gut microbiota dysbiosis and to its immunomodulatory properties. Pharmacol Res 150:104487

Wei J, Wang S, Pei D, Qu L, Li Y, Chen J, di D, Gao K (2018) Antibacterial activity of hydroxytyrosol acetate from olive leaves (Olea Europaea L.). Nat Prod Res 32:1967–1970

Wulandari L, Retnaningtyas Y, Lukman H (2016) Analysis of flavonoid in medicinal plant extract using infrared spectroscopy and chemometrics. J Anal Methods Chem 2016

Zhang H, Hao F, Liu R (2013a) Interactions of lead (II) acetate with the enzyme lysozyme: a spectroscopic investigation. J Lumin 142:144–149

Zhang Y, Li Q, Liu X, Zhu H, Song A, Jiao J (2013b) Antioxidant and micronutrient-rich milk formula reduces lead poisoning and related oxidative damage in lead-exposed mice. Food Chem Toxicol 57: 201–208

Zhang H, Liu Y, Zhang R, Liu R, Chen Y (2014) Binding mode investigations on the interaction of lead (II) acetate with human chorionic gonadotropin. J Phys Chem B 118:9644–9650

Žugčić T, Abdelkebir R, Alcantara C, Collado MC, García-Pérez JV, Meléndez-Martínez AJ, Režek Jambrak A, Lorenzo JM, Barba FJ (2019) From extraction of valuable compounds to health promoting benefits of olive leaves through bioaccessibility, bioavailability and impact on gut microbiota. Trends Food Sci Technol 83:63–77