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LEAD REPRODUCTIVE TOXICITY AND MODULATOR ROLE OF MANGO WASTES EXTRACTS IN MICE

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ABSTRACT

Lead is an environmental metal known as reproductive toxicant. Mango wastes are a good source of potential antioxidants. The current study was carried out to examine the modulatory role of mango peel (MPE) and kernel (MKE) extracts against lead productive toxicity in mice. The following endpoints were studied: sperm DNA damage, histopathological changes in testes as well as male–mediated toxicity in non-treated female mice mated with Pb-acetate-treated males and their offspring. Two doses of MPE and MKE (50 and 100 mg/kg) were used to examine the mango extract effects on the reproductive toxicity induced by Pb-acetate (500 ppm). The mango extracts significantly decreased the level of sperm DNA damage induced by Pb-acetate, modulated the histopathological alterations stimulated in the testes by Pb-acetate exposure. The mango extracts also diminished the developmental toxicity induced in females mated with Pb-acetate-treated males. It can be concluded that MPE and MKE have a modulator role against the lead reproductive toxicity in mice

INTRODUCTION

Environmental exposure to heavy metals such as lead is known to have a negative impact on reproduction and development in humans and animals, including influence on male and female fertility, abortions, malformations and birth defects (Apostoli and Catalani, 2011). In male, lead causes reproductive toxicity via suppression of spermatogenesis and induces a wide range of behavioral, biochemical and physiological effects (Falana and Oyeyipo, 2012). Evidence is accumulating to support the role of oxidative stress in the lead toxicity and the mechanisms include the lead effects on membranes, DNA, and antioxidant defense systems of cells (Ahamed and Siddiqui, 2007). Recent studies have shown that sperm DNA damage correlates with infertility, increased risk of early pregnancy loss, defective embryogenesis, congenital malformations, genetic abnormality and prenatal morbidity (Moskovtsev *et al*., 2009).

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Semen quality is influenced by a variety of lifestyle, environmental, and occupational factors. The possible causes of a the possible causes of a damaged sperm DNA include defects during spermatogenesis, abortive apoptosis and oxidative stress (Aitken and De Iuliis, 2007). High level of reactive oxygen species (ROS) is one of the main causes of DNA damage and morphologically abnormal sperm cells. Oxidative stress induces peroxidative damage to cell membrane and causes mitochondrial mutation and nuclear DNA damage (Shamsi *et al*., 2010).Sperm DNA is an important measure of fertilizing efficiency with better diagnostic and prognostic capabilities than routine semen parameters (Shamsi *et al*., 2008). Since sperm, oxidative stress and DNA damage are important factors for male infertility and in consequence, for healthy pregnancy. An experiment involving the use of antioxidants to minimize DNA damage in spermatozoa seems to be justified. Spermatozoa are particularly vulnerable to oxidative stress due to their high polyunsaturated fatty acid content, inherent deficiency in intracellular antioxidant enzyme protection and a limited capacity for DNA repair (Gharagozloo and Aitken, 2011).

Several researches have focused on the health benefits of consumption of mango fruit that mango is a natural source of bioactive compounds with promising antioxidant, anti-inflammatory and anticancer properties (El-Baroty *et al*., 2014). Recent reports demonstrated that mango byproducts are a good source of functional ingredients such as phenolic compounds that has potential antioxidant properties (Tunchaiyaphum *et al*., 2013). The total antioxidant capacity and phenolic content of mango peel and kernel are higher compared to the edible fruit parts (Vega-Vega *et al*., 2013). The objectives of the current study were to examine : 1/ lead acetate reproductive toxicity; 2/ the modulatory role of mango peel (MPE) and kernel (MKE) in the lead acetate-induced sperm DNA damage; 3/ the role of mango extracts in diminishing histopathological changes in male mice testes; 4/ the male–mediated developmental toxicity in non-treated female mice mated with males exposed to lead acetate.

MATERIALS AND METHODS

Chemicals and mango extracts preparation

Lead acetate was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate, hexane and ethanol were obtained from El-Gomhoreya Co., Cairo, Egypt. Ripe mango peels and kernels were collected as wastes after mango pulp processing (Al-Qahera Company for Agriculture Industry, Al-Obor, Egypt). Mango peels were washed to remove any dirt particles. The peels were spread thin in trays and dried at 50^{o}C using a cross flow drier for 18 h to have 10% of water content. The dried peels were powdered using a hammer mill and sieved through a 150 mm sieve. The mango seeds were washed air-dried and the kernels were removed manually from seeds. The kernels were chopped, and dried at 50 ºC and powdered (Augustin and Ling, 1987). The obtained mango powder was kept in hexane overnight (20ºC, no light, gentle shaking). Next, the defatted powder was extracted trice with 80% ethanol (1:5). The consecutive three filtrated extracts were combined and concentrated using a rotary evaporator under reduced pressure. The concentrated extracts (MPE and MKE) were lyophilized and kept at –18°C until further analysis. Lyophilized extracts of MPE and MKE were dissolved in dimethyl sulfoxide [DMSO] and next administered at two doses (50 or 100 mg/kg b. w.) to the animals with the use of feeding tube (0.1 mL/mice/day).

Determination of total phenolic content

The total phenolic content of the extracts was determined in the extracts using the Folin-Ciocalteu method (Blois, 1958). The absorbance was measured at 765 nm using a UV/VIS 1201 spectrophotometer (Shimadzu, Kyoto, Japan). The total amount of phenolic compounds was calculated and expressed as gallic acid equivalent (GAE mg/g).

DPPH (1, 1-diphenyl 2-picrylhyorazyl) free radicals scavenge assay

The measurement of antioxidant activity of the extracts was based on the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by (Singleton *et al*., 1999). Aliquot (30 µL) of each sample were mixed with 1.0 mL of 0.1 mmol/L DPPH in methanol. The control samples were devoid of the extract. The reaction mixture was shaken well and allowed to react for 20 min at room temperature. The remaining DPPH free radical was determined by absorbance measurement at 517 nm against methanol blanks. The percentage scavenging effect was calculated from the following equation:

Scavenging activity % = (control absorbance - sample absorbance)/ control absorbance x 100

Animals and experimental design

Swiss albino mice of both sexes (8-12 weeks old) were procured from the National Research Center (Dokki, Cairo, Egypt) and housed in plastic cages with steel wire tops and wood bedding. The animals were acclimatized two weeks before the experiments and were maintained in rooms with temperature of 25±2◦C, and 50-70% humidity and light cycle (12 h:12 h). The mice received a standard mouse chow and water *ad libitum*. All experimental protocols related to the use and care of laboratory animal in research were approved by the Animal Care and Use committee of National Research Centre (Dokki, Cairo, Egypt). The animals were randomly divided into eight groups (15 animals/group): 1/control group, mice received tap water and 0.1 mL DMSO (vehicle) by gavage; 2/ MPE group, mice received MPE via oral intubation (100 mg/kg b.w.); 3/ MKE group, mice received MKE via oral intubation (100 mg/kg b.w.); 4/ Pb group, Pb-acetate was given in drinking water (500 ppm) (Haleagrahara] *et al*., 2011); 5/ Pb + MPE 50 group, MPE was given via oral intubation (50 mg/kg b.w.); 6/ Pb + MPE 100 group, MPE was given via oral intubation (100 mg/kg b.w.); 7/ Pb + MKE 50 group, MKE was given via oral intubation (50 mg/kg b.w.) and 8/ Pb + MKE 100 group, MKE was given via oral intubation (100 mg/kg b.w.). All treatments were administered daily for 28 days.

Acridine orange (AOT) assay

The assay was performed according to the protocol described by Hodjat *et al*. (2008). The air-dried sperm smears were fixed in Carnoy's solution for 1 hr at 4°C and then incubated in 1 N HCl at 75°C for 15 min. The slides were stained with AO dye (0.2 mg/mL) for 10 min, washed with distilled water to remove background staining, and air-dried. At least 1000 spermatozoa per mouse were analyzed under fluorescence microscope (490/530 nm excitation/barrier filter) at 1000 X magnification. Green spermatozoa were considered to have normal double-stranded DNA, and yellow or red spermatozoa had abnormal single-stranded DNA.

Toluidine Blue (TB) assay

The air-dried sperm smears were fixed in fresh 96% ethanol-acetone (v/v) at 4°C for 30 min and then hydrolyzed in 0.1 N HCl at 4°C for 5 min. The slides were rinsed in distilled water for 2 min and finally stained with TB 0.05% (w/v), in 50% (v/v) McIlvaine citrate-phosphate buffer (2 mol/L Na_2HPO_4 and 0.1 mol/L citric acid, pH 3.5) for 10 min (Erenpreisa *et al*., 2003). A total of 1000 spermatozoa per animal was analyzed under light microscope at 100-x magnification. Spermatozoa with light blue heads were categorized as those with DNA of

normal integrity, while those with dark blue or dark purple heads were abnormal with poor DNA integrity.

The percentage of the sperm cells with abnormal chromatin was calculated using the following formula:

$$\frac{\text{No. of spermatozoa with damaged DNA}}{\text{No. of spermatozoa with intact DNA+No. of spermatozoa with damaged DNA}} \times 100$$

In addition, DNA damage inhibition rate between Pb-acetate and the mango extracts groups was calculated using the following formula:

$$\frac{\text{DNA damage in Pb treated group} - \text{DNA damage in extract treated group}}{\text{DNA damage in Pb treated group}} \times 100$$

Comet assay

This assay was performed as described by Donnelly (Donelly *et al*., 1999). Briefly, sperm were suspended in phosphate buffer (pH 7.5) and mixed with 0.5% (w/v) low melting point agarose and sandwiched between a lower layer of 1% (w/v) normal melting point agarose and an upper layer of 0.5% (w/v) low melting agarose on fully forested microscope slides. The slides were dipped in cold lysis buffer (2.5 mol/L NaCl, 100 mmol/L EDTA, 10mmol/L Tris-HCl, 1% (v/v) Triton X-100, and 10% (v/v) DMSO, pH 10) at 4ºC for 2 hr and protected from light. To decondense sperm DNA, lysis buffer was supplemented with 10 mmol/L dithiothreitol (DTT) for 1 hr at 4ºC and then incubated with 200 mg/mL proteinase K for 2.5 hr at 37ºC.

After lysis, the slide were washed with distilled water and placed in chilled alkaline solution (300mmol/L NaOH and 1 mmol/L Na_2EDTA, pH 13) for 30 min in the dark. Electrophoresis was conducted in the dark at 4ºC for 30 min at 19 V/cm and approximately 300 m A. Following electrophoresis, the slides were rinsed three times with distilled water, stained with ethidum bromide (5µL/mL) and examined under the fluorescence microscope equipped with 515-560 nm excitation filters and 590 nm barrier filters. Following electrophoresis, the slides were rinsed three times with distilled water, stained with ethidum bromide (5µL/mL) and examined under the fluorescence microscope equipped with 515-560 nm excitation filters and 590 nm barrier filters. Comet images were scored with the comet score 15-image analysis Software (TriTek Corp., Sumerduck, VA). The scored parameters included tail length, the percentage of head intact DNA and Olive tail moment. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers. The Olive tail moment is a global comet parameter expressed as [(tail mean – head mean) × (% tail DNA/100)] and used to quantify DNA damage.

Male–mediated developmental toxicity

Thirty-five days after the end of treatment, five male mice from each experimental group were mated with untreated virgin female mice (10 females/group) for two weeks. Such a design allowed to examining the effect of paternal exposure to the treatments on the parameters of reproductive performance. Each treated male mouse was housed in a separate cage with two virgin untreated females of the same strain. The day of sperm positive vaginal smear was considered as day'0' of gestation. The gravid females (n = 10 per group) which were mated with control and treated males were sacrificed by cervical dislocation on day 13-15 of gestation. Uterine content was examined to determine the number of implantation sites, fetal resorptions, dead and live fetuses and hematoma. Potency, litter size, post-implantation loss (%), weights of live fetuses and the placenta were recorded. Potency is the ability of male rat to inseminate the female rat.

$$\text{Potency} = \frac{\text{Number of females inseminated}}{\text{Number of females exposed to mating}} \times 100$$

In addition, fetal liver cells were suspended in phosphate buffer (pH 7.5) and the comet assay was performed as described above (2.3.3. chapter).

Histological Examination

Autopsy samples were taken from the testes of mice in different groups and fixed in 10% (v/v) formol saline for twenty-four hours. After washing with tap water, serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 **ºC** in hot air oven for 24 hours. Paraffin blocks were cut at 4 µm thickness, deparaffinized, stained by hematoxylin and eosin and examined under light microscope (Banchroft *et al*., 1996).

Statistical analyses

Descriptive values of data were represented as means ± standard deviation (SD). Student T test was used for the statistical analysis of total phenolic content and scavenging activity data. Statistical analysis of data of biological experiments was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with $p \leq 0.05$ being considered statistically significant. Statistical analysis was conducted with SPSS (v16; Lead Technologies, Inc., IL USA) program was used.

RESULTS

Mango kernel extract had significantly higher level of phenolics and greater free radical scavenging activity compared to mango peel extract as shown in Table 1.

Table 1. Bioactive compounds present in mango peels extract (MPE) and mango kernel extract (MKE)

Characteristic	MPE	MKE
Total phenolics (GAE	49.16 ± 0.03	78.23 ± 0.05**
Scavenging activity (%)	93.89 ± 0.02	98.58 ± 0.01*

n = 3; Data are expressed as mean ± standard deviation (SD).
(*) Significance at ($P \leq 0.05$); (**) Significance at ($P \leq 0.01$).

Sperm DNA integrity determination

Five mice from each group were sacrificed by cervical dislocation one day and 35 days (duration of spermatogenesis) after the end of treatment. The epididymis from each mouse, cleared of adhering tissues and placed on a Petri dish. The tissue was minced to allow spermatozoa swim out into the 1mL of phosphate buffer saline (PBS, pH 7.4). Sperm chromatin integrity was determined by toluidine blue and acridine orange at two different time points (24h & 35 days). In addition, sperm DNA damage was examined by comet assay.

Table 2. Sperm chromatin integrity detected by acridine orange (AO) and toluidine blue (TB) assays

Group	Sperm cells with damaged DNA (%)							
	24 h				35 days			
	AO		TB		AO		TB	
	Mean ± SD	DNA damage inhibition rate (%)	Mean ± SD	DNA damage inhibition rate (%)	Mean ± SD	DNA damage inhibitionrate (%)	Mean ± SD	DNA damage inhibition rate (%)
Control	4.2±0.4^{a}		4.9±0.5^{a}		3.8±0.7^{a}		5.1±1.4^{a}	
Mango peels extract (MPE)	5.9±0.6^{a}		5.9±0.7^{a}		5.7±1.3^{a}		6.0±0.5^{a}	
Mango kernel extract (MKE)	5.2±0.4^{a}		4.8±0.4^{a}		4.6±0.9^{a}		5.5±0.6^{a}	
Pb-acetate	33.3±0.8^{g}		27.3± 1.5^{f}		25.9±2.1^{f}		34.2±2.6^{f}	
Pb-acetate+MPE 50 mg/kg	19.8±1.1^{de}	40.5	19.1±0.9^{de}	30	17.1±0.6^{de}	33.9	21.6±1.1^{de}	36.8
Pb-acetate+MPE 100 mg/kg	15.2±0.6^{cd}	54.4	13.5±1.6^{bc}	50.5	12.0±1.4^{bc}	53.7	16.6±1.1^{c}	51.5
Pb-acetate+MKE 50 mg/kg	16.9±1.3^{cd}	49.3	16.3±1.1^{cd}	40.29	14.9±0.5^{cd}	42.5	18.8±1.3^{d}	44.9
Pb-acetate+MKE 100 mg/kg	10.7±1.7^{b}	67.9	10.8±0.2^{b}	60.4	9.3±1.0^{b}	64.1	13.3±0.4^{bc}	61.0

5 000 sperm cells were examined in five mice per group; Pb: lead; AO: Acridine Orange; TB: Toluidine Blue; MPE: Mango peels extract; MKE: Mango kernel extract. Different superscripts within the same column depict significant differences ($p \leq 0.05$).

Table 3. Sperm chromatin integrity detected by comet assay

Experimental group	Tail length (μm)	Tail DNA (%)	Olive tail moment (μm)
Control	2.33±0.04^{a}	2.07±0.04^{a}	4.83±0.18^{a}
Mango peels extract (MPE)	2.45±0.06^{a}	2.12±0.04^{a}	5.21±0.20^{a}
Mango kernel extract (MKE)	2.36±0.04^{a}	2.10±0.03^{a}	4.96±0.13^{a}
Pb-acetate	3.82±0.06^{c}	4.24±0.03^{e}	16.18±0.29^{d}
Pb-acetate + MPE 50 mg/kg	3.38±0.16^{b}	3.32±0.11^{d}	11.20±0.30^{c}
Pb-acetate + MPE 100 mg/kg	3.43±0.04^{b}	2.55±0.02^{b}	8.82±0.16^{b}
Pb-acetate+ MKE 50 mg/kg	3.60±0.04^{b}	2.90±0.17^{c}	10.47±0.74^{c}
Pb-acetate +MKE 100 mg/kg	3.39±0.08^{b}	2.49±0.03^{b}	8.52±0.26^{b}

Pb: lead; MPE: Mango peels extract; MKE: Mango kernel extract.
Values are expressed as Mean ± SD.
Different superscripts within the same column designate significant differences ($p \leq 0.05$).
TL: Tail length; TDNA: % Tail DNA; OTM: Olive Tail Moment.

Table 4. Reproductive performance of the first generation progeny at 13th-15th day of gestation

Group	Potency (%)	Post-implantation loss (%)	Litter size	Fetal weight (g)	Placental weight (g)
Control	100	10.8±5.2^{a}	8.8±1.2^{b}	0.36±.00^{b}	0.08±.00^{b}
Mango peels extract (MPE)	100	11.0±3.2^{a}	9.4±.4^{b}	0.48±.01^{cd}	0.10±.01^{bc}
Mango kernel extract (MKE)	100	11.8±3.8^{a}	9.0±.5^{b}	0.48±.01^{cd}	0.13±.01^{d}
Pb-acetate	100	80±20.0^{b}	1.8±1.8^{a}	0.03±0.02^{a}	0.03±.02^{a}
Pb-acetate+MPE 50 mg/kg	100	6.5±4.2^{a}	8.6±0.7^{b}	0.35±.05^{b}	0.12±.01cd
Pb-acetate+MPE 100 mg/kg	100	2.5±2.5^{a}	8.8±0.6^{b}	0.37±.03^{bc}	0.12±.01^{bc}
Pb-acetate+MkE 50 mg/kg	100	8.1±3.5^{a}	8.6±0.4^{b}	0.55±.07^{d}	0.10±.01^{bc}
Pb-acetate+MkE 100 mg/kg	100	8.6± 5.4^{a}	9.0 ±.8^{b}	0.75±.05^{e}	0.11±.01^{bc}

Pb: lead; MPE: Mango peels extract; MKE: Mango kernel extract. Values are expressed as Mean ± SD.
Different superscripts within the same column depict significant differences ($p \leq 0.05$);

Table 5. Chromatin integrity in fetal liver cells detected by comet assay

Group	Tail length (μm)	Tail DNA (%)	Olive Tail Moment (μm)
Control	2.27± 0.03^{a}	1.49±0.02^{b}	3.39±0.09^{a}
Mango peels extract (MPE)	2.51±0.07^{a}	1.53±0.01^{b}	3.84±0.09^{a}
Mango kernel extract (MKE)	2.34±0.01^{a}	1.28±0.01^{a}	2.99± 0.05^{a}
Pb-acetate	4.30±0.01^{d}	4.06±0.02^{d}	17.46±0.10^{d}
Pb- acetate+MPE 50 mg/kg	3.93±0.06^{c}	3.09±0.05^{c}	12.17±0.32^{c}
Pb-acetate+MPE 100 mg/kg	3.32±0.12^{b}	3.02±0.02^{c}	10.03± 0.40^{b}
Pb-acetate+MKE 50 mg/kg	3.33±0.07^{b}	3.19±0.09^{c}	10.64±0.36^{b}
Pb-acetate+MKE 100 mg/kg	3.20±0.15^{b}	3.13±0.08^{c}	9.83± 0.40^{b}

Pb: lead; MPE: Mango peels extract; MKE: Mango kernel extract. Values are expressed as Mean ± SD.
Different superscripts within the same column designate significant differences ($p \leq 0.05$).
TL: Tail length; TDNA: % Tail DNA; OTM: Olive Tail Moment.

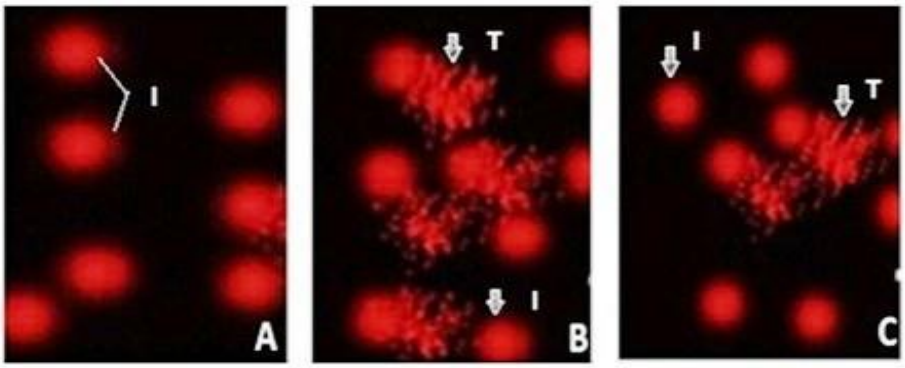

Figure 1. DNA damage assessed by comet assay in spermatozoa obtained from A/ control mice; B/ Pb-acetate treated mice and C/ Pb-acetate + mango extract-treated mice. I: intact cells, T: tailed cells

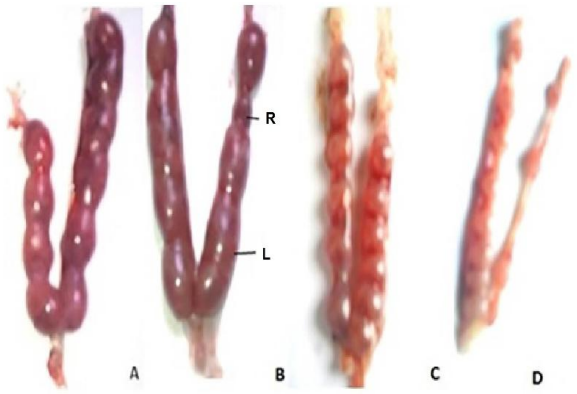

Figure 2. The images of the uterus of a control pregnant mice at day 13 of gestation (A), of the uterus with live [L] and resorbed [R] fetuses (B), and of the uteri with a more advanced fetal resorption (C and D), the B, C and D uteri were collected from females mated with lead acetate-treated males

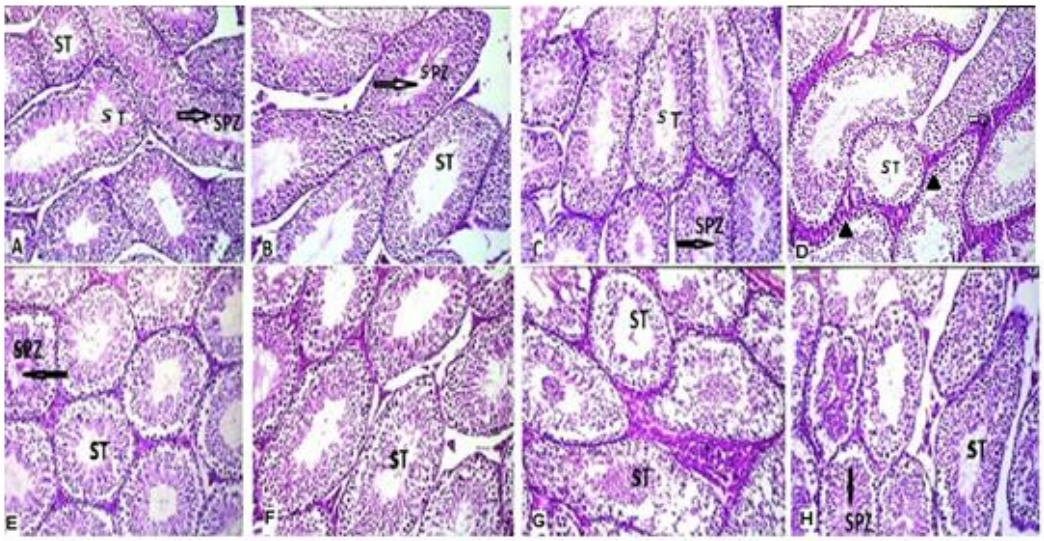

Figure 3. Microscopic images of the testis originated from control mice (A) and mice treated with Pb acetate and/or mango extracts (B-G). A/ control males show normal architecture of testis, seminiferous tubules (ST) show a clear lumen and all cell types are represented including Sertoli cells, spermatogonia, primary spermatocytes and spermatozoa (SPz); B/ Mango peel extract (MPE)-treated males – the images are similar to those of controls; C: Mango kernel extract (MKE)-treated males – the images are similar to those of controls; D/ Pb-acetate treated males – degenerative changes (disarrangement of spermatogenic cells, detachment of germinal epithelium from the basal layer (arrow head) and less spermatozoa present in the tubular lumen) are visible in seminiferous tubules; E/ Pb-acetate + 50 mg/kg MPE-treated males ; F/ Pb-acetate + 100 mg/kg MPE-treated males ; G: Pb-acetate + 50 mg/ kg MKE-treated males; and H/ Pb-acetate + 100 mg/ kg MKE-treated males- normal testis architecture, showing numerous spermatogonia arranged in rows similar to that of control (H&E, 40×)

Sperm DNA Integrity

In both methods and at the two studied time points, the percentage of spermatozoa with damaged DNA was significantly higher ($p \leq 0.05$) in Pb-treated mice than in controls as shown in Table 2. Both MPE and MKE significantly decreased the percentage of abnormal spermatozoa in Pb-acetate treated mice and the inhibition rate of sperm DNA damage was increased by increase of dose. The percentage of tail length (TL), tail DNA (%) (TDNA) and olive tail moment (OTM) were significantly ($p \leq 0.05$) penhanced in Pb-acetate animals in comparison to the control group. Administration of MPE and MKE resulted in a significant decrease in the Pb-acetate increased TL, TDNA and OTM (Table 3, Figure 1).

The MKE extract tended to exhibit higher inhibition rates than MPE extracts ($p \leq 0.05$). DNA strand breakage was also detected in mouse spermatozoa exposed to Pb-acetate and examined by means of comet assay as shown in Table 3.

Developmental toxicity

Reproductive performance of females mated with control, MPE-, MKE-, Pb-acetate-, Pb-acetate + MPE or Pb-acetate + MKE-treated males is depicted in Table 4. Pb-acetate did not affect the potency of males. Placental weight, fetal body weight and litter size of the F1 progeny in the Pb-acetate-treated group was significantly ($p \leq 0.05$) lower than in the control group. However, both mango extracts restored these lead decreased parameters to control values. The post implantation loss, in turn, was significantly higher in fetuses sired by Pb-acetate exposed males in comparison to control males (Tab. 4, Fig. 2). On the other hand, administration of MPE or MKE to the Pb-acetate-treated males significantly reduced the number of resorbed fetuses as compared to the Pb-acetate group. As was demonstrated by the results of comet assay, TL, TDNA% and OTM were significantly ($p \leq 0.05$) higher in fetal liver cells of fetuses sired by Pb-acetate-treated males in comparison to those sired by control males (Tab. 5). The mango extracts partially abolished the negative effect of Pb-acetate on DNA damage in the fetal liver cells.

Histopathological changes in the testes

Testicular sections of control mice showed normal testis architecture. The cross sections of seminiferous tubules were rounded or oval surrounded by narrow interstitial spaces containing Leydig cells. The seminiferous tubules were lined by germinal epithelium showing a clear lumen and all types of cells including Sertoli cells, spermatogonia, primary spermatocytes and spermatids. The spermatogenic cells were seen in regularly arranged rows (Figure 3A).

There was no histopathological alteration in the testis of mice treated with 100 mg/kg b.w. MPE and MKE (Figures 3B and C, respectively). Histopathological images of the testis of mice treated with Pb-acetate showed degenerative changes in some seminiferous tubules i.e., disarrangement of spermatogenic cells and detachment of germinal epithelium from the basal layer. In addition, a decrease in spermatozoa concentration in the tubular lumen was visible (Figure 3D). A significant decrease in the tubule damage was found in the testes sections obtained from Pb-acetate plus MKE and MPE both doses treated mice as compared to mice treated with Pb-acetate alone (Figurs 3E, F, G & H). The spermatogenic cells are arranged in rows and more numerous as compared to that of Pb-acetate group.

DISCUSSION

Testicular tissue is a major target for metal-induced oxidative damage because of its high content of polyunsaturated membrane lipids. The lipid peroxidation destroys the structure of lipid matrix leading to a decrease in membrane integrity and sperm motility (Chandra *et al*., 2007). Therefore, the integrity of sperm DNA is a useful biomarker for male infertility diagnosis and prediction of assisted reproduction outcomes (Lewis *et al*., 2013). In the current study, sperm DNA damage was higher in the Pb-acetate treated animals. Similarly, Al-Juboori *et al*. (2013) showed that Pb-acetate induced an increase in the percentage of sperm cells with fragmented DNA. Ahmed *et al*. (2012) reported that Pb-acetate has a direct effect on DNA causing damage and apoptosis in liver of female rabbit. In addition, several studies detected an increase in DNA strand breaks in workers exposed to Pb (Fracasso *et al*., 2002; Danadevi *et al*., 2003; Manikantan *et al*., 2010). The results of the current study showed that Pb-acetate negatively affected the testicular tissue architecture in mice. The germinal epithelium of some seminiferous tubules showed a disarrangement and detachment from the basal layer. In addition, Pb-acetate caused a decrease in sperm number in the tubular lumen. These findings are in consistent with a finding that lead exposure caused progressive vascular, tubular and interstitial testicular damage (Makhlouf *et al*., 2008; Moniem *et al*., 2010). Moreover, Ayinde *et al*. (2012) indicated that Pb-acetate reduced seminiferous epithelium and the number luminal spermatozoa. These results indicate that lead targets testicular spermatogenesis and sperm within the epididymis to produce reproductive toxicity.

The potential adverse effect of sperm DNA damage on the quality of the post-implantation embryo and spontaneous abortion was confirmed (Anjum *et al*., 2011). Sperm DNA damage precedes the loss of fertilization potential and poor embryo quality, resulting in pregnancy loss (Borini *et al*., 2006). Dorea (2004) confirmed that lead is of public health concern due to their toxic effects on vulnerable fetuses. This was clearly shown in the present study, where, embryotoxicity was observed in females mated with Pb-acetate treated males. In addition, there was significant increase in the DNA damage in liver cells of embryos sired by Pb-acetate treated males. These results are in agreement with Anjum *et al*. (2011) who revealed that significant decrease in number of pre- and post-implantation loss was observed in the females that mated with Pb-acetate treated males. In addition, significant decrease in the weight of the reproductive organs, sperm count and viable sperm were observed in Pb-acetate exposed rats. Fruits contain antioxidant compounds such as phenolics, betalains and carotenoids. Antioxidants can be defined as substances able to inhibit or delay the oxidative damage of protein, nucleic acid and lipid caused by dramatic increase of reactive oxygen species (ROS) during environmental stress (Lim *et al*., 2006). Antioxidants act by reducing free radical activity, scavenging

free radicals, potential complexion of pro-oxidant metals and quenching of singlet oxygen (Tachakittirungrod *et al*., 2006). Mango (*Mangifera indica* L.) is excellent source of the antioxidants, vitamin C and carotenoids. Several studies have shown that mango peel and seed kernel had remarkable antioxidant activity due to its high content of phenolic compounds (Abdalla *et al*., 2007; Ashoush and Gadallah, 2011; Dorta *et al*., 2012). The present study revealed that MPE and MKE possess high content of total phenolic and high scavenging activity. Interestingly, the MKE posses higher content of total phenolic than MPE. The current results showed that MKE was more potent in indicating the lead reproductive toxicity and this may be related to its high phenolic content.

The correlation between antioxidant activity and phenolic content of extracts obtained from various natural sources has been confirmed (Meyers *et al*., 2003). Several studies indicated that antioxidant treatment appears to improve sperm DNA integrity (Greco *et al*., 2005; Menezo *et al*., 2007; Tremellen *et al*., 2007). A rich daily dietary intake of antioxidants has been proposed as an alternative to improve male reproductive capacity by reducing the extent of oxidative damage in sperm cells (Song *et al*., 2006).. The antioxidant properties of flavonoids can protect the sperm DNA integrity from ROS induced by estrogenic compounds (Cemeli *et al*., 2004). Leonard *et al*. (2004) proved that toxic actions of metals are oxidative in nature and it is indicative that mango extracts was able to attenuate the toxicity of lead due to its antioxidative potential. The results of the present study demonstrated that co-administration of MPE and MKE with Pb-acetate reduced the sperm DNA damage and abnormal spermatozoa incidence induced by Pb-acetate alone. In addition, MPE and MKE relatively prevented the Pb-acetate induced histopathological changes in the testis of mice. Similarly, MPE and MKE modulated the reproductive performance of females mated with the Pb-acetate treated males showing increase of litter size and fetal body weight.

The present results were supported with several studies indicated that mango phytochemicals have antigenotoxic potential. Mangiferin (MGN), a major phenolic compound found in mango, is capable of inducing significant reduction in the level of DNA damage induced by CdCl2 (SatishRao *et al*., 2009). Rodeiro *et al*. (2012) showed that mangiferin protects against DNA damage induced by several mutagenes that would be associated with its antioxidant properties of mangiferin. El Makawy *et al*. (2015) demonstrated that mango extract reduced the DNA damage induced by Pb-acetate in liver as demonstrated by a reduction in micronuclei and decrease in tail length, tail DNA% and olive tail moment. They concluded that mango by-product have potential to protect from oxidative stress and genotoxicity of lead. Kondapalli *et al*. (2014) found that mango was demonstrated to protect DNA against UV + H2O2 and γ-irradiation induced DNA damage, confirming its protective actions in vitro and thus could be a valuable source of antioxidants. In addition, mango leaves extracts present potential use in pharmaceutical applications as chemopreventive agent of diseases associate with oxidative stress giving their numerous antioxidant and health promoting properties scientifically demonstrated (Mohan *et al*., 2013). Gil-Villa *et al*. (2009) showed that the increased intake of antioxidant rich foods improved the gestational results in a group of couples with history of recurrent embryo loss. Al-Attar (2011) indicated that the antioxidant improved the testis structures and functions of lead exposed animals. We can conclude that the extracts of mango by-product showed a relative improvement to the reproductive toxicity induced by pb-acetate in mice.

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