Polish Journal of Food and Nutrition Sciences

DE GRUYTER OPEN

Pol. J. Food Nutr. Sci., 2015, Vol. 65, No. 1, pp. 39–47
DOI: 10.1515/pjfns-2015-0009
http://journal.pan.olsztyn.pl

Original article
Nutritional Research

Evaluation of Mango Byproduct Extracts as Antioxidant Against Pb-Acetate-Induced Oxidative Stress and Genotoxicity in Mice

Aida I. El Makawy[1], Ihab S. Ashoush[2], Omaima M. Abd-Elmoneim[1]*

[1]*Cell Biology Department, National Research Centre, El Buhouth Street, Dokki, Giza, Egypt*
[2]*Food Science Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

Key words: mango byproducts, lead acetate, antioxidant activities, oxidative stress, liver dysfunction, genotoxicity

The antioxidant and antiproliferative properties of mango by-products were investigated. This study was carried out to evaluate the protective role of mango peel or kernel defatted extracts against Pb-acetate adverse effects on oxidant/antioxidant status, liver dysfunction biomarkers, histopathological changes and genotoxicity in male mice. Total phenolic content and antioxidant activity of both extracts were evaluated. Two doses of both extracts (50 and 100 mg/kg) were used to evaluate their role against the toxicity of Pb-acetate (500 ppm). Mice given mango extracts with Pb-acetate had significantly lower plasma MDA, AST and ALT and higher glutathione than mice given Pb-acetate alone. Mango extracts prevented the histopathological changes in liver induced by Pb-acetate and decreased the cytotoxicity of lead by increasing the ratio of PCE/NCE. Mango extract treatment 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. It can be concluded that mango by-product extracts have potential to protect from oxidative stress and genotoxicity of lead.

INTRODUCTION

Fruit and vegetable by-products are very rich in bioactive components, which may be a potential source of antioxidants that have a beneficial effect on health [Duda-Chodak & Tarko, 2007]. Mango (*Mangifera indica* L.) is one of the most important tropical fruits worldwide in terms of production and consumer acceptance [FAO, 2012]. Mango by-products contain significant amounts of phytochemicals, which makes them suitable to be processed for value-added applications in functional foods and nutraceuticals [Ajila *et al.*, 2007, 2010]. Mango peel contains various classes of polyphenols, carotenoids, and vitamins with different health-promoting properties, mainly antioxidant activity [Schieber *et al.*, 2003; Ajila *et al*., 2008; Manthey & Perkins-Veazie, 2009]. Mango kernels are rich sources of gallic acid, ellagic acid, ferulic acid, cinnamic acids, tannins, vanillin, coumarin, and mangiferin, all having potential to act as a source of natural antioxidants [Berardini *et al*., 2005; Soong & Barlow, 2006; Abdalla *et al.,* 2007; Ribeiro *et al*., 2008; Patthamakanokporn *et al*., 2008; Khammuang & Sarnthima, 2011; Kittiphoom, 2012].

Lead (Pb) is an environmental pollutant that can be detected in biological system and environment. Lead is known as a genotoxic agent affecting the integrity of chromosomes. It is listed as a human carcinogen on the basis of rodent tests [ARC, 1994]. Lead can inhibit the activity of many enzymes, contribute to oxidative stress, it also increases the rate of DNA single and double strand breaks, DNA–protein cross-links, induces micronuclei formation, chromosomal aberrations and causes DNA damage [Chen *et al*., 2006; Wozniak & Blasiak, 2003]. Results of some *in vitro* and *in vivo* tests detecting the genotoxic effects of lead have been reported. Poma *et al*. [2003] found Pb-acetate induced a dose-dependent increase of micronuclei and SCE in human melanoma cells. The study performed by Wozniak & Blasiak [2003] found that Pb-acetate induced DNA damage in isolated human lymphocytes by measuring the change in the comet length. Studies in humans have shown that occupational exposure to lead is associated with DNA damage as measured by micronuclei or comet assays [Fracasso *et al*., 2002; Palus *et al*., 2003; Minozzo *et al*., 2004; Olewinska *et al*., 2010].

Our objective is to assess the antioxidant activity of mango by-product extracts and to determine their ability to reduce the adverse effects of Pb-acetate in male mice. In addition, to investigate their modulator role against the liver dysfunction biomarkers, histopathological changes and genotoxicity of Pb-acetate.

MATERIALS AND METHODS

Plant

Ripe mango seeds and peels as by-products (waste) were collected after mango pulp processing from Zebda variety during the summer season of 2012 from Al-Qahera Company for Agriculture Industry, Al-Obor, Egypt.

* Corresponding Author:
E-mail: aelmakawy@yahoo.com (A. Elmakawy)

Chemicals

Pb-acetate was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate, hexane and ethanol were obtained from El-Gomhoreya Co., Cairo, Egypt. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) and Folin--Ciocalteu phenol reagent was purchased from Sigma–Aldrich Inc. (St Louis, MO, USA). While, commercial kits used for determining alanine aminotransferase (ALT), aspartate aminotransferase (AST), malonaldehyde (MDA) and reduced glutathione (GSH) were purchased from Biodiagnostic Co. Dokki, Egypt.

Defatted powder extracts preparation

Mango peels were washed with tap water to remove any dirt particles. The peels were spread thin in trays and dried at 50°C using a cross flow drier for 18 h to a moisture level around 10%. 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°C [Augustin & Ling, 1987]. The dried material was ground in a hammer mill into a powder form. Mango peels and kernel powder was defatted by steeping in hexane as a solvent overnight in dark place with gentle shaking at 20°C. The defatted powder of peels and kernel were extracted with ethanol in the ratio of 1:5; thus, 100 g of powdered was soaked overnight in 500 mL of 80%ethanol, the infusion was collected, and the sample was soaked in ethanol two more times. Each infusion was filtered and the three filtrates were combined and concentrated using a rotary evaporator under reduced pressure [Kim *et al.*, 2010]. The concentrated extracts were lyophilized and kept at –18°C until analysis for antioxidant and biological experiments start. Extracts were dissolved in DMSO to the desired concentrations (50 and 100 mg/kg) and administered by gavages as 0.1 mL/ mice/day.

Determination of total phenolic content

The total phenolic content of the extracts was determined colorimetrically, using the Folin-Ciocalteu method, as described by Singleton *et al.* [1999]. An aliquot of the samples (30 μL) was mixed with 1.8 mL of Folin–Ciocalteu reagent previously diluted with distilled water (1:10). The solution was allowed to stand at 25°C for 5 min before adding 1.2 mL of 15% sodium carbonate solution in distilled water. The absorbance at 765 nm was measured using a model UV/VIS 1201 spectrophotometer (Shimadzu, Kyoto, Japan). A blank sample consisting of water and reagents was used as a reference. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as GAE (mg/g).

Antioxidant activity (DPPH free radicals scavenge) assay

The ability of the extracts to scavenge DPPH free radicals was determined by the method described by Blois [1958]. Aliquots (30 μL) of each sample were mixed with 1.0 mL of 0.1 mmol/L DPPH in methanol. The control samples contained all the reagents except 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 decrease in absorbance against control according to the following equation:

Scavenging activity % =
= [(control absorbance – sample absorbance)/ control absorbance] x 100

Biological experiment procedure

Animals

Sixty four adult male Swiss mice, weighing 20–25 g, were obtained from the Animal House, National Research Centre, Giza, Egypt. The animals were randomly selected and housed in polycarbonate cage in temperature and humidity controlled room with a 12 h light/dark cycle and free access to standard pellet diet and drinking water. All animals were cared for and experiments were carried out in accordance with the European Community guidelines for the use of experimental animals and approved by the Ethics Committee of the National Research Centre, Egypt. Mice were acclimatized to laboratory environment during one week prior to the study.

Experimental design

Animals were divided randomly into eight groups of eight animals each as follows: (I): control group; mice received tap water as drinking water and o.1 mL DMSO by gavages. (II): Mice received tap water as drinking water and 100 mg/kg/day mango peels defatted extract (MPDE) by gavages. (III): Mice received tap water as drinking water and 100 mg/kg/day mango kernels defatted extract (MKDE) by gavages. (IV): Mice received tape water containing Pb-acetate at a dose of 500 ppm as drinking water daily for two weeks according to Nagaraja *et al*. [2011]. (V): Mice received Pb-acetate in drinking water as group (IV) and 50 mg/kg MPDE by gavages. (VI): Mice received Pb-acetate in drinking water and 100 mg/kg MPDE by gavages. (VII): Mice received Pb-acetate in drinking water as group (IV) and 50 mg/kg MKDE by gavages. (VIII): Mice received Pb-acetate in drinking water as group (IV) and 100 mg/kg MKDE by gavages. The dose of mango extracts was selected according to Pourahmad *et al*. [2010], and all experimental groups were administered daily for 14 days.

Animals were anesthetized and blood samples were collected on heparin from ocular vascular bed using capillary tubes, then centrifuged for 10 min to isolate plasma and stored at – 20°C.

Determination of malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS), the last product in lipid peroxidation pathway, in plasma at 534 nm according to Ohkawa *et al.* [1979].

Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was estimated by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to produce a yellow colored complex with absorption at 405 nm according to Beutler *et al.* [1963].

Evaluation of liver function

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method adopted by Reitman & Frankel [1957].

Histopathological investigation

Autopsy samples were taken from the liver of the euthanized mice of different groups and fixed in 10% formaldehyde saline solution. Washing was done in tap water then serial dilutions of ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin wax tissue blocks were sectioned at 4 micron thickness by sledge microtome. The sections were collected on the glass slides, deparaffinized and stained with hematoxylin and eosin for routine examination by the light electric microscope [Banchroft *et al.*, 1996].

Micronucleus assay

The micronucleus (MN) test was carried out in mice femoral bone marrow cells and frequencies of micro nucleated polychromatic erythrocytes (MNPCEs) were recorded [Chauhan *et al.*, 2000]. The femoral bone marrow cells were aspirated using syringe and needle (21G) 3.0 mL of FBS and centrifuged at 800×*g* for 10 min. The supernatant was discarded; pellet was mixed, smeared on clean glass slides and fixed in methanol for 5 min. The slides were stained with Giemsa (10%, v/v in Sorenson buffer) for 10 min and observed under light microscope.

A total number of MNPCEs among 2000 polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) per mouse was calculated to determine the induction of MN. In addition, the number of PCE/NCE ratio per animal was recorded to evaluate the cytotoxicity of Pb-acetate in bone marrow cells.

Single cell gel electrophoresis (comet assay)

The comet assay was performed as described by Tice *et al.* [2000]. Bone marrow was flushed from the femora with RPMI 1640 medium. The cell suspension (25 μL) was mixed with 250 μL of 0.5% molten low melting point agarose (LMPA) and a sample of 75 μL of the mixture were rapidly spread on comet slides pre-coated with 1% (w/v) normal melting point agarose (NMPA). Cover slips were added and the slides were allowed to gel at 4°C. After removing the cover slips, another layer of LMPA was added and allowed to solidify at 4°C. After gelling, the slides were immersed in cold freshly prepared lysis solution (2.5 mol/L NaCl, 0.1 mol/L EDTA, 10 mmol/L Tris base, 1% sodium lauryl sarcosinate and 1%Triton X-100) for 1 h at 4°C in the dark. Slides were then washed three times with neutralization buffer (0.4 mol/L Tris, pH 7.5) for 5 min and incubated in fresh alkaline buffer (0.3 mol/L NaOH and 1 mmol/L EDTA, pH<13) for 30 min at room temperature to allow the unwinding of DNA. Electrophoresis was conducted at room temperature in fresh ice-cold alkaline electrophoresis buffer for 30 min (1V/cm; 300 mA). After electrophoresis, the slides were neutralized 3 times for 5 min using Tris buffer and exposed to 70% ethanol for 5 min. After drying at room temp., slides were stained using ethidium bromide solution (25 μL/mL) and analyzed within 3–4 h using a fluorescence microscope (Olympus Optical Co., Tokyo, Japan) at 40x magnification.

Comet assay

Comet images were scored with a computerized method using comet score 15 image analysis Software (TriTek Corp., Sumerduck, VA). Scored parameters included tail length, head DNA % and Olive tail moment. The Olive tail moment is a global comet parameter expressed as [(tail mean – head mean) × (% tail DNA/100)] and used to quantify DNA damage [Olive *et al.*, 2001].

Statistical analyses

Descriptive values of data were represented as means ± standard errors. Statistical analysis 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 [Snedecor & Cochran, 1980]. Statistical analysis was conducted with SPSS software (v11.5; Lead Technologies, Inc., IL USA).

RESULTS

Bioactive compounds in MPDE and MKDE

MKDE was found to have significantly higher levels of phenolics and greater free radical scavenging activity compared to MPDE (Table 1).

Effects on lipid peroxidation

Pb-acetate group had a significantly higher level of plasma MDA than control group, and administration of either MPDE or MKDE significantly lower the plasma MDA level than that of Pb-acetate group but remained higher than control (Table 2).

Effect on glutathione concentrations

Mice treated with Pb-acetate had a significantly lower plasma glutathione (GSH) level compared to the control (Table 2), and addition of either MKDE or MPDE increased the level of plasma glutathione although no treatment reached the level of control animals.

Effects on liver function parameters

Mice treated with Pb-acetate developed significant hepatocellular damage as evident from a significant elevation in plasma activities of AST and ALT compared to control animals (Table 3). Oral administration of MKDE and MPDE concurrently with Pb-acetate resulted in a significantly reduction in AST and ALT levels.

TABLE 1. Bioactive compounds in MPDE and MKDE.

Characteristics	MPDE	MKDE
Total phenolics (mg GAE/g)	49.16 ± 0.03^{b}	78.2 ± 0.05^{a}
Scavenging activity (%)	93.89 ± 0.02^{b}	98.58 ± 0.01^{a}

Data are the mean ± SE, n = 3, Mean values in the same row bearing the same superscript do not differ significantly ($P \leq 0.05$).

TABLE 2. Effects of different treatments on plasma MDA and GSH concentrations in male mice.

Groups	MDA (nmol/mL)	GSH (mg/dL)
Control	0.13±0.004^{h}	22.92±0.32^{a}
100 mg/kg MPDE	0.21±0.003^{f}	19.24±0.21^{cb}
100 mg/kg MKDE	0.19±0.003^{g}	20.33±0.28^{b}
Pb – acetate	0.51±0.003^{a}	9.78 ±0.15^{f}
Pb – +50 mg/kg MPDE	0.37±0.006^{c}	17.24±0.42^{d}
Pb – +100 mg/kg MPDE	0.24±0.007^{e}	19.12±0.59^{cb}
Pb – +50 mg/kg MKDE	0.39±0.008^{b}	16.12±0.14^{e}
Pb – +100 mg/kg MKDE	0.28±0.005^{d}	18.33±0.14^{cd}

Data are expressed as means ± SE. Mean values in the same column within each parameter bearing the same superscript do not differ significantly (p≤0.05).

TABLE 3. Effects of different treatments on liver function in male mice.

Groups	AST (U/mL)	ALT (U/mL)
Control	53.60±0.38^{g}	41.15±0.54^{g}
100 mg/kg MPDE	55.20±0.42^{f}	43.33±0.16^{f}
100 mg/kg MKDE	57.01±0.44^{e}	45.82±0.37^{ed}
Pb – acetate	110.39±0.68^{a}	88.53±0.26^{a}
Pb – +50 mg/kg MPDE	98.20±0.71^{b}	50.13±0.56^{c}
Pb – +100 mg/kg MPDE	58.21±0.56^{e}	44.16±0.39^{ef}
Pb – +50 mg/kg MKDE	88.21±0.61^{c}	53.59±1.28^{b}
Pb – +100 mg/kg MKDE	67.01±0.33^{d}	46.42 ±0.42^{d}

Data are expressed as means ± SE. Mean values in the same column within each parameter bearing the same superscript do not differ significantly (p≤0.05).

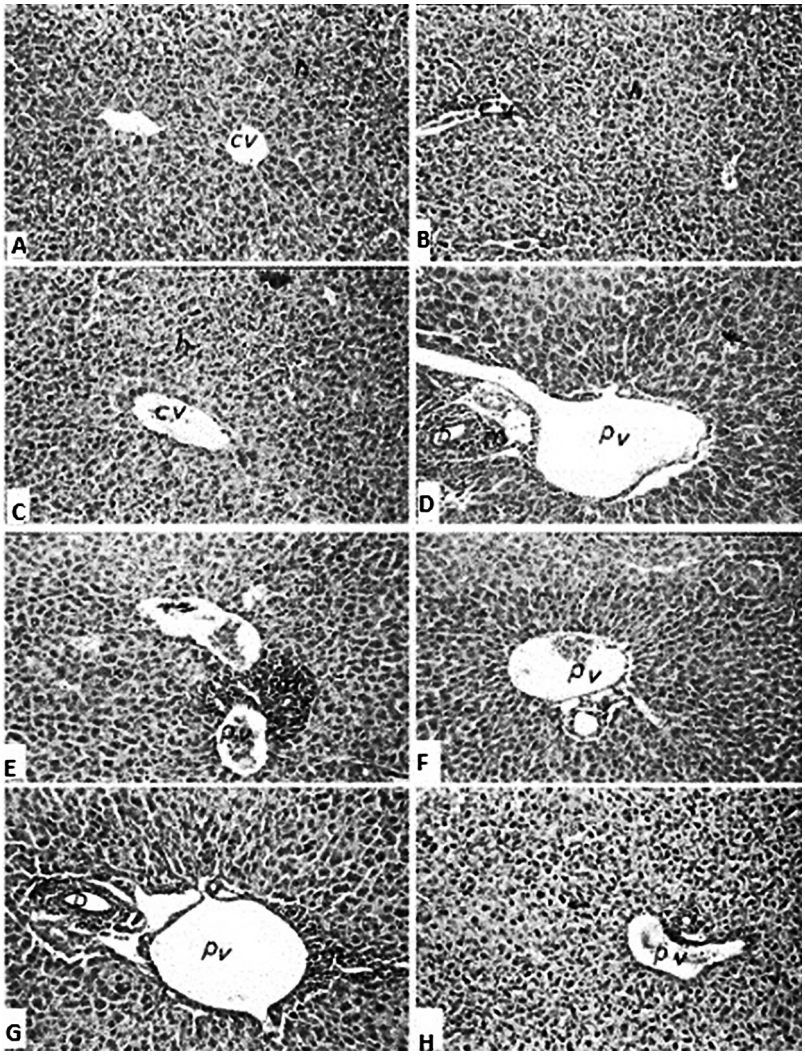

FIGURE 1. Photomicrography sections in the liver of rat in (A) control, (B) MPDE, (C) MDKE, (D) Pb-acetate, (E) Pb-acetate+50 mg/kg MPDE, (F) Pb-acetate+100 mg/kg MPDE, (G) Pb-acetate+50 mg/kg MKDE, (H) Pb-acetate+100 mg/kg MKDE groups (H & E, 40x).

TABLE 4. The severity of histopathological alterations in liver of male mice.

Treatment	Portal inflammation
Control 100mg k/g MPDE	—
100 mg/kg MPDE	—
100 mg/kg MKDE	—
Pb – acetate	++++
Pb – +50 mg/kg MPDE	+++
Pb – +100 mg/kg MPDE	—
Pb – +50 mg/kg MKDE	+++
Pb – +100 mg/kg MKDE	+

—: Nil; +: Mild; ++: Moderate; +++: Severe; ++++ Very severe effect.

Histopathological examination

Histopathological analysis of the liver found normal structure of the central vein and surrounding hepatocytes in the control mice and those receiving MKDE and MPDE alone (Figure 1A, B& C). Histopathological profiles of the liver from Pb-acetate-treated mice revealed diffuse Kupffer cells proliferation in between the hepatocytes associated with periductal inflammatory cells infiltration and severe dilatation in the portal vein in the portal area (Figure 1D) (Table 4). Liver sections of mice treated with Pb-acetate plus MPDE (50 mg/kg bw) showed massive number of inflammatory cells aggregation in the periductal tissue at the portal area in association with dilatation in the portal vein (Figure 1E). On the other hand, liver section of mice treated with Pb-acetate plus high dose of MPDE showed dilatation of the portal vein (Figure 1F). Liver sections of Pb-acetate plus MKDE (50 mg/ kg bw) treated mice showed a periductal inflammatory cells infiltration in the portal area associated with dilatation in the portal vein (Figure 1G). While, the histopathological changes in liver sections treated with Pb-acetate plus high level of MKDE revealed mild hydropic degeneration in the hepatocytes associated with dilatation in the portal vein (Figure 1H).

***In vivo* micronucleus assay**

The frequencies of micronucleated polychromatic erythrocytes (MNPCEs), the numbers of normochromatic (NCEs) and polychromatic erythrocytes (PCEs), as well as the PCE/NCE ratio for each treatment assayed in this study are shown in Table 5. Cytotoxicity evaluation of bone marrow erythrocytes showed that Pb-acetate induced significant reduction in the ratio of PCE/NCE as compared to control. While, simultaneous treatment with the extracts of mango peels and kernels resulted in a statistically higher PCE/NCE ratio when compared with Pb-acetate group. But the ratio of PCE/NCE remained lower than the control. Statistical analysis showed that there was no significant difference in the frequencies of micronuclei between the groups treated with each of MPDE and MKDE alone and the control. Nevertheless, a remarkable and statistically significant increase in the frequencies of MNPCEs was found in group of mice treated with Pb-acetate compared with control. MPDE and MKDE simultaneous treatment with Pb-acetate provides reduction in the micronuclei frequencies induced by lead as shown in Table 5.

DNA damage detection by comet assay

The comet assay results are summarized in Table 6. For Pb--acetate (Pb) tail length, tail DNA% (TDNA) and Olive tail moment (OTM) were increased ($p \leq 0.001$) relative to the control group. Pb-acetate resulted in 5.77 fold increase in TDNA value relative to control (Figure 2, Table 6). Administration of mango peel extract in two doses (50 and 100 mg/kg) concurrently with Pb-acetate resulted in 1.27 and 1.74 fold decrease in TDNA value, respectively, relative to Pb-acetate group. Meanwhile, mango kernel extract applied in two doses resulted in 1.96 and 2.06 fold decrease in TDNA value, respectively, relative to Pb-acetate group. On the same time, ingestion of DMSO and the extracts of mango peel and kernels (100 mg/kg) by the animals for 14 days did not cause any statistically significant differences.

DISCUSSION

Fruit and vegetable are the major sources of natural antioxidants and contain various antioxidant compounds. Among these compounds, phenolic compounds repre-

TABLE 5. Micronuclei frequencies and the ratio of PCE/NCE in the bone marrow cells of male mice.

Experimental groups	MNPCEs/1000 PCEs	PCEs/1000 Cells	NCEs/1000 Cells	PCE/NCE %
Control	6.40 ± 0.54^{a}	886.20 ± 1.30^{d}	113.80 ± 1.30^{b}	7.79 ± 0.10^{d}
100 mg/kg MPDE	6.00 ± 1.00^{a}	892.00 ± 2.12^{e}	106.00 ± 4.69^{a}	8.26 ± 0.18^{e}
100 mg/kg MKDE	6.60 ± 0.55^{a}	894.00 ± 4.69^{e}	108.00 ± 2.12^{a}	$8. \pm 440.42^{e}$
Pb – acetate	28.20 ± 0.83^{e}	830.00 ± 3.67^{a}	170.00 ± 3.67^{e}	4.88 ± 0.13^{a}
Pb – +50 mg/kg MPDE	21.20 ± 0.84^{d}	865.00 ± 3.96^{b}	134.00 ± 3.96^{d}	6.62 ± 0.49^{c}
Pb – +100 mg/kg MPDE	17.20 ± 0.84^{c}	875.20 ± 3.11^{c}	124.80 ± 3.11^{c}	7.02 ± 0.20^{d}
Pb – +50 mg/kg MKDE	20.20 ± 0.83^{d}	861.40 ± 5.13^{b}	138.60 ± 5.13^{d}	6.22 ± 0.27^{b}
Pb – +100 mg/kg MKDE	14.80 ± 0.83^{b}	877.80 ± 1.92^{c}	122.20 ± 1.92^{b}	7.18 ± 0.13^{d}

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

TABLE 6. Comet assay parameters in male mice bone marrow cells of all experimental groups.

Experimental groups	TL	TDNA	Fold decrease	OTM
Control	2.14±0.01 [a]	1.22±0.02[a]	5.77	2.55±0.07[a]
100 mg/kg MPDE	2.15±0.04 [a]	1.201±0.02[a]	5.82	2.58±0.05[a]
100 mg/kg MKDE	2.15±0.03 [a]	1.21±0.04[a]	5.77	2.57±0.05[a]
Pb – acetate	5.07±0.03 [f]	7.04±0.3[f]	-----	35.80± 0.02[f]
Pb-+50 mg/kg MPDE	4.93±0.02[e]	5.56±0.03[e]	1.27	27.27±0.03[e]
Pb – +100 mg k/g MPDE	4.12±0.01[c]	4.05±0.01[d]	1.74	16.65±0.02[d]
Pb – +50 mg/kg MKDE	4.28±0.02 [d]	3.60± 0.02[c]	1.96	15.48±0.03[c]
Pb – +100 mg/kg MKDE	3.58±0.02 [b]	3.42±0.02 [b]	2.06	12.25 0.02[b]

Data are expressed as means ± SE. Mean values in the same column within each parameter bearing the same superscript do not differ significantly (p≤0.05). TL: Tail length, TDNA: % Tail DNA, OTM: Olive Tail Moment.

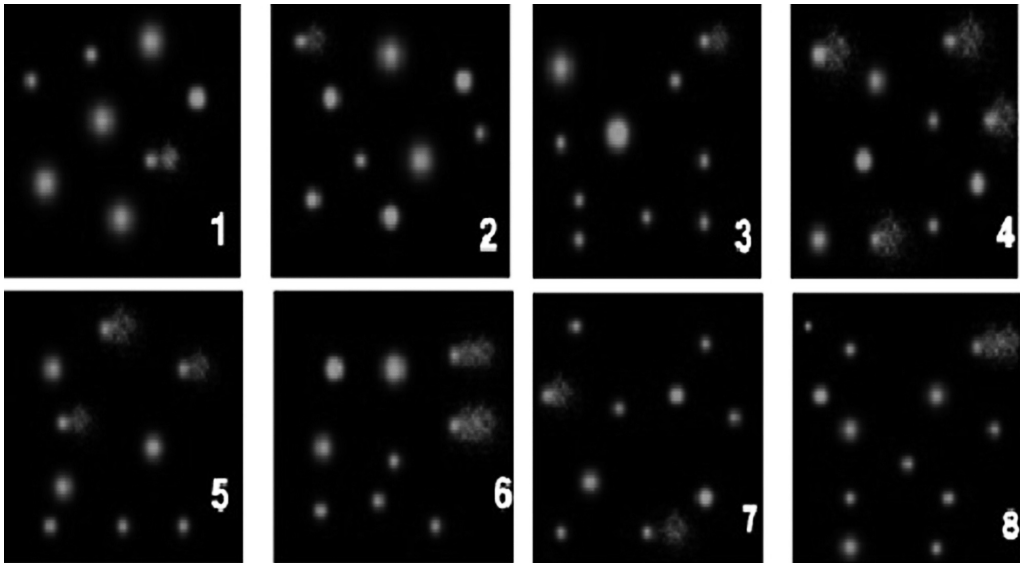

FIGURE 2. Extent of DNA damage assessed by comet assay in bone marrow cells obtained from mice of (1) control, (2) MPDE, (3) MKDE,(4) Pb--acetate (5), Pb-acetate+50 mg/kg MPDE, (6) Pb-acetate+100 mg/kg MPDE, (7) Pb-acetate+50 mg/kg MKDE, (8) Pb-acetate+100 mg/kg MKDE treated male mice.

sent a majority of natural antioxidants presently identified [Khonkarn *et al*., 2010]. They have the ability to scavenge free radicals and exhibit antimutagenic, anticarcinogenic, antiglycemic, anticholesterol, anti-inflammatory and antimicrobial properties [Friedman & Levin, 2009]. Mango peel and seed kernel had remarkable antioxidant activity due to their high content of phenolic compounds [Kim *et al*., 2010; Ashoush & Gadallah, 2011; Dorta *et al*., 2012]. The present work revealed that mango extracts possessed a high content of total phenolic compounds. These findings are in harmony with those obtained by Ribeiro *et al.* [2008]. The correlation between antioxidant activity and phenolic content of extracts obtained from various natural sources has been confirmed [Parejo *et al*., 2002; Meyers *et al*., 2003]. Under oxidative stress conditions, reactive oxygen species (ROS) have strong oxidizing ability which can induce damage to lipids, proteins and nucleic acids with the consequent change in their structures and functions [Aruoma, 2003]. Generation of highly reactive oxygen species (ROS) aftermath of lead exposure may result in systematic mobilization and depletion of the cell intrinsic antioxidant defenses [Patra *et al*., 2011]. The results of this study support the hypothesis; since there was a significant increase in the level of MDA and a significant decrease in the endogenous antioxidants following intake of Pb-acetate [Mohammad *et al*., 2008; Haleagrahara *et al.*, 2011; Aziz, 2012]. This was explained by the high affinity of lead for sulfhydryl (SH) group in several enzymes such as SOD, GSH and GPx; thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes as these enzymes are potential targets of lead toxicity [Marchlewicz *et al*., 2007]. Results of the present study illustrated that mango extracts lowered the level of lipid peroxidation, free radical generation and increased antioxidant enzymes. These results were in accordance with previous studies which demonstrated that mango extracts protected against oxidative stress induction [Ajila & Prasada Rao, 2008; Prasad *et al.*, 2008; Pourahmad *et al*., 2010; Roche & Pérez, 2012]. The mechanism responsible of lipid peroxidation inhibition may be associated with

•OH radical scavenging properties of mango extract, since •OH radicals are considered major promoters of lipid peroxidation *in vitro* and *in vivo* [Martinez *et al*., 2000; Niki, 2009]. It is known that compounds belonging to several classes of phytochemical components such as phenols, flavonoids, and carotenoids, are able to scavenge free radical such as $O2^{\bullet}$, $OH^{\bullet}$, or lipid peroxyl radical in plasma [Mandal *et al*., 2009].

Several researchers have reported that Pb-acetate developed hepatocellular damage as evident from a significant elevation in blood serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), which corroborates our findings [Mehana *et al*., 2012; Azoz & Raafat, 2012; Aziz, 2012]. Increasing of the serum activities of AST and ALT was a consequence of lead hepatotoxic effect [Todorovic, *et al*., 2005; Durgut, *et al*., 2008]. The current investigation of histopathological results also showed that Pb-acetate caused degenerative changes in liver tissue including diffuse kupffer cells proliferation in between the hepatocytes associated with periductal inflammatory cells infiltration and severe dilatation of the portal vein in the portal area. Also, several changes such as degeneration of hepatocytes, fatty changes, blood sinusoidal lumen dilatation and the appearance of infiltrated inflammatory leucocytes were observed. Aziz [2012] histological study showed that Pb-acetate caused several changes such as degeneration of hepatocytes, fatty changes, blood sinusoidal lumen dilatation and the appearance of infiltrated inflammatory leucocytes. The histological examination of liver tissue in mice treated with mango extracts revealed that most of the histological alterations induced by Pb-acetate were markedly reduced. These findings confirmed the protective effect of mango extracts against the histological changes in Pb-acetate hepatotoxicity. Also, Pourahmad *et al.* [2010] found that mango extracts possessed the potential to protect hepatocyte against all oxidative stress toxicity induced by cumene hydroperoxide (CHP).

Excess oxidative stress can lead to oxidative damage of DNA causing significant base damage, strand breaks, altered gene expression, and ultimately mutagenesis [Nisha & Deshwal, 2011]. Our results showed that Pb-acetate induced genotoxic and cytotoxic effects in male mice. Where, Pb-acetate increases the micronuclei induction and decreases the PCE/NCE. These findings were in accordance with works of other authors [Celik *et al*., 2005; Piao *et al*., 2007; Alghazal *et al*., 2008; Tapisso *et al*., 2009; Azoz & Raafat, 2012]. Also, the comet assay showed a positive effect of Pb-acetate on the induction of DNA damage, represented in increase in the comet tail length, tail DNA% (TDNA) and Olive tail moment (OTM). These results are in agreement with results of Wozniak & Blasiak [2003]; Ferraro *et al*. [2004]; Xie *et al*. [2005]; Shaik *et al*. [2006]; Osman *et al*. [2008]; and Ramsdorf *et al.* [2009] who showed a positive effect of lead on the induction of DNA damage in several tissues and organs due to the induction of DNA strand breaks. Furthermore, the present study indicated that mango extracts showed the protective role against Pb-acetate-induced DNA damage. Previous study indicated that mango peel extract protected cells from DNA damage induced by H_2O_2 [Kim *et al*., 2010]. Similar result demonstrated that mangiferin pretreatment significantly reduced the genotoxic and cytotoxic effects induced by $CdCl_2$ [Viswanadh *et al*., 2010]. In addition, mango stem bark extract (MSBE) and mangiferin showed cytoprotective effects against oxidative damage and mitochondrial toxicity induced by xenobiotics to human hepatic cells [Tolosa *et al*., 2013].

CONCLUSION

Based on the above results, it could be concluded that mango by-product extracts have the potential to protect from oxidative stress, mitigate changes in hepatic biochemical and pathological parameters and genotoxicity of lead exposure due to its antioxidant activity.

ACKNOWLEDGMENTS

This work was supported by the Ministry of State for Scientific Research, Science and Technology Development Fund, under research support grant (TC/12/ RSG /2012) grant number (4782).

REFERENCES

1. Abdalla A.E.M., Darwish S.M., Ayad E.H.E., El-Hamahmy R.M., Egyptian mango by-product 1: Compositional quality of mango seeds kernel. Food Chem., 2007, 103, 1134–1140.
2. Ajila C.M., Aalami M., Leelavathi K., Prasada Rao U.J.S., Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. Inn. Food Sci. Emerg. Technol., 2010, 11, 219–224.
3. Ajila C.M., Bhat S.G., Prasada Rao U.J.S., Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem., 2007, 102, 1006–1011.
4. Ajila C.M., Prasada Rao U.J.S., Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. Food Chem. Toxicol., 2008, 46, 303–309.
5. Alghazal M., Sutiakova I., Kovalkovicova N., Legath J., Falis M., Pistl J., Induction of micronuclei in rat bone marrow after chronic exposure to Pb-acetate trihydrate. Toxicol. Ind. Health, 2008, 24, 587–593.
6. ARC (Seventh Annual Report on Carcinogens), National toxicology program. National Institute of Environmental Health Sciences. Research Triangle Park, 1994, North Carolina.
7. Aruoma O.I., Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mut. Res., 2003, SI, 523, 9–20.
8. Ashoush I.S., Gadallah M.G.E., Utilization of mango peels and seed kernels powders as sources of phytochemicals in biscuit. World J. Dairy Food Sci., 2011, 6 35–42.
9. Augustin M.A., Ling E.T., Composition of mango seed kernel. Pertanika, 1987, 10, 53–59.
10. Aziz F.M., Protective effects of latex of *Ficus carica* L. against lead acetate-induced hepatotoxicity in rats. Jord. J. Biol. Sci., 2012, 175–182.
11. Azoz H.A., Raafat R.M., Effect of lead toxicity on cytogenicity, biochemical constituents and tissue residue with protective role of activated charcoal and casein in male rats. Aust. J. Basic Appl. Sci., 2012, 6, 497–509.

12. Banchroft J., Stevens A., Turner D., Theory and Practice of Histological Techniques. 1996, Fourth Ed. Churchil Livingstone, NewYork, London, San Francisco, Tokyo.
13. Berardini N., Fezer R., Conrad J., Beifuss U., Carle R., Schieber A., Screening of mango (*Mangifera indica* L.) cultivars for their contents of flavonol O – and xanthone C-glycosides, anthocyanins, and pectin. J. Agr. Food Chem., 2005, 53, 1563–1570.
14. Beutler E., Kelly B.M., Duron O., Improved method for the determination of blood glutathione. J. Lab. Clinic. Med., 1963, 61, 882–888.
15. Blois M.S., Antioxidant determinations by the use of a stable free radical. Nature, 1958, 181, 1199–1200.
16. Celik A., Ogenler O., Comelekoglu U., The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate. Mutagenesis, 2005, 20,411–415.
17. Chauhan L.K., Pant N., Gupta S.K., Srivastava S.P., Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbofuran exposure. Mut. Res., 2000, 465, 123–129.
18. Chen Z., Lou J., Chen S., Zheng W., Wu W., Jin L., Evaluating the genotoxic effects of workers exposed to lead using micronucleus assay, comet assay and TCR gene mutation test. Toxicology, 2006, 223, 219–226.
19. Dorta E., Lobo M.G., Gonzalez M., Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. J. Food Sci., 2012, 77, C80-C88.
20. Duda-Chodak A., Tarko T., Antioxidant properties of different fruit seeds and peels. Acta Sci. Pol. Technol. Aliment., 2007, 6, 3, 29–36.
21. Durgut R., Koc A., Gonenci R., Bal R., Celik S., Guzaf M., Altug M.E., Atesoglu O., Effects of high dose lead toxication on liver, kidney, heart, brain and blood rabbits: an experimental study. J. Appl. Biol. Sci., 2008, 2, 11–18.
22. FAO, Food and Agriculture Organization, FAOSTAT database collections, agricultural data, food and agriculture organization of the United Nations. Available from: [http://faostat.fao.org], 2012.
23. Ferraro M.V., Fenocchio A.S., Mantovani M.S., Oliveira-Ribeiro C.A., Cestari M.M., Mutagenic effects of lead (Pb II) on the fish H. malabaricus as evaluated using the comet assay, piscine micronucleus and chromosome aberrations tests. Genet. Mol. Biol., 2004, 27, 103–107.
24. Fracasso M.E., Perbellini L., Solda S., Talamini G., Franceschetti P., Lead induced DNA strand breaks in lymphocytes of exposed workers: role of reactive oxygen species and protein kinase C. Mut. Res., 2002, 515, 159–169.
25. Friedman M., Levin C., Analysis and biological activities of potato glycoalkaloids, calystegine alkaloids, phenolic compounds and anthocyanins. 2009, *in:* Advances in Potato Chemistry and Technology (eds. J. Singh, L. Kaur). Elsevier Academic Press (USA), pp. 127–161.
26. Khammuang S., Sarnthima R., Antioxidant and antibacterial activities of selected varieties of thai mango seed extract. Pak. J. Pharm. Sci., 2011, 24, 37–42.
27. Khonkarn R., Okonogi S., Ampasavate C., Anuchapreeda S., Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. Food Chem. Toxicol., 2010, 48, 2122–2129.
28. Kim H., Moon J.Y., Kim H., Lee D.S., Cho M., Choi H.K., Kim Y.S., Mosaddik A., Cho S.K., Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. Food Chem., 2010, 121, 429–436.
29. Kittiphoom S., Utilization of mango seed. Int. Food Res. J., 2012, 19, 1325–1335.
30. Manthey J.A., Perkins-Veazie P., Influences of harvest date and location on the levels of β-carotene, ascorbic acid, total phenols, the *in vitro* antioxidant capacity, and phenolic profiles of five commercial varieties of mango (*Mangifera indica* L.). J. Agri. Food Chem., 2009, 57, 10825–10830.
31. Marchlewicz M., Wiszniewska B., Gonet B., Baranowska B.I., Safranow K., Kolasa A., Glabowski W., Kurzawa R., Jakubowska K., Rac M.E., Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of mice chronically exposed to lead. Biometals, 2007, 20, 13–19.
32. Martinez G., Delgado R., Perez G., Garrido G., Nunez-Sellés A.J., Leon O.S., Evaluation of the in vitro antioxidant activity of *Mangifera indica* L. Extract (Vimang). Phytother. Res., 2000, 14, 424–427.
33. Mehana E.E., Meki A.M.A., Fazili K.M., Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Exp. Toxicol. Pathol., 2012, 64, 291–295.
34. Mandal S., Yadav S., Yadav S., Nema R.K., Antioxidants: A Review. J. Chem. Pharm. Res., 2009, 1, 102–104.
35. Meyers K.J., Watkins C.B., Pritts M.P., Liu R.H., Antioxidant and antiproliferative activities of strawberries. J. Agr. Food Chem., 2003, 51, 6887–6892.
36. Minozzo R., Deimling L.I., Gigante L.P., Santos-Mello R., Micronuclei in peripheral blood lymphocytes of workers exposed to lead. Mut. Res., 2004, 565, 53–60.
37. Mohammad I.K., Mahdi A.A., Raviraja A., Najmul I., Iqbal A., Thuppil V., Oxidative stress in painters exposed to low lead levels. Arh. Hig. Rada. Toksikol., 2008, 59, 161–169.
38. Nagaraja H., Tan J., Srikumar C., Kulur A.B., Protective effect of alpha-lipoic acid against lead acetate-induced oxidative stress in the bone marrow of rats. Int. J. Pharm., 2011,7(2), 217–227.
39. Niki E., Lipid peroxidation: physiological levels and dual biological effects. Free Radic. Biol. Med., 2009, 47, 469–484.
40. Nisha K., Deshwal R.K., Antioxidants and their protective action against DNA damage. Int. J. Pharm. Pharm. Sci., 2011, 3, 28–32.
41. Ohkawa H., Ohishi W., Yagi K., Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 1979, 95, 351–358.
42. Olewinska E.L., Kasperczyk A., Kapka L., Kozłowska A., Pawlas N., Dobrakowski M., Birkner E., Kasperczyk S., Level of DNA damage in lead-exposed workers. Ann. Agric. Environ. Med., 2010, 17, 231–236.
43. Olive P.L., Durand R.E., Banath J.P., Johnston P.J., Analysis of DNA damage in individual cells. Methods Cell Biol., 2001, 64, 235–249.
44. Osman A.G.M., Mekkawy I.A., Verreth J., Wuertz S., Kloas W., Kirschbaum F., Monitoring of DNA breakage in embryonic stages of the African catfish *Clarias gariepinus* (Burchell, 1822) after exposure to lead nitrate using alkaline comet assay. Environ. Toxicol., 2008, 23, 679–687.
45. Palus J., Rydzynski K., Dziubaltowska E., Wyszynska K., Genotoxic effects of occupational exposure to lead and cadmium. Mut. Res., 2003, 540, 19–28.

46. Parejo I., Viladomat F., Bastida J., Rosas-Romeo A., Flerlage N., Burillo J., Comparison between the radical-scavenging activity and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. J. Agric. Food Chem., 2002, 50, 6882–6890.
47. Patra R.C., Rautray A. K., Swarup D., Oxidative stress in lead and cadmium toxicity and its amelioration. Vet. Med. Int., 2011, 457327.
48. Patthamakanokporn O., Puwastien P., Nitithamyong A., Sirichakwal P.P., Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. J. Food Comp. Anal., 2008, 21, 241–248.
49. Piao F., Cheng F., Chen H., Li G., Sun X., Liu S., Effects of zinc coadministration on lead toxicities in rats. Ind. Health, 2007, 45, 546–551.
50. Poma A., Pittaluga E., Tucci A., Lead-acetate genotoxicity on human melanoma cells *in vitro*. Melanoma Res., 2003, 13, 563–566.
51. Pourahmad J., Eskandari M.R., Shakibaei R., Kamalinejad M., A search for hepatoprotective activity of fruit extract of *Mangifera indica* L. against oxidative stress cytotoxicity. Plant Foods Hum. Nutr., 2010, 65, 83–89.
52. Prasad S., Kalra N., Singh M., Shukla Y., Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice. Asian J Androl., 2008, 10, 313–318.
53. Ramsdorf W.A., Ferraro M.V., Oliveira-Ribeiro C.A., Costa J.R., Cestari M.M., Genotoxic evaluation of different doses of inorganic lead (PbII) in *Hoplias malabaricus*. Environ. Monit. Assess., 2009, 158, 77–85.
54. Reitman S., Frankel S., A colourimetric method of the determination of plasma glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 1957, 28, 56–63.
55. Ribeiro S.M.R., Barbosa L.C.A., Queiroz J.H., Knodler, M., Schieber A., Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chem., 2008, 110, 620–626.
56. Roche L.D., Pérez A.F., Protective effects of *Mangifera indica* L. extract against lipofundin induced oxidative stress in rats. Pharm. Crops, 2012, 3, 94–98.
57. Schieber A., Berardini N., Carle R., Identification of flavonol and xanthone glycosides from mango (*Mangifera indica* L. Cv. "Tomy Atkins") peels by high performance liquid chromatography–electrospray ionizations mass spectrometry. J. Agr. Food Chem., 2003, 51, 5006–5011.
58. Shaik A.P., Sankar S., Reddy S.C., Das P.G., Jamil K., Lead-induced genotoxicity in lymphocytes from peripheral blood samples of humans: *in vitro* studies. Drug Chem. Toxicol., 2006, 29, 111–124.
59. Singleton V.L., Orthofer R., Lamuela-Ravento´s R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Method Enzymol., 1999, 299, 152–178.
60. Snedecor G.W., Cochran W.G., Statistical Methods 7th Ed. 1980, Iowa State University Press, Ames, Iowa.
61. Soong Y.Y., Barlow P.J., Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longan* Lour.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. Food Chem., 2006, 97, 524–530.
62. Tapisso J.T., Marques C.C., Mathias M.L., Ramalhinho M.G., Induction of micronuclei and sister chromatid exchange in bone-marrow cells and abnormalities in sperm of Algerian mice (*Mus spretus*) exposed to cadmium, lead and zinc. Mut. Res., 2009, 678, 59–64.
63. Tice R.R., Agurell E., Anderson D., Burlinson B., Hartmann A., Kobayashi H., Miyamae Y., Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ. Mol. Mutagen., 2000, 35, 206–221.
64. Todorovic T., Dozic I., Dragana V., Pejovic J, Marjanovi M., The influence of chronic lead poisoning on the activity of some serum enzymes in rats. Acta Veterinaria (Beograd), 2005, 55, 471–482.
65. Tolosa L., Rodeiro I., Donato M.T., Herrera J.A., Delgado R., Castell J.V., Gomez-Lechon M.J., Multiparametric evaluation of the cytoprotective effect of the *Mangifera indica* L. stem bark extract and mangiferin in HepG2 cells. J. Pharm. Pharmacol., 2013, 65, 1073–1082.
66. Viswanadh E.K., Rao B.N., Rao B.S., Antigenotoxic effect of mangiferin and changes in antioxidant enzyme levels of Swiss albino mice treated with cadmium chloride. Hum. Exp. Toxicol., 2010, 29, 409–418.
67. Wozniak K., Blasiak J., *In vitro* genotoxicity of lead acetate: induction of single and double DNA strand breaks and DNA-protein cross-links. Mut. Res., 2003, 535, 127–139.
68. Xie H., Wise S.S., Holmes A.L., Xu B., Wakeman T.P., Pelsue S.C., Carcinogenic lead chromate induces DNA double-strand breaks in human lung cells. Mut. Res., 2005, 586, 160– 172.

Submitted: 9 March 2014. Revised: 8 May, 18 August, 17 September, and 23 September 2014. Accepted: 8 October 2014. Published on-line: 9 February 2015.