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Studying the Effect of Gamma-Irradiated Pomegranate Peels Aqueous Extract against Lead Toxicity in Wistar Rats

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ABSTRACT

The present study, carried out to evaluate the effect of gamma (γ) irradiation on total phenolic content and antioxidant activity of pomegranate peel powder as well as to evaluate the effect of gamma-irradiated pomegranate peels extract (GPE) against biochemical alterations of lead induced testicular and hepato-toxicity in rats. From the results, it was obtained that gamma irradiation induced significant increase in the total phenolic content and antioxidant activity of pomegranate peels powder. Concurrent treatments of lead-injected rats with GPE induced significant reduction in serum activities of liver enzymes, level of hepatic and testicular lipid peroxidation and plasma levels of caspase 3 associated with significant increase in the levels of luteinizing hormone, follicle stimulating hormone and testosterone in addition to marked improvement in the level of total antioxidant capacity and glutathione content and the activity of superoxide dismutase activity and catalase activity of the liver and testes of lead-intoxicated rats. In conclusion GPE could ameliorate the damage and apoptotic effects occurred by exposure to toxic dose of lead.

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Authors' Contribution
AMAA performed experiments. ANES contributed in the part of radiation facility. AMM performed the biological study and collected blood samples. AMAA, ANES and AMM wrote the manuscript.

Key words
Lead toxicity, Pomegranate peels, Gamma-irradiation, Apoptosis.

INTRODUCTION

Lead (Pb) is regarded as a potent toxin that used in building materials, lead acid batteries, paints, ceramic glazes and for many other purposes. Excessive dietary intake of lead has been linked with cancers of stomach, small intestine, large intestine, ovary, kidney, lungs, myeloma, all lymphomas, and all leukemia (Offor *et al*., 2017). Among the soft tissues, liver tissue is the largest depot of Pb followed by kidney cortex and medulla (Haouas *et al*., 2014). One of the possible mechanisms of Pb toxicity-induced liver injury is the disturbance of pro-oxidant and antioxidant balance by generation of reactive oxygen species (ROS) and this can induce oxidative damage of critical biomolecules such as lipids, proteins, and DNA (Bharali, 2013). Toxicity and damage induced by Pb could be reduced and recovered by administration of natural antioxidants and chelating agents that forms an insoluble complex with Pb and removes the same from Pb burdened tissue (Adikwu *et al*., 2013).

Pomegranate (*Punica granatum*) is widely used in the folk medicine of many cultures. The high antioxidant capacity of pomegranate fruit, juice and peel is related to presence of large amounts of phytochemicals compounds as polyphenols in particular, ellagitannins, condensed tannins and anthocyanins substances that able to inactivate the products of the oxidative catabolism that trigger cell disorders and damage (Ibrahim *et al*., 2016a). Moreover, pomegranate possesses radical scavenging properties in diethyl nitrosamine-induced liver injuries, reversed methotrexate toxicity in the liver by decreasing oxidative stress and liver apoptosis, and enhanced the activity of liver enzymes against toxicity- induced over production of ROS (Al-Shaaibi *et al*., 2016).

Pomegranate fruits could exposed to high risk of infestation with sucking insects and mite pests during growth which negatively affect their quality and constrain the international trade (Ananda *et al*., 2009). The application of irradiation technology in agricultural products, including fruits is considered as effective method of food processing to reduce microbial load and to extend the shelf life of product without significantly affecting the quality and chemical or sensory of food attributes (Shahbaz *et al*., 2014). Moreover, gamma (γ) irradiation (10 kGy) has also showed increased phenolic acid content in cinnamon and clove while phenolic content in nutmeg remained unaltered. Thus, the aim of this study was to investigate the effect of gamma irradiation on total phenolic content and antioxidant activity of pomegranate peel powder and also to evaluate the effect of gamma-irradiated pomegranate peels extract (GPE) against biochemical alterations of lead induced testicular and hepato-toxicity in rats.

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MATERIALS AND METHODS

Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fresh fruits of pomegranate (*Punica granatum*) were purchased from local market (Cairo, Egypt). The pomegranate peels were isolated, cleaned and air-dried. The dried pomegranate peels were grinded to coarse powder and passed through the 60 mesh screen.

Gamma irradiation treatment

The powder of dried pomegranate peels was packed in polyethylene bags and irradiated with gamma rays at dose level (10 kGy), using Indian Gamma Cell (Ge 4000 A) ^{60}Co source at a dose rate of 1.667 kGy/h at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

Preparation of aqueous extract

To obtain the aqueous extract, 700 g of either raw or gamma-irradiated dried pomegranate peels powder was boiled in 3000 mL of distilled water for 15 min with continuous stirring (Qnais *et al*., 2007). The resultant solution was filtered through a filter paper and then the filtrate was dried at 40-45°C in the incubator. Solutions were prepared by dissolving about 8.6 g of resultant powder in 100 ml distilled water.

Determination of total phenolic content and antioxidant activity

The total phenolic content (TPC) of raw and gamma-irradiated pomegranate peels was measured using by Folin-Ciocalteu colorimetric method (Yasoubi *et al*., 2007). The results were expressed as gram of gallic acid equivalents per 100 gram of dry weight (g GE/100 g DW). The antioxidant activity was measured using the ABTS ((2,2-azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid)) method (Cai *et al*., 2004). The absorbance of the reaction samples was compared to that of the Trolox standard. Trolox Equivalent Antioxidant Capacity (TEAC) was expressed in millimoles of Trolox equivalents (TE) per 100 g of dry weight (mM TE/100 g DW).

Animals and treatment

The experiment was conducted on 28 male rats (170 to 200g body weight (BWT)) purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water that were allowed *ad libitum*.

The animal were randomly divided into 4 groups, each consisted of 7 rats. Group I (Control group); rats fed on balanced diet and served as control, Group II (Pb group); rats were given lead acetate in a dose of 4mg/kg B.WT (Metwally *et al*., 2015) by orogastric tube for 30 days and Group III and IV (Pb+ RPE and Pb+ GPE); rats received lead acetate (4mg/kg B.WT) concurrently with either raw (RPE) or gamma-irradiated pomegranate aqueous peels extract (GPE) at dose level 0.43g/kg B.W. (Khalil, 2004) orally by gavage daily for 30 days.

At the end of the experiment, animals from each group were sacrificed 24 h post the last dose of treatment. Blood samples were withdrawn by cardiac puncture after slight anathesation of rats using diethyl ether and allowed to coagulate and centrifuged to get serum for biochemical analysis.

Biochemical analysis

The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957), serum γ-glutamyl transferase (GGT) was assessed according to Rosalki (1975) and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954). Enzyme-linked immunosorbent assay (ELISA) kits were used for measurement of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and caspase 3 (Wang and Keiser, 1998). Liver and testes were dissected, thoroughly washed with ice-cold 0.9% NaCl, weighed, minced and homogenized (10% w/v) using 66 mmol/L chilled phosphate buffer (pH 7.0). The tissue homogenates were centrifuged at 6000 rpm for 15 min and the supernatants were used to estimate malondialdehyde (MDA) (Yoshioka *et al*., 1979), total antioxidant capacity (TAC) (Mahfouz *et al*., 2009), glutathione content (GSH) (Beutler *et al*., 1963), superoxide dismutase activity (SOD) (Minami and Yoshikawa, 1979) and catalase activity (CAT) (Johansson and Borg, 1988).

Statistical analysis

Results were presented as mean ± SE (n = 7). Experimental data were analyzed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. The statistical analyses were performed using computer program Statistical Packages for Social Science (1998). Differences between means were considered significant at $P<0.05$.

RESULTS

The data in Table I indicated that gamma radiation processing resulted in significant increased in the total

phenolic content and antioxidant activity of pomegranate peel powder by percent change 4.2% and 13.3%, respectively.

In comparison to the control group, lead administration significantly increase the serum activities of AST, ALT, ALP and GGT and the level of hepatic and testicular MDA with an obvious reduction in the levels of testosterone, LH, FSH, TAC and GSH content and SOD and CAT activity of the liver and testes of lead-intoxicated rats. However, the concomitant administration of RPE and GPE with lead acetate significantly reduced the activities of liver enzymes and increased the levels of testosterone, LH and FSH with marked improvement in the antioxidant status and reduction in lipid peroxidation induced by lead acetate (Tables II, III). The results of this study revealed elevation in the plasma levels of caspase 3 in lead treated group when compared to control group, whereas the plasma level of caspase 3 was significantly reduced by concurrent treatment of lead-intoxicated rats with either RPE or GPE comparing to non-treated lead-intoxicated group (Fig. 1).

Table I.- Total phenolic and antioxidant activity of raw and γ-irradiated pomegranate peels powder.

Parameters	Pomegranate peels powder samples		Change
	Raw	Irradiated	
Total phenolic content (g GAE/100g DW±SD)	16.17±0.23	16.85±0.22	4.2%
Total antioxidant activity (g TE/100g DW± SD)	12.14±0.28	13.76±0.21	13.3%

Values are means of three replicates (± SD).

Table II.- Effect of raw and γ-irradiated pomegranate peels aqueous extract on serum aspartate transaminase (AST), alanine transaminase (ALT), γ-glutamyl transferase (γGT) and alkaline phosphatase (ALP) activities and testosterone (T), luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in rats treated with hepatotoxic dose of lead acetate.

Groups	Control	Pb	Pb + RPE	Pb + γPE
AST (U/ml)	36.23±0.80^{c}	63.69±1.12^{a}	49.12±0.92^{b}	47.38±0.85^{b}
ALT (U/ml)	27.16±0.57^{d}	49.26±0.82^{a}	35.28±0.76^{b}	31.91±0.72^{c}
ALP (U/100ml)	8.84±0.06^{d}	17.80±0.09^{a}	12.22±0.07^{b}	10.52±0.06^{c}
γ GT (U/ml)	4.36±0.44^{c}	7.19±0.58^{a}	5.86±0.46^{b}	5.73±0.58^{b}
T (n mol/L)	4.90± 0.15^{a}	2.39±0.11^{c}	4.15±0.14^{b}	4.23± 0.13^{b}
LH (IU/L)	0.92± 0.10^{a}	0.50±0.08^{c}	0.76±0.09^{b}	0.79±0.11^{b}
FSH (IU/L)	0.81± 0.08^{a}	0.60±0.06^{c}	0.71 ± 0.07^{b}	0.72± 0.06^{b}

Values are expressed as means ± S.E. (n=7). Values in the same column with different superscript are significantly different at P<0.05. RPE, raw pomegranate peels aqueous extract; γPE, γ-irradiated pomegranate peels aqueous extract.

Table III.- Effect of raw and γ-irradiated pomegranate aqueous extract on hepatic and testicular malondialdehyde (MDA) and antioxidant status in rats treated with toxic dose of lead acetate.

Parameters		Control	Pb	Pb + RPE	Pb + γPE
MDA (n mol/g tissue)	Liver	171.10±4.11^{c}	306.21±6.24^{a}	195.33±4.72^{b}	191.76±4.62^{b}
	Testes	131.65±4.48^{d}	232.15±5.27^{a}	174.36±4.13^{b}	163.11±4.38^{c}
TAC (U/mg protein)	Liver	0.72±0.04^{a}	0.49±0.03^{c}	0.61±0.03^{b}	0.63±0.04^{b}
	Testes	0.68±0.05^{a}	0.42±0.04^{c}	0.56±0.04^{b}	0.58±0.03^{b}
GSH (mg/g tissue)	Liver	28.45 ±2.54^{a}	18.74±1.56^{c}	25.00±0.47^{b}	24.69±0.45^{b}
	Testes	20.10±0.61^{a}	11.85±0.52^{d}	16.27±0.48^{c}	17.88±0.41^{b}
SOD (U/mg protein)	Liver	49.74 ±3.82^{a}	27.96±2.78^{c}	41.77±3.18^{b}	41.92 ±3.15^{b}
	Testes	20.76±0.68^{a}	12.56±0.51^{d}	16.75±0.68^{c}	18.08±0.57^{b}
CAT (U/mg protein)	Liver	54.27±1.57^{a}	32.26±1.96^{d}	45.15±1.36^{c}	48.22±1.71^{b}
	Testes	33.28±0.56^{a}	17.02±0.58^{d}	25.18±0.69^{c}	28.24±0.73^{b}

TAC, total antioxidant capacity; GSH, glutathione content; SOD, superoxide dismutase activity; CAT, catalase activity. For other abbreviations and statistical details, see Table II.

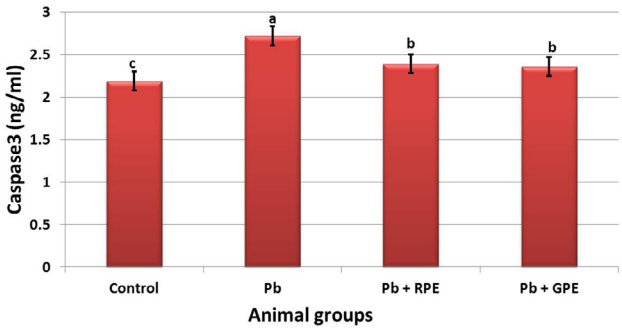

Fig. 1. Effect of raw and gamma-irradiated pomegranate aqueous extract on Caspase 3 in rats treated with toxic dose of lead acetate. For abbreviations and statistical details, see Table II.

DISCUSSION

Pomegranate (*Punica granatum*) is used for the prevention and treatment of liver diseases and can reduce tissue damage due to its high antioxidant activity. In this work, the total phenolic content of raw peel powder was determined to be 16.17 ± 0.23g GE/100 g DW in the control that was increased by 4.2% after γ-irradiation (at 10 kGy). This increase in total phenolic content could be attributed to the ability of γ-rays to induce degradation of tannins present in pomegranate peels powder having higher molecular weight into the release of simple phenolic compounds like gallic acid, tannic acid, etc. Irradiation may break this complex to facilitate release of active ingredients, which were contributed to increase the total phenolic content (Kumari *et al*., 2009; Mounir *et al*., 2019). Also, the result shows a significant increase in antioxidant activity values from raw pomegranate peels powder to γ-irradiated pomegranate peels powder (10 kGy) (12.14±0.28 and 13.76±0.21 g TE/100g DW, respectively). This increase could be attributed to enhanced free phenolics of the irradiated samples which enhance the antioxidant properties. The enhanced antioxidant capacity/activity of a plant after irradiation is mainly attributed either to increased enzyme activity (*e.g.* pheny- lalanine ammonia-lyase and peroxidase activity) (Alothman *et al*., 2009). Also, similar results were obtained by Kumari *et al*. (2009) wherein they have found out a significant increase in gallic acid concentration and total phenolics in the water extract due to irradiation that leads to increase in antioxidant property.

The data showed that lead exposure caused significant increase in the activity of serum AST, ALT, ALP and GGT suggesting hepatotoxic effect of Pb. Large part of Pb is accumulated in the liver and can induced hepatocytes damage by destroying the permeability of the cell membrane, with resultant release of cellular enzymes leading to increase their serum values (Todorovic *et al*., 2005). Furthermore, Nabil *et al*. (2012) found that the elevation of AST and ALT activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue. On the other hand, injection of RPE or GPE to lead-intoxicated rats resulted in remarkable reduction in the activity of serum AST, ALT, ALP and GGT compared to lead acetate administrated group which may be due to potent antioxidant and hepato-protective properties of pomegranate Ashoush *et al*. (2013). Salim *et al*. (2014) concluded that pomegranate administration reduced the activity of liver enzymes against dimethoat-induced hepatic toxicity due to the powerful antioxidants (polyphenols, total phenols, and total flavonoids) which present in high levels in pomegranate which allow pomegranate to do as reducing agents, metal chelators, hydrogen donors and singlet oxygen quenchers.

In this work, administration of lead acetate to male rats resulted in pituitary and gonadal dysfunctions manifested by a marked decrease in the levels of LH, FSH and testosterone. Hamed *et al*. (2014) reported that Pb administration significantly decreased the plasma levels of gonadotrophic (LH and FSH), and gonadal hormones (estradiol, progesterone and testosterone). The induction of gonadal impairment by Pb might be due to the direct effect of Pb on gonads and/or central effect on the pituitary gland (Hamed *et al*., 2014). It has been shown that exposure to environmental contaminants adversely affects testicular function by decreasing pituitary LH secretion and reducing Leydig cell steroidogenesis (Murugesan *et al*., 2007). Wang *et al*. (2013) suggested that reduction in the plasma level of testosterone was due to inhibition of testicular key steroidogenic enzyme. Treatment of rats with either RPE or GPE ameliorated the toxic effects of lead acetate and the levels of testosterone, FSH and LH were increased. Hong *et al*. (2008) found that the increase in sex hormones by pomegranate can be due to the ability of pomegranate to reduce stress hormones, such as cortisol.

In this study, a significant elevation in the level of MDA and significant reduction in the level of TAC, GSH, SOD and CAT activity were observed in the liver and testes of lead-intoxicated rats when compared to the other groups. The oxidative damage induced by Pb might be due to the direct depletion of antioxidant reserves and generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by MDA levels as the final products of lipid peroxidation (El-Nekeety *et al*., 2009). The increased MDA level observed in the present study was also due to decreased SOD activity, an indicator of oxidative stress. The possible explanation could be related to the proposed role of GSH in the active excretion of Pb through bile by

binding to the thiol group of GSH and then being excreted (El-Tantawy, 2016). However, the increased in hepatic and testicular lipid peroxidation significantly reduced and the decreased in TAC, GSH, SOD and CAT activity significantly increased when lead-intoxicated rats received RPE or GPE. The antioxidant effects and detoxification activity of pomegranate peels could be attributed to improve the immunity by activation antioxidant enzymes such as glutathione (GSH) and glutathione peroxidase (GPx) and preventing oxidative damage. In addition, pomegranate possess strong free radicals scavenging and antioxidant properties due to its high nutritive value, fibers and a variety of biologically active compounds (tannins, phenolic acids, estrogenic flavonoids) (Salim *et al*., 2014). Parmar and Kar (2007) found that administration of pomegranate peel extract in atherogenic diet-fed animals significantly reduced the tissue (hepatic, cardiac, and renal) and serum lipid peroxidation as compared to the respective values of atherogenic diet-fed animals.

The results of this study indicated that lead provokes apoptosis in the rat testis and liver and that revealed by elevation in the hepatic and testicular levels of caspase 3 in lead treated group when compared to control group. Agarwal *et al*. (2009) revealed that stimulation of caspase cascade and simultaneous extracellular signal-regulated kinase (ERK) dephosphorylation are the most significant operative pathways directly associated with apoptotic signals triggered by lead acetate in adult rat hepatic stem cells. Treatment of lead-intoxicated rats with RPE and GPE induced reduction in caspase-3 which may be attributed to the antioxidant and anti-inflammatory activities of the active constituents of the extract. Ibrahim *et al*. (2016b) concluded that pomegranate peels extract reduced apoptosis by decreasing caspas-3 level and decreases fibrosis by reducing collagen accumulation in the hepatic tissues. Na *et al*. (2015) pretreatment with pomegranate peels reduced the induction of apoptotic proteins indicating that pomegranate peels decreases apoptosis in hepatocytes.

CONCLUSION

In this work it was obtained that γ-radiation processing induced significant elevation in the antioxidant and total phenolic contents of pomegranate peel powder and that could be useful and help to boost the international marketing and consumer acceptability of this fruit. Also, this study concluded that γ-irradiated pomegranate Peels aqueous extract has therapeutic effects on oxidative damage-induced by Pb administration through its antioxidant and anti-apoptotic activity and could be used as a dietary supplement to human populations exposed to lead toxicity.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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