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Ameliorating Effect of Gamma-irradiated Moringa Leaves Against Arsenic-Induced Oxidative Damage in Rat Liver

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ABSTRACT

The objective of this study was to investigate the effect of gamma (γ)-irradiation on the microbiological quality and phenolic components of dried *Moringa oleifera* leaves (ML) powder and to compare the effect of raw and γ-irradiated ML against arsenic-induced hepatotoxicity. The results of the present study showed that gamma irradiation of ML improved its microbiological quality and increased the percentage of phenolic compound kaempferol. The results also revealed that male albino rats fed on a diet containing sodium arsenite $NaAsO_2$ (200mg/kg) for 6 weeks displayed increased activities of serum alkaline phosphatase, γ-glutamyl transferase and transaminases and increased malondialdehyde level and xanthine oxidase activity concomitant with a decrease in glutathione level and xanthine dehydrogenase, superoxide dismutase and catalase activities. The addition of ML (200mg/kg) either gamma-irradiated or not to the diet of rats has significantly ameliorated the alteration in the antioxidant/oxidant status and improving liver function enzymes. It is concluded that ML modulates hepatic toxicity associated with oxidative stress and the γ-irradiation of ML increased its hygienic quality without a significant loss in its quality attributes.

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INTRODUCTION

Spices and aromatic herbs are well known for their useful phytochemical constituents. However, these plant materials are often highly contaminated by microbes that can occur from the plants themselves, soil, water, air and dust during post-harvest storage and processing, and it could be a cause of serious food-borne illness (Seo *et al*., 2007). As utilization of the aromatic herbs in food and bio-industry increases, mass production and the supply of these materials with a high quality are required (Yu *et al*., 2004). Various conventional methods of sterilization and reduction of microbial loads are used. These methods are, however, recognized as less safe, and are now prohibited or being increasingly restricted in most countries because of loss of colour, flavour and aroma as well as deposit of chemical residues in various products (Adu-Gyamfi and Mahami, 2014). Instead microwave, gamma radiation and ozone are now widely used to reduce microbiological contamination (Zhao and Cranston, 1995).

Many studies dealing with the use of radiation technology for medicinal and aromatic herbs and spices have concluded that radiation can be considered radiologically hygienic and toxicologically safe technology (Adu-Gyamfi and Mahami, 2014). However, questions focusing on loss of phytochemical constituents, free radicals and radiolytic by-product formation, and changes of antioxidant properties during irradiation are still being debated (Suhaj *et al*., 2006). Gamma irradiation of almond skins above 4 kGy enhanced the antioxidant activity (Harrison and Were, 2007).

Arsenic (As) toxicity is a global health problem affecting millions of people and animals (Tanju and Madhuri, 2013). It is a naturally occurring metalloid, ubiquitously present in the soil and vegetation. Humans may be exposed to arsenic via ingestion through drinking water (major), inhalation and skin absorption (Gupta *et al*., 2005). Arsenic toxicity also induces generation of reactive oxygen species (ROS), which may lead to membrane damage, oxidative stress and carcinogenesis of various organs and subsequent cellular damage and organ disorders (Tanju and Madhuri, 2013).

Moringa oleifera Lam, is the most widely distributed species of the Moringaceae family throughout the world, especially in Asian and African countries, having a remarkable range of pharmacological properties in addition to significant nutritional value. The various plant parts have wide medicinal applicability for the treatment of cardiovascular diseases. The roots, leaves, gum, flowers and seed infusion contains important bioactive constituents, which are thought to be responsible for their diuretic, cholesterol lowering and anti-ulcer properties (Fakurazi *et al*., 2012). Moringa leaves contain a rich and rare combination of zeatin,

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quercetin and kaempferom that have shown potent anticancer, anti-inflammatory and antifungal activities (Patel *et al*., 2010). For that, *M. oleifera* leaves were used as nutritional supplement and growth promoters (Sanchez *et al*., 2006).

Therefore, the aim of this study was to study the effect of γ-irradiation on microbiological and total phenolic contents of dried moringa leaves (ML) and also to determine the effect of raw and γ-irradiated ML against arsenic-induced liver oxidative damage in rats.

MATERIALS AND METHODS

Gamma irradiated ML

Dried ML were purchased from herbal market (Cairo, Egypt) ground to fine powder, sieved through No. 20 mesh size and stored in an air tight container. Sodium arsenite ($NaAsO_2$) and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Dried ML were transferred into polyethylene bags and treated with 10 kGy of gamma rays, using a Cobalt-60 source (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), located at the National Center for Radiation Research and Technology (NCRRT), in Nasr City, Cairo, Egypt. The dose rate of the irradiation process was 2.50 kGy/hour calculated according to the dosimeter department in the NCRRT.

Microbiological analysis

Samples of either raw or γ-irradiated dried ML were analyzed for total viable cells, coliforms, moulds and yeasts, *Escherichia coli, Salmonella* spp. and *Pseudomonas* spp. by using the serial dilution and pour plate methods (APHA, 1976). Ten grams of each samples was added to 90-ml peptone water (1% peptone + 0.5% NaCl) and placed on a mechanical shaker for 15 min. The mixture was then allowed to settle for about 5 min to allow coarse material to settle down. Microbial load determination was carried out on the supernatant by using serial dilution and pour plate methods. Total viable cells were estimated on plate count agar at 37°C for 48 h, colifoms on violet red bile agar at 37°C for 48 h. *E. coli* was estimated on eosin methlyene blue agar at 37°C for 48 h. *Pseudomonas* spp. was enumerated on *Pseudomonas* agar base and C-N supplement at 37°C for 48 h. Moulds and yeast were estimated on oxytetracycline glucose yeast extract agar with incubation at 28°C for 48 h. The presence of *Salmonella* spp. was shown by plating on xylose lysine deoxycholate agar with incubation at 37°C for 48 h. For each sample, duplicate plating was carried out. Three independent experiments were conducted.

For data analysis the microbial counts (colony forming units, cfu/g) were transformed into logarithms (log_{10}) and means were determined.

Determination of total phenolic compounds

The water extract of raw and γ-irradiated ML was prepared by adding 10 g of dried *Moringa* into 100 mL of hot water for 15 min with constant stirring and was vacuum-filtered twice and centrifuged for 10 min.

The total phenolic contents of raw and γ-irradiated ML water extract were determined by using the Folin-Ciocalteau calorimetric method (Tsantili *et al*., 2010). The total polyphenol content was compared to that of gallic acid standard curve previously prepared covering the concentration 20 to 100 µg/mL. Samples were measured in triplicate analysis.

Determination of phenolic compounds of raw and treated Moringa

Ground dry ML powder (10 g) was weighed in a test tube, to which 100 ml of 80% aqueous methanol was added and the suspension was slightly stirred and sonicated twice for 15 min then one left at room temperature (~20°C) for 24h. The extract was centrifuged for 10 min at 1500 x g, and supernatants were filtered through a 0.2 µm Millipore membrane filter. The filtrate (1-3 ml) was used for analysis of phenolic compounds with high performance liquid chromatography (HPLC) according to the method of Goupy *et al*. (1999). The phenolic standards from Sigma Chemical Co (St. Louis, MO, USA) were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for calculation of phenolic compounds concentration by the data analysis of Hewllet packared software.

Biological study

Animals

Male albino Sprague Dawley rats (10±2 weeks old; 120±20 g) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimatized to controlled laboratory conditions for two weeks. Rats were maintained on stock rodent diet and tap water *ad libitum*. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Experimental design

The animals were randomly divided into four groups each of seven rats. Group I served as normal control and were fed on standard commercial diet. Group

II (As) rats were fed on commercial diet containing sodium arsenite ($NaAsO_2$) (200mg/kg) for 6 weeks. Group III (As+ rML) rats were fed on commercial diet containing $NaAsO_2$ (200mg/kg) and raw ML powder (200mg/kg) for 6 weeks. Group IV (As+IRR ML) rats were fed on commercial diet containing $NaAsO_2$ (200mg/kg) and γ-irradiated ML powder (200mg/kg) for 6 weeks.

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. Also, liver was removed for biochemical investigation.

Biochemical analysis

Liver was 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 homogenate was centrifuged (200×g) for 20 min at 4°C and the supernatants were used to estimate the level of malondialdehyde (MDA) according to Yoshioka *et al*. (1979). Hepatic xanthine oxidase (XO) and xanthine dehydrogenase (XDH) were determined according to Kaminski and Jewezska (1979). Hepatic glutathione content (GSH) and the activity of superoxide dismutase (SOD) and catalase (CAT) were measured by the method of Gross *et al*. (1967), Minami and Yoshikawa (1979) and Aebi (1984), respectively.

The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957), serum gamma glutamyl transferase (γGT) according to Rosalki (1975) and serum alkaline phosphatase activity (ALP) was estimated according to Kind and King (1954).

Statistical analysis

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 (SPSS) (SPSS, 1998). Differences between means were considered significant at $P < 0.05$.

RESULTS

The results showed no microorganisms detected in irradiated ML (Table I). The total phenolic content of raw and γ-irradiated ML was 121±3 and 126±3 mg Gallic acid equivalent (mg GAE) /g ML, respectively (Table II). The phenolic compounds were identified by HPLC against standard compounds and the results listed in Table II. Kaempferol was found to be the major bioactive compound of raw ML (4.752mg/100g) and its concentration was increased by γ-irradiation to 5.326 mg/100g (Table II).

Table I.- Microbiological counts (Mean±SD) of raw and γ-irradiated dried *Moringa oleifera* leaves powder (ML) (log10 cfu/g).

	Raw ML Mean ± SD (n=3)	γ-Irradiated ML (10 kGy) Mean ± SD (n=3)
Total viable count	6.10± 0.11	not detected
Coliforms	6.46±0.07	not detected
Moulds and yeasts	2.73±0.68	not detected
E. coli	1.29±0.19	not detected
Pseudomonas sp.	3.44±0.23	not detected

Table II.- Phenolic compounds of raw and γ-irradiated dried *Moringa oleifera* leaves powder (ML) (mg/100g).

Phenolic compounds	Raw ML	γ-irradiated ML
Gallic	0.908	1.256
3,4 dihydroxybenzoic	0.134	0.172
Kaemperfrol	4.752	5.326
Catechin	0.239	0.368
Chlorogenic	0.318	0.776
Caffeic	---	0.113
Vanillic	0.027	0.025
Caffeine	0.041	0.044
Ferulic	0.117	0.131
P. coumaric	0.561	0.573
Apigenin	0.032	0.028
Hesperidin	0.116	0.111
Cinnamic	0.052	0.077
Chrysin	0.017	0.034
Total Phenolic Content (mg gallic acid equivalent/g ML)	**121 ± 3**	**126 ± 3**

The activity of ALT, AST, ALP and γGT in the serum was significantly ($P<0.05$) increased in the arsenic-treated rats compared to control (Table III). Concomitant administration of ML with arsenic has significantly improved the activity of these liver enzymes.

Administration of arsenic to the diet of rats led to a significant depletion in XDH, SOD and CAT activities and GSH content in the liver of rats (Table IV). These changes were accompanied by a significant increase in the concentration of MDA and the activity of XO. On the

Table III.- Effect of raw and γ-irradiated dried *Moringa oleifera* leaves powder (ML) on alanine and aspartate transaminases (ALT and AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) activity in the serum of As-fed rats.

Parameters	Control	As	As+ ML	As+γ-irradiated ML
AST (U/ml)	27.71±2.23[a]	49.61±2.78[d]	36.51±2.31[c]	32.22±1.96[b]
ALT (U/ml)	17.63±1.91[a]	38.86±2.25[d]	29.21±1.69[c]	24.49±2.01[b]
ALP(U/dl)	9.75±1.72[a]	16.49±2.36[d]	13.11±1.62[c]	11.86±1.93[b]
γ-GT (U/ml)	3.55±1.25[a]	6.82±1.69[c]	4.82±1.38[b]	4.11±1.29[b]

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.D. (n=7).

Table IV.- Effect of raw and γ-irradiated dried *Moringa oleifera* leaves powder (ML) on oxidants and antioxidant levels in the liver of arsenic fed rats.

Parameters	Control	As	As+ ML	As+γ-irradiated ML
MDA (nmol/g tissue)	186±13.54[a]	394±13.94[d]	255±12.14 [c]	237±11.89[b]
XO (mU/mg protein)	2.48±0.21[a]	3.80±0.24[d]	2.91±0.19[c]	2.62±0.16[b]
XDH (mU/mg protein)	3.21±0.48[a]	1.63±0.37[d]	2.77±0.42[c]	2.95±0.39[b]
GSH (mg/g tissue)	27.76±2.49[a]	15.91±1.88[c]	22.97±2.17[b]	23.57±1.93[b]
SOD (U/mg protein)	45.37±2.94[a]	31.23±2.54[d]	39.28±2.23[c]	42.83±2.36[b]
CAT (U/g protein)	3.33±0.08[a]	1.82±0.05[c]	2.78±0.08[b]	2.91±0.08[b]

Means in the same column with different superscripts are significantly different at (P<0.05), Values are expressed as mean ± S.D. (n=7).
CAT, catalase; GSH, glutaathine; MDA, malondialdehyde; 4, SOD, superoxide dismutase; XDH, xanthine dehydrogenase; XO, xanthione oxidase.

other hand, co-administration of ML with arsenic has significantly improved these biochemical alterations (Table IV).

DISCUSSION

The arsenic toxicity is mediated via generation of ROS, which in turn lead to the development of carcinogenesis and other cytotoxic effects. ML contain different classes of phytocompounds, vitamins and carotenoids and these compounds mainly contribute to the antioxidant properties as well as other biological activities.

The results of the present study indicated that γ-irradiation significantly improved the microbiological quality of dried ML powder. These findings are in agreement with the study of Adu-Gyamfi and Mahami (2014) that no survivors of viable cells, coliforms, moulds and yeasts, *E. coli* and *Pseudomonas* spp were detected in the samples of dried ML irradiated with 10 kGy. These findings are expected since γ-irradiation has been shown to be generally effective in reducing the microbial load of dehydrated ingredients, spices and dried herbal products (Adu-Gyamfi, 2005). It is important to further note that γ-irradiation overcomes shortcomings such as chemical residues, loss of heat-sensitive active ingredients and discoloration associated with conventional technologies such as the use of chemical preservatives, heat, fumigants (Farkas, 1998).

The mechanism by which radiation kills microorganisms is by splitting water molecules into hydrogen (H^+), hydroxyl (OH^-) and oxygen (O^{-2}) radicals. These radicals react with and destroy or deactivate microbial components such as DNA, proteins and cell membranes (Niemira and Sommers, 2006).

Moreover, the present results indicated that γ-irradiation of ML resulted in an increase in the total phenolic content. Villavicencio *et al.* (2000) concluded that γ-irradiation could increased the total phenolic contents compared with raw samples and that might be due to the decomposition of some insoluble phenolic compounds or could be attributed to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by γ-irradiation (de Camargo *et al.*, 2012).

The liver is the major target organ for sodium arsenite induced hepatotoxicity because metabolism of As involved only in liver (Muthumani and Milton Prabu,

2013). The present study illustrated that As administrated to rats caused a significant increase in the activity of AST, ALT, ALP and GGT which could be due to severe damage of hepatocyte membranes. Arsenic is known to exert its toxicity through the generation of ROS, that can modify liver membrane lipid through peroxidation which may eventually lead to liver membrane damage (Oyewole, 2011).

In the present study, liver marker enzymes were significantly reduced after treatment with leaf of *M. oleifera* in animals with As toxicity. It was reported that moringa decreased hepatic markers enzymes against As induced toxicity in rats (Kumar and Pari, 2003).

The hepatoprotective action of ML may be due to its natural antioxidants content (Kanter, 2010) that protect the structural integrity of hepato cellular membrane, or via healing of hepatic parenchyma and the regeneration of hepatocytes (Ahmed and Khater 2001) thus preventing enzymes leakage into the blood circulation and consequently enhance the recovery from hepatic damage (Anwar *et al*., 2007).

In the present study, arsenic treatment has provoked oxidative damage in liver tissues demonstrated by significant increases in the level of MDA content and XO activity accompanied by significant decreases in the level of GSH content and the activity of XDH, SOD and CAT. The antioxidant enzymes both preserve cellular stability and play an important role in keeping free radicals away (Kavasoğlu *et al*., 2015). The results corroborate the findings of Flora *et al*. (2002) that As administration induced lipid peroxidation in blood, liver and kidney of rats. MDA production could be due to the impairment of cells' natural protective system and could be directly related to the GSH depletion.

The decrease of GSH content could be due interpreted by the fact that As interacts with SH groups in GSH leading to their oxidation and thus damaging proteins and enzymes requiring SH groups (Tanju and Madhuri, 2013). The decrease in SOD and CAT activity following As administration may be attributed to enhanced superoxide radical production during As metabolism and increase in superoxide radicals inhibits catalase activity (Bhattacharjee *et al*., 2013).

On the other hand, addition of either raw or of γ-irradiated ML to the diet containing As modulates liver damage noticed by a remarkable reduction in the level of MDA content and XO activity in the liver of rats associated with significant increment in the concentration of GSH content and the activity of XDH, SOD and CAT. The amelioration may be attributed to its free radical scavenging activity, decreasing lipid peroxides and enhancing antioxidants (Sharma and Rajani, 2011). Moreover, the leaves of *M. oleifera* (raw or irradiated) are rich in different classes of phytocompounds, ascorbic acid (vitamin C) and flavonoids as carotenoids, alkaloids, proanthocyanidins (Das and Kanodia, 2012). Pari and Kumar (2002) hypothesized that the β-carotene of moringa is responsible for the hepatoprotective activity as β-carotene may exhibit a good radical trapping antioxidant activity. Besides carotene, moringa is also reported to contain antioxidants such as vitamin C that might be responsible for the antioxidant and radio-protective properties of moringa in rats subjected to whole body gamma irradiation (Rao *et al*., 2001).

Gamma irradiation can be considered effective to improve the quality of plant materials in terms of reduction of microbial contamination (Harrison and Were, 2007). Gamma irradiation significantly improved the microbiological quality of dried ML and increased the percentage of some phenolic compound such as kaempferol. Flavonoids are phenolic substances which have antioxidant property that counteracts the oxidative stress (Sharma and Rajani, 2011).

Therefore, in order to meet national and international standards, it is recommended that γ-irradiation should be integrated into processing protocols of moringa products to ensure acceptable microbiological quality for both domestic and export markets.

Based on the results obtained in the current study, it could be concluded that raw as well as γ-irradiated *M. oleifera* leaves may protect against hepatotoxicity associated with oxidative stress.

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