Original Article

Protective Effect of *Moringa oleifera* on γ-Radiation-Induced Hepatotoxicity and Nephrotoxicity in Rats

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ABSTRACT

Ionizing radiation generates reactive oxygen species that induce oxidative stress, which generates free radicals, which associated with many degenerative diseases. The present study has been designed to evaluate the ameliorative role of aqueous moringa oleifera leaf extract (MO) against γ-radiation (IRR)-induced oxidative stress in hepatic and renal tissues in rats. Twenty four male albino rats were divided into four groups, (1) control group injected with the vehicle, (2) MO treated group, (3) IRR group, (4)MO/ IRR treated group. Biochemical and ultra structural examinations were utilized for evaluation of the oxidative stress, hepatotoxicity and nephrotoxicity. IRR (6Gy) caused a significant increase in liver and kidney malondialdehyde (MDA) and total nitrate/nitrite (NO (x)) levels and significant decrease in superoxide dismutase (SOD), catalase (CAT) activities and glutathione (GSH) content. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and the levels of urea nitrogen and creatinine in serum were increased. Administration of MO (300 mg/kg, i.p.) for 15 days prior to IRR ameliorated the hepatotoxicity and nephrotoxicity induced by IRR. Ultra structural examination of liver and kidney tissues confirmed the biochemical data. The present results revealed that MO has a protective effect against IRR-induced hepatotoxicity and nephrotoxicity through its free radical scavenging activity and enhancement of the antioxidant defense mechanisms.

Keywords: Moringa oleifera; Gamma radiation; Oxidative stress; Hepatotoxicity; Nephrotoxicity.

INTRODUCTION

Radiotherapy is an essential therapeutic modality of a wide variety of tumors, but its acute side effects on the normal tissues limit the effectiveness of therapy. Ionizing radiation absorption by living cells can directly disrupt atomic structures, producing chemical and biological modifications. It can also act indirectly through radiolysis of water, thereby generating reactive oxygen species (ROS) that may induce oxidative damage to essential biomolecules such as proteins, DNA, lipoproteins and lipids[1,2]. The generation of these free radicals induced oxidative stress, which associated with many degenerative diseases, including athero-sclerosis, cancers, asthma, arthritis, heart attack, kidney damage, liver injury and induction of apoptosis[3]. The direct and indirect effects of radiation initiate a series of biochemical and molecular signaling events that may repair the damage or culminate in permanent physiological changes or cell death[4,5].

Moringa oleifera (MO) Lam (Family: Moringaceae) is a highly valued plant in tropic and subtropical countries where it is mostly cultivated[6]. The leaves are highly nutritious, being a good source of protein, β-carotene, vitamins A, B, C and E, riboflavin, nicotinic acid, folic acid, pyridoxine, amino acids, minerals and various phenolic compounds[6,7]. MO leaves are highly nutritious, being a significant source of beta-carotene, vitamins, protein, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolic[7,8]. Almost all the parts of these plants have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders[9].

MO possesses antitumor, anti-inflammatory, antihypertensive, cholesterol lowering, antioxidant, antidiabetic and hepatoprotective activities[7,10]. Leaves of Moringa oleifera have been reported to regulate thyroid status and possess radioprotective effect[11]. The plant has also been reported to be hepatoprotective against antitubercular drug and acetaminophen[12,13]. Therefore, the objective of this study is to evaluate the possible protective effect of aqueous extract of moringa oleifera against liver and kidney damage induced by gamma-radiation in rats.

MATERIALS AND METHODS

Animals

Male adult Wistar albino rats, weighing 120-150 g were obtained from the experimental animal house of the National Cancer Institute (NCI), Cairo University. Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water ad. Libitum. Animals were kept under a controlled lighting condition (light: dark, 13 h: 11 h). The animals' treatment protocol has been approved by the animal care committee of the National Center for Radiation Research and Technology, Cairo, Egypt, following the guidelines of NIH.

Chemicals

Moringa oleifera was obtained Genesis Today, Inc., USA. All other chemicals and solvents used were of the highest purity grade available.

Irradiation

Whole-body γ-irradiation was performed at the National Centre for Radiation research and Technology (NCRRT), Cairo, Egypt, using an AECL Gamma Cell-40 biological irradiator.

Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.012 Gy/s.

Experimental Design

Animals were divided at random into 4 groups, 6 animals each. The first group: rats were injected i.p. with saline solution for 15 consecutive days, this group served as a control. The second group: rats were i.p. injected with MO (300 mg/kg) for 15 consecutive days[14]. The third group: rats were injected i.p. with saline solution for 15 consecutive days, 1 hour after the last treatment, rats were exposed to a single dose of whole body gamma rays (6 Gy).The Fourth group: rats were injected i.p. with MO (300 mg/kg) for 15 consecutive days, 1 hour after the last injection, rats were exposed to a single dose of whole body gamma rays (6 Gy).Twenty-four hrs. After the last dose of the specific treatment, animals were anesthetized with ether, and blood samples were obtained by heart puncture. Serum samples were separated for the measurement of indices of hepatotoxicity and nephrotoxicity.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated according to the method of Reitman and Frankel[15]. Serum urea nitrogen and creatinine were determined according to the methods of Hallet and Cook[16] and Bonsnes and Taussky[17], respectively.

The animals were then sacrificed by decapitation after exposure to ether in a desiccator kept in a well-functioning hood. Livers and kidneys were removed and washed with ice-cold saline, blotted with a piece of filter paper and homogenized using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenates were centrifuged at 800 x g for 5 min at 4°C to separate the nuclear debris. The supernatants were centrifuged at 10,500 xg for 20 min at 4°C to get the post mitochondrial supernatant which was used to assay superoxide dismutase (SOD) activity. In the liver and kidney homogenates superoxide dismutase (SOD) and catalase (CAT) activities were determined according to the methods of Minami and Yoshikawa[18] and Aebi[19], respectively. Reduced glutathione (GSH), malondialdehyde (MDA), an index of lipid peroxidation, and total nitrate/nitrite (NOx) levels were determined according to the methods of Ellman[20], Buege and Aust[21] and Ignarro *et al*[22]., respectively.

Ultra structural studies

For electron microscopic examination, the liver and kidney were cut into small pieces, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) and post in 1% osmium tetroxide in 0.3M cacodylate buffer. The specimens were then dehydrated and embedded in Epon 812[23]. Ultrathin sections were cut and stained according to Reyonold.[24] Sections were examined with JoelXL 100 transmission electron microscope at NCRRT, Cairo, Egypt.

Statistical analysis

Differences between obtained values (mean ± S.E.M. n = 6) were carried out by one way analysis of variance (ANOVA) followed by the Bonferroni's multiple comparison test. A P value of 0.05 or less was taken as a criterion for a statistically significant difference.

RESULTS

Biochemical Results

Table (1) showed the effects of *Moringa oleifera* (MO), gamma-irradiation (6Gy) (IRR) and their combination on the levels of serum urea nitrogen, creatinine and the activities of liver enzymes. Gamma-irradiation induced a significant increase in

(602.9% and 501.4%) in the levels of serum creatinine and serum urea nitrogen respectively compared to the control group. Prior treatment with MO for 15 consecutive days before irradiation significantly reduced the levels of creatinine and urea in serum by 78.9% and 78.6% respectively compared to the irradiated group.

Gamma-irradiation (6 Gy) induced a significant increase (163.4% &82.7%) in the serum activities of ALT & AST, respectively, compared with the control group. Administration of MO prior to irradiation induced a significant decrease in ALT and AST activities compared to the irradiated group (Table 1), but still significantly higher than the control value ($P < 0.01$) .

In table 2, administration of MO for 15 consecutive days induced no change in all the parameters in the kidney tissues compared to the control group. Gamma-irradiation (6 Gy) induced a significant increase (61.1% & 52.8%) in MDA and NO(x) levels, respectively compared to the control group ($P < 0.001$). Administration of MO prior to irradiation induced a complete restoration of MDA and NO(x) levels to the control level. Gamma-irradiation (6 Gy) induced a significant decrease (57%, 40.4% & 48.7%) in the activities of SOD and CAT and the content of GSH in the kidney tissues, respectively, compared to the control group ($P < 0.001$). Pre-administration of MO to radiation induced significant amelioration in the activities of SOD and CAT and GSH content compared to the irradiated group ($P < 0.001$).

Gamma-irradiation (6 Gy) induced a significant increase (66.3% &90.2%) in MDA and NO(x) levels, and significant decrease (54.5%, 31% &81.7%) in the activities of SOD and CAT and the content of GSH in the hepatic tissues, respectively compared to the control group ($P < 0.001$). Administration of MO prior to irradiation induced a significant amelioration of MDA and NO(x) levels and the activities of SOD and CAT and GSH content compared to the irradiated group ($P < 0.001$) (Table 3).

Ultra structural results

The ultra-structure of a normal hepatocyte is presented in (Fig 1a) revealed that each hepatocyte exhibited rounded large central nucleus limited by a double membrane envelope interrupted by nuclear pores. The nucleus possesses euchromatin and prominent nucleoli. Numerous mitochondria. The cytoplasm of hepatocyte contained also cisternae of rough endoplasmic reticulum arranged in parallel stacks beaded by numerous fine ribosomes. Glycogen granules aggregated in rosette shaped. Examination of hepatocytes of the MO group showed healthy mitochondria, well developed cisternae of RER. Glycogen granules appeared as coarse electron dense granules aggregated in rosettes shaped and bile canaliculus showing the microvilli in its lumen (Fig 1b). Hepatocytes exhibited a normal distribution of its euchromatin and prominent nucleus. The cytoplasm is rich in healthy mitochondria with double walled membrane and intact cristae. Moreover, the presence of well-developed cisternae of RER. Glycogen granules were also seen, (Fig 1c). Hepatocytes of irradiated group showed histopathological alterations referred to irregular outlines shaped nucleus with large center nucleolus, there was fragmented cisternae of rough endoplasmic reticulum. Mitochondria were swollen, lost their cristae (Fig1d). Transmission electron microscopic examination of liver sections of group Moringa, radiation liver in Fig 1e. Hepatocytes regained their normal appearance as their cytoplasm contained numerous mitochondria but still degenerated and of variable shape and size. Regeneration in the rough endoplasmic reticulum. Also, the nuclear membrane of the hepatocytes

probably exhibited normal regular appearance (Fig 1e.)

Electron microscopic examination of a control group revealed normal ultra structural of the distal convoluted tubules, normal basal invagination, normal basement membrane, normal spherical nuclei and numerous mitochondria (Fig. 2a). Electron microscopic examination of the MO group revealed the appearance of vacillation, few lysosomes with numerous apical thin microvilli, normal central nuclei and multiple mitochondria in between basal infoldings (Fig. 2b & 2c). Electron micrograph showed ultra structural changes in different proximal and distal convoluted tubules in renal kidney cortex components exposed to gamma-radiation revealed focal degeneration of cytoplasmic matrix accompanied by different sized scattered lysosomes and few vacuoles (Fig 2d). Also dilated in the lumen showed few microvilli. In addition, demonstrating nuclei are dark small with condensed chromatin (Fig. 2e). Ultra structurally, treatment of rats with MO prior to IRR revealed different histopathological alterations. These alterations were manifested as destruction of the apical microvilli of proximal convoluted tubules, thickening of the basement membrane, abnormal distribution of mitochondria accompanied by irregular nuclear envelope of the nucleus and many large lysosomes scattered in different size (Fig 2f &2g). In addition, the cytoplasmic fragments appeared in the lumen. The nucleus appeared moderate electron density with loss of heterochromatin. The cytoplasm appears vacuolated with loss of normal architecture of cells, a common feature was shown in Fig 2 g.

DISCUSSION

Ionizing radiation produces harmful effects on the organisms and due to wide spread use of radiation in therapy and industry, natural products could be the most potent strategy to ameliorate the deleterious effect of ionizing radiation[25]. Ionizing radiation induces significant elevation in the physiological and metabolic processes, as well as, disorders in blood biochemical parameters and causing chain-reaction of oxidation[26].

In the present study, gamma-irradiation (6 Gy) induced a significant increase in the activities of ALT, AST and the levels of creatinine and urea in serum. This increase is in agreement with the previous findings of Ibrahim[27] and Mansour[28]. This increase might be due to the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in the aminotransferase activities in blood serum[29]. Increase in serum urea was due to increase in glutamate dehydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetase activity leading to increase in urea concentration[30].

In this study, treatment with MO before IRR extract suppresses α-irradiation induced AST and ALT elevations. Concurrently, significant preservation of liver histology was observed in the groups that were pretreated with MO. Recovery towards normalization of the enzymes following MO pretreatment suggested that the plant extract has some roles in preserving the structural integrity of hepatocellular membrane, thus prevented enzymes leakage into the blood circulation. Our results are consistent with the generally accepted hypothesis that transaminase level return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes[31]. Concurrently, this study showed that treatment with MO for 15 days

prior to irradiation reduced the levels of raised serum urea and creatinine suggesting that the contents of MO not only protected the integrity of kidney but also increased its regenerative and reparative capacity.

In the present study, oxidative injury, evidenced by the elevated MDA and NOx levels, and depleted GSH levels and SOD and catalase activities, as well as morphological changes and impairments in hepatic and renal functions due to γ-irradiation were improved by MO pretreatment. Administration of MO leaf extract prior to γ-irradiation significantly decreased hepatic marker enzymes, urea, creatinine, total nitrate/nitrite and lipid peroxidation with a significant increase in the level of antioxidants.

In the present study, increased MDA level in the liver and kidney tissues of whole-body irradiated rats, indicating the presence of radiation-induced oxidative damage. Peroxidation of membrane lipids can disrupt membrane fluidity and cell compartmentation, which may contribute to impaired cellular function and necrosis[32]. The present findings have shown that irradiation impaired the renal and hepatic functions, while MO pretreatment prevented elevations in tissue MDA and attenuated the impairments in the liver and kidney functions.

In agreement with the present study, Nakagawa *et al*[33]. Reported that, γ-irradiation enhances endogenous NO biosynthesis in liver, intestine, lung, kidney, brain, spleen or heart of the animals. The enhancement of NO production following exposure to a high dose (6 Gy) of gamma rays was attributed to high levels of expression of the inducible nitric oxide synthase[34]. On the other hand, IRR could act as a priming signal which synergizes a second signal such as interferon Á or lipopolysaccharide to induce NO production in macrophages[35].

CAT is one of three families of primary antioxidant enzymes in mammalian cells which are critical to peroxide removal. Therefore, the reduction in the enzymatic activity of CAT may be due to the increased utilization of this antioxidant to counteract lipid peroxidation production[36]. On the other hand, the significant decrease in the activities of SOD and CAT could be attributed to the excess of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation[27,28].

The reduction in GSH content might be due to the inhibition of GSH synthesis or due to the lack of amino acids required for GSH formation[32]. On the other hand, the decrease in tissue GSH levels after irradiation might be due to its consumption during the oxidative stress induced by ionizing radiation[28,37].

Phytochemical analysis showed that MO possess various phytochemicals such as ascorbic acid, phenolics (catechin, epicatechin, ferulic acid, ellagic acid, myricetin) etc., which may play the key role in prevention of lipid peroxidation by scavenging radiation-induced free radicals[38,39]. On the other hand, Verma *et al*[9] concluded that the MO possess high phenolic content and potent antioxidant properties, which may be mediated through direct trapping of the free radicals and also through metal chelation. MO inhibited the Fenton reaction-generated free radical activity *in vitro* in a concentration dependent manner[11].

The leaves of MO contain nitrile glycosides such as niazirin and niazirinin, and mustard oil glycosides. These glycosides are reported to have antioxidant activities[40]. Niaziridin, a nitrile glycoside, is a bioenhancer for drugs and antioxidative nutrients, including vitamins A and C[41]. Previous study has shown that administration of an extract from MO for

two weeks elevates the level of glutathione and prevents acetaminophen and paracetamol-induced liver injury[13,42].

Therefore administration of MO prior toγ-radiation protected against γ-radiation-induced liver and kidney damage. The protection may be attributed to its free radical scavenging activity, decreasing lipid peroxides and enhancing antioxidants[43].

The present results revealed that, the kidney and liver of gamma-irradiated rats exhibited varying lesions that included in kidney degeneration of cytoplasmic matrix accompanied by different sized scattered lysosomes, vacuolation, dilated lumen, demonstrating nuclei and condensed chromatin. In addition, liver exhibited varying lesions that included irregular outlines shaped nucleus with large nucleolus, fragmented rough endoplasmic reticulum and swollen rupture in its cristae. Similar results were documented by many authors on different experimental animals exposed to gamma-radiation[29,44], who reported that, radiation-induced alterations and apoptosis in mouse kidney. Also, Zhao and Robbins[45] suggested that, IRR-induced late normal tissue injury, tissue dysfunction and failure associated with atrophy, fibrosis and/or necrosis as well as vascular injury.

The present study described the alterations in the histological structure of rat renal convoluted tubules and hepatocytes treated with MO leaf extract revealed little or no damage. The present results showed that, pretreatment of irradiated rat with MO attenuated the adverse effects of radiation exposure. The normal architecture of the liver was restored, whereas, proximal convoluted tubules exhibited normal appearance. Pari and kumar[12] hypothesized that the β-carotene of MO is responsible for the hepatoprotective activity as β-carotene may exhibit a good radical trapping antioxidant activity. Besides carotene, MO is also reported to contain antioxidants such as vit C[8]. Rao *et al*[11]. Reported that vit C present in MO might be responsible for the antioxidant and radioprotective properties of the extract in rats subjected to whole body gamma irradiation.

Nevertheless, proximal convoluted tubules were abnormal with either vacuoles or few lysosomes in the MO kidney ultra structural. Furthermore, in MO-irradiated group, the kidney was abnormal with either destruction of the apical microvilli, thickening of the basement membrane, abnormal distribution mitochondria, or irregular nucleus envelope and scattered lysosomes.

Prakash Babu *et al*[42]. Reported that low doses of MO extract had moderate protective effect compared to the high dose of the extract indicating a dose-dependent effect. Prakash Babu *et al*[42]. Concluded that, Methanolic extracts of *Moringa oleifera* lam roots was found to distort the histo-architecture of both liver and kidneys of guinea pigs. These effects are time-dependent and dose-dependent. The liver and kidney of guinea pigs in the reversal group retained histo-architectural distortions. Buraimoh *et al*[46]. Showed that ethanolic leave extract of *Moringa oleifera* has an appreciable ability to prevent damage to the liver.

In conclusion, the current study has demonstrated that irradiation-induced oxidative damage of the liver and kidney tissues is ameliorated by the aqueous extract of MO leaf. This effect is attributed to its free radical scavenging activity and antioxidant activity.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

FIGURE LEGENDS

Figure 1

Electron photomicrograph of (1a) control liver cells shows spherical nucleus with thin peripheral rim of heterochromatin, the cytoplasm contain rough endoplasmic reticulum (RER), mitochondria (M), lysosomes (Ly) and glycogen rosettes (G). (X20000), (1b): moringa liver cells show that, the cytoplasm is rich in healthy mitochondria (M), the presence of well-developed cisternae of RER, Glycogen granules (G), bile canaliculus showing the microvilli in its lumen. (X20000), (1c): moringa liver cells showing the nucleus of the hepatocyte (N) with normal distribution of its euchromatin and prominent nucleolus. The cytoplasm is rich in healthy mitochondria (M) with double walled membrane and intact cristae. Notice the presence of well- developed cisternae of RER. Glycogen granules (G) are also seen. (X8000), (1d): section of hepatocyte of irradiated albino rats liver cells showing irregular outlines hepatocyte shaped nucleus (N) with large center nucleolus. Fragmentation of rough endoplasmic reticulum (thick arrow), swollen mitochondria (SM) and rupture in its cristae. (X8000), (1e): section of hepatocyte of Moringa-irradiated albino rats liver cells showing improvement in the hepatocytes, normal mitochondria (M) and regeneration in the endoplasmic reticulum (E). Rough shape nucleus with regular nuclear membrane and found. (X8000).

Figure 2

Fig. 2a: An electron micrograph of an ultrathin section of renal cortex of control rat showing a part of distal convoluted tubule with normal spherical nucleus (N) and numerous mitochondria (m) in between basal invaginations and normal basement membrane (BM) (8000X). Fig. 2b: An electron micrograph of kidney of MO-treated rat showing a part of proximal convoluted tubule revealing numerous apical thin microvilli (MV) with normal central nuclei (N) and multiple mitochondria (m) in between basal infoldings, demonstrating few vacuoles (V) surrounding the nuclei (Fig. 2c) and few lysosomes (X10000). Fig. 2d: An electron micrograph of an ultrathin section of renal cortex of an irradiated rat showing a part of proximal convoluted tubule revealing different sized scattered lysosomes (ly) and few vacuoles (V) (X 5000).Fig. 2e: An electron micrograph of an irradiated rat showing a part of proximal convoluted tubule dilated in its lumen*, ↑demonstrating nuclei are dark small with condensed chromatin (X8000) *showing few microvilli (MV). Fig. 2f: An electron micrograph of an ultrathin section of renal cortex of a MO- irradiated rat showing a part of proximal convoluted tubule demonstrating destruction of the apical microvilli (arrows), thickening of the basement membrane (BM), abnormal distribution mitochondria (M). The nucleus appears irregular of its nuclear envelope (arrow) and many large different sized scattered lysosomes (ly) (X4000). Fig. 2g: An electron micrograph of an ultrathin section of renal cortex of a MO- irradiated rat showing a part of proximal convoluted tubule demonstrating destruction of the apical microvilli (arrows), the cytoplasm shows destroyed mitochondria (m) and abnormal distribution. Note, presence of detached cytoplasmic fragments in the lumen (L) and thickening of the basement membrane (BM). The nucleus (N) appears moderate electron density with loss of heterochromatin. The cytoplasm appears vacuolated, loss of normal architecture of cells with increase in the different sized scattered lysosomes (ly) are seen as well. (X2500).

REFERENCES

1. Hall EJ, Giaccia AJ. Radiobiology for the Radiologist, 6th ed., Lippincott Williams & Wilkins, Philadelphia, PA, 2006.
2. Giardi MT, Touloupakis E, Bertolotto D, Mascetti G. Preventive or potential therapeutic value of nutraceuticals against ionizing radiation-induced oxidative stress in exposed subjects and frequent fliers. *Int. J. Mol. Sci*.2013; 14: 17168-17192.
3. El-Bahr SM. Biochemistry of free radicals and oxidative stress. *Science International*. 2013; 1: 111-117.
4. Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. *Cancer Metastasis Rev*. 2004; 23: 311–322.
5. Azzam EI, Jay-Gerin JP, Pain D. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters* 2012; 327: 48–60.
6. Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, El-Deeb FE, El-Shemy HA. Active principle from Moringa oleifera Lam leaves effective against two leukemias and a hepatocarcinoma. *African Journal of Biotechnology* 2010; 9: 8467–8471.
7. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with multiple medicinal uses. *Phytother Res*. 2007; 21(1): 17-25.
8. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. *J Agric Food Chem* 2003; 51: 2144–55.
9. Verma AR,Vijayakumar M, Mathela CS, Rao CV. *In vitro* and *In vivo* antioxidant properties of different fractions of Moringa oleifera leaves. *Food Chem. Toxicol.* 2009; 47: 2196–2201.
10. Tiloke C, Phulukdaree A, Chuturgoon AA. The antiproliferative effect of Moringa oleifera crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC Complementary and Alternative Medicine* 2013; 13:226- 234.
11. Rao AV, Devi PU, Kamath R. *In vitro* radioprotective effect of Moringa oleifera leaves. *Indian J. Exp. Biol*. 2001; 39 (9): 858–863.
12. Pari L, Kumar NA. Hepatoprotective Activity of Moringa oleifera on Antitubercular Drug-induced Liver Damage in Rats. *J Med Food*. 2002; 5: 169-175.
13. Fakurazi S, Hairuszah I, Nanthini U. Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level. *Food and Chemical Toxicology*. 2008; 46: 2611–2615.
14. Mbikay M. Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in Pharmacology*. 2012; 3: 1-12.
15. Reitman S, Frankel SA. A colourimetric method for the determination of serum oxaloaetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol*. 1957; 28: 56-63.
16. Hallet CJ, Cook JG. Reduced nicotinamide adenine dinucleotide-coupled reaction for emergency blood urea estimation. *Clin. Chem. Acta*. 1971; 35: 33-37.
17. Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffe Reaction. *J. Biol. Chem*. 1945; 158: 581-591.
18. Minami M, Yoshikawa H. A simplified assay method of superoxide dismutase activity for clinical use. *Clin Chim Acta* 1979; 92:337–42.
19. Aebi H. Catalase *in vitro*. *Methods Enzymol*. 1984; 105: 121-126.
20. Ellman GL. Tissue sulfhydryl groups. *Arch. Biochem. Biophys*. 1959; 17: 214-226.
21. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol*. 1978; 52: 302-310.
22. Ignarro L, Buga G, Wood K, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and veins is nitric oxide. *Proc. Nat. Acad. Sci*. 1987; 84: 9265-9269.
23. Hayat MA. Basic technique for transmission electron microscopy. New York, Harcaurt Brace, Jonamovich. 1973.

24. Reynold AS. The use of lead citrate at high PH as an electron opaque stain in electron microscopy. *J. Cell. Bio*. 1963; 17: 208.
25. Jagetia GC. Radioprotective potential of plants and herbs against the effects of ionizing radiation. *J. Clin.Bioch.Nutr*. 2007; 40:74-81.
26. Ammar AA. Evaluation of the protective role of wheat germ oil in irradiated rats. *Isotope and Rad. Res*. 2009; 41: 911-920.
27. Ibrahim NK. Possible protective effect of kombucha tea ferment on cadmium chloride induced liver and kidney damage in irradiated rats. *International Journal of Biological and Life Sciences*. 2013; 9(1): 7-12.
28. Mansour HH. Protective effect of ginseng against gamma-irradiation-induced oxidative stress and endothelial dysfunction in rats. *EXCLI Journal*. 2013; 12: 766-777.
29. Gaur A, Bhatia AL. Modulation of phosphatase levels in mice liver by genistein treatment against radiation exposure. *Pharmacognosy Res*. 2009; 1(2): 72-79.
30. Ramadan LA, Shouman SA, Sayed-Ahmed MM, El-Habit OH. Modulation of radiation-induced organs toxicity by cremophorel in experimental animals. *Pharmacol Res*. 2001; 43:185-191.
31. Ahmed MB, Khater MR. Evaluation of the protective potential of Ambrosia maritime extract on acetaminophen-induced liver damage. *J. Ethnopharmacol*. 2001; 75: 169-174.
32. Sener G, Kabasakal L, Atasoy BM, Erzik C, Velioğlu-Oğünç A, Cetinel S, Contuk G, Gedik N, Yeğen BC. Propylthiouracil-induced hypothyroidism protects ionizing radiation-induced multiple organ damage in rats. *J Endocrinol*. 2006; 189(2), 257-69.
33. Nakagawa H, Ikota N, Ozawa T and Kotake Y. Dose- and time-dependence of radiation-induced nitric oxide formation in mice as quantified with electron paramagnetic resonance. *Nitric Oxide Biol Chem*. 2001; 5: 47-52.
34. Gisone P, Dubner D, Pérez MD, Michelin S, Puntarulo S. The role of nitric oxide in the radiation-induced effects in the developing brain. *In vivo*. 2004; 18: 281-292.
35. McKinney LC, Aquilla EM, Coffin D, Wink DA and Vodovotz Y. Ionizing radiation potentiates the induction of nitric oxide synthase by interferon-gamma and/or lipopolysaccharide in murine macrophage lines. Role of tumor necrosis factor alpha. *Ann N Y Acad Sci*. 2000; 899: 61-68.
36. Kalpana C, Menon VP. Modulatory effects of curumin on lipid peroxidation and antioxidant status during nicotine induced toxicity. *Polish Journal of Pharmacology*. 2004; 56(5): 581-586.
37. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292: 18-36.
38. Sinha M, Das DK, Bhattacharjee S, Majumdar S, Dey S. Leaf extract of Moringa oleifera prevents ionizing radiation-induced oxidative stress in mice. *J Med Food*. 2011; 14(10):1167-1172.
39. Sinha M, Das DK, Datta S, Ghosh S, Dey S. Amelioration of ionizing radiation induced lipid peroxidation in mouse liver by Moringa oleifera Lam. leaf extract. *Indian J Exp Biol*. 2012; 50(3): 209-215.
40. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH. Isolation and structure elucidation of new nitrile and mustard oil glycosides from Moringa oleifera and their effect on blood pressure. *Journal of Natural Products*. 1994; 57: 1256-1261.
41. Shanker K, Gupta MM, Srivastava SK, Bawankule DU, Pal A, Khanuja SPS. Determination of bioactive nitrile glycoside(s) in drumstick (Moringa oleifera) by reverse phase HPLC. *Food Chemistry* 2007; 105: 376–382.
42. Prakash Babu B, Ravi Kumar P, Bharavi K., Venkateswarlu U, Rama Devi V, Srilatha Ch. Protective Effect of Moringa oliefera Lam Leaf Extract in Paracetamol Induced Hepatotoxic Rat Model. *IJPI'S Journal of Pharmacology and Toxicology*. 2011; 1(5): 24-34.
43. Ashok Kumar N, Pari L. Antioxidant action of Moringa oleifera Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. *J Med Food*. 2003; 6(3): 255-259.

44. Ghassah M, Labejof L, Berry JP, Galle P. Early ultra structural lesions of apoptosis induced *In vivo* in two varieties of tissues (thymus and kidneys) after a single whole-body-gamma irradiation of adult mice. *Cell Mol Biol* (Noisy-le-grand). 1997; 43(8):1197-204.
45. Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr Med Chem.* 2009; 16(2):130-43.
46. Buraimoh AA, Bako G, Ibrahim FB. Hepato protective effect of ethanolic leave extract of *moringa oleifera* on the histology of paracetamol induced liver damage in wistar rats. *International Journal of Animal and Veterinary Advances.* 2011; 3(1): 10-13.

Table 1. Effect of *Moringa oleifera* (MO), γ-irradiation (IRR) and their combination on the serum urea and creatinine levels and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in male albino rats

Parameters	Creatinine (mg/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)
Control	0.68±0.04	14.50±1.6	31.86±0.4	59.95 ± 1.7
MO	0.62±0.02^{b}	16.80±0.38^{b}	37.22±0.6^{ab}	62.86± 1.9^{b}
IRR	4.78±0.05^{a}	87.20±2.1^{a}	83.92± 1.1^{a}	109.5 ± 3.4^{b}
MO+IRR	1.01 ± 0.13^{b}	18.70±1.2^{b}	42.37± 0.5^{ab}	69.04 ± 1.6^{ab}

Data are presented as mean ± SE of six rats.

^{a,b} Statistically significant ($P < 0.05$) compared to control rats group and irradiated rats group, respectively at $p \leq 0.05$ using ANOVA followed by Bonferroni's Multiple comparison test as a post ANOVA test.

Table 2. Effect of Moringa oleifera (MO), γ-irradiation (IRR) and their combination on the levels of malondialdehyde (MDA), total nitrate/nitrite (NOx) and reduced glutathione (GSH), and the activities of superoxide dismutase (SOD) and catalase (CAT)in rat kidney tissue

Parameters	Control	MO	IRR	MO+IRR
MDA	171.6 ±11.9	180.2±8.12^{b}	276.5±12.02^{a}	176.5 ± 0.13^{b}
NOx	47.4 ± 4.4	51.47 ± 2.1^{b}	72.45 ± 2.6^{a}	48.46 ± 4.9^{b}
SOD	85.85 ± 3.33	77.2 ± 0.42^{b}	37.0 ± 3.63^{a}	79.68 ± 4.41^{b}
GSH	0.152 ± 0.005	0.157 ± 0.006^{b}	0.078 ± 0.003^{a}	0.149 ± 0.004^{b}
CAT	74.43 ± 1.62	77.59 ± 0.87^{b}	44.37 ± 0.91^{a}	70.97 ±1.80^{b}

Data are presented as the means ± SE of 6 rats.

MDA content and NO(x) concentration are expressed in nmol /g tissue, SOD activity is expressed in µg /g tissue, catalase activity is expressed in mmol/g tissue and GSH content is expressed in µmol/ g tissue. a,b Statistically significant ($P < 0.05$) compared to the control and irradiated groups, respectively at $p \leq 0.05$ using ANOVA followed by Bonferroni's Multiple comparison test as a post ANOVA test.

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Table 3. Effect of Moringa oleifera (MO), gamma-irradiation (6Gy) (IRR) and their combination on the levels of malondialdehyde (MDA), total nitrate/nitrite (NOx) and reduced glutathione (GSH), and the activities of superoxide dismutase (SOD) and catalase (CAT)in rat hepatic tissue

Parameters	Control	MO	IRR	MO+IRR
MDA	114.9 ±2.78	109.6±2.3^{b}	191.1±9.9^{a}	119.0 ± 2.0$^{.3b}$
NOx	54.00 ± 5.9	45.06 ± 2.6^{b}	102.7 ± 1.5^{a}	64.30 ± 4.3^{b}
SOD	68.07 ± 1.6	66.27 ± 1.9^{b}	31.10 ± 1.4^{a}	60.47 ± 1.02^{ab}
GSH	0.388 ± 0.015	0.410 ± 0.005^{b}	0.071± 0.005^{a}	0.398 ± 0.005^{b}
CAT	158.9 ± 3.5	163.7 ± 3.4^{b}	109.6 ± 1.1^{a}	157.8 ±1.80^{b}

Data are presented as the means ± SE of 6 rats.

MDA content and NO(x) concentration are expressed in nmol /g tissue, SOD activity is expressed in µg /g tissue, catalase activity is expressed in mmol/g tissue and GSH content is expressed in µmol/ g tissue. a, b Statistically significant ($P < 0.05$) compared to the control and irradiated groups, respectively at $p \leq 0.05$ using ANOVA followed by Bonferroni's Multiple comparison test as a post ANOVA test.

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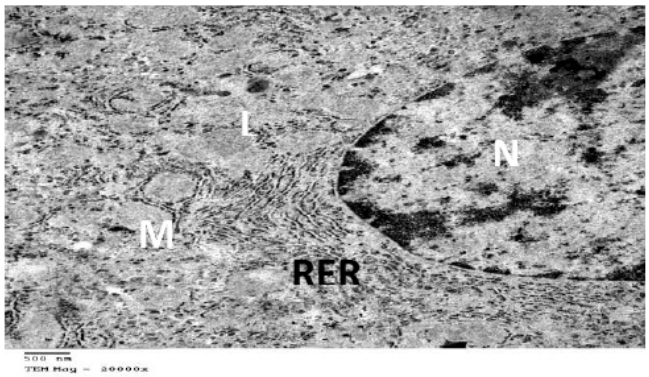

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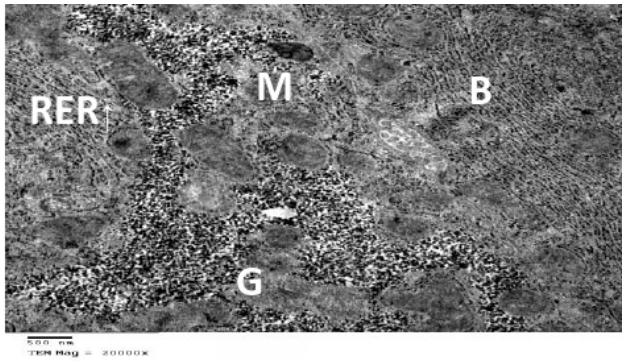

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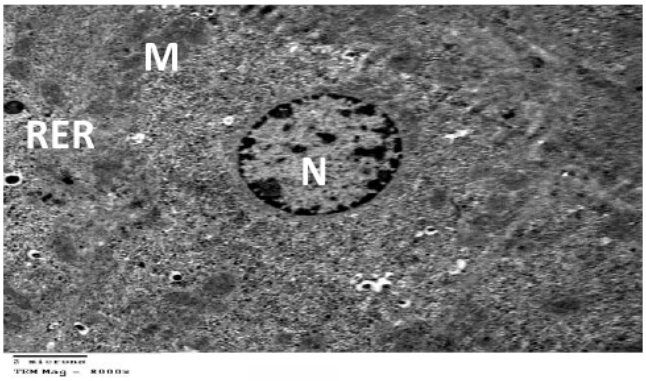

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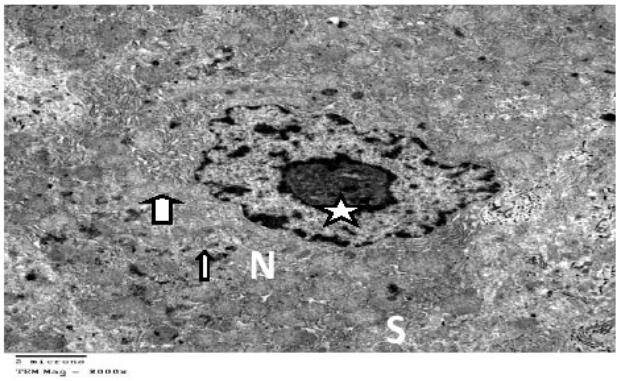

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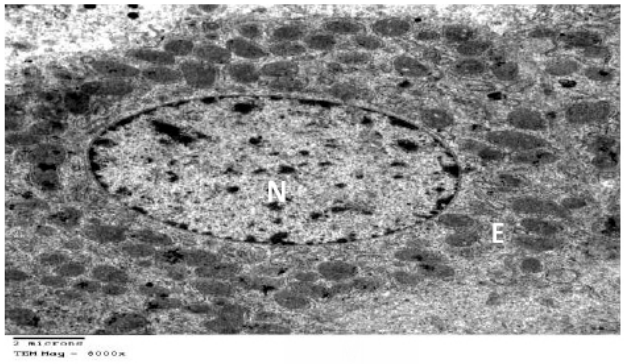

1e

Figure 1. Electron photomicrograph of (1a) section of control liver cells (X20000), (1b): section of moringa liver cells (X20000), (1c): moringa liver cells (X8000), (1d): section of hepatocyte of irradiated albino rats liver cells (X8000), (1e): section of hepatocyte of Moringa-irradiated albino rats liver cells (X8000)

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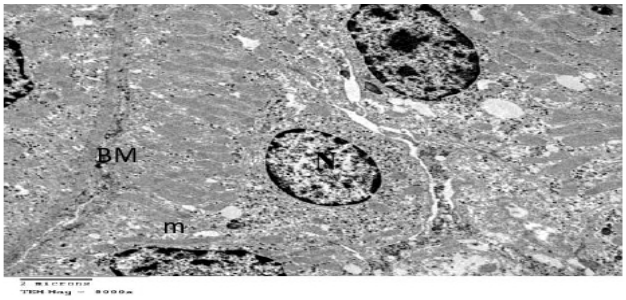

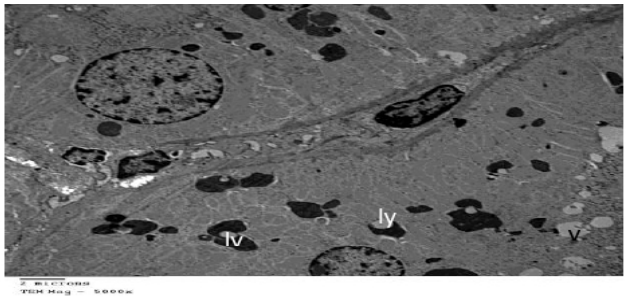

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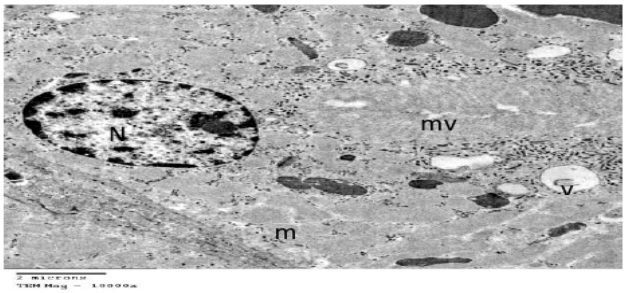

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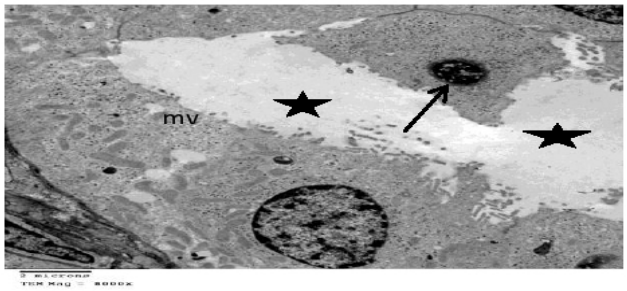

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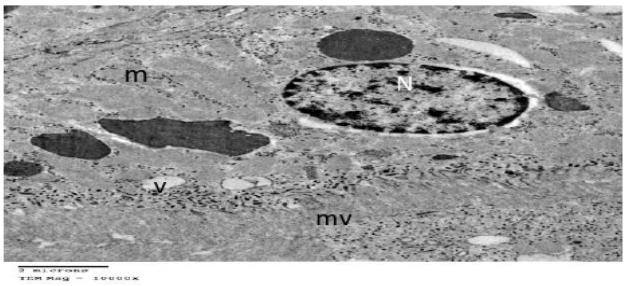

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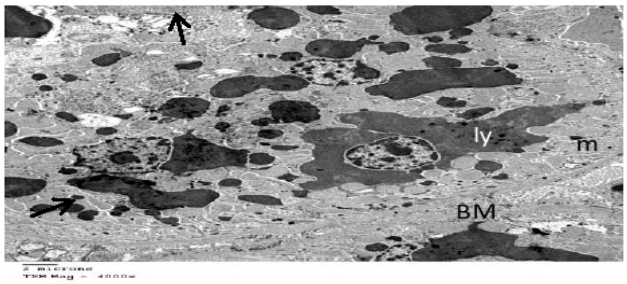

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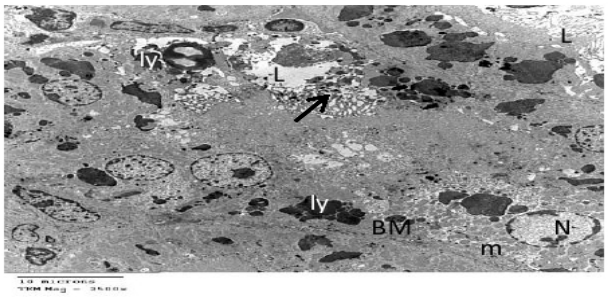

2g

Figure 2. (2a) An electron micrograph section of renal cortex of control (8000X),(2b, 2c) Electron micrograph sections of kidney of MO-treated rat (X10000), (2d, 2e)Electron micrograph sections of renal cortex of irradiated rat (X 5000), (X8000), (2f, 2g) Electron micrograph sections of renal cortex of a MO-irradiated rat (X4000), (X2500)