Middle-East Journal of Scientific Research 16 (10): 1303-1315, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.16.10.7659

Role of *Moringa oleifera* Leaves on Biochemical and Genetical Alterations in Irradiated Male Rats

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Abstract: Radiations has tremendous therapeutic benefits for humans; It is used as radiotherapy for different malignant diseases. However, it is also associated with the risk of serious adverse effects. The deleterious effects of ionizing radiation are associated with alterations in oxidizing systems. The present study clarifies the role of *Moringa oleifera* as a protector in male rats against oxidative stress induced by gamma irradiation. Fifty male albino rats were randomly divided into five groups. Group I; animals without any treatment, as control. Group II; animals exposed to 4Gy, low dose of gamma irradiation. Group III; animals exposed to 6Gy, high dose of gamma irradiation. Group IV; animals exposed to 4Gy gamma irradiation then gavage with 50 mg/kg body weight daily of *Moringa oleifera* water extract for one month. Group VI; animals exposed to 6Gy gamma irradiation then gavaged with 50 mg/kg body weight daily of *Moringa oleifera* water extract for one month. The evaluated haematological parameters were, iron, vitamin B_{12} and folic acid. AST, ALT, ALK.Ph. and malondialdehyde (MDA) activity and level were analyzed respectively. The genetic parameters were evaluated by means of DNA fragmentation, micronucleus test and comet assay. The results revealed a significant decrease in RBCs, Hb, Ht% and platelets, however, MCV and MCH increased significantly after exposure to low and high doses of irradiation. *Moringa oleifera* treatment ameliorated these effects, especially in the low gamma irradiation dose and improved the anemia. Gamma irradiation also decreased significantly: WBCs, lymphocytes, monocytes, neutrophils, esinophiles and basophiles. The decrease was more pronounced in the 6Gy high dose than 4Gy low dose. After treatment with *Moringa oleifera* at the low or high doses, lymphocytes, monocytes and basophiles counts increased more with the low dose than with the high dose. Iron, vitamin B_{12} and folic acid decreased significantly after exposure to gamma irradiation with 4Gy and 6Gy doses. After *Moringa oleifera* treatment, the low dose group restored their levels close to the control values. The AST, ALT and ALK. Ph. activities increased proportionally with the gamma irradiation dose. *Moringa oleifera* treatment decreased these levels below the normal control levels in the 4Gy dose. However, at the 6 Gy dose, ALK.Ph. activity still slightly increased, while AST and ALT levels approached the control levels. *Moringa oleifera* recovered the hepatic damage. Malondialdehyde (MDA) increased with low and high doses of irradiation and *Moringa oleifera* decreased these effects by lowering oxidative damage. The genotoxicity study revealed that exposure of male rats to gamma irradiation increased the DNA fragmentation, frequency of the micronucleated polychromatic erythrocytes (MnPCEs) formation and the DNA damage. These effects were more pronounced with the high dose of irradiation than with the low dose. However, the DNA fragmentation, frequency of the MnPCEs formation and the DNA damage decreased after the treatment of irradiated male rats with *Moringa oleifera* leaf extract.

Key wards: Gamma irradiation · *Moringa oleifera* · Biochemical changes · DNA damage

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INTRODUCTION

Exposure to ionizing radiation represents a genuine increasing threat to mankind and our environment. The steadily increasing applications of radiation in clinical practice, industrial and agricultural activities, on top of residual radio-activity resulting from nuclear test explosions, have a measurable impact contributing to possible radiation hazards in humans. Control of radiation hazards is considered as one of the most important challenges in order to protect our life from radiation damage [1]. Gamma radiation also known as gamma rays and denoted as ã, are electromagnetic rays of high frequency and hence high energy. Gamma rays are ionizing radiation and thus are biologically hazardous [2]. All ionizing radiation causes similar damage at a cellular level. Gamma rays and neutrons are more penetrating, causing diffuse damage throughout the body (e.g. radiation sickness, cell's DNA damage, cell death due to damaged DNA, increasing incidence of cancer) rather than burns. The most biological damaging forms of gamma radiation occur in the gamma ray window, between 3 and 10 MeV [3]. Gamma rays are also used for diagnostic purposes in nuclear medicine in imaging techniques [4]. An acute full-body equivalent single exposure dose of 1Gy causes slight blood changes, but 2.0–3.5 Gy causes very severe syndrome of nausea, hair loss and hemorrhage and can cause death. A dose of 5 Gy is considered approximately the LD_{50} (lethal dose for 50% of exposed population) for an acute exposure to radiation even with standard medical treatment [5, 6].

Medicinal plants have over the years been the lifeline of many indigenes which do have access to, or cannot afford pharmaceuticals. In recent years the shift from synthetic drugs to natural products is considered a panacea to wellness that is cutting across all socio-economic barriers [7]. The inhibition to the widespread use of medicinal plants is gradually being eroded by the inability of orthodox drugs to cure long standing and common ailments such as malaria, diabetes and hypertension. Additionally, anecdotal evidence of efficacy of medicinal plants has boosted confidence in the use of such products. The coupling agent of acceleration to the use of medicinal plants, herbal plants, natural products etc., is the emergence of nutraceutical plants [8]. These are natural plant products that link nutritional plants and medicinal plants. One 21st century plant that has taken center-stage and promises to be the catalyst towards the achievement of the millennium development goals of reducing poverty, disease and malnutrition is *Moringa oleifera*.

Moringa oleifera had antimicrobial, antihypertensive, anti-inflammatory, antidiabetic and anti-cancer activities [9]. However, most studies done so far are not *in vivo* animal studies. The drive to introduce *Moringa oleifera* as a drug is still in abeyance.

Moringa oleifera is an angiosperm plant, native of the Indian subcontinent, where its various parts have been utilized throughout history as food and medicine. It is now cultivated in all tropical and sub-tropical regions of the world. The nutritional, prophylactic and therapeutic virtues of this plant are well known. Dietary consumption of its parts is taken into mind as a strategy of personal health preservation and self-medication in various diseases [10]. The enthusiasm for the health benefits of *Moringa oleifera* is variably labeled as Miracle Tree, Tree of Life, the Best Friend, God's Gift to Man, Savior of the Poor. In many regions of Africa, it is widely consumed for self-medication by patients affected by diabetes, hypertension, or HIV/AIDS [11- 13].

Moringa oleifera is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers fruits and seeds [14]. Scientific analysis has shown and confirmed that moringa leaves are indeed a powerhouse of essential macro- and micro-nutrients [15]. The leaves are believed to contain approximately 46 types of antioxidants, 90 nutrients, 18 amino acids (among which 8 are the essential ones). It is on record that, gram of moringa leaves contain: more vitamin C than oranges, more vitamin A than carrots, more calcium than milk, more potassium than banana, more iron than spinach; and the protein quality can equate with those in milk and eggs, as well as antioxidants such as â-carotene, vitamin C and flavonoids [16, 17].

A recurring explanation for the therapeutic actions of *Moringa oleifera* medication is the relatively high antioxidant activity of its leaves, flowers and seeds [18, 19]. Among the major classes of phytochemicals found in the plant, flavonoids appear to carry most of this activity [20]. *Moringa oleifera* has anti-inflammatory, anticancer and antioxidant potential. Moringa reduced air way inflammation, suggesting it might help as an asthma treatment [21]. Because of the chemical complexity of the *Moringa oleifera* medicinal formulations, their apparent therapeutic effects could be due to the combined actions of various bioactive components found in the plant,

including trace metal ions, vitamins, alkaloids, carotenoids, polyphenols, fats, carbohydrates and proteins [22]. Some compounds may collectively affect broad aspects of physiology, such as nutrient absorption and processing, redox state, or immunity [23].

In the present study we measured the therapeutic effect of *Moringa oleifera* on haematological indices, some vitamines and liver functions after exposure to gamma irradiation. The possible genotoxicity of ionizing radiation was also measured by means of the DNA fragmentation, micronucleus test and comet assay in the spleen, bone marrow and liver tissues of rats after irradiation exposure. In addition, the protective role of moringa against radiation induced genotoxicity was investigated.

MATERIALS AND METHODS

Irradiation: The two used doses were; 4Gray (4Gy) and 6Gray (6Gy). Gamma irradiation was performed using the cobalt-60 cell 3500 of the Middle Eastern Regional Radioisotope Center for the Arab Countries (MERRCAC) in Dokki, Giza, Egypt.

Plant Extraction: *Moringa oleifera* dried leaf powder was obtained from the National Research Center (NRC), Dokki, Giza, Egypt. The powder was extracted with boiled distilled water (50 mg/kg) for 10 minutes and then left to cool. After stirring, the mixture was kept for gavage administration. The extracted moringa leaf powder was prepared freshly every day.

Experimental Animals: Fifty male albino rats weighing 130±20 g were obtained from the animal house of the National Research Center, Dokki, Giza. 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*.

Experimental Design: Rats were randomly divided into five equal groups (ten rats in each group): Group1; served as normal control. Group II; low dose irradiated group, the animals were subjected to whole body gamma irradiation at a single dose of 4 Gy. Group III; high dose irradiated group; the animals were subjected to whole body gamma irradiation at a single dose of 6 Gy. Group IV; low dose of 4 Gy irradiated group, then the animals were gavaged 50 mg/kg *Moringa oleifera* water extract once daily for one month. Group VI; high dose of 6 Gy irradiated group, then the animals were gavaged 50 mg/kg *Moringa oleifera* water extract once daily for one month. At the end of the experiment, blood samples were withdrawn by cardiac puncture after slight anathesation of rats using diethyl ether. A portion of the blood was collected using an EDTA anticoagulant for the haematological parameters study. Another portion of the blood was collected in a test tube and centrifuged at 3000 gx to get serum for biochemical analysis.

Blood Analysis: Blood hematology was evaluated by using Genesis instrument (AD Alicia Diagnostics Incorporation USA). Levels of vitamin B_{12} and folic acid were determined using radioimmunoassay procedures [24]. Iron levels were assayed in serum by Anderson *et al*. [25] method. The serum AST and ALT activities were determined using colorimetric method [26]. Serum ALK.Ph. activity was analyzed according to the standard procedure of Kind and King [27]. Malondialdehyde was also determined by the colorimetric method [28].

Genetical Analyses:

DNA Fragmentation Analysis

DNA Fragmentation Using Diphenylamine Reaction Procedure: Spleen tissues of rats were used to determine the quantitative profile of the DNA fragmentation using Diphenylamine reaction procedure. Briefly, spleen samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM tris-HCl (pH 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 r.p.m. (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. Half ml, of 25% trichloroacetic acid (TCA) was added to the pellets (P) and the supernatants (S) and incubated at 4°C for 24 h. The samples were centrifuged for 20 min at 10 000 r.p.m. (Eppendorf) at 4°C and the pellets were suspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample, 160 ml of DPA solution [150 mg DPA in 10 ml glacial acetic acid, 150 ml of sulfuric acid and 50 ml acetaldehyde (16 mg/ml)] was added and incubated at room temperature for 24 h [29]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

%Fragmented DNA= [OD (S)/ OD (S) + OD (P)] X 100

DNA Fragmentation Using Gel Electrophoresis Laddering Assay: Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA [30]. Briefly, spleen tissues were homogenized, washed in PBS and lysed in 0.5 ml of DNA extraction buffer (50 mM Tris–HCl, 10 mM EDTA, 0.5% Triton and 100 µg/ml proteinase K, pH 8.0) for overnight at 37°C. The lysate was then incubated with 100 µg/ml DNase-free RNase for 2h at 37°C, followed by three extractions of an equal volume of phenol/chloroform (1:1 v/v) and a subsequent re-extraction with chloroform by centrifuging at 15,000 rpm for 5 min at 4°C. The extracted DNA was precipitated in two volumes of ice-cold 100% ethanol with 1/10 volume of 3 M sodium acetate, pH 5.2 at -20 °C for 1h, followed by centrifuging at 15,000 rpm for 15 min at 4 °C. After washing with 70% ethanol, the DNA pellet was air-dried and dissolved in 10 mM Tris–HCl/1 mM EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with ethidium bromide in Tris/acetate/EDTA (TAE) buffer (pH 8.5, 2 mM EDTA and 40 mM Tris–acetate). A 100-bp DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transillumination.

Micronucleus Test: The bone marrow cells of rats were resuspended in a small volume of fetal calf serum on a glass slide and used for smear preparation. The smear of bone marrow cells was prepared from each rat. After air-drying, the slides were fixed in methyl alcohol for 10 min and stained with 5% Giemsa stain for 10 min. Three slides were prepared for each animal and were coded before observation and one was selected at random for scoring. From each coded slide, 2,000 polychromatic erythrocytes (PCEs) were scored for the presence of micronuclei under oil immersion at high power magnification. In addition, the percentage of micronucleated polychromatic erythrocytes (%MnPCEs) was calculated on the basis of the ratio of MnPCEs to PCEs [31].

Comet Assay for DNA Strand Break Determination: Isolated hepatic cells of all groups of rats were subjected to the modified single-cell gel electrophoresis or comet assay [32, 33]. A small piece of the liver was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm^3 pieces while immersed in HBSS, with a pair of stainless steel scissors to obtain the cells. After several washings with cold phosphate-buffered saline (to remove red blood cells), the minced liver was dispersed into single cells using a pipette [34]. In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were then treated with alkali for 20 min to denature the DNA and electrophoresis under alkaline conditions (30 min) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence microscope (Olympus BX60 F-3) with a green filter at × 40 magnification. For each experimental condition, about 100 cells were examined to determine the percentage of cells with DNA damage that appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus [35, 36]. A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.

Statistical Analysis: All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [37] followed by Duncan, multiple range tests for biochemical data and Scheffe-test for Genetical data respectively to assess significant difference between groups.

RESULTS

Table1. shows the effect of gamma irradiation and *Moringa oleifera* on the haematological parameters in male rats. There are significant decreases ($P \leq 0.05$) in RBCs, Hb and Ht% in groups exposed to 4Gy and 6Gy gamma irradiation when compared to the control (-16.67, -14.81 and -15.3) for low and (-26.6, -25.6 and -26.1) for high gamma irradiation doses, respectively. More reductions were observed with the high dose of gamma irradiation. Administration of *Moringa oleifera* increased RBCs, Hb and Ht% with low dose of gamma irradiation above the control levels. However, with high irradiation dose, they increased above the irradiated group but they

Table 1: Effect of *Moringa oleifera* on controlling the changes induced by gamma irradiation on some haematological parameters in male rats.

	Groups				
Parameters	Control	Low dose (4Gy)	High dose (6Gy)	Low dose +Moringa	High dose +Moringa
RBCS(10^6 /ml)	4.74±0.23[b]	3.95±0.24[d]	3.48±0.25[e]	5.26±0.40[a]	4.4±0.35[c]
Mean±SE % change	-16.7	-26.6	11.0	-7.2	
Hb (g/dl)	13.3±0.50[b]	11.3±0.55[d]	9.9±0.53[e]	14.5±0.90[a]	12.5±0.77[c]
Mean±SE % change		-14.8	-25.6	8.65	-6.0
Ht% Mean±SE	39.9±1.50[b]	33.8±1.30[d]	29.5±1.20[e]	43.1±2.90[a]	37.4±2.43[c]
%change		-15.3	-26.1	8.0	-6.16
MCV (fl) Mean±SE	82.2±1.60[d]	90.2±1.70[b]	92.3±3.60[a]	81.8±1.60[d]	85.8±2.22[c]
%change		9.73	12.27	-0.47	3.59
MCH (pg) Mean±SE	27.5±0.50[c]	29.7±1.25[a]	29.7±1.10[a]	27.5±0.60[c]	28.7±0.75[b]
%change		8.0	8.0	0.0	4.36
MCHC (%) Mean±SE	32.8±0.10[ab]	32.7±0.44[b]	32.6±1.35[a]	33.43±0.20[b]	33.4±0.19[a]
%change		-0.21	-0.49	1.95	1.83
Platelets(10^3/ml)	374±21.30[a]	359.5±10.90[a]	241.5±35.40[c]	365±19.10[a]	317.5±24.40[a]
Mean±SD %change		-3.9	-35.42	-2.41	-15.0

Data are presented as means±SE.

a, b, c, d and e/ means in the same row followed by different superscripts are significantly different ($P \leq 0.05$).

Table 2: Effect of *Moringa oleifera* on controlling the changes induced by gamma irradiation on WBCS counts and its differential counts in male rats.

	Groups				
Parameters	Control	Low dose (4Gy)	High dose (6Gy)	Low dose +Moringa	High dose +Moringa
WBCs(10^3/ml) Mean±SE	16.1±1.20[a]	14.1±0.80[b]	9.5±1.22[d]	15.3±0.80[a]	13.1±0.80[c]
%change		-12.15	-40.81	-4.98	-18.38
Lymphocytes(10^3/ml)	8.37±0.66[a]	7.15±0.50[b]	4.8±0.80[c]	8.1±0.49[a]	7.0±0.50[b]
Mean±SE %change		-14.6	-43.1	-3.22	-16.13
Monocytes(10^3/ml)	0.38±0.04[a]	.326±0.03[b]	0.23±0.02[d]	0.36±0.03[a]	0.29±0.02[c]
Mean±SE %change		-13.1	-38.7	-4.0	-22.7
Neutrophiles(10^3/ml)	6.46±0.65[a]	5.82±0.45[b]	3.24±0.49[d]	5.66±0.50[b]	4.56±0.38[c]
Mean±SE %change		-9.9	-49	-13.6	-29.4
Esinophiles(10^3/ml)	0.56±0.03[a]	0.45±0.03[c]	0.34±0.04[e]	0.5±0.02[b]	0.42±0.03[d]
Mean±SE %change		-19.1	-38.6	-10.71	-25.0
Basophiles(10^3/ml)	0.29±0.03[a]	0.24±0.02[b]	0.18±0.03[c]	0.28±0.04[a]	0.27±0.03[a b]
Mean±SE %change		-17.24	-37.93	-2.41	-8.62

Data are presented as means±SE.

a, b, c, d and e/ means in the same row followed by different superscripts are significantly different ($P \leq 0.05$).

still below the control group (Table 1). MCV and MCH increased significantly ($P \leq 0.05$) with the low and high irradiation doses compared to the control group. Treatment with *Moringa oleifera* decreased the irradiation effects as shown in (Table1). MCHC% did not change significantly in most treated groups. Platelets count decreased significantly ($P \leq 0.05$) in the group exposed to high irradiation dose (6Gy). *Moringa oleifera* ameliorated this decrease by (-2.4 and -15.0) and the platelets count were partly recovered.

Data presented in Table 2. Showed that WBCs count was significantly decreased ($P \leq 0.05$) in rats treated with low (4Gy) and high (6Gy) of gamma irradiation. The higher dose (6Gy) caused a significantly higher reduction in the WBCs counts and became even significantly lower than the low dose (4Gy) of irradiation. Moreover, the lymphocytes count, monocytic count and neutrophiles exhibited the same significant ($P \leq 0.05$) level observed with the WBCs count. *Moringa oleifera* treatment ameliorated these effects in both the low and the high irradiation doses. *Moringa oleifera* treatment, at the low dose level resulted in a complete recovery of the lymphocytes and monocytes. However, at the high irradiation dose, the recovery was not complete. In regard to the neutrophiles, esinophiles and basophiles, they all decreased significantly at both irradiation exposure levels with a significant dose effect (Table 2). *Moringa oleifera* treatment increased, significantly, the neutrophiles count

Table 3: Effect of *Moringa oleifera* on controlling the changes induced by gamma irradiation on iron, vitamin B_{12} and folic acid levels in male rats.

	Groups				
Parameters	Control	Low dose (4Gy)	High dose (6Gy)	Low dose +Moringa	High dose +Moringa
Iron (mg/l) Mean±SE	2.28±0.2 [b]	1.85±0.3 [c]	1.19±0.2 [d]	2.6±0.2 [a]	2.25±0.3 [b]
%change		-18.86	-47.81	14.0	-1.32
B_{12} (pg/ml) Mean±SE	112.3±13.0 [a]	97±8.9 [b]	63.5±8.8 [c]	119.5±14.4 [a]	95.0±9.4 [b]
%change		-13.62	-43.5	6.41	-15.41
Folicacid(ng/ml)	19±1.6 [b]	12.5±2.0 [d]	7.1±1.7 [e]	22.9±3.0 [a]	16.9±2.3 [c]
Mean±SE %change		-34.2	-62.63	20.53	-11.1

Data are presented as means±SE.

a, b, c, d and e/ means in the same row followed by different superscripts are significantly different ($P \leq 0.05$).

Table 4: Effect of *Moringa oleifera* in controlling the changes induced by gamma irradiation on AST, ALT Alkaline phosphatase and malondiadehyde levels in male rats

	Groups				
Parameters	Control	Low dose (4Gy)	High dose (6Gy)	Low dose + Moringa	High dose+ Moringa
AST(U/L)	237±10.9 [c]	266±13.3 [b]	325±22.2 [a]	217±15.7 [d]	234.5±18.8 [c]
Mean±SE %change		12.24	37.1	-8.43	-1.1
ALT(U/L)	77.5±6.8 [c]	96±7.75 [b]	138.5±17.8 [a]	67.5±7.9 [d]	80±7.5 [c]
Mean±SE %change		23.9	78.7	-12.9	3.23
Alk.Ph.(U/L)	139±12.0 [c]	168.5±14.9 [b]	259.5±33.3 [a]	111.5±10.3 [d]	174.5±13.0 [b]
Mean±SE %change		21.2	86.7	-19.8	25.54
MDA(mmol/ml)	11.2±0.9 [c]	13.3±0.8 [b]	17.3±0.7 [a]	9.6±0.8 [d]	13.1±0.7 [b]
Mean±SE %change		18.8	54.6	-23.1	17.1

Data are presented as means±SE.

a, b, c and d/ means in the same row followed by different superscripts are significantly different ($P \leq 0.05$)

at the high dose gamma irradiation. *Moringa oleifera* treatment, with low dose gamma exposure restored the count of esinophiles and basophiles almost to the control level. At the high dose of irradiation, *Moringa oleifera* treatment also caused a significant increase in esinophiles and basophiles above their normal levels after exposure to gamma rays.

Data presented in Table (3) showed a significant decrease ($P \leq 0.05$) in iron, vitamin B_{12} and folic acid levels due to 4Gy or 6Gy irradiation exposure. The percentage of decreases in iron, vitamin B_{12} and folic acid in irradiated rats were (-18.9 and -47.8%), (-13.62 and -43.5%) and (-34.2 and -62.6%) respectively compared to the control. After *Moringa oleifera* treatment in animals exposed to low dose of gamma rays exhibited significant increase ($P \leq 0.05$) in iron, vitamin B_{12} and folic acid above even the control group. While *Moringa oleifera* treatment in animals exposed to the high dose of gamma irradiation, inecreased the values and reduced the% of changes to become (-1.32, -15.41 and -11.1%) respectively, but they did not return to the control level.

Table (4) demonstrates that the AST, ALT and Alk.Ph. activities were significantly increased ($P \leq 0.05$) in low dose and high dose gamma irradiation exposure with a significant dose effect, compared to the control. *Moringa oleifera* reduced the gamma irradiation effects on AST, ALT and Alk.Ph. activities. Comparing to the control, *Moringa oleifera* decreased the gamma irradiation effects. The pronounced decrease was observed in the group exposed to the low dose of gamma irradiation. However the level of alkaline phosphatase in the high dose gamma irradiation group was still significantly higher than the control group. Also the results presented in Table 4, revealed that there was a significant increase ($P \leq 0.05$) in Malondiadehyde (MDA) levels after exposure to low and high doses of gamma irradiation (18.8 and 54.6%) as compared to the control level. *Moringa oleifera* decreased significantly ($P \leq 0.05$) MDA level in the low dose irradiation group. After *Moringa oleifera* treatment, the levels were decreased to be less than the controls by -23.1% in the low dose groups. However, in the high dose groups, they still had slightly higher levels (17.1%) as shown in Table 4.

Genetic Assay

DNA Fragmentation

Diphenylamine Reaction: The toxicity effect was investigated by quantitative DNA fragmentation analysis. The fragmented DNA was examined in spleen tissue collected from treated animals using diphenylamine reaction procedure (Figure 1). The results revealed that fragmented DNA in untreated control animals was lower than all other treated groups. However, rate of DNA fragmentation in irradiated animals was significantly higher than the control rats (Figure 1). Where, the fragmented DNA in animals treated with radiation was 3

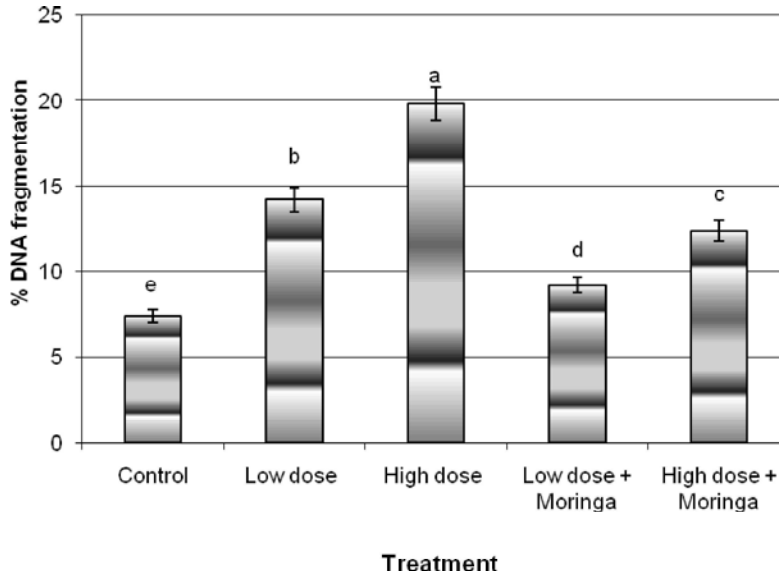

Fig. 1: DNA fragmentation in spleen tissues of male rats irradiated with 4Gy (low) or 6 Gy (high) gamma rays and treated with *Moringa oleifera* extracts analyzed by diphenylamine reaction procedure. Data are presented as means±SE. a, b, c d and e means in the same row followed by different letters are significantly different ($P \leq 0.05$).

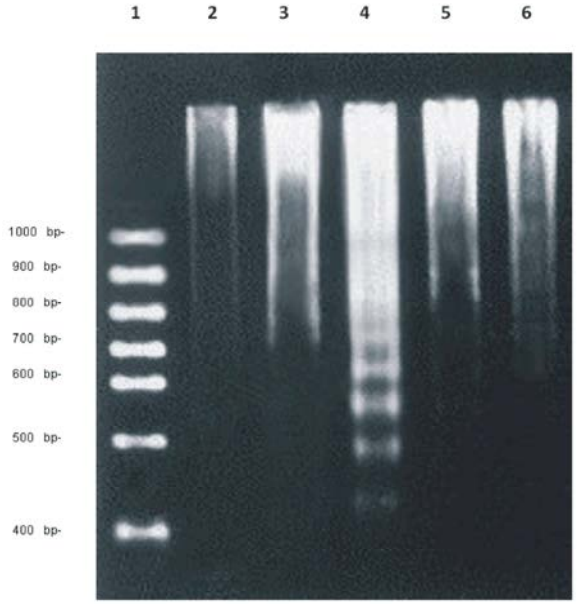

Fig. 2: DNA fragmentation detected with agarose gel of DNA extracted from liver tissues of male rats by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lane 2 represents control rats. Lane 3 represents low dose of irradiation. Lane 4 represents high dose of irradiation. Lane 5 represents rats exposed to low dose of irradiation and treated with moringa. Lane 6 represents rats exposed to high dose of radiation and treated with moringa.

times with the low dose and 4 times with the high dose of irradiation compared with the control animals. On the other hand, treatment of irradiated male rats with *Moringa oleifera* extracts decreased significantly the rate of DNA fragmentation compared with the irradiated animals (Figure 1). The rate of DNA fragmentation decreased due to *Moringa oleifera* extract treatment by approximately 35% and 38% compared for the low and high doses of irradiation, respectively (Figure 1).

DNA Fragmentation Using Gel Electrophoresis Laddering Assay: DNA fragmentation was determined in male rats exposed to low and high doses of irradiation. The DNA damage was investigated in the liver tissue collected from rats using gel electrophoresis laddering assay (Fig. 2). The results of gel electrophoresis laddering assay showed that treatment with low dose of irradiation caused slightly more DNA damage as compared to the control rats. However, rats treated with the high dose of irradiation had more bands compared with the control or low dose treated rats. *Moringa oleifera* extracts treatment decreased the DNA fragmentation damage compared to the irradiated rats.

Micronucleus Test: The effects of gamma irradiation and *Moringa oleifera* treatment on micronucleated polymorphic erythrocytes (MnPCEs) formation in the bone marrow cells of male rats are summarized in Table (5). The results revealed that MnPCEs formation was lower in

Table 5: Micronucleated polychromatic erythrocytes (MnPCEs) of male rats exposed to gamma irradiation and *Moringa oleifera* (mean±SEM).

Treatment	MnPCEs / 3000 PCEs
Control	6.1±0.1[d]
Low dose (4Gy)	17.2±0.3[b]
High dose (6Gy)	24.3±0.2[a]
Low dose + Moringa	10.6±0.4[c]
High dose + Moringa	16.8±0.6[b]

Data are presented as means±SE.

a, b, c and d/ means in the same row followed by different superscripts are significantly different (P≤0.05).

Table 6: Visual score of DNA damage in rats exposed to gamma irradiation and treated with *Moringa oleifera*.

	No. of cells		Class**				
Treatment	Analyzed*	Comets	0	1	2	3	DNA damaged cells (%mean)
Control	100	6	94	5	1	0	6[d]
Low dose (4Gy)	100	19	81	11	5	3	19[b]
High dose (6Gy)	100	32	68	8	11	13	32[a]
Low dose + Moringa	100	11	89	7	2	2	11[c]
High dose +Moringa	100	23	77	7	7	9	23[b]

*: Number of cells examined per a group, **: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.

a, b, c and d/ means in the same row followed by different superscripts are significantly different (P≤0.05).

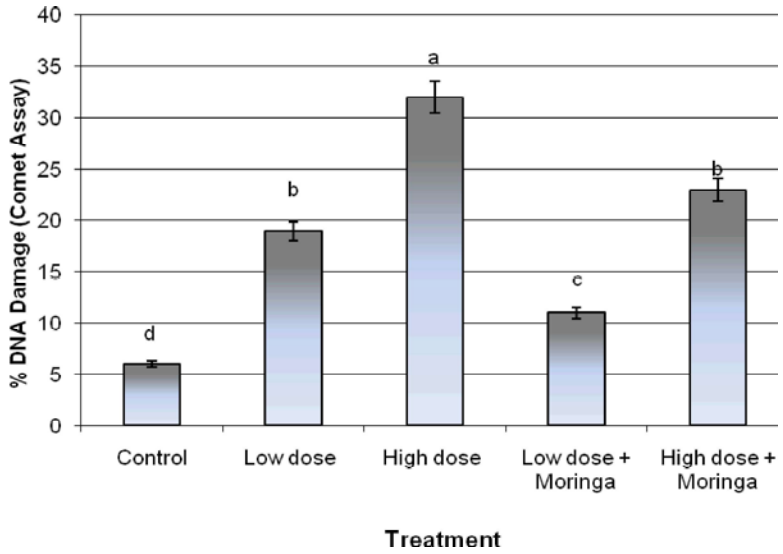

Fig. 3: DNA damage in liver cells of male rats exposed to gamma irradiation and treated with *Moringa oleifera* extract analyzed by the comet assay. Data are presented as means±SE. a, b, c and d/ means in the same row followed by different letters are significantly different (P≤0.05).

control rats than those in all treated groups. Irradiation of male rats with low or high doses increased, significantly, the formation of MnPCEs (Table 5). The increases in the rates of MnPCEs formation were 300% at low dose 4Gy and 400% at the high dose 6Gy of irradiation in comparison to the controls (Table5).

Rats exposed to low dose of gamma irradiation and treated with *Moringa oleifera* extract had significantly lower incidence of MnPCEs compared to the untreated group. The reduction in MnPCEs formation was 38.4%. Similar trend was observed when rats were exposed to the high dose of irradiation then treated with *Moringa oleifera* extract. However, the MnPCEs formation was significantly (P≤0.05) lower (30.8%) than in rats exposed to the high dose of gamma irradiation (Table 5).

Comet Assay: The results of comet assay for DNA strand break in nuclei from individual liver cells of male rats exposed to low or high doses of gamma irradiation showed considerable DNA damage (Fig. 3 and Table 6).

These results showed that DNA damaged cells were significantly ($P \leq 0.05$) higher in rats exposed to low or high doses of gamma irradiation compared to the control rats with a pronounced dose effect.

DNA damaged cells were categorized in class 3 according to the tail length of damaged cells. Rats exposed to high dose of irradiation had higher comet assay score than those exposed to the low dose of irradiation and all the other groups. DNA damaged cells in rats exposed to gamma irradiation and then treated with *Moringa oleifera* extract were significantly lower in comparison to rats treated with either low or high doses of gamma irradiation alone (Fig. 3 and Table 6).

DISCUSSION

Animal models are powerful tools for investigating and understanding the complexity of radio frequency radiation pathogenic potency, since the mechanisms involved are, without doubt, multifactorial. The advantage of such model is that one can precisely design the experiment, keep the relevant parameters under strict control and imitate the pathological course to be studied. This is on contrast to the many well-known, mostly ethical limits that are encountered during a human study [38, 39]. It is known that active or developing physiological systems are, in general, more sensitive to noxious stimuli than static systems. Because erythropoiesis is an ongoing process, there is a continuous progression of cells from erythroblasts to immature and mature erythrocytes, which is balanced in the steady-state condition. As bone marrow is the most proliferative tissue in the body, it could be presumed that the haematopoietic system could reveal radiation effects, even subtle ones [38, 39].

The formation of superoxide partially accounts for the well known oxygen enhancement of radiation induced biochemical changes and cell damage. After whole body gamma irradiation at a dose level of 6 Gy, a significant decline in RBCs, Hb, Ht% and WBCs count was observed in rats [40]. Treatment with *Moringa oleifera* for 21 days resulted in increased Ht% and WBCs count. The WBCs changes affected the differential counts of lymphocytes, neutrophils, monocytes but not esinophils [10]. It was reported that moringa powder, after 3 months of treatment, was effective to increase Hb concentration but iron remained unchanged, MCH was increased significantly but MCV was not changed in anemic subjects [41]. Also daily consumption of dried leaf powder did not improve iron status in anemic subjects. Moringa contains iron but its bioavailability was low and could not cover iron requirements [41]. Moreover, it was found that in subjects suffer from anemia, treatment with *Moringa oleifera,* which is reported to contain alkaloids, flavonoids can induce increase in RBCs, Hb, Ht%, MCV, MCH and MCHC. Freshly blanched drumstick leaves showed a mild positive relationship in the improvement of anemia [42].

Gamma irradiation results in a decrease of the total count of WBCs, Lymphocytes, monocytes, neutrophils, basophils and esinophils [43]. The results are consistent with the previous findings that irradiation induced leucopenia [44] and reduces lymphocytes, neutrophils and monocytes count [43, 45]. The decreases could be attributed to high radio sensitivity of haematopoietic tissue [46] and a reduction in the viability of spleen hematopoietic stem cells [47]. The integrity of the immune system depends upon the normal functioning of the lymphoid organs so that the alterations in the homeostasis of spleen tissues will affect immune responses. Spleen plays an important role in immune functions by proliferating lymphocytes [48]. The decrease in lymphocytes might result from oxidative damage in the spleen tissues. Furthermore studies have demonstrated that lymphocytes are considered to be the most sensitive type of blood cells and the earliest blood change induced following whole body irradiation is lymphopenia [49].

Folic acid, is one of the B vitamins acts as cofactor, severe folate deficiency causes megaloblastic anemia. Folate intake further confounded by the presence in these foods of additional potential anticancer micronutrients such as vitamin C, vitamin E and carotenoids. Folic acid deficiency alters normal DNA methylation and by inducing an imbalance in DNA precursors leading to modified DNA synthesis repair. Deficiency of vitamins increased exposure to endogenous and exogenous reactive oxygen species [50]. Folate deficiency causes chromosome breaks in human genes. Vitamin B_{12} deficiency would be expected to cause chromosome breaks by the same mechanism as folate deficiency [51]. Supplementation of drumstick leaves may increase the iron status as measured by hematological indices. Also drumstick leaves can thus be an important source of vitamins, folic acid and vitamin B_{12} [52].

Moreover, it was found that the treatment of rats with a single dose of 6 Gy gamma irradiation induced a hepatic damage and a significant elevation in activities of ALT and AST [53]. *Moringa oleifera* flowers or leaves extracts showed a significant reduction in the severity of the liver damage by decreasing the activities of ALT after exposure the rats to toxic liver injury [54]. The results strongly indicated the therapeutic properties of mohyroethanolic

extracts against acute liver injury and thereby scientifically support its traditional use. *Moringa oleifera* is a rich source of essential minerals and antioxidants, it has been used in human and animal nutrition [55]. Liver marker enzymes were significantly reduced after treatment with flower and leaf extracts of *Moringa oleifera* in animals with acetaminophen toxicity. It was reported that *Moringa oleifra* extract decreased hepatic markers enzymes against drugs induced toxicity in rats [56]. It has a protective effect by decreasing liver lipid peroxides and enhancing antioxidants. It was also reported that *Moringa oleifera* is used in Indian folk medicine for the treatment of various illness [57]. They have evaluated the hepatoprotective effect of an ethanolic extract of leaves on liver enzymes ALT, AST and Alkaine phosphatase. *Moringa oleifera* showed a protective action by the effect of its leaves on these enzymes after liver damage induced by anti-tubercular drugs; it enhances the recovery from hepatic damage [7].

The exposure of animals to radiation led to induction of lipid peroxidation as evidenced by increased malondialdhyde [53]. Also, it was confirmed that exposure to ionizing radiation whether occupational or during radiotherapy leads to serious systemic damage to various cellular and subcellular structures [43]. Radiation exposure creates free radicals causing oxidative stress and an increase in lipid peroxidase level. It was also found that the oxidative damage marker (MDA) was decreased after treatment with *Moringa oleifera* flowers and leaves hydroethanolic extracts that act against liver injury and thereby scientifically support their traditional use [54].

Overviews of the literature suggested that radiofrequency radiation induce genetic toxicity. Furthermore, exposure to non-ionizing radiation was able to cause a micronucleus frequency increase in human lymphocytes [58]. The induction of SCEs in human blood after exposure to radiation was explored by Maes *et al*. [59]. An increase of SCEs in cells exposed to radiation and then treated with mitomycin C has been reported. Chronic *in vivo* studies by Vijayalaxami *et al*. [60] have revealed a significant increase in micronucleus frequency in the bone marrow and peripheral blood of mice exposed to 20 h/day, 7 days/week, for 18 months. An increase of 0.05% in MN frequency not correlated with a carcinogenic outcome could not be of biological relevance [61]. When gamma radiation breaks DNA molecules, a cell may be able to repair the damaged genetic material, within limits. However, a study of Rothkamm and Lobrich [62] has shown that this repair process works well after high-dose of exposure but is much slower in the case of a low-dose exposure. In agreement with these findings, our results revealed that exposure to radiation increased DNA fragmentation micronucleus formation and DNA damage.

Similar to our results, it was also reported that supra-supplementation of 3000 mg/kg b.wt aqueous *Moringa oleifera* leaf extract did not elicit hepatorenal toxicity after an acute *in vivo* exposure [63]. Similarly, nothing adverse was found haematologically. Phenomenally, the biological mechanism of *Moringa oleifera* to reduce the genetic toxicity is not well known. Natural antioxidants have an important role in the prevention of many age-related diseases and promotion of health. Among natural antioxidants from plants, flavonoids and other phenolic compounds are potent antioxidants and chelating agents [18]. Therefore we can suggest that the anti-genotoxic effetcs of *Moringa oleifera* are mainly attributed to the antioxidants existing in its leaves.

CONCLUSION

A possible therapeutic action of *Moringa oleifera* leaf extract, through protecting tissues from ionizing gamma irradiation hazards, was observed. On the biochemical level, all hematological parameters and some vitamins levels were enhanced after *Moringa oleifera* treatment. Also *Moringa oleifera* reduced hepatic damage through improving AST, ALT, ALK. Ph. and malondialdehyde activities. On the genetic level, it reduced DNA fragmentation, frequency of the micronucleated polychromatic erythrocytes formation and DNA damage. This may be through its antioxidant activity that antagonizes alterations in the oxidizing systems that resulted from gamma irradiation exposure.

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