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The radioprotective effects of methanolic extract of *Moringa oleifera* leaves against gamma irradiation in rats

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Moringa oleifera is a highly valued plant. It has an impressive range of medicinal uses with high nutritional value. The purpose of this study was to explore the capability of polyphenolic-rich Moringa oleifera leaf extract against the hazards of gamma radiation. Adult albino rats were irradiated with 5 Gy gamma rays in the presence or absence of *Moringa oleifera* leaves (150 mg per kg body weight). The blood serum was collected at days 1, and 15 post-irradiation. A decrease in glutathione level was showed in the irradiated control as positive control group, while a recovery pattern was showed in treated animal groups. The normal value of glutathione level was recovered by day 15 post-treatment by *Moringa oleifera leaves*. In serum of irradiated rats, an increase in lipid peroxidation level above normal was scored. Also, a significant decrease was noticed in *Moringa oleiferaleaves* pre and after treated groups. A noticed improvement was seen in proliferating cell nuclear antigen (PCNA) and interlukin 10 levels post treated groups. The present study suggests the possible radio protective role of *Moringa oleiferaleaves* extracts.

Keywords: *MORINGA OLEIFERA*, gamma irradiation, radioprotector and Methanolic extract.

INTRODUCTION

Radiation travels from a source as waves or as energized particles. We found that radio waves and microwaves are generally considered harmless (Mohamed et al., 2014). Radiation has a health risk, Where, it damages tissues, genes and DNA causing severe damage for the biological systems. Radiation-induced the simultaneous release of inflammatory cytokines as a consequence of the generation of free radicals (Reed, 2011).

Free radicals are believed to play a role in more than sixty different health conditions, including the ageing process, cancer, radiation damage, atherosclerosis, etc. Fortunately, there are many plant derived natural antioxidants that are capable of converting free radicals into stable compounds before they inflict major damage to cellular macromolecules (Mohammad and Holly, 1997). “Oxidative stress” results from an imbalance between the defense provided by cell antioxidants and the formation of reactive oxygen species (ROS) (Sandra, 2013). When the antioxidant defence in the human body becomes overwhelmed, oxidative stress to the cellular components often occurs, inducing inflammatory, adaptive, injurious, and reparative processes (Mohammad and Holly, 1997).

On other hand, lifestyle and nutrition might play an important role against environmental oxidant exposure and damage (Borut and Rok, 2014; Cross, 2002). Antioxidants have the ability to reduce the energy of the free radicals. The role of free radical scavenging antioxidants is to remove reactive the free radicals before they attack biologically important lipids, proteins, and DNA(Chan et al.,2011).

Plants especially with free radical scavenging, antioxidant properties, and immunostimulatory properties have been evaluated for their radioprotective effects and studies carried out in the past two decades have shown that some of the commonly used dietary agents(Manjeshwar et al., 2016; Uma, 2006). Recently, the scavenging ability of certain plant extracts containing several polyphenols, flavones,catechins, and procyanidins against ROS and their inhibitory effects against X- and ç-ray-induced cell transformation were previously reported, both in vivo and in vitro (Benavente-Garcia et al., 2002; Sandhya et al., 2013).

Moringa oleifera is a highly valued plant. The leaves are highly nutritional value rich in amino acids, vitamins A, B, C and E, riboflavin, folic acid, nicotinic acid, minerals, protein and various phenolic compounds (Khalafalla et al., 2010; Anwar et al., 2007). All the parts of Moringa oleifera plants have been used for various diseases in the natural medicine, including the treatment of infectious and inflammation diseases in addition to hematological, gastrointestinal and hepato-renal disorders (Verma et al., 2009).

Moringa Oleifera (MO) Leaves have been possess radio-protective effect, anti-tumor, anti-inflammatory, anti-oxidant, anti-diabetic and hepatoprotective effects (Tiloke et al., 2013).

Oxidative stress results from the production of oxygen radicals in excess. These radicals are potential agents of oxidative stress. The major endogenous antioxidant enzymes are Glutathione, Lipid peroxidase, Superoxide dimutase (SOD) and Catalase. The exogenous antioxidant like carotenoids, vitamins A, C and flavonoids found in food (Asma et al., 2005). Moringa oleifera are very rich in exogenous antioxidant. Their leaves are known to have a high content of vitamins and antioxidants (Jahn,. 1988).

Moringa oleifera leaves and seeds (Lalas and Tsaknis, 2002)are rich sources of antioxidants. MO extract has protective effects by increasing antioxidants and decreasing liver lipid peroxides (Ashok and Pari, 2003).

In the scientific literatures, Moringa extracts have been cited as having anti-inflammatory, antibiotic, hypotensive, antiulcer, hypo-cholesterolemic and hypoglycemic activities (Fahey, 2005). Antioxidant activity of ethanolic extract of *Moringa oleifera leaves* leaves was measured by DPPH and hydrogen peroxide free radicals scavenging method (Andréa et al., 2012).

The ethanolic extract of Moringa oleifera leaves was showed antioxidant activity stronger than that in the saline extract. The ethanolic extract of MO leaves has antioxidant properties and showed the best scavenging capacity to DPPH radical (Andréa et al., 2012). Evidences support that *Moringa oleifera leaves* have radiomodulatory effect against gamma exposure in rats(Moron et al., 1979). Depending upon anti-oxidative properties of *Moringa oleiferaleaves*; the present study has focused on their radiomodulatory effect of plant extract against gamma exposure in rats.

MATERIALS AND METHODS

Animals

The subjects of this research were sixty, Male Wister rats, 6-8 weeks old, weighing 130-180 g. These rats were kept under controlled conditions of temperature and light (Light: dark, 12 hrs: 12 hrs.), with free access to food and water. All the ethical protocols and guidelines for animal handling and treatment were were performed according to the guidelines set by the World Health Organization (WHO) and the Mansoura University, Egypt.

Preparation of methanol extract

Fresh leaves of Moringa oleifera were shade-dried over a two week period and thereafter reduced to powder form. 630g of the powdered leaves were obtained and exhaustively extracted with 250ml of 70% methanol for 72 hours. The mixture was preserved in a brown bottle and shaken every day to ensure proper diffusion and circulation. The crude concentrated extracts were filtered and exposed in the absence of sun for the methanol to dry off and also for the extract to solidify for easy measurement.

Determination of optimum dose.

A dose selection of *Moringa oleifera leaves* was carried out on the basis of drug tolerance study. For this purpose, various doses of plant extract (200, 150, 100, 50, 25 mg/kg body weight) were dissolved in bidistiled water and tested for their tolerance (once in a day for 15 consecutive

days) in rats. Thus, the most optimum and tolerable dose of *Moringa oleiferaleaves* (150 mg/ kg b. wt.) was obtained and used for further detailed experimentation.

Irradiation

Cobalt teletherapy unit (Co-60) (The National Centre for Radiation Sciences), Radiotherapy Department, & Cobalt unit for irradiation. Un anaesthetized animals were restrained in well-ventilated Perspex boxes and exposed to gamma radiation at a distance (SSD) of 80 cm from the source at a dose rate of 5 Gy /min.

Experimental design

The rats were divided into four equal groups, 15 rats each. Rats in group I served as negative control giving neither extract nor radiation. Group II served as positive control exposed to gamma radiation at a dose rate of 5 Gy /min. Group III and Group IV injected with *Moringa oleiferaleaves* extract (0.5 ml/animal at a dose of 200 mg/ kg body weight for 5 consecutive days) either before (Group III) or after (Group III) exposure to radiation. Animals were necropsied on days 1, 15 post-treatment intervals to evaluate hematological and biochemical parameters.

Hematological study

Blood was collected in a vial containing 0.5 M EDTA. Total number of erythrocytes (RBC), leucocytes (WBC), hematocrit (Hct) and hemoglobin (Hb) percentage were determined by Advia 120® Hematology System (Bayer Diagnostic Division, Tarrytown, NY, USA)

Biochemical determinants

The level of glutathione (GSH) was determined according to the methods of Moron et al., (1979) (23). The lipid peroxidation (LPx) level in the serum was measured by the assay of thiobarbituric acid reactive substances (TBARS) following the method of Ohkhawa et al., (1979) (24). Serum IL-10 was measured with an immunosorbent assay ELISA kit (Cat. No. EL10027, ANOGEN Immunotech; Ontario, Canada).

Immuno-histochemistry for Detection of tissue proliferating cell nuclear antigen (PCNA):

Tissue sections 5 µm thick were mounted on polylysine-coated slides, deparaffinized, rehydrated, and then heated with 10 mM citrate buffer (pH 6) in a 95 _C water bath for 15 min. After two washings with PBS, a primary antibody of PCNA (Zymed Lab. Inc., San Francisco, CA, USA, 1:100 dilutions) was added to the slides and incubated at 4 _C overnight. After washing three times, tissue sections were incubated for 1 h with 3 µg/ml biotinylated goat anti-mouse antibody (Vector Laboratories, Burlingame, CA, USA). The slides were then incubated with 3% hydrogen peroxide to quench endogenous peroxidase activity.

After three washings with PBS, slides were incubated with an avidin biotin complex reagent containing horseradish peroxidase (Vector Laboratories) in PBS and color development was achieved by incubation using the aminoethyl cabazole substrate kit (Zymed Lab. Inc.).

The tissue sections were counterstained with hematoxylin. To determine the percentage of PCNA-positive cells, PCNA-staining cells and non-staining cells in high-powered fields were counted in different areas of luminal epithelium. Positive and negative control slides for each marker were included within each session. As a negative control, liver tissue section was processed in the above mentioned sequences but the omission of the primary antibody and PBS was replaced.

Sections were examined under Zeiss light microscopy at x40, the proliferation index (P.I) is the number of positive nuclei stained by PCNA in 1000 hepatocytes then the mean of them calculated, and the mean for each group is taken according to Akyol et al., 1998, Zero% was given to unstained sections.

RESULTS

The heamatological parameters of blood of different groups are tested and the results are compared in Table 1. The results showed that red blood cell counts (RBCs) were significantly lower in all groups compared with healthy control ($P<0.05$). While, red blood cell counts (RBCs) were extremely significantly higher in post-irradiated *Moringa* group compared with irradiated control ($P<0.01$). Also, WBC, HB & HCT were extremely significantly higher in post-irradiated *Moringa* group compared with irradiated control ($P<0.01$). ALL groups showed significantly lower levels of RBCs, WBC, HB & HCT compared with healthy controls.

Effect of *Moringa oleiferaleaves* extract on antioxident and inflamatory markers (Glutathione, Lipid peroxidation, Interleukin 10) are compared in different groups in Table 2. The results showed that Glutathione were significantly lower in all groups compared with healthy control.

While, Glutathione were extremely significantly higher in post-irradiated *Moringa oleiferaleaves* group compared with irradiated control (P<0.01). Also, the results showed that Interleukin 10 were extremely significantly lower in Prophylactic *Moringa* and post-irradiated *Moringa* compared with irradiated control (P<0.01).

Table (1): Effect of *Moringa oleiferaleaves* extract on heamatological characteristics before and after radiation

Groups	Healthy Control	Irradiated Control	Prophylactic *Moringa*	Post-Irradiated *Moringa*
RBC $\times 10^6$ (/mm^3)	5.92 ± 0.30	4.1 ± 0.28*	4.42 ± 0.32** ^	4.69 ± 0.26** ^
WBC $\times 10^3$ (/mm^3)	6.7± 0.49	4.05 ± 0.45	4.61 ± 0.48**	4.96 ± 0.46** ^
HB (mg/dl)	14.90 ± 0.53	9.88 ± 0.53**	10.52 ± 0.47** ^	11.05 ± 0.71**
HCT (%)	44.2 ± 1.60	33.40 ± 1.75**	35.1 ± 1.36** ^	38.00 ± 1.83** ^

Data are expressed as mean ± SD
*p<0.05 compared to healthy control. ** p<0.01 compared to healthy control
^ p<0.05 compared to irradiated control. ^^ p<0.01 compared to irradiated control

Table (2): Effect of *Moringa oleiferaleaves* extract on antioxident and inflamatory markers before and after radiation

Groups	Healthy Control	Irradiated Control	Prophylactic *Moringa*	Post-Irradiated *Moringa*
Glutathione	1.10 ± 0.29	0.32 ± 0.15**	0.58 ± 0.14** ^	0.91 ± 0.18* ^^
Lipid peroxidase	1.77 ± 0.52	2.15 ± 0.60	1.92 ± 0.63* ^	1.73 ±0.55 ^
Interleukin 10	26.5 ± 4.3	41.6 ± 9.1**	33.5 ± 5.2* ^	28.8 ± 4.8 ^^

*p<0.05 compared to healthy control. ** p<0.01 compared to healthy control
^ p<0.05 compared to irradiated control. ^^ p<0.01 compared to irradiated control

Figure 1 & 2 showed that. Liver section from Post Irradiated *Moringa* group preserved hepatic architecture with normal hepatocytes arranged in thin plate compared with irradiated control (P<0.01). While, liver sections from Prophylactic *Moringa* showed partial loss of hepatic architecture with moderate hydropic degeneration of hepatocytes compared with healthy control (P<0.01). Effect of extract on proliferating cell nuclear antigen (PCNA) are compared in different groups in Table 3 , figure (3). The results showed that proliferating cell nuclear antigen (PCNA) was significantly lower in Prophylactic *Moringa* & Post Irradiated *Moringa* groups compared with irradiated control (P<0.05).

We found in normal control preserved hepatic lobular architecture with normal hepatocytes arranged in thin plates in comparison with liver sections from irradiated control which showed partial loss of hepatic architecture with moderate hydropic degeneration of hepatocytes, congested central vein and sinusoids with p <0,05.

liver section from Prophylactic *Moringa* showed preserved hepatic architecture with normal hepatocytes arranged in thin plates, congested, dilated sinusoids (arrows) .

(H&E,x200), D) liver section from Post irradiated *Moringa* showed preserved hepatic architecture with almost normal hepatocytes arranged in thin plates, congested, dilated sinusoids black arrows),collectionof lymphocytes (red arrow) .

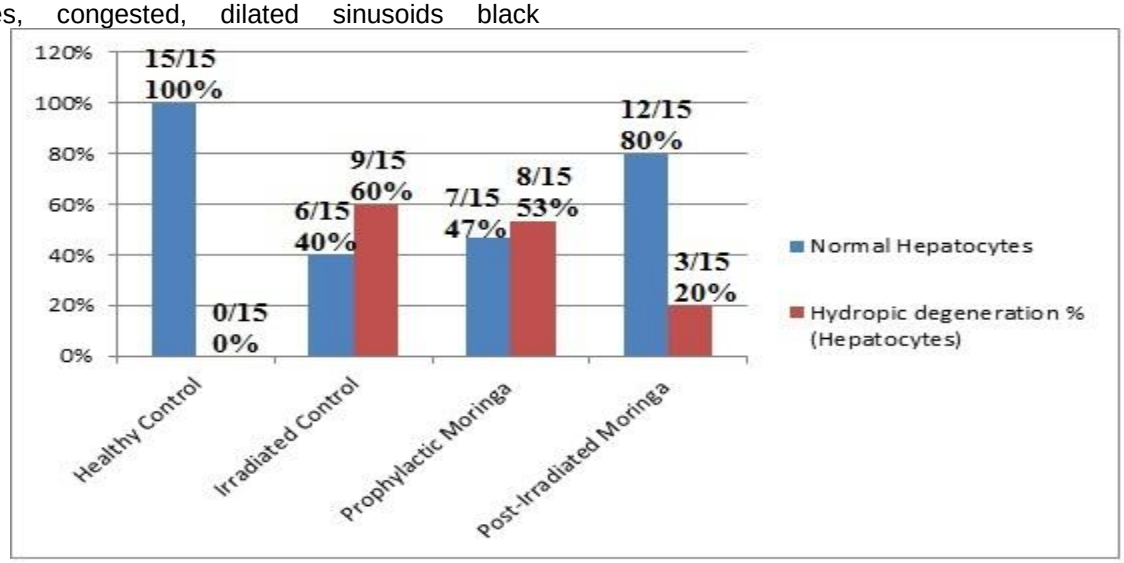

Figure (1): Effect of *Moringa oleiferaleaves* extract on Hepatocytes before and after radiation.

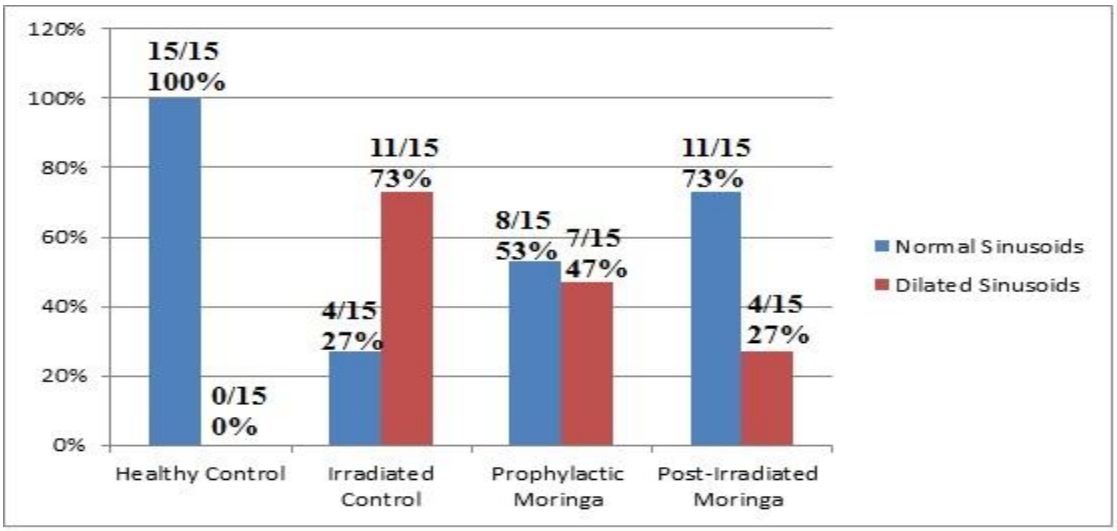

Figure (2): Effect of *Moringa oleiferaleaves* extract on Sinusoids before and after radiation.

Table (3): PCNA scoring before and after radiation.

	PCNA-Proliferation index (Mean±S.D)
Healthy Control	**2.6 ± 1.54**
Irradiated Control	**31.42 ± 3.76****
Prophylactic *Moringa*	**12.43 ± 4.76**^**
Post Irradiated *Moringa*	**13.65 ± 4.14**^**

Cross table, chi square

***p<0.05 compared to healthy control. ** p<0.01 compared to healthy control**

^ p<0.05 compared to irradiated control. ^^ p<0.01 compared to irradiated control

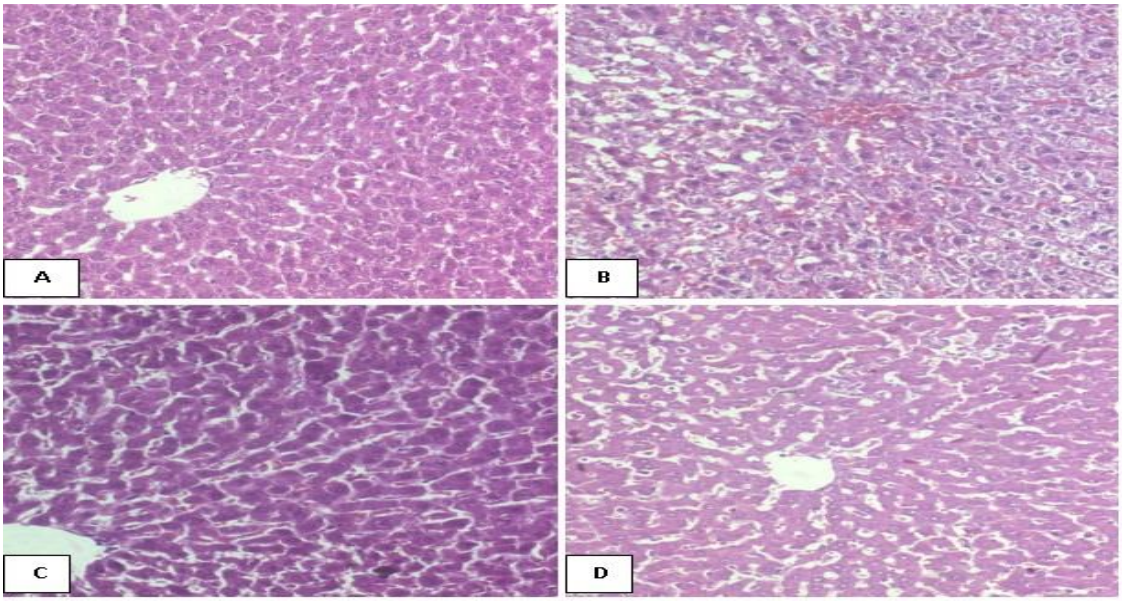

Figure (4): liver section (H&E,x200)A) from normal control showed preserved hepatic architecture with normal hepatocytes arranged in thin plates. B) irradiated control showed partial loss of hepatic architecture with moderate hydropic degeneration of hepatocytes, congested central vein and sinusoids, C) liver section from Prophylactic *Moringa* showed preserved hepatic architecture with normal hepatocytes arranged in thin plates, congested, dilated sinusoids, D) Post irradiated *Moringa* showed preserved hepatic architecture with almost normal hepatocytes arranged in thin plates.

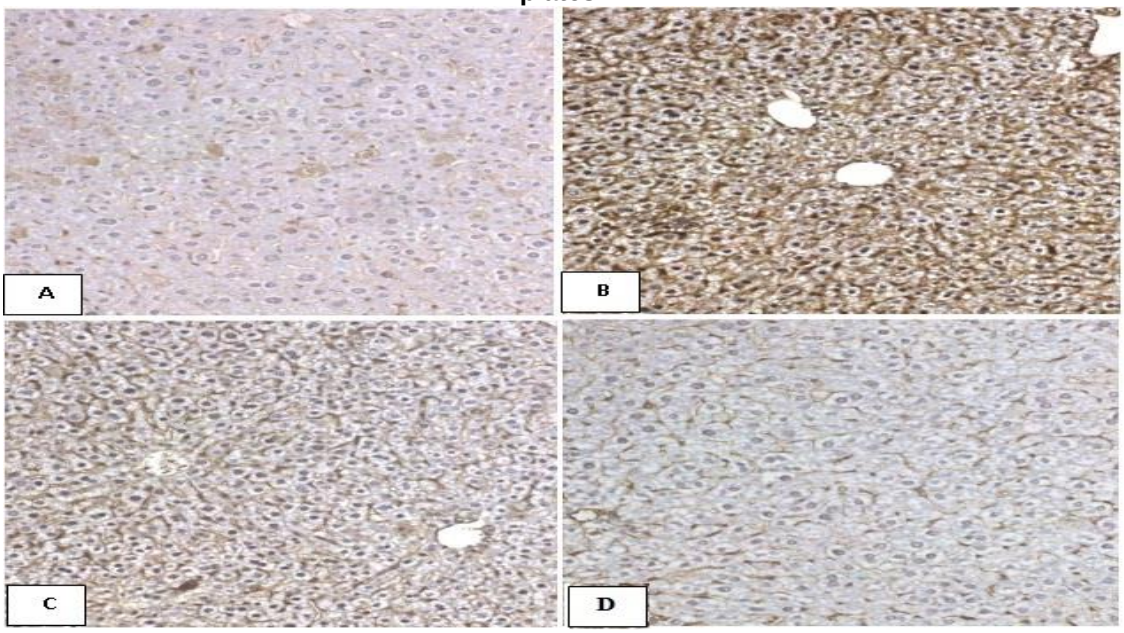

Figure (5) : liver section with IHC (DAB,IHC,x200) A) normal control negative expression of PCNA, B)Irradiated control showing positive brown nuclei expression of PCN, C) Prophylactic *Moringa* showing positive brown nuclei expression of PCNA, D) Post irradiated *Moringa* showing positive brown nuclei expression of PCNA.

DISCUSSSION:

The treatment of malignant tumors depends mainly on radiotherapy. So, effective radioprotectors are essential in this process depending on the generation of ROS to eradicate tumors (Mishra, 2002; Delanian et al., 2005). The radio-protectors must be capable of protecting normal tissues from radiation induced ROS (Benderitter et al., 2003).

Levels of MDA were analyzed as an additional index of oxidative stress in irradiated CHO cells with antioxidant pretreatment or without. The studies showed elevated levels of MDA and thiobarbituric acid reactive substances (TBARS) content in cell models exposed to ionizing radiation (Prasad, Gowda, 2005; Arora and Goel, 2000).

In one of these studies, TBARS was seen to be elevated in cultured human lymphocytes irradiated with radiation doses as low as 1 to 4 Gy (Arora and Goel, 2000; Arora et al., 2004).

A number of medicinal plants examined for their radio protective efficacy to show their protection role against the damaging effects of ionizing radiation (Jagetia GC, Baliga, 2002; Kamat et al., 1999). The medicinal plants have active compounds including anti-inflammatory compounds, antioxidants, anti-microbial compounds, etc (Maharwal et al., 2003; Casarette, 1968)..

Moringa oleiferaleaves was able to return MDA (elevated levels) to normal levels. They were also partially effective in preventing lipid peroxidation. The antioxidant treatment dose was suitable for combating radiation-induced oxidative stress, as evidenced by their ability to prevent glutathione and cysteine.

The effect of *Moringa oleiferaleaves* extracts on heamatological parameters of rats (RBC, WBC, Hb and Hct) were examined before and after exposure to radiation. RBC, WBC, Hb and Hct levels of Post Irradiated *Moringa oleiferaleaves* group were significantly elevated ($4.89 + 0.26$, $p < 0.01$), ($4.96 + 0.46$, $p < 0.01$), ($11.05 + 0.71$, $p < 0.01$) & ($38.00 + 1.83$, $p < 0.01$) respectively, compared to Irradiated Control group.

RBCs count showed a decrease after exposure to 24Hr 5 Gy gamma irradiation, which may be due to inhibition of new cells entering into the blood or loss of cells through hemorrhage (Daga et al., 1995). A similar depression occurs in Hemoglobin level as in RBC. Daga et al., (1995) reported similar results in Swiss albino mice exposed to gamma rays. Nunia et al., (2007) reported that after 6 Gy exposures, a decrease in the hematocrit value was occured. The present study is in agreement with the results of (Nunia et al., 2007)

An initial decreasing in the RBCs counts of gamma irradiated mice was observed in the present study which is due to the decline of lymphocytes in peripheral blood that are the most radiosensitive (Kosower and Kosower, 1976; Uma et al., 1999). *Moringa oleiferaleave* pretreatment led to an increase in the leucocyte counts in comparison to group II animals (Ahamed et al., 2011).

The effect of *Moringa oleiferaleaves* on the biochemical parameters of rats (i.e., lipid peroxidation, Glutathione level and Interleukin 10) were examined before and after exposure to radiation. Glutathione level of Post Irradiated Moringa oleiferaleaves group was significantly elevated ($0.91 + 0.18$, $p < 0.001$) compared to Irradiated Control group ($0.32 + 0.15$). Lipid peroxidation level of Post Irradiated *Moringa oleiferaleaves* group shows significantly Decreasing (1.73 0.55, $p < 0.001$) compared to Irradiated Control group ($2.15 + 0.60$).

Glutathione (GSH) and a free radical scavenger are the natural antioxidant system of the body to defend against lipid peroxidation (Vamanu and Nita, 2013). GSH acts as substrate (cofactor) for the antioxidant enzymes (GSH transferase, GSH peroxidase and reductase) that are involved in the peroxidation termination (Vamanu and Nita, 2013).

A significant reduction in glutathione level after radiation exposure in Swiss albino mice was reported by Uma Devi et al., (1999).

In our study, glutathione level decreased as compared to *Moringa oleiferaleaves* protected group as a result to the enhanced utilization of the antioxidant system due to the detoxification of the free radicals generated by radiation.

Radiation induced lipid peroxidation is a free radical process that is a highly destructive process where cellular organelles loose biochemical function that leads to damage of the cell. The peroxidation of cellular membrane lipids is believed to be due to the effect of radiation exposure (Leyko and Bartosz, 1986; Agarwal and Kale, 2001; Joyeux et al., 1990).

In the present study *Moringa oleiferaleaves* reduced the lipid peroxidation in terms of malondialdehyde production.

Furthermore, results showed that the antioxidant effects of *Moringa oleiferaleaves* extract are due to their ability to trigger the

endogenous glutathione level and inhibit lipid peroxidation. The role of *Moringa oleiferaleaves* may be due to stimulating or protecting hematopoiesis in bone marrow (Sotelo-Felix et al., 2002). Since significant protection is obtained at a non-toxic low dose, *Moringa oleiferaleaves* may have an advantage over the known radioprotectors (Frost and Tyler, 2000).

In the current study the level of PCNA protein significantly elevated post irradiation compared with normal control group, which reflect the high susceptibility of the liver cells to radiation damage .Accordingly treatment with *Moringa oleiferaleaves* extract either before and after radiation was proved to be potent enough to encounter the uncontrolled increment of PCNA (proliferating cell nuclear antigen) reversing it back to lower levels.

Moringa oleiferaleaves has a number of antioxidant and anti-inflammatory components that participate in its radio protective efficiency (Ai-Hsiang et al., 2002). Further investigations to study the exact mechanism of action and clinical applicability (chemoprevention and pharmacological applications) of *Moringa oleifera leaves* as a radioprotector are in progress.

The results of our present research prove that the effect of *Moringa oleiferaleaves* extract to restore the original values of heamatological and biochemical parameters.

The results also demonstrate that post-treatment of *Moringa oleiferaleaves* leaves extract improves the lethal effects of radiation exposure without changes in hematology, behavior and body weight of experimental rat.

CONCLUSION

In conclusion, The normal value of glutathione level was recovered by day 15 post-treatment by Moringa oleiferaleaves. Also, a significant decrease in lipid peroxidation level was noticed in Moringa oleiferaleaves pre and after treated groups. A noticed improvement was seen in proliferating cell nuclear antigen (PCNA) and interlukin 10 levels post treated groups.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

TEH & NMA designed and also wrote the manuscript. HAH and HAO performed chemical analysis and data analysis. HAE and TEM designed experiments and reviewed the manuscript. ME collected the samples. All authors read and approved the final version.

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