

The Curative Role of Moringa Leaf Extract Nanomaterial and Low Doses of Gamma Irradiation on Ehrlich Carcinoma Bearing Mice

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Abstract: Globally, The most prevalent reason for death is cancer. This study aimed to evaluate the effect of *Moringa oleifera* (MO) leaves extract-loaded PLGA-PEG nanoparticles or/ and minimal dose of γ -radiation on Ehrlich solid tumor (EST) bearing mice. Seventy-five adult female *Swiss* albino mice were separated into 5 groups. Group 1: normal control NC. Group 2: EST triggered by intramuscular inoculation of 0.2 ml of Ehrlich ascites carcinoma (EAC) containing 2.5×10^6 cells in the right thigh of mice. Group 3: EST-bearing mice received MO (50 mg/kg body weight/mouse) daily for 2 weeks. Group 4: EST-bearing mice exposed to 0.5Gy of gamma radiation two times weekly for 2 weeks. Group 5: EST-bearing mice received both MO and γ -radiation. The results revealed that EAC injection caused a significant increment in muscle size, oxidative stress marker (MDA), pro-inflammatory cytokines (IL-6, NF-kB, and TNF- α), MMP-2, MMP-9, VEGF, and a significant decline in antioxidant status (SOD and CAT) activities. There was damage in muscle tissue during the histopathological studies. Treatment with nano moringa or/ and a minimal dose of γ -radiation caused amelioration in biochemical parameters besides histological studies in muscle tissue. So, nano moringa and a minimal dose of γ -radiation could be used as a safe and effective cancer treatment.

Keywords: EST; moringa nanoparticles; γ -radiation; NF-kB; MMP-2; MMP-9; VEGF.

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1. Introduction

Today, cancer is a major important global issue [1]. It is mostly leading to death [2]. Among all cancers, breast cancer is one of the most prevalent types that affect individuals worldwide [3]. Experimental tumors play a crucial role in modeling. We can now examine both the behavior and the mechanisms of tumor progression by using animal models of cancer that resemble human natural tumors [4]. Ehrlich ascites carcinoma (EAC) is a highly prevalent experimental tumor and is used extensively in modeling. It is defined as an undifferentiated carcinoma [5]. In addition, it is known to be characterized by 100% tumor malignancy without a tumor-specific transplantation antigen (TSTA), strong transplant ability, no regression, fast development, and a brief lifespan [6].

In the field of drug discovery research, many medicines were produced from natural sources, especially plants; these plants are still utilized to cure a variety of illnesses, either by themselves or as a component of herbal medicines [7]. Using natural herbs and their

constituents as alternative cancer treatments is thought to have a significant impact on cancer therapy and prevention [8]. Numerous therapeutic herbs have been used to treat and prevent various forms of cancer [9].

Moringa oleifera is a wonderful herb with numerous dietary and therapeutic benefits [10]. Numerous vital nutrients, including beta-carotene, minerals, amino acids, and vitamins, are prevalent in it [11,12]. The protein content of Moringa is comparable to that of milk and eggs, and it is higher in calcium than milk, iron than spinach, potassium than bananas, vitamin C than oranges, and vitamin A than carrots [13]. *Moringa oleifera* is rich in proteins, vitamins A, D, C, and E, as well as other nutrients like antioxidants, flavonoids, isothiocyanates, and important amino acids [14,15]. *Moringa oleifera* extract has been shown to have a number of pharmacological properties, including blood lipid-lowering, neuroprotective, anticancer, hepatoprotective, and anti-inflammatory effects [16]. Furthermore, research has been done on its potential medical benefits for the treatment of the management of pain, diabetes, rheumatoid arthritis, atherosclerosis, infertility, and depression [14].

Recently, the synthesis of nanoparticles (NPs) has been achieved by utilizing special phytochemical extracts from plant sources. Several plant metabolites, including alkaloids, phenols, and tannins, are necessary for nanoparticle formation by reducing metal ions [17]. Natural product-loaded nano-composites are suggested as a way to improve cancer targetability and decrease negative effects on normal cells. Since NPs can have great stability and high carrier capacity, their shape, variable size, and surface properties make them very useful in several fields of oncology. Because of this ability, NPs may incorporate hydrophilic and hydrophobic materials [18]. Also, NPs are promising for the regulated release of medicines and nutrition, avoiding disturbing normal cells [19].

PLGA is one type of polymer nanoparticle that has good drug release control, homogeneous particle size, high drug loading rate, high biocompatibility, and high biodegradability [20]. Extract of *Moringa* leaves loaded PLGA-PEG (poly D, L-lactic-co-glycolic acid-polyethylene glycol) is used because herbal medicines have low stability, solubility, and bioavailability. PLGA-PEG solves these issues of herbal medications due to an increase in the solubility, stability, bioavailability, and pharmacological activity of herbal medications [21,22].

Radiation therapy (RT) is widely used to treat solid human tumors because it can harm biological macromolecules, especially DNA, which effectively causes localized cell death and growth inhibition in exposed tumor cells [23]. A low dose of ionized radiation (LDIR) exposure positively affects biological systems so that repair mechanisms in the body are stimulated and activated [24]. Interestingly, several investigations employing animal and human models demonstrated that a minimal dose of radiation can promote normal cell proliferation, enzymatic repair, and tissue repair, increase immune response, slow down aging, and even stop or inhibit cancer development [25,26]. The present study aimed to assess the possible therapeutic and protective impacts of minimal dose γ -radiation and *Moringa* nanoparticles against the carcinogenic effects of EST.

2. Materials and Methods

2.1. Materials.

2.1.1. Experimental animals.

Seventy-five mature female Swiss albino mice were used as test animals in this investigation. Mice were received from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). The average age of the animals was 8–10 weeks, and they weighed between 22 and 25 g. To minimize problems during the experiment, animals were given a 14-day pre-experimentation time to adapt to laboratory conditions. Animals were housed in 15 mice per cage under typical laboratory conditions, including all hygiene precautions and continuous lighting. They were maintained in metabolic cages and kept in conditions of good ventilation, typical temperature ranges, and humidity levels throughout the experimental period. Every day, fresh supplies of water and food were provided to mice.

Every animal procedure was performed in compliance with the Public Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996)². This research protocol has been approved by the research ethics committee (REC), which follows the 3Rs principles for animal experimentation (replace, reduce, and refine). It has been prepared and carried out in accordance with the CIOMS and ICLAS International Guiding Principles for Biomedical Research Involving Animals 2012.

2.1.2. Ehrlich ascites carcinoma cell line.

Throughout the study, Ehrlich ascites carcinoma (EAC) cells were used. The cells were bought from the National Cancer Institute (NCI; Cairo University, Egypt). Each week, 2.5×10^6 EAC cells were intraperitoneally transplanted into each mouse during the experiment [27].

2.1.3. Ehrlich solid carcinoma transplantation.

Every mouse's right thigh was intramuscularly injected with 0.2 ml of EAC cells, containing 2.5×10^6 viable EAC cells. Calliper was used to measure the solid tumor development rate in each of the experimental groups. Tumor volume measurements were initiated for all groups at the beginning of pulp appearance in the Ehrlich group and continued until the tumor volume reached 1 cm^3 .

2.1.4. *Moringa oleifera* (MO) leaves extract.

We obtained *Moringa oleifera* from the National Research Centre, Dokki, Giza, Egypt, located in the Scientific Society of Moringa.

2.1.5. Moringa leaves extract-loaded PLGA-PEG nanoparticles.

National Research Centre, Giza, Egypt, produced *Moringa* leaves extract (ML)-encapsulated PLGA-CS-PEG nanocomposites (MLn), which were used for medicinal applications.

2.1.6. Urethane.

All mice were anesthetized by using urethane before being sacrificed. Urethane was purchased from STARCHEMIE, Cairo, Egypt. It is a crystal, white color, and water-soluble compound. The empirical formula of urethane ($C_3H_7NO_2$) was used. It is Also called ethyl carbamate and ethyl urethane.

2.1.7. Radiation facility.

At the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Cairo, Egypt, the whole body of mice was exposed to gamma irradiation. The Canadian Gamma Cell-40 (^{137}Cs) was the indoor shielded radiation source, which confirmed a uniform distribution of doses over the entire irradiation tray. The experimental animals were located in a particularly created acrylic container with good ventilation as well as entire body γ -radiation at a dose of 0.5 Gy of gamma radiation twice weekly for 2 weeks after 8 days of tumor inoculation according to the following procedure. Mice received 1 Gy/week for two weeks (1 Gy/ week X 2), meaning the total body radiation for two weeks is 2 Gy. The duration of one dose of γ -radiation is 1.5 min.

2.2. Preparation of *Moringa oleifera* leaf extract.

200 g of dry, grounded *Moringa* leaves were mixed with distilled water (1:10) and boiled for 40 minutes at $90 \pm 2^\circ C$. The extract was filtered with a vacuum filter, and its filtrate was kept in a dark bottle. The extract was kept at $2.3 \pm 1^\circ C$ in the refrigerator until use.

2.3. Preparation of *Moringa* leaves extract-loaded PLGA-PEG nanoparticles.

A slight modification was performed on the method for the nanoparticles, according to Parveen and Sahoo [28]. Quickly, In order to create the primary emulsion of poly D, L-lactide-co-glycolide (PLGA) nanoparticles, 100 mg of PLGA polymer was dissolved in 3 ml of chloroform. Using a microtip probe sonicator (VC 505, Vibracell Sonics, Newton, USA), the product was further emulsified in an aqueous polyvinyl alcohol (PVA) solution (12 ml, 2% w/v) to create an O/W emulsion.

In order to prepare the polyethylene glycol-blended polylactic acid (PLGA-PEG) nano-void, three different nano-formulations with three different PLGA-PEG ratios (1:2, 2:1, and 1:1) were prepared, then before being mixed with the PLGA polymer, added to the aqueous solution of PVA to produce PLGA-PEG nanoparticle. For eight hours, the mixture was stirred regularly to facilitate the organic solvent evaporation. The following day, an extra amount of PVA was eliminated by ultracentrifugation for 20 minutes at 50, 602 \times g at $4^\circ C$ (Sorvall Ultraspeed Centrifuge, USA). After that, two washes with double-distilled water were performed.

For medicinal purposes, A certain concentration of *Moringa* leaf extract (ML)-was added before emulsification in order to prepare *Moringa* extract nanoparticles (MLn).

2.3.1. Transmission electron microscopic (TEM) of PLGA-PEG.

Transmission electron microscopy (TEM, Philips CM-10, FEI Inc., Hillsboro, OR, USA) was used to examine the particle appearance of the NPs. Following the complete drying process, 100 μ g/ml of the nano-suspensions were dropletted into copper grids coated with

Formvar, and the samples were stained using 2% w/v uranyl acetate (Electron Microscopy Services, Ft. Washington, PA). Soft Imaging Viewer, besides Digital Micrograph, was used for image capture and analysis.

2.3.2. Zeta potential measurement and particle size analysis of PLGA-PEG.

The zeta potential and particle size of the PLGA-PEG NPs were measured by photon correlation spectroscopy (PCS) utilizing a Zeta Sizer (Nano ZS, Malvern Instruments, UK), with a red laser of wavelength $\lambda_0=633$ nm (He-Ne, 4.0 Mw). To determine the size, 1 mg of the NPs was added to 1 ml of water, which was further diluted 10 times with water. The measurement was done for at least 120 seconds. To find the zeta potential, samples were also put in an electrophoretic cell with a voltage of ± 150 mV. The temperature of the nanocomposites was kept constant at $25.0 \pm 0.1^\circ\text{C}$.

2.4. Measurement of tumor size.

On day seven following tumor implantation, the size of the solid tumor was measured with a Vernier caliper. According to Schirner *et al.* [29] and Kisker *et al.* [30], the following formula was used to measure the size of the tumor:

$$\text{Tumor size (mm}^3\text{)} = (\text{width})^2 \times \text{length} \times 0.52 \quad (1)$$

2.5. Experimental design.

The experimental mice were separated into 5 groups (n=15). Group 1 (Cont): The mice in this group served as normal control mice because they weren't given any medication or radiation. Group 2 (EST): Every female mouse's right thigh in this group was intramuscularly inoculated with 0.2 ml of EAC containing 2.5×10^6 cells. Group 3 (EST+MOE): A group of mice was given Moringa oleifera extract nanomaterials (50 mg/kg body weight/mouse) every day for 14 days after eight days of tumor inoculation. Group 4 (EST+IR): A group of mice were exposed to 0.5 Gy of γ - radiation 2 times weekly for 2 weeks after eight days of tumor inoculation. Group 5 (EST+MOE+IR): A group of mice was gavaged Moringa oleifera extract (50 mg/kg body weight/mice) daily for 14 days and exposed to 0.5 Gy of gamma radiation twice weekly for 2 weeks after eight days of tumor inoculation. The animals in all groups were sacrificed within 24 hours of the termination of the experiment.

2.6. Blood and tissue sampling.

All of the animals were sacrificed within 24 hours of the end of all experimental treatments. All mice were anesthetized by using urethane before being sacrificed, and disposable plastic syringes were used to puncture their hearts in order to collect blood. Blood was collected in glass tubes to obtain serum to measure biochemical parameters. Sera were produced by centrifuging blood at 300 rpm for 15 minutes. The right thighs of female mice were cut off directly, which was used for histopathological studies and biochemical parameters.

2.7. Assessment of oxidative stress and antioxidant enzyme activities.

Malondialdehyde (MDA) in tissue was assessed calorimetrically based on the procedure described by Kei [31] and Ohkawa *et al.* [32]. The colorimetric kit was bought from Bio Diagnostic Company. Superoxide dismutase (SOD) in tissue was measured

calorimetrically following the procedure described by Nishikimi *et al.* [33]. The colorimetric kit was bought from Bio Diagnostic Company (Giza, Egypt). Catalase (CAT) in tissue was measured calorimetrically following the method described by Fossati *et al.* [34] and Aebi [35]. The colorimetric kit was purchased from Bio Diagnostic Company.

2.8. *Assessment of Inflammatory response markers.*

The quantitative level of tumor necrosis factor-alpha (TNF- α) in serum was assayed using the ELISA technique by utilizing the TNF- α ELISA Kit (CSB-E11987r, Houston, Texas) following the guidelines provided by the manufacturer. ELISA kit purchased from CUSABIO company.

2.9. *Quantitative Real-Time (qRT-PCR) for interleukin-6 (IL-6) and nuclear factor-kappa B (NF-kB).*

A total RNA Isolation system (Thermo Scientific, USA) was used to extract total RNA from tissue homogenate, and a high-capacity cDNA reverse transcription kit (#K4374966, Thermo Fisher Scientific, USA) was used to convert the extracted RNA into cDNA. An Applied Biosystem with software version 3.1 (StepOne™, USA) was used to carry out real-time qPCR amplification and analysis. The qPCR assay with the primer sets is listed in Table 1.

Table 1. Primers sequence of IL-6 and NF-kB.

Gene	Primer sequence
Interleukin-6 (IL-6)	Forward primer 5'- GCCCTTCAGGAACAGCTATGA-3',
	Reverse 5'- TGTCACAACATCAGTCCCAAGA-3',
Nuclear factor kappa B (NF- κ B)	Forward 5' CATGAAGAGAAGACACTGACCATGGAAA3
	' Reverse 5' TGGATAGAGGCTAAGTGT AGACACG 3'

2.10. *Determination of metastasis enzymes(MMP-2 and MMP-9).*

The quantitative level of Serum Matrix metalloproteinase-2 (MMP-2) was assessed by the ELISA technique using the MMP-2 ELISA Kit (MBS2515523, San Diego, US) according to the manufacturer's recommendations and instructions. ELISA kit bought from MyBioSource company. Serum Matrix metalloproteinase-9 (MMP-9) was assayed by ELISA technique using the MMP-9 ELISA Kit (MBS722532, San Diego, US) in accordance with the manufacturer's instructions. ELISA kit bought from MyBioSource company.

2.11. *Assessment of angiogenesis markers (VEGF and TGF- β 1).*

The quantitative level of serum Vascular Endothelial Cell Growth Factor (VEGF) was measured by ELISA technique using VEGF ELISA Kit (CSB-E04757r, Houston, Texas, United States) following the manufacturer's guidelines. ELISA kit purchased from CUSABIO company. The quantitative level of serum Transforming Growth Factor Beta (TGF- β 1) was assayed by the ELISA method using TGF- β 1 ELISA Kit (MBS260302, San Diego, US) according to manufacturer instructions and guidelines. ELISA kit purchased from MyBioSource company.

2.12. *Histological study.*

The tumor tissues were instantly taken from the mice after they were sacrificed and cut into appropriate-sized pieces after being washed in a saline solution. They were then left for a full day in 10% neutral buffered formalin. Following fixation and dehydration in a series of increasing alcohol concentrations, the tissue samples were cleaned in xylene and embedded in paraffin at 60°C. A sliding microtome was used to cut a section with a thickness of 5 μm. After obtaining tissue slices, they were placed on glass slides and stained with hematoxylin and eosin dye for a histological examination under a light microscope [36,37].

2.13. *Statistical analysis.*

The results obtained for this work were shown in tables as mean ± standard error. A one-way analysis of variance (ANOVA) was utilized in the statistical study to examine the significance of different treated groups according to [38]. At $P \leq 0.05$, differences between means are considered statistically significant; at $P \leq 0.01$ and $P < 0.001$, they are considered highly and very highly significant, respectively. Each statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The percentage difference indicating the percent of dissimilarity for the corresponding control group and applied in accordance with the following rule:

$$\%Difference = \frac{\text{treated value} - \text{control value}}{\text{control value}} \times 100 \quad (2)$$

3. Results

3.1. *Characterization of nano-Moringa particles.*

Three distinct Moringa leaf (MLn) nanocomposites were synthesized by altering the PEG concentrations that were attached to the PLGA nanoparticles. The MLn (formulation 1; F1) has nano-size (190.137 ± 6.5 nm; Figure 1), nano-stability with zeta potential (-13.23 ± 3.32 mV) and polydispersity index (PDI) = 0.5 ± 0.02 , and well-fitted correlation data (Figure 1, 2 and 3) as shown in Table 2.

Table 2. The influence of PLGA: PEG ratios on the particle size and zeta potential of MLn- based PLGA-PEG nanoparticles.

Formulation	PEG	PLGA	Nano-size (nm)	PDI	Zeta potential (mV)
F1	2	1	190.137 ± 6.5	0.5 ± 0.02	-13.23 ± 3.32
F2	1	2	341.995 ± 15.4	0.6 ± 0.03	-5.27 ± 1.29
F3	1	1	141.772 ± 14.5	0.05 ± 0.01	-39.60 ± 3.52

PLGA; poly D-L-lactide-co-glycolide, PEG; polyethylene glycol, MLn; Moringa leaves extract nanoparticles, F1; formula 1 (ratio 1:2), F2; formula 2 (ratio 2:1), and F3; formula 3 (ratio 1:1), PDI; polydispersity index, S.E.; standard error.

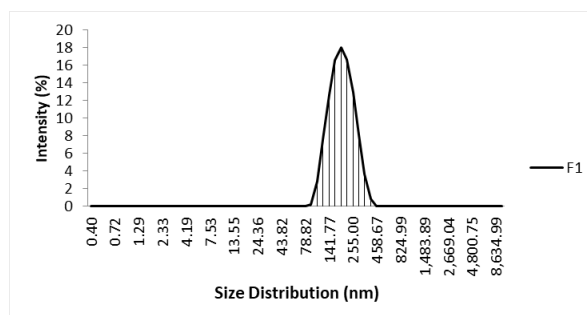


Figure 1. Size distribution of formula 1 (F1).

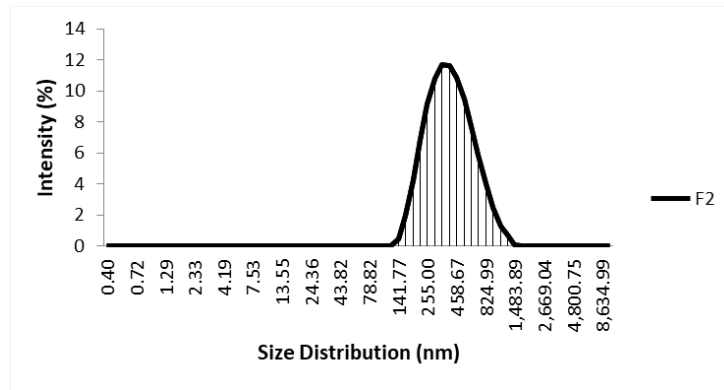


Figure 2. Size distribution of formula 2 (F2).

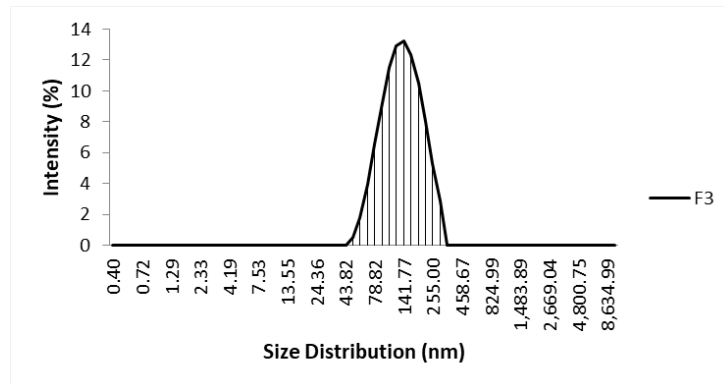


Figure 3. Size distribution of formula 3 (F3).

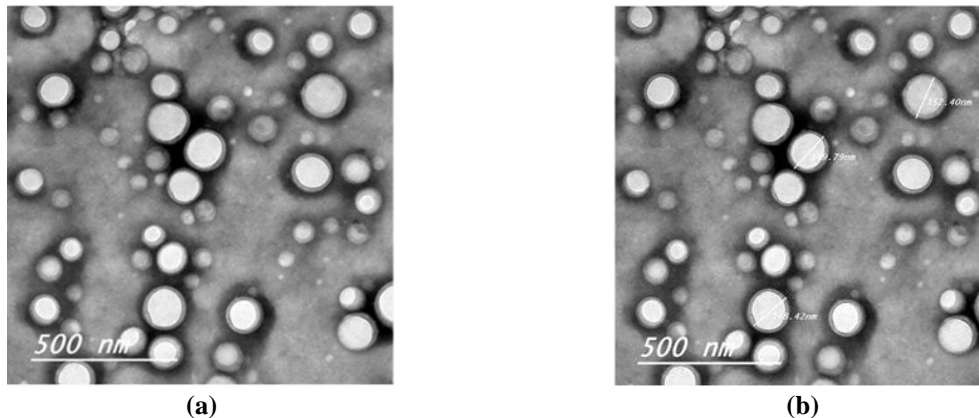


Figure 4. Characterization of F3 nanoparticles: (a) TEM of F3 nanoparticles showing spherical particles with a core and a capsule surrounding them; (b) TEM of F3 nanoparticles showing some sizes of selected particles ranging from 148.42 to 152.40nm.

3.2. The influence of *Moringa oleifera* (MO) leaves extract-loaded PLGA-PEG nanoparticles or/ and a low dose of gamma radiation on Ehrlich Solid Tumor (EST) bearing-mice.

3.2.1. Monitoring of Ehrlich carcinoma tumor size.

As demonstrated in Figure 5, it was clear that on the 7th day after tumor inoculation (ATI), a solid tumor with a mean size of $225.5 \pm 9 \text{ mm}^3$ formed in the right thigh of mice due to injection of 2.5×10^6 EAC cells in 0.2 ml physiological saline. EC tumor size overrides $372.33 \pm 35 \text{ mm}^3$ on the 14th day ATI. The increment in Ehrlich tumor size progresses over days, reaching $1406.3 \pm 123 \text{ mm}^3$ on the 21st-day ATI.

A group of experimental animals was given nano *Moringa oleifera* extract (50 mg/kg body weight/mouse) every day for 14 days after eight days of tumor inoculation, revealing

continuous and significant delay of EC size. The tumor size was recorded as $168 \pm 31 \text{ mm}^3$ on the 14th day ATI and $209 \pm 23 \text{ mm}^3$ on the 21st day ATI. However, after eight days of tumor injection, the animals bearing tumors were subjected to 0.5 Gy of γ -radiation two times weekly for two weeks, which resulted in a notable delay in the development of EC. The tumor size was recorded as $242 \pm 27 \text{ mm}^3$ on the 14th day ATI and $215 \pm 17 \text{ mm}^3$ on the 21st day ATI. The administration of nano *Moringa* and low dose γ -radiation to EAC mice resulted in a notable delay in EC progression. The tumor size recorded $294 \pm 19 \text{ mm}^3$ and $201 \pm 24 \text{ mm}^3$ on the 14th and 21st day ATI, respectively.

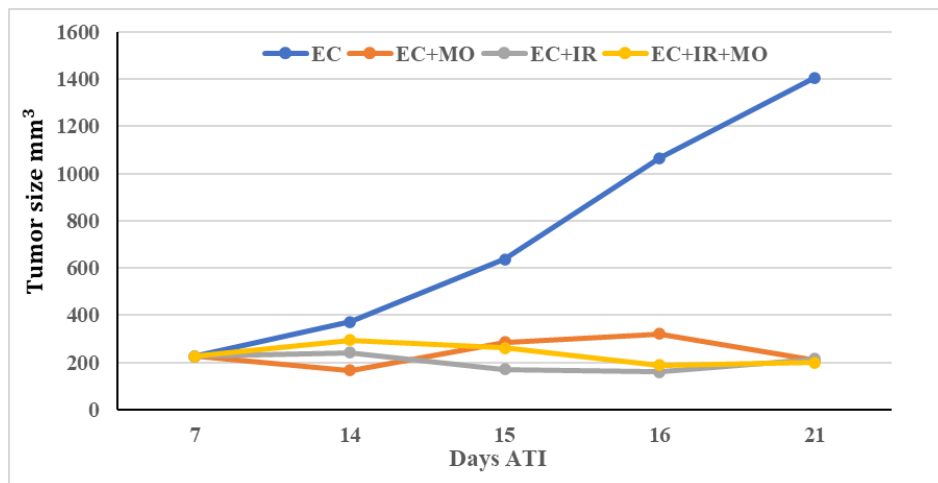


Figure 5. Impact of nano *Moringa oleifera* extract and/or γ -radiation on tumor size of EC.

3.2.2. Oxidative stress marker and antioxidant enzymes.

Lipid peroxide (MDA) level, SOD, and CAT activities in muscle tissue of female mice bearing Ehrlich solid tumor (EST) were shown in Table 3. MDA level ($163.44 \pm 0.56 \text{ nmol/g}$ tissue) revealed a significant ($p < 0.001$) elevation in the EST group when compared to the NC group ($37.52 \pm 0.32 \text{ nmol/g}$ tissue). However, nano moringa (MO) or/and a low dose of γ -irradiation (IR) treatment produced a remarkable ($p < 0.001$) decline in the level of MDA in comparison to the EST group. SOD and CAT were remarkably ($p < 0.001$) reduction in the EST group when compared to the normal group. At the same time, MO or IR treatment produced a significant ($p < 0.001$) elevation in activities of SOD and CAT. The co-administration of MO extract and IR after EST formulation caused amelioration in SOD and CAT activities.

Table 3. The influence of MO, or/and IR on the level of MDA (nmol /g.tissue), SOD (u/gprotein in tissue), and CAT (u/gprotein in tissue) in female mice.

Parameters \ Groups	Cont	EST	EST +MO	EST +IR	EST +IR+MO
MDA	$37.52 \pm 0.32^{b\ddagger}$	$163.44 \pm 0.56^{a\ddagger}$	$79.59 \pm 0.62^{a\ddagger b\ddagger}$	$118.72 \pm 0.32^{a\ddagger b\ddagger}$	$62.72 \pm 0.32^{a\ddagger b\ddagger}$
SOD	$46.24 \pm 0.42^{b\ddagger}$	$14.32 \pm 0.32^{a\ddagger}$	$31.21 \pm 0.53^{a\ddagger b\ddagger}$	$22.55 \pm 0.47^{a\ddagger b\ddagger}$	$35.86 \pm 0.34^{a\ddagger b\ddagger}$
CAT	$221.26 \pm 0.76^{b\ddagger}$	$99.94 \pm 0.72^{a\ddagger}$	$193.80 \pm 0.58^{a\ddagger b\ddagger}$	$181.40 \pm 0.40^{a\ddagger b\ddagger}$	$210.04 \pm 0.24^{a\ddagger b\ddagger}$

Means \pm Standard Error (M \pm SE), where n=6. a \ddagger : The values exhibit statistical significance when compared to Cont at ($P < 0.001$). b \ddagger : The values exhibit statistical significance against EC at ($P \leq 0.001$) where NC: Normal control group, EC: Ehrlich carcinoma group, MO: *Moringa oleifera* group, and IR: Irradiated group.

3.2.3. Pro-inflammatory cytokines (TNF- α , IL-6, and NF-kB).

The measurement of the TNF- α level is shown in Table 4. The EST group revealed a significant elevation ($P < 0.001$) in TNF- α level recorded ($97.64 \pm 0.33 \text{ pg/mL}$) in comparison

to the NC group (17.44±0.25 pg/mL). On the other hand, EST +MO, EST + IR, and EST + MO+ IR groups showed a notable reduction (P < 0.001) in TNF-α evel (38.28±0.49 pg/mL), (60.1±0.40 pg/mL) and (29.42±0.29 pg/mL) respectively, when compared to EST group. Meanwhile, a remarkable increment (P < 0.001) in IL-6 and NF-kB in the EST group were observed. Moreover, mice in (EST +MO) group, (EST + IR) group, and (EST +MO+ IR) group showed a notable decline in IL-6 and NF-kB levels when compared to the EST group.

Table 4. Influence of nano *Moringa*, γ-irradiation either alone or combined on pro-inflammatory cytokines (IL-6, NF-kB and TNF-α).

Parameters	Groups	Cont	EST	EST +MO	EST +IR	EST +IR+MO
TNF-α (pg/mL)		17.44±0.25 ^{b‡}	97.64±0.33 ^{a‡}	38.28±0.49 ^{a‡ b‡}	60.1±0.40 ^{a‡ b‡}	29.42±0.29 ^{a‡ b‡}
IL-6		1.02±0.01 ^{b‡}	7.66±0.23 ^{a‡}	3.22±0.08 ^{a‡ b‡}	4.37±0.10 ^{a‡ b‡}	2.66±0.06 ^{a‡ b‡}
NF-kB		1.03±0.01 ^{b‡}	6.41±0.04 ^{a‡}	2.76±0.06 ^{a‡b‡}	3.36±0.06 ^{a‡b‡}	2.05±0.02 ^{a‡b‡}

Means ± Standard Error (M±SE) where n=6. a‡: The values exhibit statistical significance compared to Cont at (P ≤ 0.001). b‡: The values exhibit statistical significance against EC at (P ≤ 0.001).

3.2.4. Metastasis enzymes (MMP-2and MMP-9).

Table 5 explains that, in comparison to the Control group, the EC group showed a significant rise (P < 0.001) in MMP-2 and 9 activities. However, (EST +MO) group, (EST+ IR) group, and (EST +MO+ IR) group revealed a remarkable decline in the activities of MMP-2 and MMP-9 when compared to the EC group.

Table 5. Impact of nano *Moringa*, γ-irradiation either alone or combined on metastasis enzymes (MMP-2 and MMP-9) activities.

Parameters	Groups	Cont	EST	EST +MO	EST +IR	EST +IR+MO
MMP-2 (ng/ml)		2.37±0.12 ^{b‡}	11.56±0.30 ^{a‡}	5.72±0.06 ^{a‡ b‡}	7.07±0.12 ^{a‡ b‡}	4.59±0.04 ^{a‡ b‡}
MMP-9 (ng/ml)		3.38±0.08 ^{b‡}	14.34±0.33 ^{a‡}	6.61±0.15 ^{a‡b‡}	8.57±0.28 ^{a‡b‡}	5.98±0.10 ^{a‡b‡}

Means ± Standard Error (M±SE) where n=6. a‡: The values exhibit statistical significance when compared to Cont at (P ≤ 0.001). b‡: The values exhibit statistical significance against EC at (P ≤ 0.001).

3.2.5. Angiogenesis markers (VEGF and TGF-β1).

The values of VEGF and TGF-β1 are illustrated in Table 6. In the EST group, the VEGF level (283.60±0.30 pg/ml) revealed significant (p<0.001) elevation compared to the NC group (115.52±0.47 pg/ml). However, MO and/or IR treatment produced a significant (p<0.001) decline in the VEGF level compared to the EST group. TGF-β1 was notably (p<0.001) elevated in the EST group compared to the control group. However, MO and/or IR treatment notably (p<0.001) reduced the TGF-β1 level compared to the EST group.

Table 6. Effect of nano *Moringa*, γ-irradiation either alone or combined on angiogenesis enzymes (VEGF and TGF-β1).

Parameters	Groups	Cont	EST	EST +MO	EST +IR	EST +IR+MO
VEGF (pg/ml)		115.52±0.47 ^{b‡}	283.60±0.30 ^{a‡}	135.06±0.33 ^{a‡ b‡}	190.12±0.30 ^{a‡ b‡}	129.82±0.25 ^{a‡ b‡}
TGF-β1 (pg/ml)		120.36±0.43 ^{b‡}	325.20±0.22 ^{a‡}	162.98±0.16 ^{a‡b‡}	196.84±0.28 ^{a‡b‡}	148.84±0.37 ^{a‡b‡}

Means ± Standard Error (M±SE) where n=6. a‡: The values exhibit statistical significance when compared to Cont at (P ≤ 0.001). b‡: The values exhibit significance against EC at (P ≤ 0.001).

3.2.6. Histological study.

The histological analysis under a light microscope showed that the mice's normal muscle histology (Figure 6A) does not appear to have Ehrlich carcinoma (EC). Solid Ehrlich

carcinoma (EST) in muscle tissue had compact and EC cell aggregation spread throughout the muscle fibers. EST cells are composed of big, spherical, polygonal cells with different appearances, hyperchromatic nuclei, and various degrees of nuclear and cellular pleomorphism (Figures 6 B, C). Mice administered nano-Moringa (Figures 7 A, B, and C, D) or exposed to low doses of γ -radiation (Figures 7 C, D) showed many vacuolated areas, EST tumor cell remnants, and big areas containing necrotic cells. In contrast, administering nano-Moringa to mice with EST and exposing them to low doses of γ - radiation resulted in Ehrlich tumor cell remnants and a big area containing necrotic cells (Figure 8 A, B).

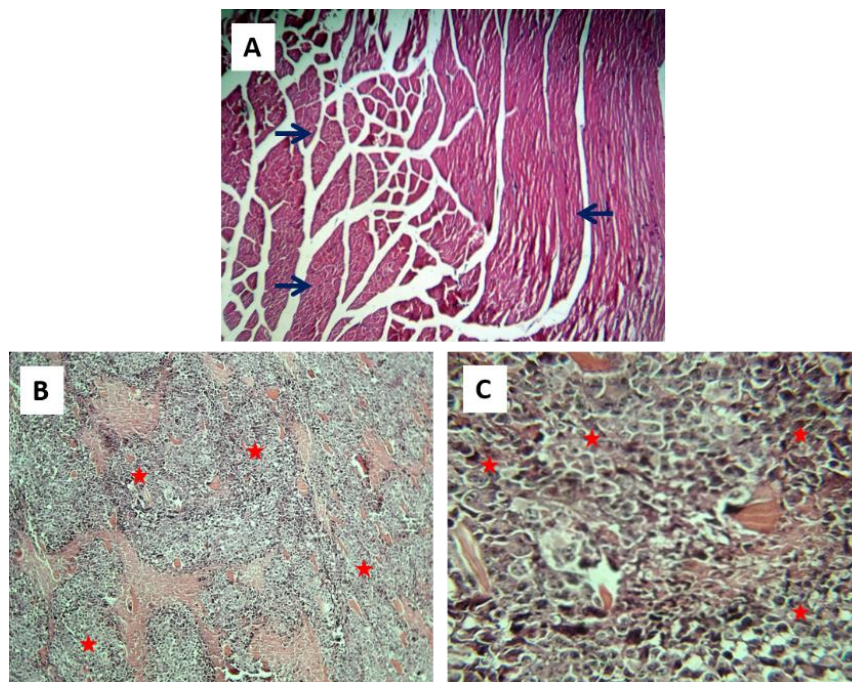


Figure 6. Photomicrographs within EC sections. (A) Normal muscle fiber is shown by the section of normal control muscle in Albino mice (\uparrow). (B) and (C) Control EC. Note: Muscle tissue was invaded by EC cells, and the muscle cells were encircled by tumor cells (stars). (H and E stain, A&B X100- C X 400).

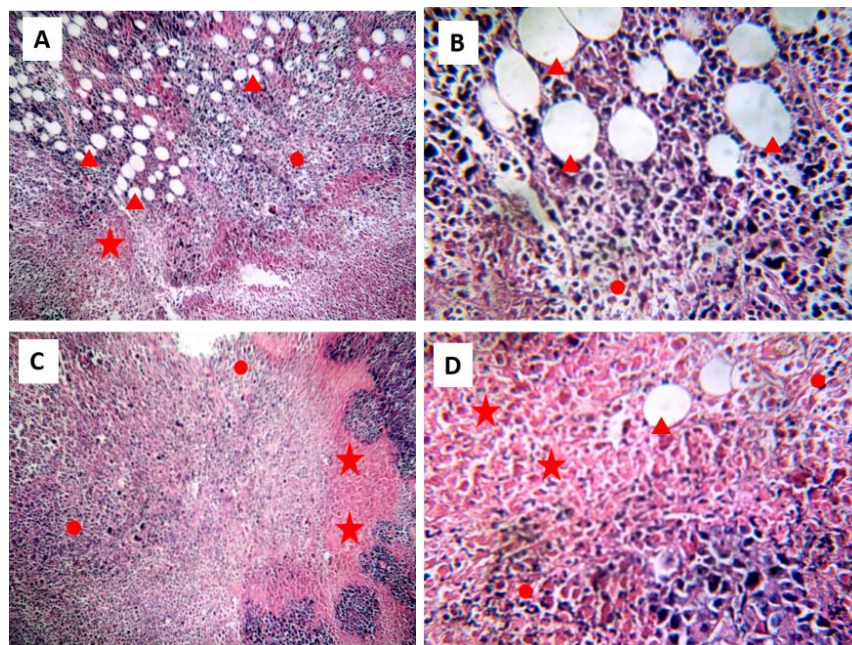


Figure 7. Photomicrographs within EC sections of mice treated with nano-Moringa (A) and (B) or exposed to low doses of γ - radiation (C) and (D) indicated many vacuolated areas (\blacktriangle), Ehrlich carcinoma tumor cell remnants (\bullet) and large area contain necrotic cells (star). (H and E stain, A&B X100- B &D X 400).

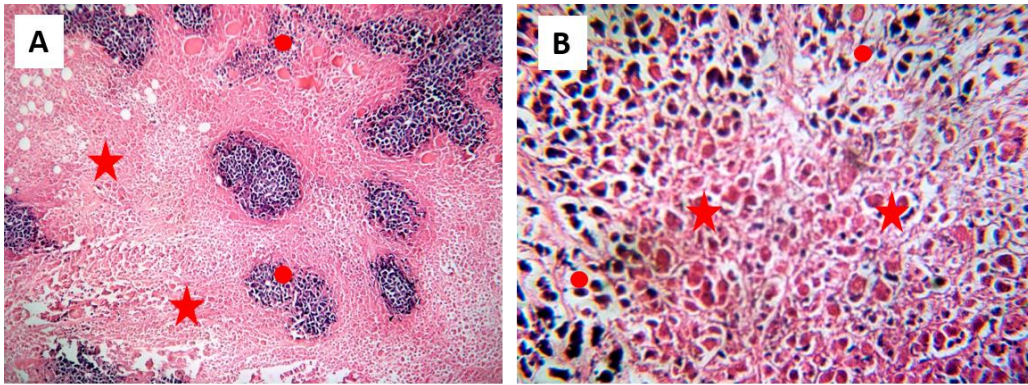


Figure 8. Photomicrographs within EC sections of mice treated with nano-*Moringa* and exposed to low doses of γ - radiation (A) and (B) demonstrated Ehrlich carcinoma tumor cells remnants (●) and large area containing necrotic cells (star). (H and E stain, A X100- B, C, D&D X 400).

4. Discussion

Cancer is the chief reason of death in the globe. It is the second greatest reason for death [39]. The death rate from cancer is still high, and there is currently no effective medication to stop tumor growth despite advances in the molecular basis, detection, and treatment of the disease [40]. Ehrlich ascites carcinoma (EAC) is a particularly aggressive tumor that grows very quickly. It can grow in almost all mouse strains [41]. It is also used as an experimental tumor because it is appropriate for investigation in a mouse model, and many human tumors resemble it. It is also used to induce subcutaneous tumor mice [42]. Fecchio *et al.* [43] reported that when Ehrlich ascitic tumor cells are implanted, local inflammatory response and increased vascular permeability are automatically induced. This causes a significant amount of edema to form, cellular migration, and gradual buildup of ascitic fluid. Researchers have effectively used Ehrlich cancer, especially in its solid form (EST), as a model in vivo research because of its rapid development and spread [44]. Additionally, it is utilized to investigate the anti-tumor properties of synthetic compounds, natural products, and nanoparticles [45-47].

A better therapeutic alternative with fewer side effects is essential for preventing the spread of cancer. Using natural or artificial substances (either alone or in combination) to slow the growth of tumors is a very promising modern strategy [40-48]. Therefore, Anti-cancer drugs derived from herbs are becoming more and more important in the assessment of cancer treatments because they have fewer or no side effects and are widely available [49]. *Moringa oleifera* leaves methanolic extract MOLME is suggested as a possible source of anticancer drugs due to the suppression of cell proliferation, reduction in tumor weight, and increased survival time [39]. Many parts of *Moringa oleifera* have different therapeutic properties, including anti-tumor, antiulcer, antioxidant, antibacterial, antifungal, antidiabetic, antispasmodic, antipyretic, anti-inflammatory, antihypertensive, antipyretic, antiepileptic, and hepatoprotective effects [50,51].

A low dose of ionized radiation (LDIR) exposure has a positive effect on biological systems so that repair mechanisms in the body are stimulated and activated [52]. Interestingly, several animal and human models studies showed that LDIR could promote normal cell proliferation, enzymatic repair, and tissue repair, increase immune response, slow down aging, and even stop or delay carcinogenesis/cancer development [25].

In the present work, the intramuscular injection of EAC cells led to changes in the muscle size of the right thigh of female mice; within the muscle fibers compact and aggregation, various degrees of cellular and nuclear pleomorphism of the EC cells spread inside

the muscle fibers. Our finding is consistent with Abd Eldaim *et al.* [53] and Teaima *et al.* [54], who noted that this inoculation-produced formation of tumor mass could be due to cell proliferation and apoptosis resistance, which led to the formation of a tumor mass. So, numerous tumor cells were found inside muscle tissue, leading to an increment in the muscle size [55,56].

Furthermore, the current work revealed that EC cell inoculation produced an increment in the level of MDA and a reduction in antioxidant enzymes, for instance, SOD and CAT [57,58]. The increment in the MDA level might be the result of EC, which has the capacity to excessively generate free radicals in the cells, leading to oxidative stress, disorder in antioxidant status, and acceleration of lipid peroxides production. Other authors reported that lipid peroxidation (LPO) is a major indicator of oxidative injury caused by ROS and impairs membrane function. It was explained that LPO causes an elevation in MDA level, which happens as a result of ROS attacking the lipids in the cell membrane [59,60]. Also, El-Bolkiny *et al.* [61] and Ali *et al.* [62] postulated that the EC inoculation caused a marked elevation in lipid peroxidation products, which are indicators of oxidative stress. This elevation was due to a disturbance in oxidative/antioxidant status. ROS, or reactive oxygen species, are more abundant in tumor cells compared to normal cells, which increase oxidative stress and destroy cell components such as lipids, proteins, and DNA [63,64]. According to their results, lipid peroxidation content and MDA levels were elevated in the EAC animals group. SOD is a key component of the defense against ROS; as reported [65], the current reduction in activity of SOD in EAC mice compared to the NC group might be related to increased lipid peroxides levels triggered by activation of metabolism. Still, SOD and CAT are crucial enzymes that block or suppress ROS, and oxidative damage occurs during tumor development, which leads to the degradation of ROS-scavenging enzymes [66,67].

Tumor necrosis factor-alpha (TNF- α) is a cytokine that causes inflammation and regulates various signaling processes [68]. This cytokine has been found to affect tumor development through multiple mechanisms, including promoting epithelial-mesenchymal transition (EMT), enhancing cell proliferation, and facilitating angiogenesis [69,70].

Moreover, the EC inoculation caused a remarkable rise in TNF- α levels. These results are consistent with Aldubayan *al.* [71], who observed that the disturbance in the systemic inflammatory response and elevation in the free radicals production is associated with an increment in over-gene expressions of TNF- α .

Interleukin-6 (IL-6) is a cytokine with pleiotropic properties, which not only controls the immune response of cells but also stimulates the growth of tumors by initiating several carcinogenic pathways. Tumor initiation and growth are stimulated by IL-6 through activating STAT3 (a signal transducer and activator of transduction-3). The pathway of IL-6/STAT3 stimulates the reduction of target genes to induce drug resistance, stimulate tumor angiogenesis, accelerate proliferation of tumor cells, invasion, and metastasis, and protect cancer cells from apoptosis [72]. Our data is in line with Abd El-Salam *et al.* [73]. They noted that Pro-inflammatory cytokines such as IL-6 and NF- κ B have an important effect on the rate of growth and development of Ehrlich carcinoma *in vitro*.

Also, the data in this investigation are reliable with the findings of Takada *et al.* [74], who revealed that NF- κ β activation controls the transcription of genes linked to invasion, antiapoptosis and cell growth in cancerous cells. Consequently, NF- κ β activation decreases cytokine-induced apoptosis while increasing tumor growth and metastasis.

The matrix metalloproteinases (MMPs) are a class of enzymes that are necessary for various physiological processes related to the restructuring of the extracellular matrix (ECM). It has been shown that they are required for angiogenesis, tumor cell invasion, and metastasis [75]. The data of our investigation also revealed that EC tumor caused an increment in MMP-2 and 9 activities. These results are reliable, as are the findings of [76], who noted that over-expressions of MMP-2 and 9 (also known as gelatinases A and B) have been observed in numerous invasive tumor cells. Also, these enzymes have an important role in angiogenesis. In addition, [77] revealed that MMP-2 and 9 activity were enhanced in cancer cells by TNF- α . TNF- α level may be responsible for the substantial rise in MMP-2 and 9 activity in EAC-bearing mice. MMPs released by tumors cause elevation of lymphatics circulation and peritoneal spaces in the organ.

VEGF is one of the most powerful angiogenic agents implicated in the development and spread of tumors. It stimulates endothelial cell growth, migration, and tube formation by binding to their expressed VEGFR-1 and VEGFR-2 tyrosine kinase (RTK) receptors [78]. Furthermore, our work revealed an elevation in the EC group. The current work agrees with the results of Dolcet *et al.* [79], who noted that NF- κ B also promotes matrix metalloproteinases related to cell invasion and induces several angiogenic factors, such as VEGF.

TGF- β has several functions in the advancement of cancer. There is growing proof that TGF- β is actively released by tumor cells throughout the later stages of cancer development. Also, TGF- β promotes cell proliferation, invasion, and metastasis [80]. There is a matching between the obtained data in the present work as a result of inoculation of EC in animals (mice) and those by [81], who observed that elevated TGF- β 1 expression indicated a probable link to the later stages of cancer development. This cytokine promotes epithelial-mesenchymal transition (EMT), metastasis, angiogenesis, and autophagy associated with immune suppression. However, TGF- β signaling suppresses epithelial proliferation in healthy tissues and accelerates the growth of tumor cells in tissues that have advanced malignancy [80].

Remarkably, the findings of the existing investigation exhibited that the administration of nano Moringa and/or γ -irradiation to EC mice caused a reduction in tumor size, represented histopathologically, many vacuolated areas, remnants of EST tumor cells and big area has necrotic cells, lipid peroxidation (MDA) and increment in SOD and CAT activities. This could be attributable to the administration of Moringa leaf extract delaying tumor cell progress, triggering apoptosis, and reducing reactive oxygen species (ROS) in tumor cells, indicating that leaves of Moringa have the ability to reduce the development and invasion of cancer cells [82]. In addition, low doses of gamma radiation can prevent tumor cell progression in mice with Ehrlich tumors by inhibiting the JAK1/STAT3 pathway [83]. Also, a low dose of radiation exposure promotes cell cycle arrest, pathway of p53, and apoptosis [84].

Using an aqueous extract of Moringa leaves as treatment caused inhibition of MDA levels while raising antioxidant levels [85]. This could be due to many types of antioxidant components inside Moringa leaves, such as phenolics, flavonoids, and carotenoids, so MOL serves as a reliable source of natural antioxidants [86]. Moreover, exposure to γ -irradiation produced a drop in free radicals, which resulted in a reduction in oxidative stress and MDA levels and improved antioxidant enzymes such as SOD and CAT when compared to the (EC) group [87].

Conversely, nano Moringa and/or γ -irradiation therapy caused a reduction in TNF- α . This could be attributable to the powerful anti-inflammatory action of Moringa, which acts as a strong antioxidant and immunomodulatory agent. In addition, it prevents the production of

macrophage cytokine (TNF- α , IL-6, and IL-8) [88]. Kumar *et al.* [89] revealed that administration of MO before γ -radiation provided protection from tissue damage caused by γ -radiation. This protection is owing to its ability to remove free radicals, reduce lipid peroxides, and boost antioxidants. This finding is in accordance with that of Hafez *et al.* [90], who discovered that STAT-3 inactivation suppresses inflammation associated with cancer and reduces the immune-suppressive tumor microenvironment.

Our investigation showed that nano Moringa and/or γ -irradiation treatment remarkably declined the toxic impact of EC by lowering the IL-6 and NF- κ B levels. This could be attributed to supplementation of MO leaf powder triggered different expression of 65 genes in tissue, including TNF, IL-1 β , INF- γ , IL-2, and IL-6. MO demonstrated chemopreventive effects against the development of cancer and the inflammatory response by downregulating pro-inflammatory mediators [91]. Jaja-Chimedza *et al.* [92] observed that Moringa has several useful components, including isothiocyanates (ITCs), which have the capacity to control a number of signal transduction pathways by either activating the master regulator Nrf2 or blocking the pro-survival pathway of NF- κ B. Also, the extract of *M. oleifera* suppresses the formation of inflammatory cytokines and controls inflammation by blocking NF- κ B. A remarkable decrease in IL-6 level was revealed compared to EC mice as the administration of doses ranged from 0.3 to 0.7Gy of γ -radiation. Has been found to potentially trigger effects of anti-inflammation and promote the secretion of anti-inflammatory cytokine [93]. Ehrlich tumor tissue strongly increased NF- κ B oncogene expression, but γ -radiation modulated the expression of this gene that aid in the progress as well as proliferation of tumor cells. When transcription factors like NF- κ B (which is critical for the invasion, migration, and the ability of tumor cells to survive) are inhibited, caspases 3 and 9 are activated in tumor cells [94].

Furthermore, the current result showed that treatment with nano Moringa and/or γ -irradiation remarkably decreased MMP-2 and 9 levels in tumor-bearing mice. This was consistent with Xie *et al.* [95], who recorded that Moringa oleifera has unique compounds called isothiocyanate (ITC) and glucosinolate. ITC may have anti-cancer and chemoprotective properties. It could be possible to inhibit the angiogenesis of tumors by the blockage of specific factors. Also, ITC and its derivatives are designed to prevent cell adhesion, invasion, and migration and decrease metastasis *in vivo* by downregulating matrix metalloproteinase (MMP) and upregulating matrix metalloproteinase inhibitors (TIMP). El Bakary *et al.* [96] reported that when EAC-bearing mice were subjected to γ -irradiation, their tumor volume was significantly delayed. This was due to the reduction of angiogenic regulator expression, the inhibition of MMP activities, and apoptosis induction.

According to the data of this investigation, treatment with nano Moringa and/or γ -irradiation caused a significant decline in VEGF and TGF- β levels. Aqueous MOL extracts have been demonstrated to hinder tumor cell development and trigger cell death to stop the development of human tumors. Mice treated with MO had much lower levels of VEGF proteins, which may indicate that MO reduces the growth of endothelial cells or blocks the influences of VEGF on endothelial cells [97]. TNF- α significantly decreased following treatment with Moringa, which plays a crucial role in liver fibrosis and inflammation; it triggers NF- κ B, which controls inflammation and activates the main fibrogenic molecule TGF- β [98]. Furthermore, MOE reduced the gene expression of TGF- β 1, boosted antioxidant enzyme activity, and inhibited lipid peroxidation. The reduction expression of collagen type IV and TGF- β 1 genes might be one of the methods of MOE to improve biological parameters. Since free radicals have the ability to trigger TGF- β 1 expression, MOE has antioxidant activity that

contributes to the inhibition of TGF- β 1 expression [99]. LDR has antioxidant effects and causes cancer cells to undergo apoptosis. Furthermore, earlier research showed that animals exposed to low-dose γ -rays, either singularly or in fractions, revealed a decline in the development of primary and/or metastatic tumors. Also, Pajonk and McBride [100] and Chen *et al.* [101] revealed that VEGF expression was dramatically reduced by LDR. This could be owing to suppression of NF- κ B. In addition, NF- κ B controls VEGF expression [102,103]. Therefore, suppression of VEGF activity by LDR could be due to a reduction in the activity of NF- κ B [104]. In addition, The anti-tumor immune response was enhanced in EAC-bearing mice by gamma radiation vaccination, which resulted in a considerable overexpression of IFN- γ and downregulation of TGF- β [105]. This could be due to radiation causing the elevation in expressing of CD44, which could generate more TGF- β , a cytokine that has been demonstrated to support the invasion of several tumors, including breast cancer, and to aid in the epithelial-mesenchymal transition (EMT) [106].

5. Conclusions

Finally, our findings discovered that the Ehrlich carcinoma inoculation to adult female Swiss albino mice induced biochemical and histological changes. Treatment with nano *Moringa* and a low dose of gamma radiation have relatively enhanced these abnormalities. Moreover, the co-treatment of MO and LDR was more efficient than nano *Moringa* or a low dose of gamma radiation alone, which could be attributed to *Moringa's* antioxidant, anticancer, anti-metastatic, and anti-inflammatory activities that reduce side effects of radiation.

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Conflicts of Interest

The authors declare no conflict of interest.

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