# Molecular and Biochemical Studies of Gamma Irradiated Rates Treated by Mesenchymal Stem Cells and Natural Antioxidant

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**Research Article**

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## Abstract

**Purpose:** Therapeutic effect of mesenchymal stem cells transplantation (MSCs) and natural antioxidant such as silymarin have been postulated as hepato-protectors against ionizing radiation induced harm. The present study was undertaken to evaluate the protective effect of (MSCs) and silymarin to ameliorate damage caused by gamma radiation.

**Materials and methods:** Three days before irradiation, rats were given silymarin at a dose of 70 mg/kg dissolved in distilled water and continued for another 21 days. One day after gamma irradiation at a dose level of 4Gy, male rats were transplanted by bone marrow mesenchymal stem cells (BMMSCs) through intravenous injection.

**Results:** One and three weeks after irradiation, irradiated animals receiving transplanted mesenchymal stem cells (TMSCs) and silymarin seperatly or with each other exhibited a pronounced elevation in liver antioxidant such as Glutathione (GSH) superoxide dismutase (SOD), glutathione –S-transferase (GST), total antioxidant capacity (TAC), catalase (CAT) and glucose-6-phosphate dehydrogenase (G-6-PDase) activity accompanied with significantdecline in lipid peroxidation and hydrogen peroxide levels in comparing with irradiated rats. Moreover, RAPD-PCR with primers OP-B10 and OP-B14 exhibited strong and different banding patterns in all treated rats compared to untreated control rats after 1 and 3 weeks of treatment.

**Conclusion:** Treatment with MSCs and silymarin possess a radio protective capacity against ionizing-radiation induced oxidative stress and organ injury.


## Introduction

Stem cells are a type of progenitor cell that can differentiate into a variety of different types of cells in the body. When stem cells divide, each new cell has the option of remaining a stem cell or transforming into a different type of cell with a different purpose (**Pittenger*et al.,* 2019).** All stem cells unrelatedly to their origin have two important features: ***first,*** stem cells are unspecified cells that can be differentiated into specified cells.***Second;*** stem cells are being abled to split and regenerate for long times **(Zakrzewski *et al.,* 2019).**

Most therapeutic applications of stem cells in human diseases are repairing injured heart by reducing inflammation, collagen deposition and remolding **(Guo *et al.,* 2020),** Leukemia **(Dessie*et al.*, 2020),** Neurodegenerative diseases **(Yao*et al.,* 2020),** Parkinson's disease **(Liu** and **Cheun g, 2020**), Alzheimer's disease **(Si** and **Wang, 2021),** repair injured liver **(Lee*et al.,* 2021),** and Type 1 diabetes (**Chen*et al.,* 2020; Abdullah** and **Alshammary, 2020**). Moreover, stem cells are generally used in experimental models in curative uses for the curing of musculoskeletal damages in mammalian animals **(Przadka*et al.,*2021)** and promoting fertility potential in sterile male rats **(Qa mar*et al.,*2021** and **Zhankina*et al.,* 2021).**

Recently, interest for using natural occurring antioxidant in nutrition or medical materials increased instead of artificial antioxidants, which are being limited because of their lateral effects such as carcinogenicity (Lourenço*et al.,* 2019). Silymarin is a mix. of flavonoid components that is isolated from the Silybummarianum (L.) Gaertn (milk thistle) plant and is well known as a natural antioxidant. It belongs to the Asteraceae family of Angiosperms, which also includes daisies, asters, and sunflowers (Azoz*et al.,* 2019). Phenols and flavonoids are natural antioxidants exhibited protective actions in various examples of toxin-produced oxidative stress (Kaurinovic and Vastag, 2019). Silymarin has curative applications such as hepatoprotection for over 2000 years (Mukhtar*et al.,* 2021 and MacDonald-Ramos *et al.,* 2021), anticarcinogenic effects (Fallah*et al.,* 2021) and have diverse impacts on numerous organs, such as the liver, pancreas, and gastrointestinal tract, as well as a potent antioxidant against oxidative stress (Khazaei*et al.,* 2021). Silymarin also, has effect on hepatic cells as protein biosynthesis and cell regeneration were increased, as well as anti-lipid peroxidation properties, ability to scavenge oxygen free radical, stabilizing cell membrane (Yang*et al.,* 2021), regulates the intracellular levels of the reduced GSH, chelates metal ions (iron and copper) (Bouhalit and Kechrid, 2018); promoter of ribosomal RNA synthesis (Khazaei*et al.,* 2021) and stimulation of protein synthesis, leading in the formation of new liver cells to replace those that have become damaged (Vargas-Mendoza*et al.,*2014).

Ionizing radiation is a high-energy radiation emitted by radionuclides, which are elements in unstable forms. It can cause structural changes leading to biological damage (**Jia*et al.,* 2021**). The severity of ionizing radiations relies on the kind, amount and density of radiation and period of exposure **(Choudhary, 2018).** Ionizing radiation consisted of both particles such as (electrons, protons, neutrons, beta and alpha) relying on their atomic characteristics and electromagnetic radiation (x-rays and γ –rays) which are the most common electromagnetic radiation producing cellular modifications such as mutations, chromosome aberration and cellular damage **(Tanaka** and **Furuta, 2021).** Gammaradiation can lead to damage in two ways either directly or indirectly or by both effects. In direct interaction which is the main harmful effect by affecting the cell as a whole, either by killing the cell or mutating the DNA through hitting a cell's macromolecules such as proteins or DNA **(Ahmed *et al.,* 2020).** Also, ionizing radiation affects indirectly, via the formation of free radicals which may either inactivate cellular mechanisms or interact with the genetic material (DNA**) (Smith*et al.,* 2017).**

## Materials And Methods

***Experimental animals:***

In this experiment, fifty male albino rats (Rattusrattus) with an average weight of 130±5 g and the same age were procured from the Egyptian Organization for Biological Products and Vaccines in Helwan city. Rats were setteled in stainless steel cages in a room with an autonomously adjusted temperature (22-25°C) and relative humidity of 55 ±5%, with a 12-hour light-dark cycle and free access to commercial balanced feed and tap water.The rats were given human care, and the current study followed the instructions's guidelines. The tests were designed by local committee, and the procedure follows the National Institutes of Health's criteria (USA).

***Silymarin drug:***

SEDICO (South Egypt Drug Industries Company) created Silymarin, which was bought. The experimental dose was dissolved in distilled water (70mg/kg body weight) **(Mahmoud*et al.,* 2019).**

***Preparation of boneMarrow mesenchymal stem cells (BMSCs):***

Eight mature male albino rats weighing 130-140 g were used to isolate bone marrow mesenchymal stem cells. Isolated bone marrow mesenchymal stem cells (BMSCs) can adhere to plastic surfaces and expand easily during in vitro culture as follows: the animal was placed in an anaesthesia chamber and halothane was used to anaesthetize it for around five minutes. Place the animal on an operation table and use cervical dislocation to kill it. Remove the skin and muscles from the back limbs and cut off the femurs and tibias. Dissect the femurs and tibias and soak them in 70% isopropanol for a few seconds before transferring them to sterile Petri dishes with 1X D-PBS. The femurs and tibias were transferred to a 10cm dish in a biosafety cabinet. Tweezers were used to hold each bone while the two ends were sliced open with a scissor. Fill a 3ml syringe with complete medium (CM) (Dulbecco's modified eagle's medium {DMEM} with 10% fetal bovine serum {FBS}, 1% Pen-Strep, and 1% L-glutamine), then flush the marrow into a 50ml tube by inserting a 22G needle into one open end of the bone. Repeat for each bone 2-3 times.Spinnung down the cells once we have collecte from all of the marrows at 200 xg and 4°C for 5 minutes. The supernatant was aspirated and the cells were resuspended in 25mL of CM. Four sterile petri dishes were used. Each of the four dishes dishes was seeded by 10ml cell suspension. For 4 weeks, the culture dishes were kept at 37°C, 5% $CO_2$ incubator. Every 2-3 days, the media was replaced. The adherent cells were trypsinized by applying 4-5ml trypsin-EDTA to each dish when the cells had reached roughly 90% confluency. The dishes were returned to the incubator and leaft there for 3 minutes to allow the cells to detach. The detached cells were washed in PBS to remove the trypsin-EDTA. The detachment cells were checked under a microscope on a regular basis. Cells became separated and rounded. The flask was shaked slightly to help the cells separation. This procedure should take no more than 5 minutes. Cell suspension was collected in a 15ml tube and cells were spinnded down for 5 minutes at 200 xg and 4°C. BMSCs were resuspended in FBS at a concentration of 1.4 x $10^7$ cells/kg in cell pellets **(Almundarij*et al.,* 2020).**

***Irradiation:***

Rats were positioned in a specific intended well-ventilated acrylic chamber and the rats body at whole were exposed to 4 Gy (gray), by a dose rate of 0.713 rad/sec for 9.33 min. from the biological irradiator cesium-137 source achieved by Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt **(Eshak** and **Osman, 2013).**

***Experimental design:***

Rats were randomly assigned into seven groups (each with six animals) after a one-week acclimatisation period: The normal control (NC) group consisted of animals that were fed a typical diet without supplementation. For three weeks, the second group (SI) was given a commercial balanced diet along with an oral dose of silymarin (70 mg/ kg diluted in distilled water). The third group (ST) received a single dose of BMSCs (about 1.4 x $10^7$cells/kg) injected into the tail vein. The fourth group (IRR) was given a total body dose of radiation (4Gy).The fifth group (SIR) received a single dose (4Gy) of radiation after receiving silymarin (70 mg/ kg) orally for three days before to radiation exposure and continuing until the end of the experiment. The sixth group (STR) received a single dosage of BMSCs (1.4 x $10^7$cells/kg) in the tail vein after being irradiated with a single dose of 4Gy. The seventh group (SSR) was given silymarin (70 mg/ kg) orally for three days before to irradiation, and then a single dosage of BMSCs was injected into the tail vein after irradiation.

***Tissue sampling:***

By the end of experimental periods, rats were fasted for 12 hrs then slaughtered by cervical dislocation after numbed by ether anesthesia. Livers were separated quickly, cleared from any particles and cut into pieces. Tissue samples from a definite fraction of the livers were weighed exactly and grind homogenously in a 10 – fold volume of ice – cold distilled water. The homogenates were centrifuged at 860 xg for 20 min. Finally the supernatants were separated and stored at 4˚C until subsequent biochemical analysis. Another specimen of liver was weighed and kept at -80˚C for later molecular analysis.

***Biochemical analysis:***

Reduced glutathione (GSH) was evaluated in tissue homogenate based on the method adopted by **Prins** and **Losse (1969).** Glutathione-S-trasferase (GST) activity was assayed spectrophotometrically using 1-chloro-2-4dinitrobenzene (CDNB) and glutathione as described by **Habig*et***

*al.* (1974). Superoxide dismutase (SOD) activity was assayed as described by Nishikimi*et al.* (1972). Catalase (CAT) activity was determined according to the method of Bock *et al.* (1980), based on the ability of CAT to decompose $H_2O_2$.The level of malondialdehyde (MDA) was determined as thiobarbituric acid reactive substance (TBARS) according to the modified method of Ohkawa*et al. (*1979). The method of H2O2 concentration was carried out according to the colorimetric method of Aebi (1984). Glucose -6- phosphate dehydrogenase (G-6-PDase) activity was estimated by the method of Kornberg *et al.* (1955). While the total antioxidant capacity (TAC) in liver was determined according to the technique of Koracevic*et al.* (2001), using colorimetric kit purchased from Bio-diagnostic Company, Taher St., Dokki, and Giza, Egypt.

*Molecular analyses:*

*DNA extraction:*

Sections of liver were put in test tubes and washed twice with 10ml sterile deionized water by vortexing for two minutes, followed by centrifugation for 5 minutes and the supernatant was decanted. 2 ml sterile deionized water was added to the washed tissue section and homogenized by tissue homogenizer. DNA extracted according to the following method of Gene jet genomic DNA purification kit supplied by Thermo Scientific Company (Elmohandsean, Cairo, Egypt).

*Random Amplified Polymorphic DNA-PCR (RAPD PCR): Williams et al. (1990):*

a- Mixed primers

2µg of pure genomic DNA and 15 pm of each primer (operon kit- B) are mixed with two PCR beads (to examine the kit efficiency), and the volume was completed to 50µl with sterile distilled water. The mixture was mixed by many inversions and collected again by spin at maximum speed in Eppendorf centrifuge for 5 seconds. Two drops of mineral oil was added.

b- Single primer of operon kit B in the mix.

1µg of pure genomic DNA and 15 pm of the selected primer Op B-10 or Op B-14 [that produced successful PCR reaction with stem cells and irradiated DNA samples (Table I); while the reminder of Op-B primers failed to produce successful PCR] were mixed with one PCR bead in 200 µl sterile eppendorf and the final volume was completed to 25 µl with sterile deionized water. The mixture was mixed by many inversions and collected again by spin at maximum speed in eppendorf centrifuge for 5 seconds. Two drops of mineral oil was added.

For primers amplification, PCR mixture was transferred to thermal cycler programmed for 35cycles as follows: 1 min at 94 $^{o}$C for DNA denaturing, 1 min at 48 $^{o}$C for annealing and 2 min at 72 $^{o}$C for new DNA extension, followed by final DNA extension at 72 $^{o}$C for 7 min . PCR product and 1 kbp DNA ladder (Gene Ruler$^{TM}$ ) were mixed with bromophenol blue loading dye (6x) then analyzed by electrophoresis on 2% agarose gel mixed with star gel and electrophoresed at 80V for 1h and the amplified DNA bands were visualized under UV light (Sambrook*et al.*, 1998).

**Table I: Nucleotide sequences of Operon RAPD-PCR kit B.**

| Name | Sequence |
|---|---|
| OP. B-07 | 5-*GGTGACGCAG*- 3 |
| OP. B-08 | 5-*GTCACACGG*- 3 |
| OP. B-09 | 5-*TGGGGGACTC*- 3 |
| OP. B-10 | CTGCTGGGAC-3`-5` |
| OP. B-11 | 5-*GTAGACCCGT*- 3 |
| OP. B-12 | CCTTGACGCA-3`-5` |
| OP. B-13 | 5- T CCCCGCT- 3 |
| OP. B-14 | 5-*TCGCTCTGG*- 3 |
| OP. B-15 | 5-*GGAGGGTG* T - 3 |
| OP. B-16 | 5- T *TGCCGGA*- 3 |
| OP. B-17 | 5-*AGGG*A *CGAG*- 3 |
| OP. B-18 | CCACAGCAGT-3`-5` |
| OP. B-19 | ACCCCCGAAG-3`-5` |

***Statistical analysis***

Using SPSS statistical package version 17.00 software, data were statistically analysed using one-way analysis of variance (One-way ANOVA) and post comparison with (LSD) test. The results were presented as means ±SE, with P≤0.05 being regarded statistically significant **(Snedecor and Cochran, 1980).**

# Results

***1-Liver biochemical parameters results:***

As shown from the present findings, MSCs and silymarin groups are tended to exhibit significant elevation in GSH content, GST, SOD, CAT, TAC and G-6-PDase activities.This goes with significant reduction in LPOand $H_2O_2$content in comparison to NC animals. However, γ- irradiation exposure (at single dose 4Gy) tended to produce significant elevation inhepatic values of LPO and $H_2O_2$ content accompanied with pronounced reduction in the measured antioxidant parameters compared to their respective NCgroups. On other hand, irradiated rats administrated silymarinor/ andMSCs succeeded to restore alterations in theses parameters comparing to their relative irradiated group with more evident effectswere obviously obsereved in most parameters after 3 weeks of treatment (Table 1).

**Table 1: liver tested biochemical parameters of both control and experimental groups.**

| Tested parameters | | Animal groups | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Liver | | NC | SI | ST | IRR | SIR | STR | SSR |
| **Reduced glutathione (GSH)** *mg/100g wet tissue* | One week | 22.63$^{a}$±0.34 | 32.88$^{b}$±0.18 +45.29 | 26.66$^{c}$±0.39 -17.81 | 8.96$^{d}$±0.23 -60.41 | 15.95$^{efg}$±1.09 -29.52 | 14.34$^{ef}$±0.93 -36.63 | 16.49$^{eg}$±0.49 -27.13 |
| | Three weeks | 28.59$^{a}$±0.10 | 33.09$^{b}$±0.78 +15.74 | 31.76$^{b}$±0.42 +11.09 | 12.91$^{c}$±0.85 -54.84 | 20.10$^{d}$±0.41 -29.70 | 19.59$^{d}$±0.45 -31.55 | 23.94$^{e}$±0.17 -16.26 |
| **Glutathione-S-trasferase (GST)** *µmol/min./g wet tissue* | One week | 22.27$^{a}$±0.34 | 24.83$^{b}$±0.41 +9.56 | 22.70$^{a}$±0.54 +1.93 | 10.17$^{c}$±0.13 -54.33 | 17.10$^{d}$±0.46 -25.15 | 19.88$^{e}$±0.34 -10.73 | 19.93$^{e}$±0.45 -10.51 |
| | Three weeks | 29.76$^{a}$±0.31 | 35.28$^{b}$±0.59 +18.55 | 33.97$^{b}$±0.20 +14.15 | 15.92$^{c}$±0.48 -46.51 | 25.02$^{d}$±0.76 -15.93 | 26.50$^{d}$±0.95 -10.95 | 28.98$^{a}$±0.44 -2.62 |
| **Superoxide dismutase (SOD)** *U/g wet tissue* | One week | 138.47$^{a}$±2.37 | 168.87$^{b}$±1.80 +21.95 | 147.40$^{c}$±1.76 +6.45 | 90.54$^{d}$±1.97 +34.61 | 104.80$^{e}$±2.17 -24.32 | 99.05$^{e}$±1.43 -28.47 | 105.28$^{e}$±1.06 -23.97 |
| | Three weeks | 149.00$^{a}$±1.71 | 174.87$^{b}$±0.99 +17.36 | 169.54$^{c}$±1.77 +13.79 | 78.63$^{d}$±2.36 -74.23 | 126.69$^{e}$±0.85 -14.97 | 120.70$^{f}$±1.48 -18.99 | 131.15$^{e}$±1.75 -11.98 |
| **Catalase (CAT)** *µmol/sec./g wet tissue* | One week | 24.33$^{a}$±0.85 | 32.88$^{b}$±0.43 +35.14 | 27.92$^{c}$±0.06 +14.76 | 10.44$^{d}$±0.75 -57.09 | 19.27$^{e}$±0.15 -20.80 | 16.39$^{f}$±0.58 -32.63 | 19.71$^{e}$±0.52 -18.99 |
| | Three weeks | 29.67$^{a}$±1.23 | 39.95$^{b}$±0.14 +34.65 | 37.82$^{c}$±0.76 +27.47 | 16.54$^{d}$±0.30 -44.25 | 25.78$^{e}$±0.68 -13.11 | 23.45$^{f}$±0.19 -20.96 | 27.83$^{a}$±0.81 -6.20 |
| **Glucose -6-phosphate dehydrogenase (G-6PD)** *mU/g wet tissue* | One week | 35.88$^{a}$±0.49 | 44.51$^{b}$±1.11 +24.05 | 44.20$^{b}$±0.60 +23.19 | 19.24$^{c}$±0.13 -46.38 | 27.55$^{d}$±1.28 -23.22 | 29.22$^{de}$±0.49 -18.56 | 30.95$^{e}$±1.05 -13.74 |
| | Three weeks | 36.86$^{a}$±0.72 | 42.23$^{b}$±0.08 +14.57 | 45.56$^{c}$±0.54 +23.68 | 13.69$^{d}$±0.73 -62.86 | 32.61$^{e}$±0.64 -11.53 | 33.41$^{e}$±0.97 -9.36 | 34.24$^{e}$±1.06 -7.11 |
| **Lipid peroxidation (LPO)** *nmol/g wet tissue* | One week | 226.39$^{a}$±2.40 | 186.24$^{b}$±2.32 -17.73 | 179.15$^{b}$±3.10 -20.87 | 293.69$^{c}$±2.07 +29.73 | 244.73$^{d}$±4.58 +8.10 | 251.70$^{d}$±3.24 +11.18 | 243.63$^{d}$±1.55 +7.62 |
| | Three weeks | 208.97$^{a}$±0.74 | 184.09$^{b}$±2.69 -11.91 | 160.11$^{c}$±0.93 -23.38 | 269.10$^{d}$±7.21 +28.77 | 236.86$^{e}$±3.36 +13.35 | 228.51$^{e}$±1.82 +9.35 | 216.52$^{a}$±2.73 +3.61 |
| **Hydrogen peroxide ($H_2O_2$)** *mM/g.wet tissue* | One week | 0.23$^{a}$±0.002 | 0.17$^{b}$±0.002 -28.51 | 0.17$^{b}$±0.003 -26.81 | 0.41$^{c}$±0.01 +72.77 | 0.25$^{a}$±0.01 +7.66 | 0.30$^{d}$±0.02 +26.81 | 0.26$^{a}$±0.004 +8.94 |
| | Three weeks | 0.25$^{a}$±0.003 | 0.22$^{b}$±0.01 -14.51 | 0.22$^{c}$±0.002 -15.69 | 0.40$^{d}$±0.01 +58.04 | 0.31$^{e}$±0.01 +20.78 | 0.30$^{e}$±0.01 +17.65 | 0.27$^{a}$±0.01 +5.88 |
| **Total antioxidant capacity (TAC)** *ng/g wet tissue* | One week | 21.61$^{a}$±0.28 | 34.27$^{b}$±0.61 +58.58 | 29.23$^{c}$±0.37 +35.26 | 11.75$^{d}$±0.68 -45.63 | 17.40$^{e}$±0.22 -19.48 | 16.05$^{f}$±0.21 -25.73 | 19.87$^{g}$±0.20 -8.05 |
| | Three weeks | 25.44$^{a}$±0.30 | 40.16$^{b}$±0.11 +57.86 | 39.52$^{b}$±0.44 +55.35 | 15.40$^{c}$±0.48 -39.47 | 24.48$^{a}$±0.75 -3.77 | 24.53$^{a}$±0.63 -3.58 | 25.38$^{a}$±0.58 -0.24 |

Results are presented as mean ± SE (n of 6 rats for each group) and % of change as compared to respective control group.

-Values superscripts with different letters. (a-g) were significantly different (P≤ 0.05), but with the same letters were insignificantly different.

NC: normal control SI: Silymarin ST: Stem cells IRR: Irradiated with gamma

SIR: Silymarin + irradiated STR: Stem cells + irradiated SSR: Silymarin + Stem cells+ irradiated.

***2- Molecular Results:***

Genomic DNA of liver tissues of treated and control animals after one and three weeks were successfully extracted at a molecular weight 20kpb (**Fig. 1a &1b)**. Random Amplified Polymorphic DNA-PCR (RAPD-PCR) of genomic DNA is performed as a genetic marker.

RAPD-PCR analysis carried out on the selected DNA liver samples of treated and control animal groups after one and three weeks to investigate the presence and/or absence of definite bands when comparing the normal RAPD-PCR pattern with RAPD- PCR patterns of other treated groups using the OP-B10 and OP-B14 primers and the results are documented in **Fig. 2a& 2b**. After one week of treatment RAPD-PCR with primer OP-B10 produced two control marker bands in normal control sample (NC) and silymarin group (SI) at molecular weights 4000 and 950 base pair. While, DNAs of control stem cells (ST) group, irradiated rats treated with silymarin (SIR) and irradiated rats treated stem cells (STR) produced one control marker band only (4000 bp) and the second control marker band (950 bp) is disappeared. Moreover, irradiated group (IRR) appeared two new bands (1650 and 2000 bp) beside one control marker band (4000 bp) and disappearing of the second control marker (band 950 bp). Silymarin plus stem cell in irradiated groups (SSR) produced one normal marker band 4000bp and appearing of a new band (2150bp), while the second control marker band (950 bp) is disappeared. After three weeks RAPD-PCR of NC group was amplified using primer OP-B10 and one major control marker band at molecular weights 4000 bps is appeared. ST, STR and SSR groups produced the same profile of NC group. RAPD-PCR of SI group produced the same control marker band (4000 bp) and another new band (1000 bp) is appeared. Similarly, RAPD-PCR of IRR group produced the same marker normal control band (4000 bp) and another new band (1650bp) is appeared. On other hand, SIR produced two new bands 950 and 2500 bp and the major control marker band is disappeared **(Fig.2a**).

Moreover, The RAPD-PCR with primer OP-B14 after one week produced two marker control bands which appeared in NC samples at molecular weights 4000 and 1000 base pair. While, the RAPD-PCR of SI group produced one marker control band (4000bp) and another new band (1200 bp) is appeared.On the other hand ST group produced news even major bands (3000, 2500, 2000, 1700, 1500, 1300 and 1200 bps) and the two marker control bands are disappeared. Moreover IRR group, the two marker control bands are disappeared and one new band (1300 bp) is appeared. In case of RAPD-PCR of SIR group, one new band (1500 bp) is appeared and one marker control band (4000bp) is appeared while the second control band (100 bp) is disappeared. STR and SSR groups RAPD-PCR produced new five bands (3000, 2500, 2000, 1700 and 1500 bps) beside one marker control band (4000bp) while the second control band (100 bp) is disappeared. Also, the RAPD-PCR of NC group after hre weeks was amplified using primer OP-B14; one major normal control marker band with molecular weights 1000 bp is appeared. Whereas, RAPD-PCR product of SI group produced one new band (1500 bp) while the normal control marker band (1000 bp) is disappeared. In ST group the normal control marker band (1000 bp) is disappeared and a new five band (2600, 2500, 2000, 1700 and 1500 bps) are appeared. Also, in case of RAPD-PCR of IRR group did not have any bands when comparing with NC group. On other hand, SIR group the normal control marker band (1000 bp) is disappeared and a new one band (1300 bps) is appeared. While, STR and SSR groups produced the same ST banding pattern profile in which the normal control marker band (1000 bp) is disappeared and a new five band (2600, 2500, 2000, 1700 and 1500 bps) are appeared **(Fig. 2b).**

## Discussion

Ionizing radiations are recognized to stimulate oxidative stress by the production of reactive oxygen species (ROS) such as superoxide ($O_2 \cdot$), ($OH \cdot$) and hydrogen peroxide ($H_2O_2$) (Jameel and Mohammed, 2021) causing a lopsidedness in the prooxidants and antioxidants in the cells (Nasr*et al.,* 2020). Numerous mechanisms may cause cellular injury after radiation exposure but the formation of oxygen free radicals followed by LPO which may be one of the main reasons in the sequential of events (Wang*et al.,* 2019). Radiation generates ROS that combined with cellular molecules, including DNA, lipids, and proteins (Jia*et al.,* 2021).

The current data registered a pronounced decline in the antioxidant defense system concomitant with the growing lipid and hydrogen peroxides levels in liver tissues following γ-radiation exposure. In compatible with the present study of **Zakaria2017; Sarhan** and **Naoum 2020** who registered a decline in the activities of SOD, GST and GSH content which may be due to the formation of ROS that reacst with the enzymes molecules causing denaturation and limited inactivation of enzymes. Under ordinary situations, lipid peroxidation occurs in narrow range in body tissues while, the exaggerated formation of free radicals forming peroxidative modifications that at the end promotes LPO **(Sack*et al.,* 2017; Akhigbe** and **Ajayi, 2021),** which is ascribed to the oxidation of the polyunsaturated fatty acids by generation of free radical causing structure and function modifications to molecules cellular membrane by direct way, through transporting energy or by indirect way, through formation of dominant oxygen derived free radical ($OH \cdot$), superoxide ($O_2 \cdot$) and nitric oxide ($NO^{\cdot}$) **(Abd-Ellatef*et al.,* 2017** and **Iuchi*et al.,* 2019)** or to the deficiency in antioxidants levels which have the ability to hunt peroxy radicals after radiation exposure **(Forman** and **Zhang, 2021).**

Also, **Sun *et al.*(2018)** and **Olivares*et al.*(2020)** reported that radiation exposure exhibited decline in GSH content through minimizing the efficacy of GSH-Rx or reducing the activity of G-6-PD leading to scarcity of NADPH which is essential to modify oxidized glutathione (GSSG) to its reduced form GSH **(Xiong*et al.,* 2020)** or through oxidative stress which caused a decrease in glutamate levels, which has been estimated to be in relation with decline intracellular GSH levels, as GSH vied with glutamate for the glutamate binding site of the gamma glutamyl cysteine synthetase, the

primary and controlling step of GSH synthesis or caused malfunction of cellular membranes uncontrolling GSH mobilization **(Forman*et al.,* 2009)** or elevated the usage of GSH (acts a reductant for peroxides and free radicals) to neutralize the excess of free radicals **(Olivares*et al.,* 2020).**

Besides, the current work showed that irradiated rats exhibited a pronounced reduction in the efficacy of hepatic antioxidant enzymes such as SOD and CAT. The reported decline in SOD efficiency is probably due to rise of $O_{2\cdot}$ generation or suppression by the $H_2O_2$, which results in the reduction in the CAT efficacy, which is responsible for degeneration of $H_2O_2$ (Wang *et al.,* 2018). While, decline in CAT efficiency may be a result of excessive usage to face off LPO formation, besides detoxifying $H_2O_2$ into $H_2O$ and $O_2$ (Rajput*et al.,* 2021). Another related finding, the present study showed that RAPD-PCR analysis succeeded in demonstration of the genetic damages induced by irradiation (IRR) by using of both primers OP-B10 and OP-B14 in one and three weeks periods. RAPD-PCR pattern with OP-B10 primer of whole body γ-irradiated rats at 4 Gy after one week produced differences represented in disappearing of half of the marker bands. While, additional new bands beside the control marker bands are appeared in the irradiated group after three weeks of treatment. This goes in agreement with Ahmed *et al.* 2020 who suggested that the vanishing or emrging of an amplified RAPD segment is most likely due to DNA damage and mutations at the primer-template interaction point, as well as unfair mitotic recombination or other structural effects that have sped up primer hybridization.

Also, γ-radiation can induce cellular DNA damage by both straight and vicarious ways. Straight way inducing by destruction of chemical bonds in DNA molecules, while vicarious way resulting by the formation of ROS such as $OH^{\cdot}$ and $O_2^{\cdot-}$ radical leading to DNA fragmentation by causing single- and double-stranded DNA breaks, mutations and LPO leading to cellular injury causing cellular death **(Ahmed*et al.* 2020).**

On other hand, the present results showed that, MSCs/BM extract; markedly inhibited the harmful effect of γ-radiation on antioxidant defense system. As it reduced LPO in parallel with elevated the efficacy of CAT, SOD and GSH-Px in liver tissue. The *in vivo* protection and particular replies of MSCs to oxidative stress may act an important role in adjusting tissue homeostasis as well as renewal of tissues after oxidative damage **(Sagaradze*et al.,* 2020** and **Yan*et al.,* 2021**) through a straight hunting and deactivation of the free radical or production of the endogenous antioxidant enzymes such as CAT and SOD**. Angeloni*et al.* 2020** Showed that MSCs implantation could restore the imbalance between ROS and the antioxidant defense system by elevating the antioxidant capacity as well as modifying LPO.

Also, In other researches human MSCs transplantation markedly reduced oxidative stress following radiation exposure **(Hu*et al.,* 2019)** by inducing a transcription factor, nuclear related factor 2 (Nrf2) which is essentially adjusted the main and inducible level of cytoprotective genes. Its activation is a type of protection against oxidative stress through SOD production leading to ROS reduction in liver **(Zhou*et al.,* 2020).**

In addition, the current results showed that MSCs usage is highly succeeded in healing the decomposition of cells DNA of the irradiated rats (STR) when it used alone or with silymarin supplement (SSR). The results reflects the greet efficiency of the MSCs when it used alone or combined with silymarin supplement in repairing the genetic changes due to irradiation. In agreement with this **Vaz*et al.* 2021** who showed that BM/MSCs pretreatment were markedly reduced the number of cells with chromosomal mutations and exhibited the perfect outcomes by reducing chromosomal mutations towards the normal, but still markedly elevated when compared to healthy control group. This explained as MSCs can restore alterations in DNA genome and keep the liver tissues safe from apoptosis following γ-radiation exposure **(Ezquer*et al.,* 2017).** This defense is multifactorial including modifying the oxidative stress reaction, organ injury and renovation.

Moreover, Natural antioxidants have a constantly and essential role for inhibition of ROS, with keeping little amount important to adjust normal cell function **(Aziz *et al.,* 2019).** Along with this, the present study showed that silymarin administration prior to exposure of rats to ionizing radiation greatly normalized all the hepatic antioxidant parameters such as SOD, CAT, and GST as well as LPO and $H_2O_2$ levels. The present results are in line with previous studies of **Abdelazim 2017; Al-Hazmi 2020** and **Ghonaim*et al.*,(2021)** who showed the very antioxidant capacity of silymarin which is eligible for catching ROS.

This could be through silymarin elevation of the antioxidant potential of cells by improving the harmful effects of free radical reactions **(El-Maddawy** & **Gad, 2012** and **Faraji*et al.,* 2019)**, protect hepatocytes (and other cells in the body and brain) from free radical reactions by catching of free radicals such as $OH^{\cdot}$ type **(Gillessen** and **Schmidt, 2020)** with an effect on DNA-expression through inhibition of nuclear factor NF- B & inhibiting LPO **(Li *et al.,* 2012)**, stimulates hepatocytes protein production and reduces the oxidation of GSH **(Kwon *et al.,* 2013).** Moreover, silymarin has metabolic and cell adjusting effects, called carrier mediated regulation of the fluidity of hepatocytes microsomes and the liver mitochondrial membrane **(Vahabzadeh*et al.,* 2018).**

Additionally, the present results showed that silymarin supplementation (SI) itself succeeded in producing a banding profile identical to the control after one week only, but it produced a negative effect by damaging the DNA when the period prolonged to three weeks. While, it produced a profile near to the control groups in silymarin treated irradiated rats after one week only. This goes in agreement with **El Mesallamy*et al.* 2011** and **Adhikari*et al.* 2013** who showed that silymarin administered 1 hr before irradiation (moderated radiation) and for 7 and 14 days following γ-irradiation induced changes in nucleic acids in its target organs; liver, spleen and bone marrow through producing modifications in RNA and DNA concentrations and inhibit nuclear DNA injury in male rats.

Moreover, silymarin regulates lopsidedness between cell regeneration and cell death through interfering with the expressions of cell cycle controllers and proteins implicated in cell death **(Kim*et al.,* 2021).** Silymarin reacts with the estradiol receptor and activate it in the hepatocytes cells, and the activated receptor could elevate the liver endonuclear RNA polymerase I efficacy and the number of ribosomes in intracytoplasm, activate the transcription of ribosome RNA and the production of enzyme, structure protein and cellular DNA indirectly, which are useful to hepatic cytothesis **(Karimi*et al.,* 2011;** and **Hajiaghamohammadi*et al.,* 2012).**

## Conclusion

Whole body gamma radiation exposure exhibited a harmful effect on liver antioxidant defense system and liver DNA molecules. While treatment with a natural antioxidant such as silymarin with transplanted MSCs improved these changes .So we recommended adminstration of silymarin pre and post gamma radiation exposure beside MSCs transplantation which showed a pronounced effect together towards changes in antioxidant defense system and liver DNA mutation.

## Declarations

We declare that the present research paper is our original work and no part of it has been published anywhere else in the past.

- **Ethical Approval and Consent to participate**

The study is ethically approved.

- **Human Ethics and Animal Ethics**

The research is carried out on male albino rats according to the basal declaration society outlines.

- **Consent for publication**

Not applicable

- **Availability of supporting data**

We confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

- **Competing interests**

We declare that we have no competing interests in the subject matter or materials discussed in this manuscript but it is a personal competing interest.


- **Funding**

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- **Authors' contributions**

Dr/Ashraf Z Mahmoud wrote the manuscript and performed the analysis and Dr/ El.Sawi, M. R. conceived & designed the analysis, while Dr/ Habza, M. N. collected the data.


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## Figures

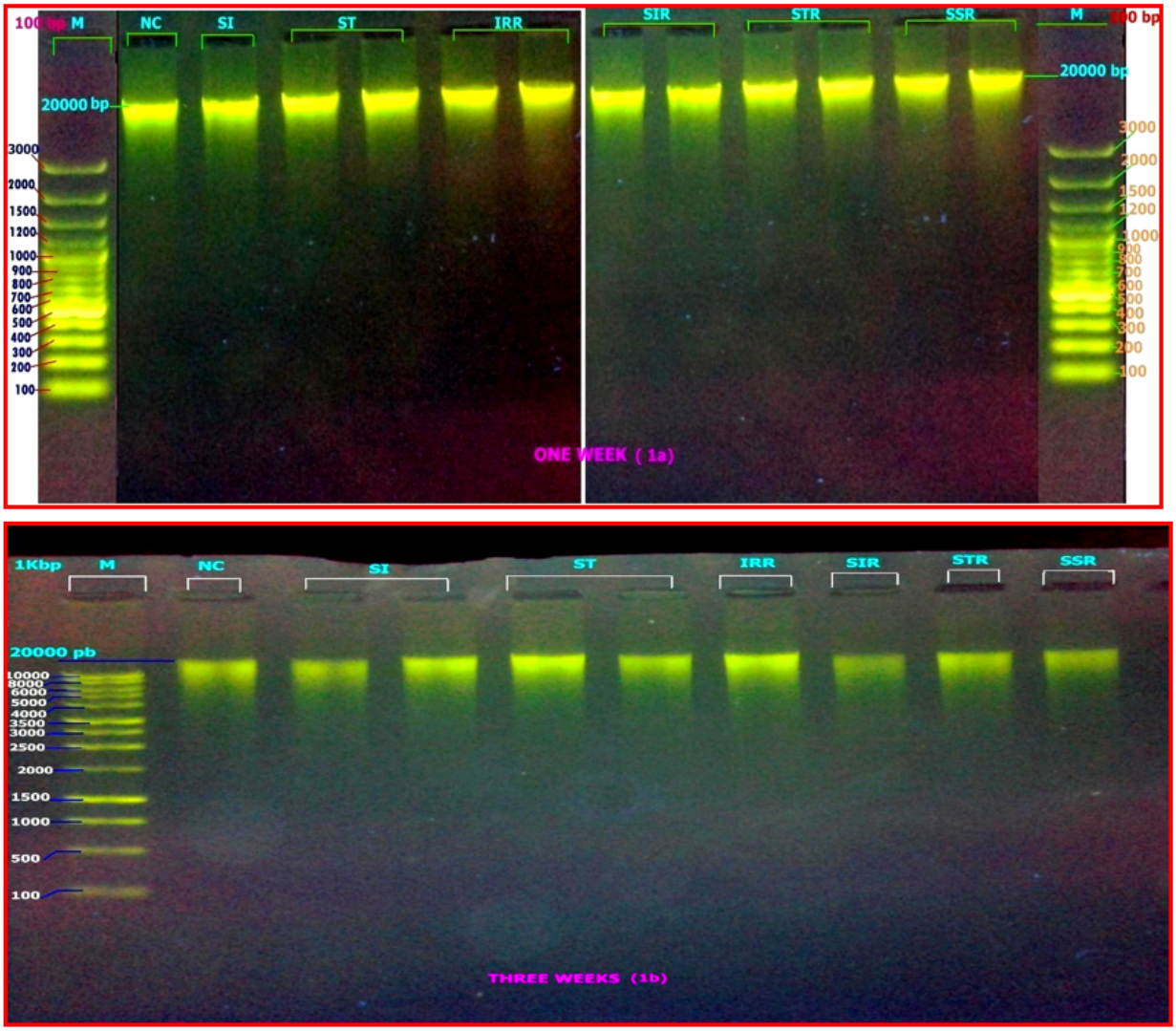


**Figure 1**

**a**: Agarose gel electrophoresis of genomic DNA of liver tissue in different groups after one week. Lane 1 is control (NC), lanes 2 is silymarin (SI), lanes 3, 4 are stem cells (ST), lanes 5, 6 are irradiated (IRR), lanes 7, 8 are irradiated with silymarin ( SIR), lanes 9, 10 are irradiated with stem cells

(STR) and lanes 11, 12 are silymarin plus stem cell in irradiated groups( SSR) respectively. Lane M is 100 bp ladder "100- 3000 bp" DNA marker.

**b:** Agarose gel electrophoresis of genomic DNA of liver tissue in different groups after three week. Lane 1 is control (NC), lanes 2,3 are silymarin (SI), lanes 4, 5 are stem cells (ST), lane 6 is irradiated (IRR), lane 7 is irradiated with silymarin ( SIR), lane 8 is irradiated with stem cells (STR) and lane 9 is silymarin plus stem cell in irradiated groups (SSR) respectively. Lane M is 1K bp ladder "100- 10000 bp" DNA marker.

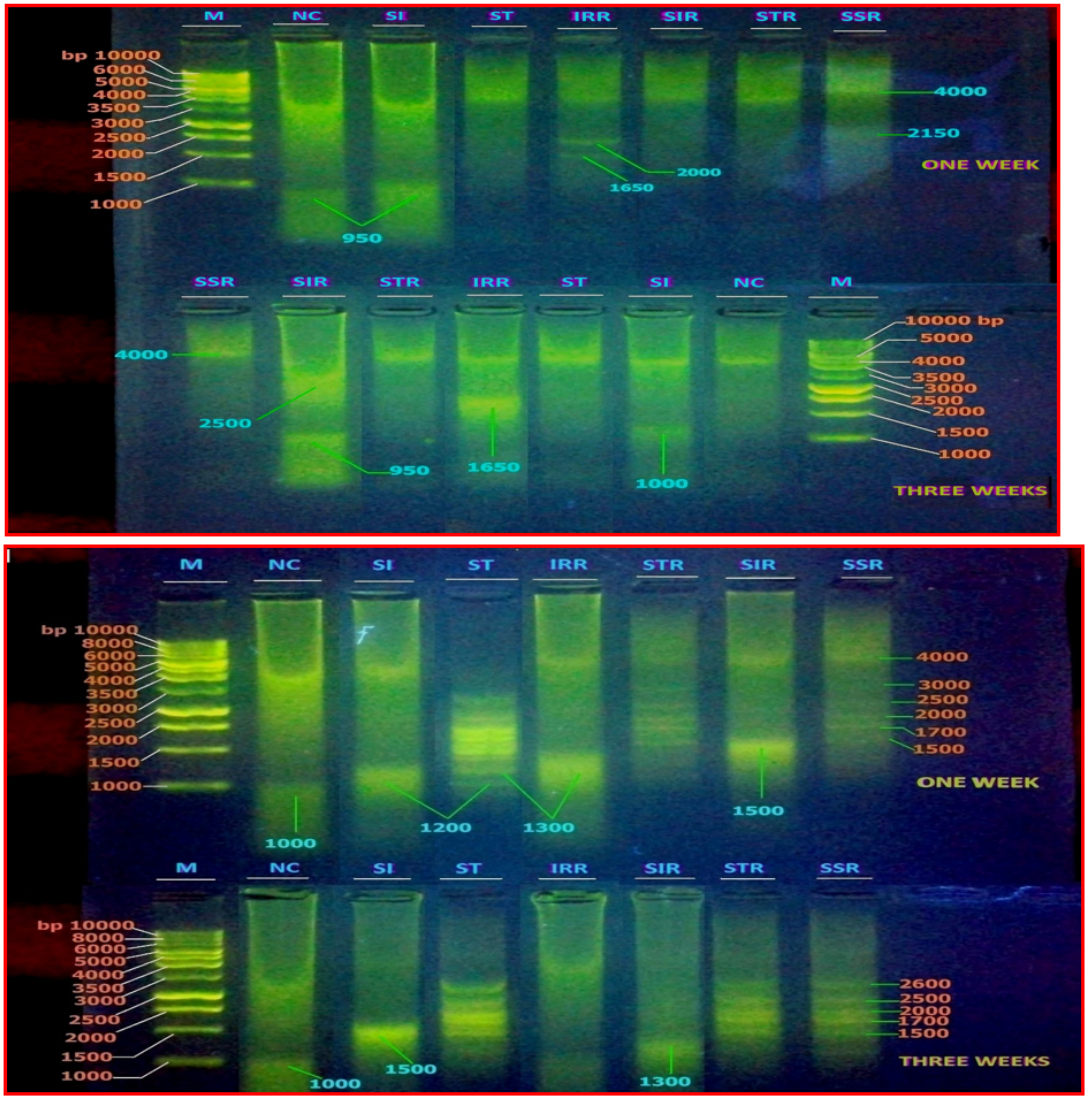


**Figure 2**

**a:** Agarose gel electrophoresis of RAPD-PCR product for primer (Op B-10) from liver tissue in different treated groups after one and three weeks. Control group (NC), silymarin (SI), stem cells (ST), irradiated (IRR), irradiated with silymarin ( SIR), irradiated with stem cells (STR) and silymarin plus stem cell in irradiated groups (SSR) respectively.. Lane M is 1kb ladder "100- 10000 bp" DNA marker.

**b:** Agarose gel electrophoresis of RAPD-PCR product for primer (Op B-14) from liver tissue in different treated groups after one and three weeks. Control group (NC), silymarin (SI), stem cells (ST), irradiated (IRR), irradiated with silymarin ( SIR), irradiated with stem cells (STR) and silymarin plus stem cell in irradiated groups (SSR) respectively.. Lane M is 1kb ladder "100- 10000 bp" DNA marker.