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Unveiling the mitigating effect of matcha-silver nanoparticles on radiation-induced ovarian injury in rats

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Radiotherapy, an essential cancer treatment, poses significant risks to ovarian tissue, especially during whole-body irradiation treatments. This study aims to evaluate the efficacy of silver nanoparticles derived from matcha green tea (M-AgNPs) inmitigating oxidative stress and apoptosis caused by gamma radiation in rat ovaries. The thirty-six rats were partitioned into six groups. Group 1: control; Group 2: 3; normal rats received matcha and M-AgNPs (10 mL/kg) orally for 14 days respectively; Group 4: rats subjected to 6 Gy whole-body gamma radiation. Groups 5 and 6 of irradiated rats received daily oral administrations of matcha and M-AgNPs (10 mL/kg) for a duration of 14 days, respectively. Biochemical and histological investigations were conducted to evaluate oxidative stress, apoptosis, and ovarian tissue architecture. Radiation significantly ($P< 0.05$) decreased total antioxidant capacity (TAC) and elevated 8-OHdG levels, indicating oxidative damage. The treatment with M-AgNPs mitigated these effects by reducing the concentration of 8-OHdG and enhancing TAC, thereby restoring antioxidant balance. Furthermore, radiation markedly ($P< 0.05$) elevated p53 and ERK1 expression levels, whereas M-AgNPs treatment significantly ($P< 0.05$) diminished p53 and ERK1 expression levels in ovarian tissue. Radiation causes several forms of DNA damage, while the treatment with M-AgNPs mitigated this damage. Histopathological evaluations exhibited considerable improvements in the histological characteristics of ovarian tissue following the administration of M-AgNPs in comparison to irradiated rats. According to these findings, M-AgNPs represent one of the most promising methods for mitigating radiation-induced ovarian damage. Additional work is necessary to clarify particular molecular pathways and their long-term effects.

Keywords: 8-OHdG, Antioxidant, ERK1, Gamma radiation, p53

Multiple cancer types commonly utilize radiotherapy as a cytotoxic therapy[1]; nonetheless, it frequently harms healthy tissues, reducing its efficacy against malignancies. The ovaries are vulnerable to substantial exposure during cancer treatments that include total body irradiation[2]. Furthermore, Kim *et al*. showed that radiation can adversely affect the ovarian tissue of prepubescent girls and young women[3]. The extent of radiation-induced tissue injury is proportional to the radiation dose, fractionation schedule, and the patient's age at the time of treatment[4-6]. Research estimates that 2 Gy of radiation is sufficient to eradicate half of the primordial follicles in the ovary[7]. Damage to the follicular pool from radiation can accelerate reproductive aging, leading to premature menopause, subfertility, or infertility[8].

Radiation therapy induces reactive oxygen species (ROS) *via* water radiolysis. Both normal and malignant cells undergo oxidative stress from these ROS, which contribute to radiation-induced gonadotoxicity[9,10]. The resulting oxidative stress plays a critical role in activating the intrinsic mitochondrial pathway of apoptosis, primarily through the activation of p53[11]. This triggers a cascade that leads to cell death by releasing cytochrome c, activating caspases, and cleaving DNA repair enzymes[11,12]. Since ionizing radiation generates DNA-damaging free radicals, compounds capable of neutralizing these radicals could protect normal tissues from radiation damage[13].

Nanomaterials, particularly those with significant antioxidant properties, represent a promising approach for developing novel protective agents. Among these, silver nanoparticles (AgNPs) are widely studied for their medical applications[14]. The green creation of metal nanoparticles using medicinal plant extracts is a crucial area of research, especially in the context of pharmaceutical applications[15]. Plants include numerous bioactive chemicals that facilitate the creation of metal nanoparticles by acting as

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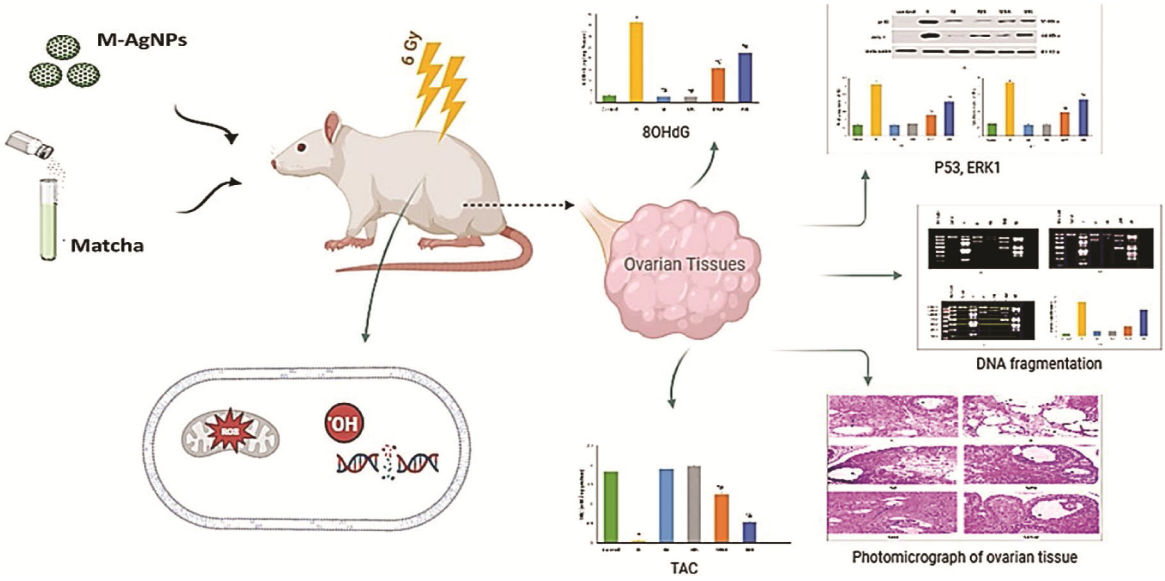

Graphical Abstract

reducing and stabilizing agents[16]. Moreover, plants, known for their antioxidant properties, offer an eco-friendly and effective route for synthesizing nanoparticles[17]. Matcha (M), or powdered Japanese green tea, has gained popularity due to its high catechin content, which imparts potent antioxidant and anti-inflammatory properties[18,19]. Catechins, a class of phenolic molecules, are believed to be responsible for these effects[20].

Our previous research utilized liquid chromatography-mass spectrometry (LC-MS) to analyze matcha. The LC-MS analysis detected many bioactive substances. Six phenolic compounds, six flavonoids, and two alkaloids have been identified[21].Previous studies have demonstrated that polyphenolic compounds, such as those found in matcha, can stimulate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, leading to enhanced production of antioxidant enzymes and offering cellular defense against oxidative stress. Xie *et al*. reported that epigallocatechin-3-gallate (EGCG), a primary polyphenol in green tea, activated the transcription factor Nrf2, thereby enhancing the synthesis of antioxidant enzymes[22]. Furthermore, Odson *et al*. demonstrated that Nrf2 is regarded as a primary response to oxidative stress[23].In our previous study, we assessed the antioxidant capability of matcha and M-AgNPs using the DPPH (2,2-diphenyl-1-picryl ydrazyl) assay[24]. The results revealed that matcha exhibited free radical scavenging activities; moreover, M-AgNPs exhibited further significant free radical scavenging activities. This may be due to the coating of polyphenolic residues on the surface of AgNPs, which enhances the interaction and capacity of matcha green tea polyphenols to donate hydrogen to free radicals[25].Therefore, this study aims to explore the potential of M-AgNPs in mitigating oxidative stress and apoptosis in ovarian tissues damaged by radiation.

Materials and Methods

Chemicals, Preparation, and characterization of M-AgNPs

We bought matcha from ILEAF NATURLS Company in the United States. We acquired all the additional compounds from standard commercial sources, ensuring their composition was of analytical grade. In accordance with our prior research method, M-AgNPs were synthesized[24]. M was utilized in the biogenic synthesis and green sonochemical method to produce AgNPs. M-AgNPs were characterized by various techniques, including DLS, TEM, FTIR, and TGA[24]. Furthermore, we evaluated the toxicity of matcha and AgNPs by the MTT method to determine the cell survival of the normal lung fibroblast cell line WI38.

Experimental animal groups

We purchased 36 healthy female Wistar albino rats, weighing between 180 and 200 grams. We randomly distributed six (n = 6) groups of rats, as shown in (Fig. 1): Rats in Group 1 (control; C) were healthy, untreated rats. Group 2 (Matcha; M) rats received

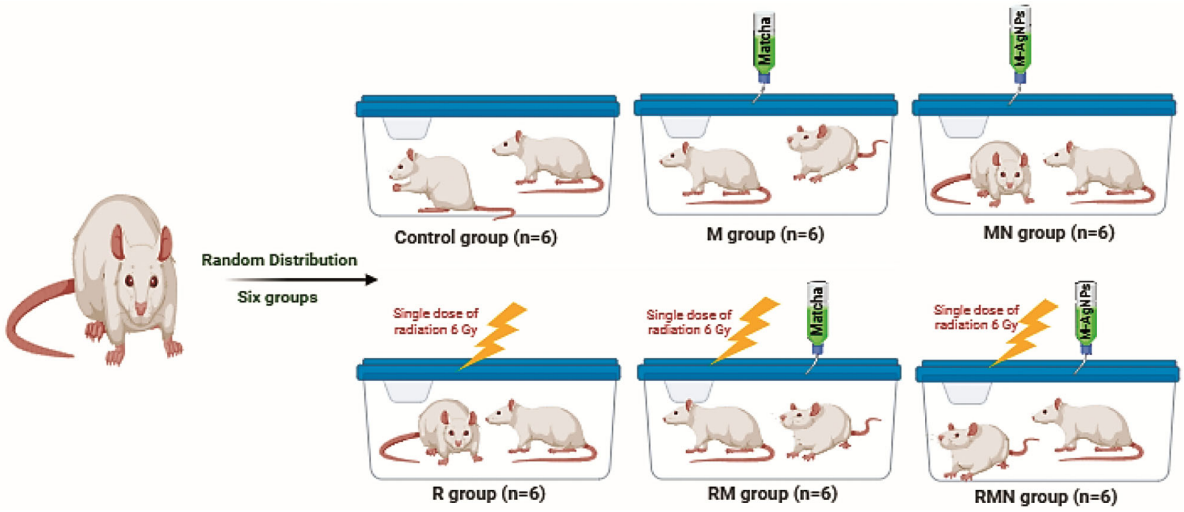

Fig. 1 — The experimental design for the oral administration of Matcha and M-AgNPs to irradiated rats

matcha orally for 14 consecutive days at a dose of 10 mL/kg/day[26]. Rats in Group 3 (Matcha-silver nanoparticles; MN) were given 10 mL/kg/day of M-AgNPs orally for 14 consecutive days, while rats in Group 4 (radiation, R) received a single dose of whole-body gamma radiation (6 Gy), after which they were left untreated for the duration of the experiment. Group 5 (MR) rats received a single dose of whole-body gamma rays, followed by the oral administration of matcha (10 mL/kg/day) for 14 consecutive days; Group 6 (MNR) rats received a single dose of whole-body gamma rays (6 Gy), followed by the oral administration of 10 mL/kg/day of M-AgNPs for 14 consecutive days. The ethics research committee of the National Center for Radiation and Technology of the Egyptian Atomic Energy Authority revised the protocol and granted its sanction (REC-NCRRT-10A/22).

Total antioxidant capacity (TAC) evaluation

TAC of the ovary sample was analyses by using colorimetric method[27]. Briefly the cell lysate from the ovary tissues was prepared before analysis. Cell homogenate was collected by centrifugation for 10 min at 2000 rpm. The pellet was sonicated on ice in 1-2 mL cold Phosphate buffer and again centrifuged for 15 min at 4000 rpm. The supernatant was collected for the analysis and analyzed according to the procedure provided with the kit (Biodiagnostic assay kit). Using the following formula, the total antioxidant concentration was determined: Total antioxidant concentration mM / L =AB-ASA x 3.33

(Where Absorbance of blank is represented as AB and sample as ASA)

8-Hydroxy-2-deoxyguanosine (8-OHdG) measurement

From the ovarian tissues, a cell homogenate was prepared, and the 8-OHdG test was conducted using the supernatant obtained from centrifugation. Using the 8-OHdG ELISA Kit (Biovision) procedure, the amount of 8-OHdG was determined.

DNA fragmentation assay

We extracted and purified whole genomic ovary samples using the GeneJet Genomic DNA Purification Kit (Thermo Scientific, K0721), following the manufacturer's procedure. We determined the length of the fragments using a DNA ladder consisting of 2 kbp DNA (PeqGold 2 kb, Peqlab, GMH). We put genomic fragments on a 1.5% agarose gel, colored them with ethidium bromide, separated them using 75 volts and 150 milliamperes of electrophoresis, and looked at them on a UV plate. We utilized the Gel documentation system (Geldoc-it, UVP, England).

Western Blot analysis

The Ready Prep™ total protein extraction kit was procured from Bio-Rad and was utilized as per the manufacturer's guidelines, with the kit added to homogenized tissue samples from various groups. For quantitative protein analysis, the Protein Assay Kit (Bradford) from Bio Basic was employed. The assay was conducted to measure protein concentrations in each sample. Total of 20 µg of protein from every sample was combined with 2x Laemmli sample buffer, which contained, 20% glycerol, 10% 2-mercaptoethanol, 4% SDS, 0.004% bromophenol blue, and 0.125 M Tris HCl, the pH was adjusted to 6.8. To denature the protein the samples were heated at 95°C for 5 before electrophoresis. SDS-PAGE was

conducted using the Acrylamide Kit from purchased from Bio-Rad Laboratories. The gel preparation followed the manufacturer's instructions.

For the transfer, a sandwich setup was created in the following order from bottom to top: first filter paper, then PVDF membrane, after that gel, and then filter paper. This assembly was kept in a transfer tank which was filled with transfer buffer (1x) containing 25 mM Tris, 20% methanol along with 190 mM glycine, The transfer was performed for 7 min at 25 V to facilitate the movement of protein bands from the gel to the membrane. After the transfer, the membrane was blocked for 1 hour at RT in TBS with Tween 20 (TBST) containing 3% BSA. Primary antibodies against p53 and ERK, which were procured from external sources, were diluted in TBST as per the manufacturer's instructions and incubated for overnight at 4°C with the blot. The membrane was then rinsed 3-5 times for 5 min each with TBST. Next, Goat anti-rabbit IgG-HRP were applied for 1 hour at room temperature, followed by repeated rinsing for at least 3-5 rinses with TBST. Finally, the chemiluminescent substrate was applied to the blot in accordance with the instructions. This required combining equal amounts of peroxidase solution and western luminal/enhancer solution. The resulting chemiluminescent signals were recorded using an imager based on a CCD camera, examined to determine the intensity of the target protein band, and compared to a control sample (beta-actin).

Histopathological studies

Samples of ovaries were taken from every animal group and kept in neutral buffered formalin (10%). Following this, the fixed specimens underwent trimming, washing, and dehydration in increasing alcohol grades. After that, they were washed in xylene, fixed in paraffin, then sectioned at a thickness of 4-6 µM, finally stained with hematoxylin and eosin in accordance with[28]. The prepared slide was then examined using a light digital microscope (Olympus XC30, Tokyo, Japan).

Statistical analysis

The results were displayed as mean values plus standard error. Next, employing one-way analysis of variance (ANOVA) tests, we statistically examined a number of evaluations. This was followed by a post-hoc test using Tukey's HSD multiple comparisons. We declared the results statistically significant if the p-value was less than 0.05. The software known as SPSS statistical version 20 (SPSS® Inc., USA) carried out every single statistical analysis.

Results and Discussion

Effects of M-AgNPs on TAC

Based on our research, the R group showed a noteworthy decrease in TAC concentrations when compared to the control group (Fig. 2). Reductions in the quantity and activity of antioxidant enzymes are one of the detrimental effects of IR on the antioxidant system[29]. Moreover, the results shown in Figure 2 indicate that the MR and MNR groups had a significantly higher TAC concentration than the irradiated group. According to the research findings, matcha and M-AgNPs are potent antioxidants that can reduce gamma radiation-induced oxidative stress in the ovaries and enhance the cells' antioxidant defenses. These results suggest that matcha could serve as a valuable source of antioxidants[30], including flavanonols, flavanols, and phenolic acids. Moreover, our results are similar to those of Mora *et al*. who found that giving Italian Mediterranean buffaloes green tea extracts for 90 days increased antioxidant levels and improved the developmental potential of oocytes recovered through ovum pickup[31]. Furthermore, our results showed that M-AgNPs had greater antioxidant activity than M alone. In line with what Abdel-Aziz *et al*. found, plant extracts that contained AgNPs had higher amounts of total flavonoids and total phenolic compounds than plant extracts that were used alone[32].

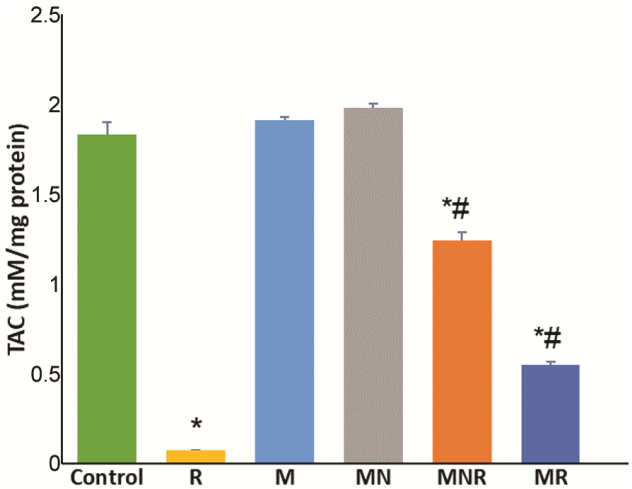

Fig. 2 — Illustrates the total antioxidant capacity (TAC) of various studied groups. The study presented the data as means ± SE for each group. * Significant at $P< 0.05$ level in comparison to the control group, while # Significant at $P< 0.05$ level in comparison to the irradiated group

Effects of M-AgNPs on 8-OHdG level

Our findings indicated that the radiation group had a significantly higher level of 8-OHdG compared to the control group (Fig. 3). The oxidation of guanine from damaged DNA creates 8-OHdG, a significant indicator of oxidative stress[33]. Several factors, including radiation, contribute to tissue oxidative stress. IR reacts with water molecules inside the cells to produce free radicals[34]. These free radicals have the potential to damage cellular DNA and other organelles within the cell. The data presented in Figure 3 clearly indicates that the MR and MNR groups significantly mitigated the rise in 8-OHdG when compared to the irradiated group. These results highlight the potential of matcha as a beneficial source of bioactive substances known for their positive health impacts and antioxidant qualities, including flavonoids and polyphenols. According to Megahd & Gabal, orally administered matcha enhanced both biochemical and microscopic findings by lowering DNA damage to cells and protecting ovarian and uterine tissues from oxidative stress and cell death[35]. Furthermore, the MNR group had lower levels of 8-OHdG compared to the MR group. These findings showed that the potent antioxidant activity of M-AgNPs against radiation can strengthen cellular defenses and protect cellular DNA from damage.

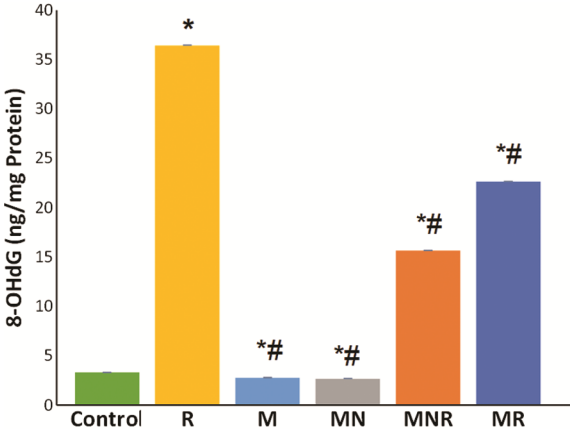

Fig. 3 — Illustrates the levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in various groups under study. The study presented the data as means ± SE for each group. * Significant at $P< 0.05$ level in comparison to the control group, while # Significant at $P< 0.05$ level in comparison to the irradiated group

DNA fragmentation assay

Further evidence of DNA fragmentation was obtained by electrophoresing DNA samples from rat ovarian tissues on an agarose gel. IR creates different types of DNA damage, most notably double-strand breaks, altered nucleotides, crosslinks, and single-strand breaks[36]. The DNA fragmentation assay (Fig. 4) confirmed both necrosis and apoptosis in the

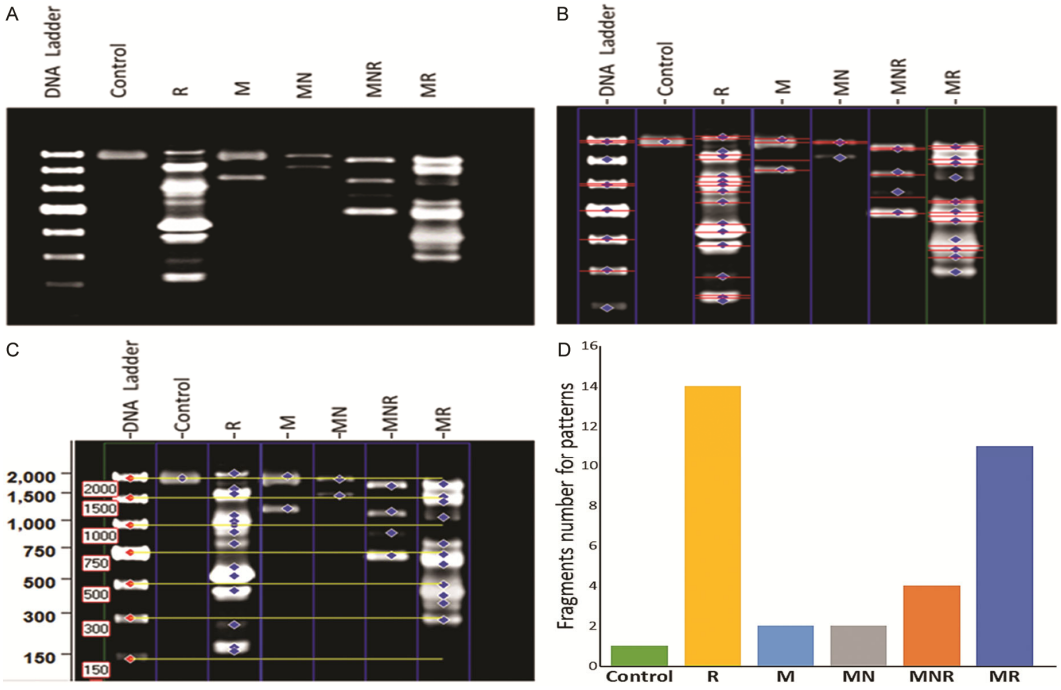

Fig. 4 — The examination of DNA fragmentation in the different groups: (A) fragmentation patterns for ovary samples; (B) computerized detection for fragmentation patterns for ovary samples; (C) computerized fragment length calculation of patterns for ovary samples; and (D) fragment number for patterns for ovary samples

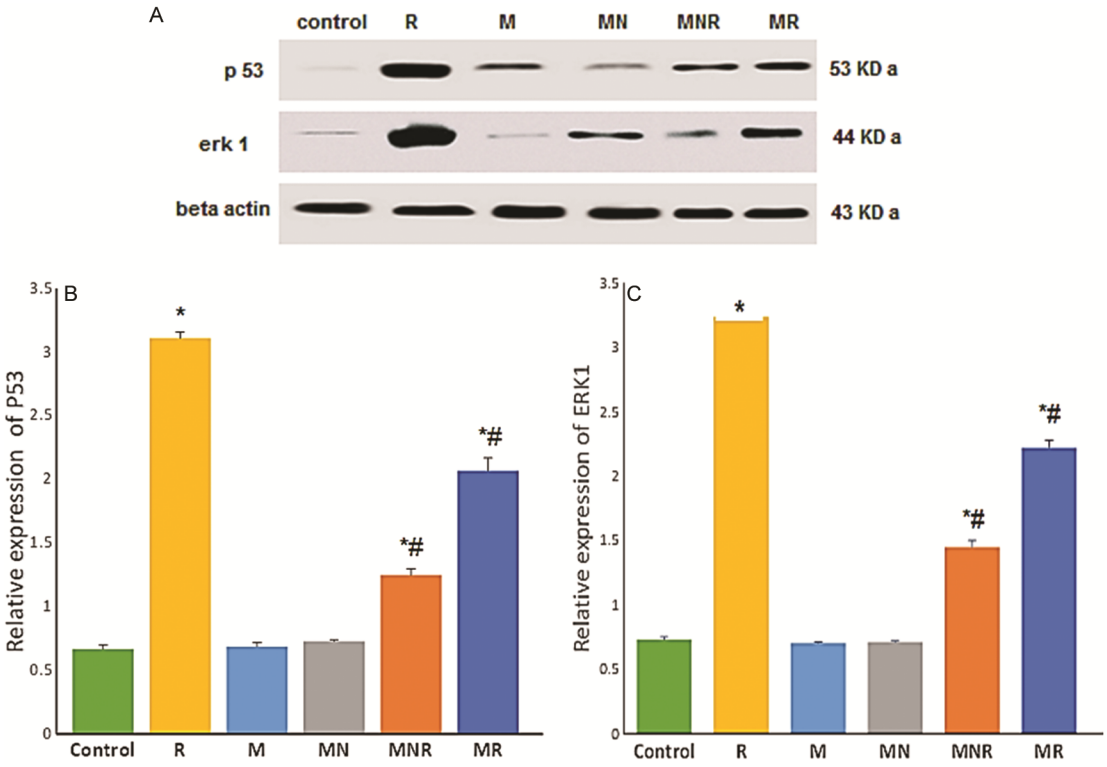

Fig. 5 — p53 and ERK1 expression levels in ovarian tissue in the different experimental groups (Western blot analysis). The study presented the data as means ± SE for each group. * Significant at $P< 0.05$ level in comparison to the control group, while # Significant at $P< 0.05$ level in comparison to the irradiated group.

ovaries of radiation-treated rats. These findings align with Can *et al*. who reported necrosis in the ovaries of irradiated rats[37]. Apoptosis was observed in the M and MN groups, while DNA from the MNR group demonstrated a significant reduction in the necrotic effects of radiation, with apoptosis being induced. This indicates a potential protective effect of the treatment against radiation – induced DNA damage.

Western blot analysis

The expression of both p53 and ERK1 was significantly high in irradiated group tissues compared to the control group (Fig. 5). Ghosh *et al*. reported that radiation exposure elevates and localizes the p53 gene in the cell nucleus, a finding that aligns with our results[38]. Furthermore, activation of the ERK1/2 pathway has been linked to radiation response, modulating cell survival, and apoptosis, often working in conjunction with p53 during stress responses[39]. This aligns with our observation of increased ERK1 and p53 in irradiated tissues.

The cellular response to radiation is masterfully regulated by the tumor suppressor p53[40,41]. When cells are exposed to radiation, the DNA damage response gets activated, which in turn causes an increase in the amount of p53 protein. This is achieved mainly by encouraging the translation of proteins and preventing their destruction[42].

The accumulation of p53 protein in the nucleus triggers multiple signaling pathways. These pathways control how the cell reacts to physical or chemical stress[43]. Activating p53-mediated signaling can either start the intrinsic pathway of apoptosis and cell senescence, which both make cell death more likely[44], or it can stop the cell cycle and let DNA repair happen, which is beneficial for cell survival. As a result, p53 is an essential component in the process of determining the fate of cells following irradiation. In the current study, we found that both the MR and MNR groups were able to modulate p53 and ERK1 levels in comparison to the R group. Notably, the MNR group showed better results than the MR group, indicating that the nanoparticles enhance the effectiveness of matcha.

Histopathological studies

The control group's ovarian tissue section (Fig. 6-C) showed a normal histological structure with no significant pathological alterations. Ovarian

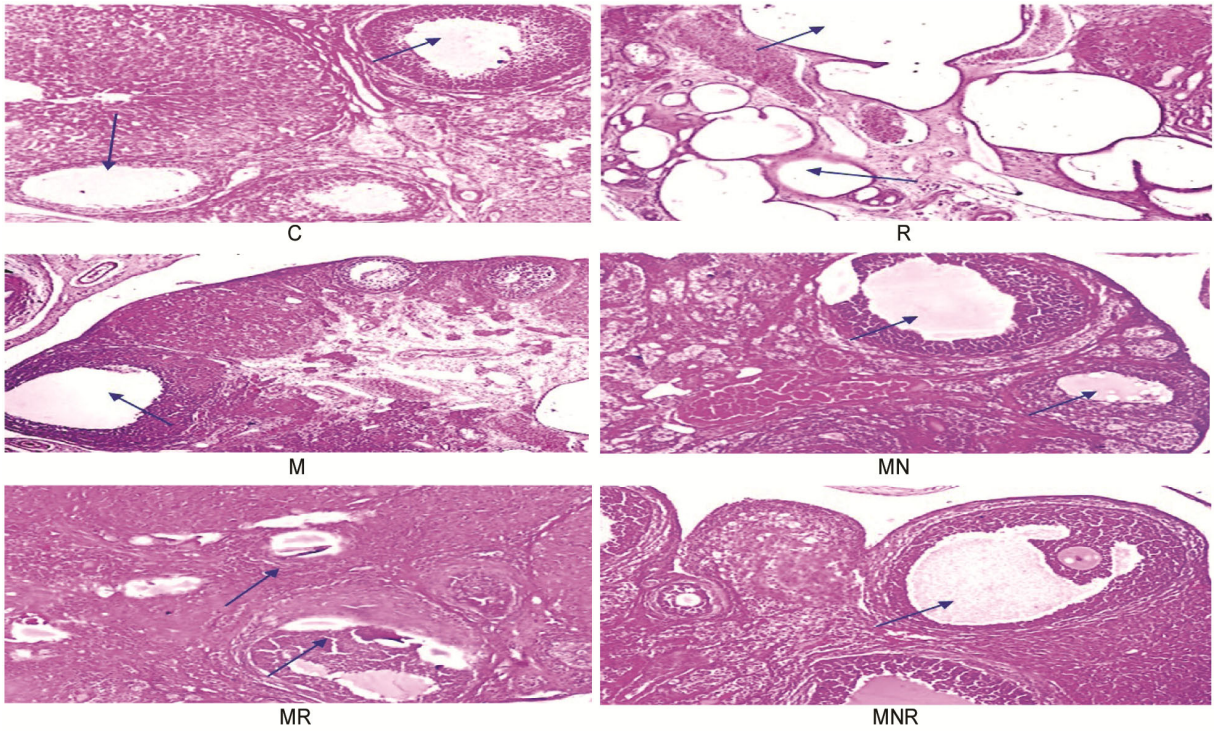

Fig. 6 — Photomicrograph of ovarian tissue section showing; (C) ovarian follicles in various stages of development; (R) multiple different-size follicular and corpora luteal cysts; (M & MN) ovarian follicles in various stages of development and with normal appearance in the ovarian cortex; (MR) large corpora luteal cysts with hyperplasia of the ovarian interstitial tissue stroma; (MNR) multiple follicles in many different stages and well organized graffian follicle with oocyte (H & EX 200)

follicles are in various stages of development in the ovarian cortex. A regular arrangement of follicular cells surrounds the oocyte. The ovarian follicles, both primary and tertiary, were normal. In contrast, rats exposed to radiation (Fig. 6-R) exhibited multiple different-size follicular and corpora luteal cysts. We observed hyperplasia of the ovarian interstitial tissue stroma. Mature graffian follicle presents with a centrally located oocyte, with disruption of granulosa cells in some follicles also noticed. The ovarian tissue sections of M and MN rat groups had a normal ovarian structure with no significant pathological alterations. The ovarian cortex displayed ovarian follicles at various stages of development, all with normal appearances. The follicular cells were arranged in a regular manner around the oocyte. Normal ovarian follicles, including primary and tertiary follicles, and also graffian follicles present with centrally located oocytes were seen (Fig. 6-M and MN).

The group ovarian tissue section of the MR rats revealed different stages of ovarian follicles. Multiple ovarian and large Corpora luteal cysts were observed. We observed hyperplasia of the ovarian interstitial tissue stroma. Mature graffian follicles present with a centrally located oocyte with disruption of granulosa cells in some follicles were also noticed (Fig. 6-MR). On the other hand, the animals' group MNR revealed marked improvement in the ovarian tissue, including multiple follicles in many different stages and well-organized graffian follicles with oocytes. Mature graffian follicles displayed a normal arrangement of granulosa cells with a centrally located oocyte (Fig. 6-MNR).

Conclusion

Oxidative stress plays a significant role in radiation-induced organ damage, contributing to various disorders. In this study, we investigated the potential effects of M-AgNPs against oxidative stress and apoptotic events in radiation-induced ovarian injury in rats. The findings indicated that the administration of M-AgNPs for two weeks following exposure to radiation is more efficacious in diminishing oxidative stress, suppressing apoptotic markers, and alleviating radiation-induced DNA damage in ovarian tissue compared to the standard M treatment. The distinctive phytochemical and physicochemical properties of M-AgNPs augment their biological activity. Furthermore, the amalgamation of matcha's antioxidant-laden phytochemicals with the

potent attributes of AgNPs may yield a synergistic effect, in which the whole therapeutic result surpasses the individual effects of each constituent.

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Conflict of interest

All authors declare no conflict of interest.

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