Benha Journal of Applied Sciences (BJAS)
Vol. (10) Issue (9) (2025), (1- 15)

print: ISSN 2356–9751
online: ISSN 2356–976x

Benha Journal of Applied Science (BJAS)
Applied and Basic Science
https://bjas.journals.ekb.eg/

BENHA JOURNAL OF APPLIED SCIENCES
مجلة بنها للعلوم التطبيقية

Potential effects of low-dose gamma radiation on Bax and p53 gene expression in albino mouse fetuses

Hagar Rashad[1,2], Mohammed Hussein Awwad[1], Gamal Ahmed[3], Amr M. Abdelhady[2] and Mervat K. Iskandar[1]

[1] Zoology Department, Faculty of Science, Benha University, Qalyubia, Egypt
[2] Department of Radiology and Medical Imaging Technology, Misr University for Science and Technology, Giza Egypt
[3] Department of Physics, Al-Azhar University, Cairo, Egypt

E-mail: Hagar.Rashad@must.edu.eg

Received 23-4-2025
Accepted for publication 25-6-2025
Published 23-12-2025

Abstract

Exposure to ionizing radiation from both natural and human-made sources poses significant risks to living organisms, particularly during critical embryonic developmental stages. Ionizing radiation generates free radicals, inducing oxidative stress and damaging essential biomolecules, including DNA. This study examines the effects of low-dose gamma irradiation on fetal development using albino mice as a model organism. Pregnant mice were exposed to varying doses of gamma radiation (5, 10, and 50 mGy) on gestational day 15. The study assessed the effects on fetal weight, length, and the gene expression of Bax and p53, along with histopathological changes in the lungs, heart, and liver. The results revealed a dose-dependent decrease in fetal weight and length, with the higher doses (50 mGy) causing significant developmental impairments. Furthermore, gamma irradiation significantly upregulated the expression of Bax and p53, indicating increased pro-apoptotic activity and DNA damage response. Histopathological analysis showed structural abnormalities in the lungs, heart, and liver of irradiated fetuses. These findings underscore the importance of caution regarding radiation exposure during pregnancy, as it may lead to profound and long-term health consequences.

Keywords: Gamma irradiation, fetal development, Bax gene, p53 gene, albino mice

1. Introduction

Living organisms are continuously exposed to ionizing radiation from natural sources, such as radon decay products and cosmic rays, as well as human activities including nuclear weapons testing, fuel reprocessing, and nuclear accidents (Hurem et al., 2017). Ionizing radiation interacts with matter by exciting and ionizing molecules, generating free radicals that lead to the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive compounds can induce oxidative stress, disrupt cellular membranes, and damage biological molecules particularly DNA through bond cleavage and structural alterations (Ping et al., 2020).

Both humans and animals are particularly susceptible to ionizing radiation throughout critical

Doi: http/10.21608/bjas.2023.88220.1003

early life phases, including gametogenesis, embryogenesis, and organogenesis. Ionizing radiation can impact all organs and biological systems, potentially causing both non-cancerous consequences and malignancy. Experimental investigations have demonstrated that exposure to ionizing radiation during crucial developmental phases may modify differentiation signals, resulting in enduring harmful effects that may manifest later in life. (Mattsson et al., 2021; Tokpinar et al., 2024). Permanent (irreversible) "developmental programming" is, among other mechanisms, ascribed to epigenetic alterations in gene transcription (Sia et al., 2020; Toprani et al., 2024). In particular, exposure to gamma radiation resulted in low birth weight, short stature, and ossification in fetuses due to oxidative stress (Alwood et al., 2017; Sia et al., 2020; Tokpinar et al., 2024).

The integration of radiography, computed tomography (CT), nuclear medicine, ultrasonography (US), and magnetic resonance imaging (MRI) in contemporary healthcare is such that women with confirmed or undetected pregnancies are likely to undergo examination using any of these modalities (Mattsson et al., 2021). Most diagnostic medical imaging procedures in radiography, computed tomography (CT), conventional fluoroscopy, and nuclear medicine expose the embryo or fetus to absorbed doses of 0.1 Gy or less to guarantee fetal safety (Brent, 2015; Gomes et al., 2015; Mainprize et al., 2023).

Ionizing radiation, such as X-ray or gamma ray, damages tissue by creating an unbalanced free radical and disrupts molecules, such as DNA, producing mutations that can lead to cancer (Elkady & Ibrahim, 2016). The human body possesses intrinsic defensive mechanisms against radiation, including p53, recognized as the custodian of the genome, which functions in DNA damage response and cancer suppression (Mukherjee et al., 2022; Okazaki, 2022). Moreover, it is essential for the activation of genes associated with cell cycle arrest and death, rendering the cells radioresistant (Okazaki, 2022). Furthermore, p53 enhances the expression of pro-apoptotic mediators, thus disturbing the complex equilibrium between cellular anti-apoptotic and pro-apoptotic proteins. p53 disassembles the complex formed by the anti-apoptotic Bcl2 and the pro-apoptotic Bax. Released Bax induces apoptosis by promoting the release of cytochrome c from mitochondria, resulting in cell death.

Animal experimental research suggests that acute or chronic low-dose ionizing radiation (LDIR) (≤100 mSv) or low-dose-rate ionizing radiation (LDRIR) (<6 mSv/h) exposures could be detrimental. Furthermore, it demonstrated that fetal weight, placental weight, and fetal morphometric parameters were statistically considerably reduced. The expression of the p53 gene was demonstrated to be induced by a minimum of 10 mGy of total body irradiation in mice. p53 enhances the expression of pro-apoptotic mediators, thereby disturbing the complex equilibrium between anti-apoptotic and pro-apoptotic proteins within the cell. Furthermore, it disassociates the complex formed by the anti-apoptotic Bcl2 and the pro-apoptotic Bax. Bax induces apoptosis by promoting the release of cytochrome c from mitochondria (Mukherjee et al., 2022; Okazaki, 2022). Experimental studies indicate that ionizing radiation produces two categories of impacts and dangers: (1) short-term deterministic risks and (2) long-term stochastic risks. Deterministic short-term effects manifest as prenatal mortality during the implantation phase and cellular death during organogenesis, leading to growth retardation, deformities, and neurological consequences (Mattsson et al., 2021).

The work aims to investigate the effect of low-dose gamma radiation on the fetuses of albino mice. The work explores the impact of radiation on fetal weight, length, and molecular changes, including the genes that appear during and after irradiation, such as p53 and Bax, which are responsible for apoptosis and cellular stress. Besides that, it discusses the histopathological tissue changes for critical organs such as lungs, hearts, and livers due to irradiation of low doses of gamma irradiation. The outcome of this work is to explore the potential effect of radiation on the fetus's development and its overall impact on human health.

2. Materials and methods

2.1 .Animal Acquisition, Housing, and Ethics

Twenty virgin female and fertile male BALB/c mice (40–53 g) were obtained from the Theodor Bilharz Research Institute (Giza) and housed at 25–30°C with free access to food and water. After a one-week acclimation period, during which they were

monitored for health and stress, 8–9-week-old females were used for breeding. All procedures were approved by Benha University's ethics committee (ZD/FSc/BU-IACUC/2024-24; BUFS-REC-2025-364 Zoo).

2.2 .Breeding Procedure

Three females were housed overnight with one male, and mating was confirmed by the presence of a vaginal plug the next morning. Pregnant females were housed individually and monitored throughout gestation for behavior, weight, and health.

2.3. Radiation Exposure

Pregnant mice were randomly assigned to a control group (A) or one of three radiation groups: B (5 mGy), C (10 mGy), and D (50 mGy). Gamma radiation (662 keV) was delivered on gestational day 15 at 10 mGy/min using a cesium-137 source. Exposure times were 30 seconds (B), 1 minute (C), and 5 minutes (D). Food and water were withheld during irradiation.

2.4. Dissection and Measurements

On day 20 of pregnancy, mice were euthanized and uteri dissected. Maternal body weight was recorded pre- and post-dissection; fetal weight and length were measured to assess developmental effects. Figures 1 and 2 show the experimental setup and fetal outcomes.

2.5. RNA Extraction

Total RNA was extracted from 30 mg fetal liver tissue using the RNeasy Mini Kit (Qiagen), following the manufacturer's protocol. Samples were homogenized in lysis buffer with β-mercaptoethanol, centrifuged, and processed through spin columns. RNA was eluted in RNase-free water.

2.6. PCR Master Mix Preparation

RNA was reverse-transcribed into cDNA using RevertAid Reverse Transcriptase (Thermo Fisher). Quantitative PCR was performed with SYBR Green PCR Master Mix (Qiagen) in 25 µl reactions containing cDNA, primers for β-actin, p53, and Bax, and RNase-free water. Primers were obtained from Metabion (Germany).

2.7. Primer Sequences

Primer sequences were adopted from previous studies. For p53: forward 5'-GTATTTCACCCTCAAGATCC-3', reverse 5'-TGGGCATCCTTTAACTCTA-3' (Tohidi et al., 2015); for Bax: forward 5'-CTCAAGGCCCTGTGCACTAA-3', reverse 5'-GAGGCCTTCCCAGCCAC-3' (Jalili et al., 2017); and for β-actin: forward 5'-CTCAAGGCCCTGTGCACTAA-3', reverse 5'-GAGGCCTTCCCAGCCAC-3' (Sisto et al., 2003).

2.8. Real-Time PCR Conditions

PCR was performed on a Stratagene MX3005P system. Cycling included reverse transcription at 50°C, denaturation at 94°C, and 40 amplification cycles. Annealing was at 55°C for β-actin and 60°C for p53 and Bax.

2.9. PCR Data Analysis

Ct values were obtained and analyzed using the ΔCt method. Relative gene expression was calculated using the 2^-ΔΔCt formula (kareim et al., 2022).

2.10. Histopathological Examination

Formalin-fixed embryonic tissues were processed using an automated tissue processor. Fixation was performed in 10% buffered formalin for 48 hours, followed by washing in distilled water. Tissues were dehydrated through graded alcohols (70% for 2 hours, 90% for 90 minutes, and two cycles of 100% alcohol for 1 hour each), then cleared with xylene (50% alcohol/xylene for 1 hour, then pure xylene for 1.5 hours). Samples were embedded in paraffin, sectioned at 4–5 µm, and stained with hematoxylin and eosin (Suvarna et al., 2018). Sections were examined for circulatory disturbances, inflammation, degeneration, apoptosis, necrosis, and other pathological changes.

2.11. Statistical Analysis

Data were analyzed using SPSS v20 and expressed as mean ± standard error. Group differences were evaluated using the Kruskal-Wallis H test, followed by Dunn's post hoc test for multiple comparisons.

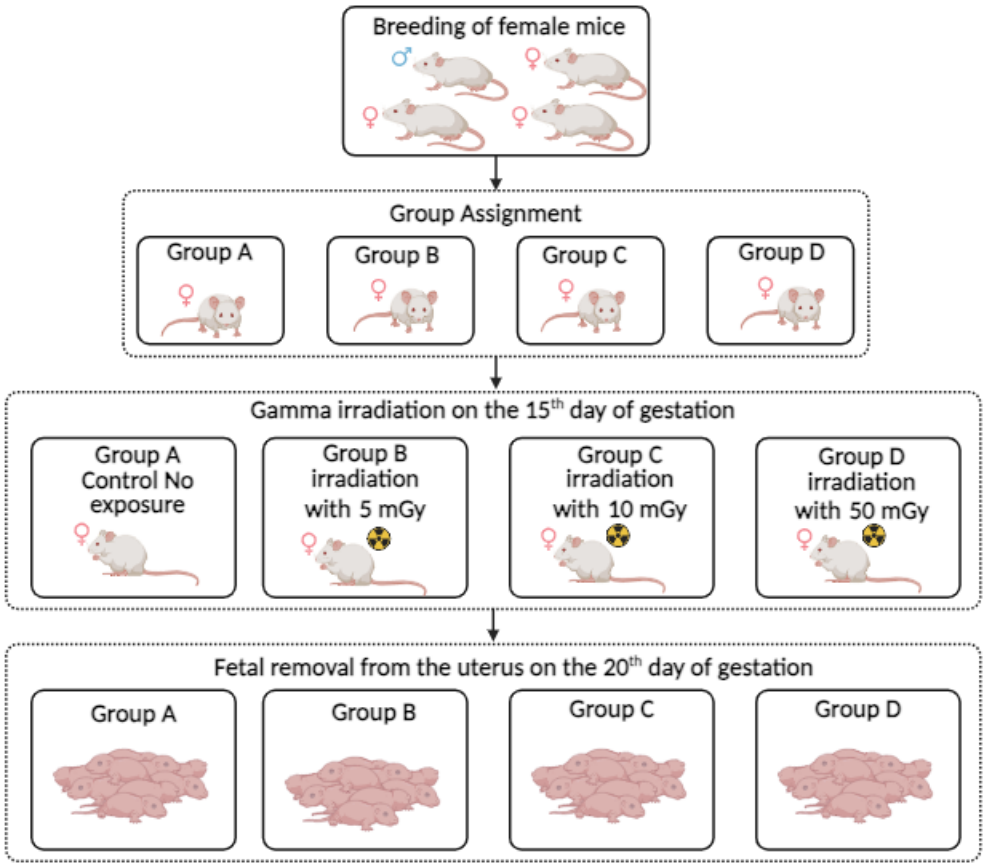

Figure 1: The experimental design.

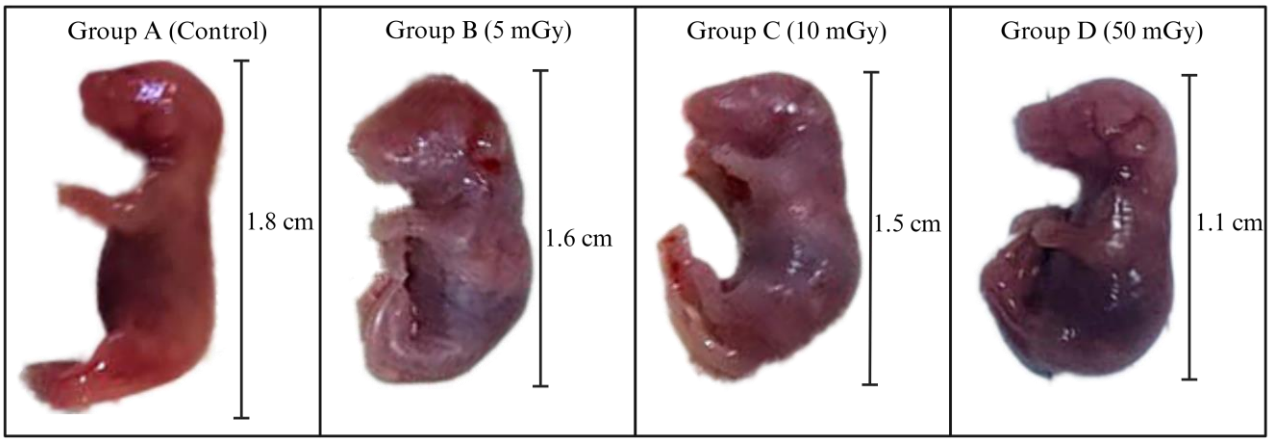

Figure 2: Dissected Fetuses from Irradiated and Control Groups on Day 20 of Pregnancy.

3. Results

3.1. Mortality rate

All four groups survived the experiment. The control, 5 mGy, 10 mGy, and 50 mGy groups showed normal growth, as illustrated in **Figure 3**.

3.2. Effect of Gamma Irradiation on Fetal Weight

Figure 4 illustrates a dose-dependent decline in fetal weight across irradiated groups compared to the control. The Kruskal-Wallis H test showed a significant difference among groups ($\chi^2(3) = 40.16$, $p < 0.001$), with mean rank scores of 74.44 (control), 64.38 (5 mGy), 32.02 (10 mGy), and 35.61 (50 mGy). Post-hoc Dunn's test ($\alpha = 0.001$) revealed significant differences between control vs. 10 mGy and 50 mGy, and between 5 mGy vs. 50 mGy, indicating the 10 mGy and 50 mGy groups were most affected.

3.3. Effect of Gamma Irradiation on Fetal Length

Figure 5 shows fetal length also varied significantly across groups ($\chi^2(3) = 35.13$, $p < 0.001$). Mean rank scores were 62.5 (control), 56.67 (5 mGy), 64.63 (10 mGy), and 25.94 (50 mGy). The marked decrease in the 50 mGy group indicates a strong dose effect. Dunn's test confirmed significant differences between control, 5 mGy, and 10 mGy groups compared to 50 mGy, with the 50 mGy group showing the greatest reduction.

3.4. The effect of gamma irradiation on Bax and p53 gene expression (fold change)

Figures 6 and **7** show a dose-dependent upregulation of Bax and p53 gene expression following gamma irradiation. The control group showed baseline expression (fold change = 1). In the 5 mGy group, Bax increased to 2.29 ± 0.20 and p53 to 3.14 ± 0.32. At 10 mGy, Bax rose to 4.77 ± 0.50 and p53 to 5.17 ± 0.25. The highest expression levels were observed in the 50 mGy group, with Bax at 9.83 ± 0.26 and p53 at 14.16 ± 0.20, indicating strong dose-responsive induction.

Dunn's post-hoc test (α = 0.001) confirmed significant differences between control vs. 10 mGy and 50 mGy, and between 5 mGy vs. 50 mGy. The 50 mGy group showed the most substantial gene expression changes.

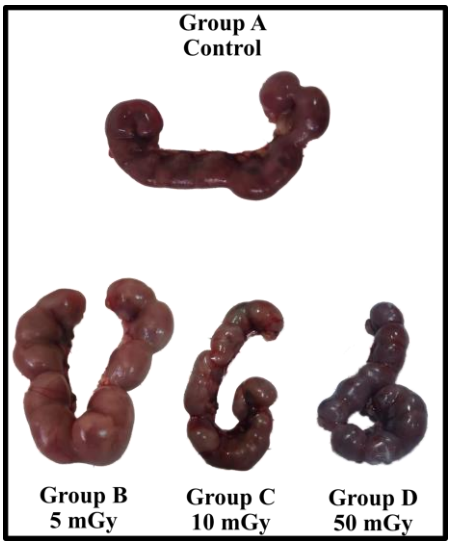

Figure 3: Representation of mice uterus-ovary complexes.

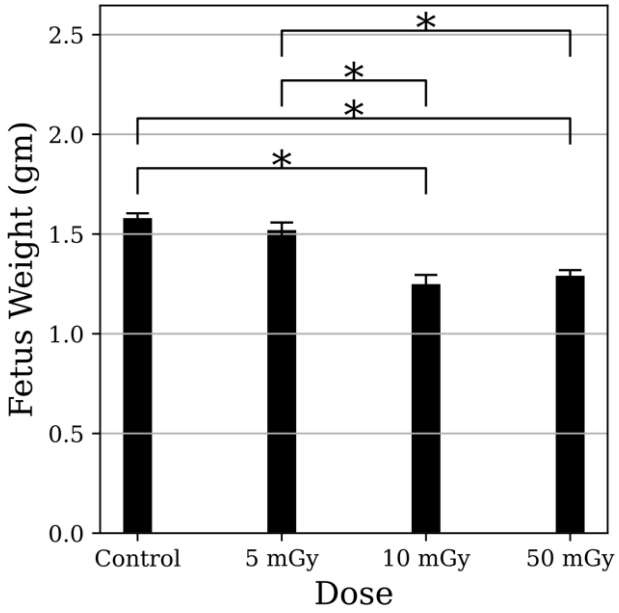

Figure 4: Fetal body weight. Data are presented as mean ± standard error of the mean (SEM). All group pairs marked with * above the line differ significantly at $P \leq 0.001$.

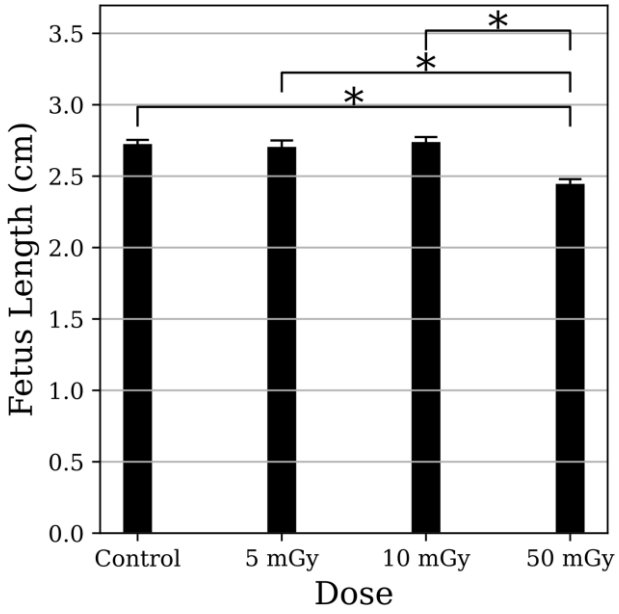

Figure 5: Fetal body length. Data are presented as mean ± standard error of the mean (SEM). All group pairs marked with * above the line differ significantly at $P \leq 0.001$.

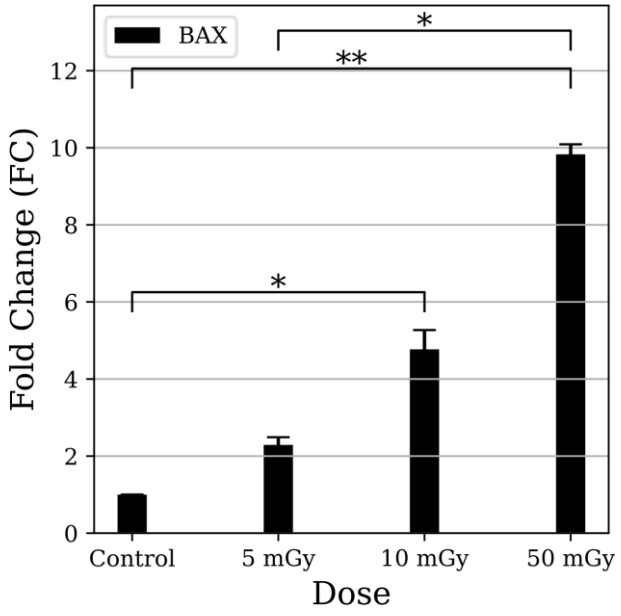

Figure 6: Dose-dependent increase in Bax Gene Expression. Data are presented as mean ± standard error of the mean (SEM) with significance levels indicated as follows: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

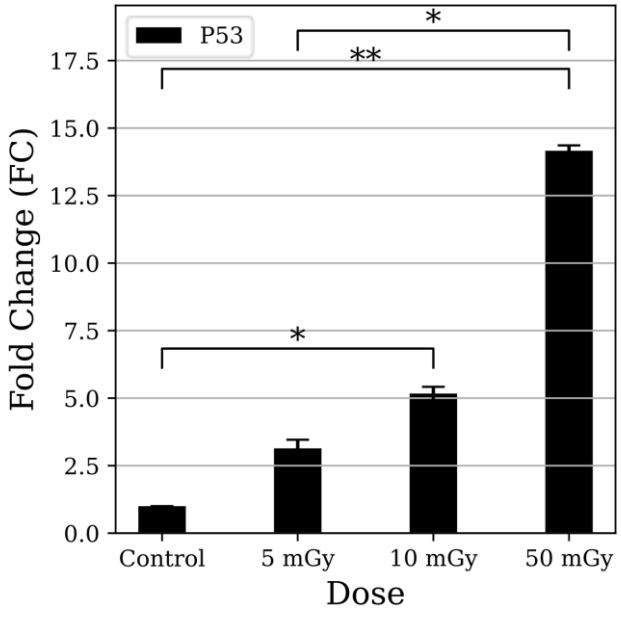

Figure 7: Dose-dependent increase in p53 Gene Expression. Data are presented as mean ± standard error of the mean (SEM) with significance levels indicated as follows: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

3.5. Histopathological findings in the liver following gamma radiation exposure

On gestational day 20, control fetal liver tissue appeared normal, with organized hepatocyte cords, clear cytoplasm, and intact sinusoids **(Figure 8a)**.

At 5 mGy, mild hepatic stress was observed, including slight vascular dilation and minimal cytoplasmic vacuolation, though architecture remained intact **(Figure 8b)**.

At 10 mGy, tissue showed increased necrosis, apoptosis, nuclear fragmentation, and cytoplasmic disintegration, along with moderate vascular dilation **(Figure 8c)**.

At 50 mGy, liver damage was severe, with extensive necrosis, vacuolation, and disrupted architecture, indicating a strong dose-dependent hepatotoxic response **(Figure 8d)**.

3.6. Histopathological findings in the lung following gamma radiation exposure

Control lung tissue showed normal broncho-alveolar structures, with well-formed alveoli, bronchioles, and septa **(Figure 9a)**.

At 5 mGy, mild inter-alveolar septal thickening, focal necrosis, and slight bronchio-alveolar expansion were noted, with minor immune cell infiltration **(Figure 9b)**.

At 10 mGy, alveolar collapse, vascular dilation, and epithelial hyperplasia appeared, indicating moderate injury and compensatory regeneration **(Figure 9c)**.

At 50 mGy, lungs showed severe damage, including alveolar wall edema, vascular congestion, and intense inflammation, compromising lung structure and function **(Figure 9d)**.

3.7. Histopathological findings in the heart following gamma radiation exposure

Control fetal heart sections revealed normal cardiomyocytes, central nuclei, and a supportive mucoid matrix **(Figure 10a)**.

At 5 mGy, early myocardial stress appeared, including focal necrosis, apoptosis, and moderate

mucoid deposition, with overall structure preserved **(Figure 10b)**.

At 10 mGy, cardiomyocytes exhibited pyknosis, vacuolization, and architectural disruption, reflecting degenerative changes **(Figure 10c)**.

At 50 mGy, heart tissue showed widespread necrosis, nuclear condensation, and fragmented muscle fibers, indicating substantial radiation-induced cardiac damage **(Figure 10d)**.

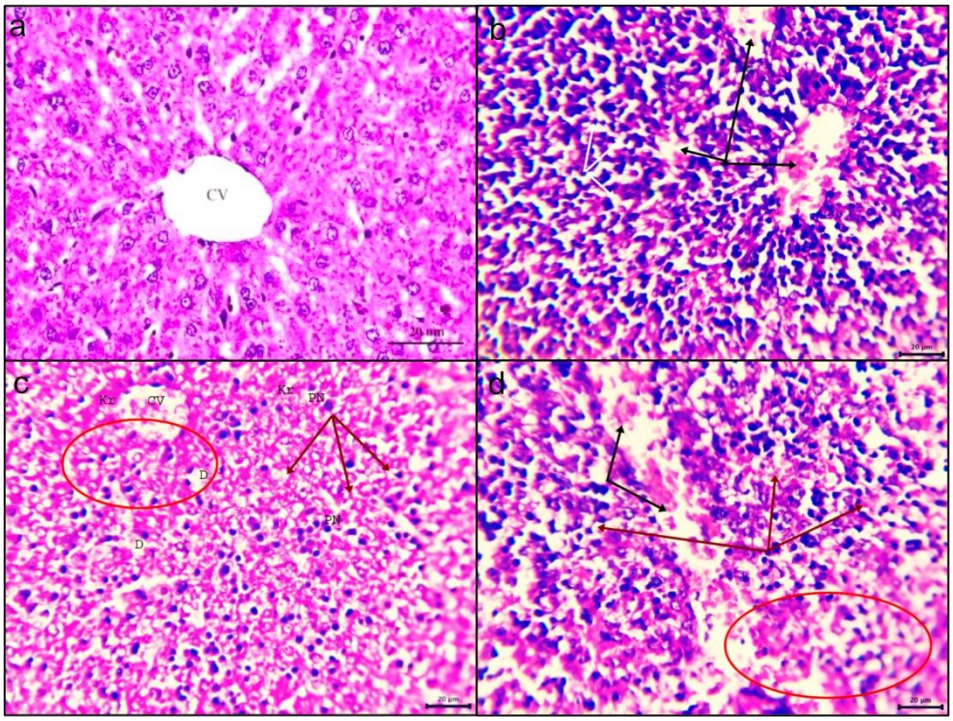

Figure 8: Histopathological liver changes in 20-day-old mouse fetuses post gamma radiation. **(a)** Control showing normal hepatocyte cords around central veins (CV). **(b)** 5 mGy: mild central vein dilation (black arrows) and minimal degeneration (white arrows). **(c)** 10 mGy: hepatocellular degeneration (D), nuclear pyknosis (PN), karyorrhexis (Kr), apoptosis, necrosis, and vascular dilation (red markers). **(d)** 50 mGy: extensive necrosis, apoptosis, nuclear condensation, cytoplasmic vacuolation, and vascular dilation (red circle, brown/black arrows).

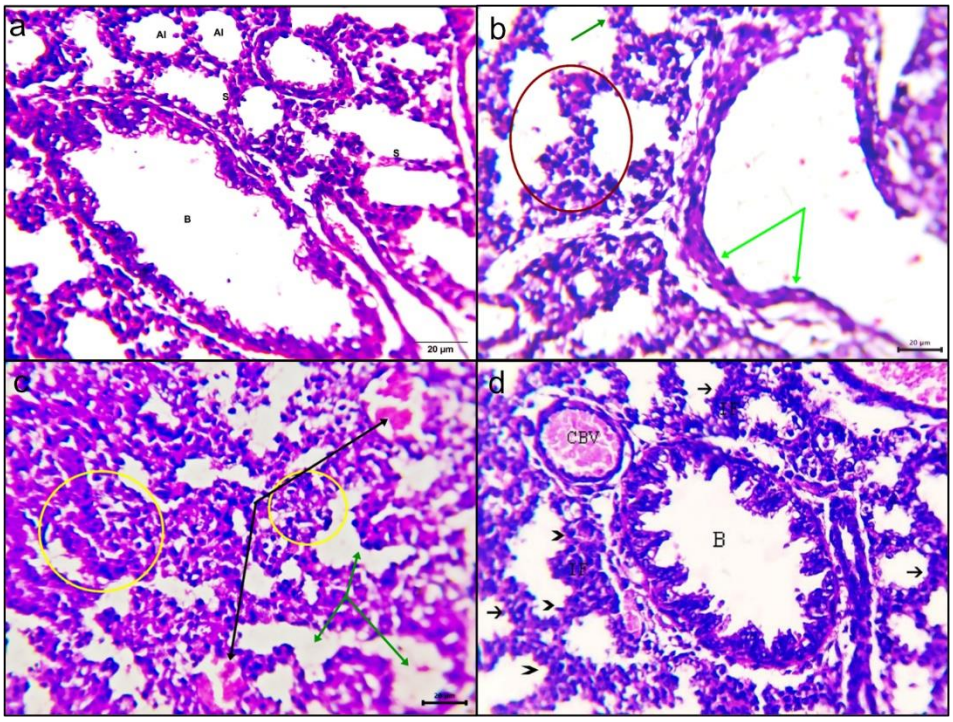

Figure 9: Lung histopathology in 20-day-old mouse fetuses post gamma radiation. **(a)** Control: normal bronchioles (B), alveolar sacs (Al), and septa (S). **(b)** 5 mGy: focal apoptosis/necrosis, septal thickening (circle), and bronchio-alveolar expansion (arrows). **(c)** 10 mGy: alveolar collapse, inflammation (yellow circle), vascular dilation (black arrows), and expansion (green arrows). **(d)** 50 mGy: severe damage—congestion (CBV), thickened/alveolar walls (black arrows), edema (arrowheads), and inflammation (IF) around thickened bronchus (B).

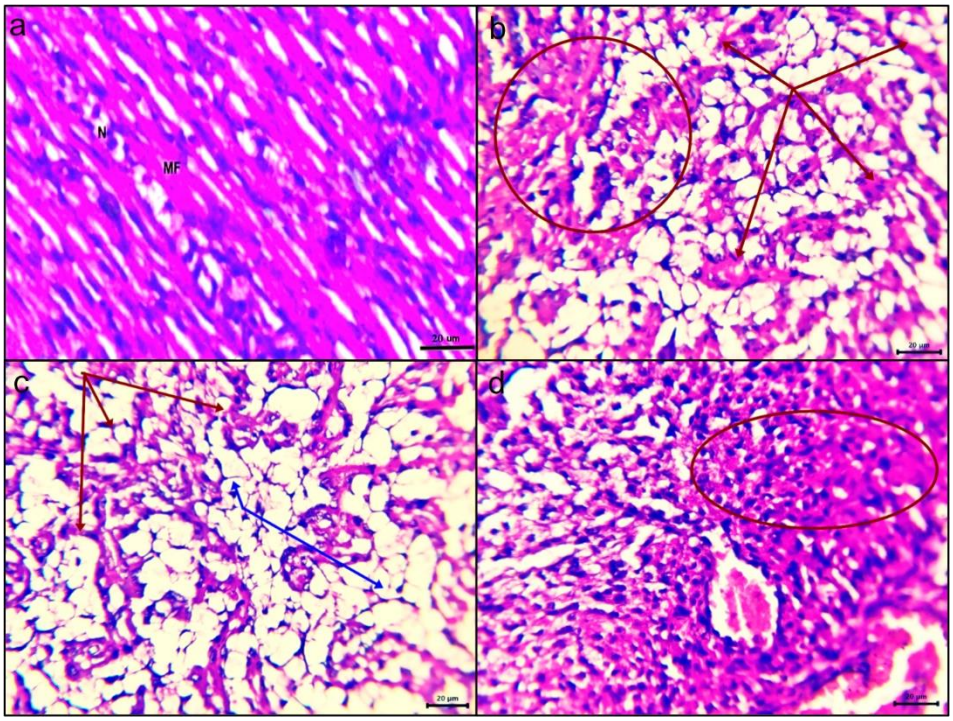

Figure 10: Cardiac histopathology in 20-day-old mouse fetuses post gamma radiation. **(a)** Control: normal myocardial fibers (MF), central nuclei (N), and mucoid matrix. **(b)** 5 mGy: focal apoptosis/necrosis, interstitial mucoid deposition, and atrophic changes (red circle/arrows). **(c)** 10 mGy: nuclear pyknosis (red arrows) and

marked cardiomyocyte degeneration (blue arrows). **(d)** 50 mGy: extensive necrosis and pyknotic nuclei (red circle), indicating severe cardiac damage.

4. Discussion

The findings of this study provide critical insights into the dose-dependent effects of gamma irradiation on fetal development, particularly how exposure influences the regulation of key genes involved in cellular stress responses, DNA repair, and apoptosis (Tharmalingam et al., 2017). Ionizing radiation at low levels generates free radicals, increasing cellular oxidative stress. Growing evidence shows that oxidative stress affects epigenetic regulators, altering gene expression and inducing phenotypic changes that impact physiological outcomes (Tharmalingam et al., 2017). The elevation of apoptosis-related genes is particularly emphasized. ROS and antioxidants play key roles in early development, including gametogenesis, organogenesis, fertilization, and embryogenesis. Excessive ROS can damage sperm DNA via lipid peroxidation, threatening the embryo's genetic integrity (Swain et al., 2022).

These findings add to the understanding of risks posed by ionizing radiation during pregnancy, showing that even minimal exposure can trigger biological responses that may affect fetal health. Given the sensitivity of developing embryos to environmental stressors, this study reinforces the need for strict radiation safety protocols, especially in medical settings involving diagnostic or therapeutic procedures during pregnancy. It also highlights the need for further research into the molecular mechanisms of radiation-induced developmental toxicity. Clarifying these pathways is essential for improving radiation safety guidelines and developing protective strategies. By advancing our understanding of low-dose radiation impacts, this research supports evidence-based policies that prioritize maternal and fetal health.

4.1. Mortality

All experimental groups—including control, 5 mGy, 10 mGy, and 50 mGy—showed a 100% survival rate throughout the study, indicating that these low radiation doses did not induce mortality. This finding aligns with previous studies demonstrating that low-dose gamma radiation does not significantly affect survival in experimental models. For instance, Fujimichi et al. (2023) reported no mortality associated with low-dose exposure, with notable shifts in age at death only occurring at much higher doses (1.9 Gy), depending on the timing of exposure. Similarly, Matsuya et al. (2014) found that survival fraction curves, modeled mathematically, supported the presence of dose-rate effects related to DNA lesion kinetics, suggesting that effective DNA repair mechanisms mitigate damage at low exposure levels. Additionally, Pathak et al. (2019) demonstrated that exogenous administration of recombinant thrombomodulin enhanced radiation protection, promoting white blood cell recovery and bone marrow regeneration, particularly when combined with other treatments. These results collectively support the notion that low-dose gamma radiation does not compromise survival, likely due to the body's effective cellular defense and repair systems.

4.2. Effect on fetal weight

One of the most significant findings of this study is the dose-dependent reduction in fetal weight, underscoring the detrimental effects of gamma irradiation on fetal development. This pattern parallels the results of Nakahira et al. (2021), who utilized chronic in utero irradiation for 18 days during gestation at medium dose rates (200–400 mGy/day). Medium-dose-rate radiation caused modest general development inhibition, seen by diminished body weight from birth (average body weight at 10 weeks was 89–103% of that of nonirradiated animals). However, this study differs by focusing on a single acute radiation event at gestational day 15, offering a clearer evaluation of how one-time exposure impacts fetal weight. Acute exposure at sensitive developmental stages can lead to more pronounced effects, as repair mechanisms may not respond quickly enough to mitigate damage (Chang et al., 2021; Nakahira et al., 2021)

A fundamental difference between this study and Nakahira et al. (2021) is the method of exposure. Pregnant dams subjected to continuous whole-body gamma-ray irradiation for 22 hours daily from gestational day 0 to 18 experienced mild general growth retardation. However, even a single acute radiation exposure at a crucial developmental phase can lead to significant and immediate decreases in fetal weight. This indicates that irradiation may

reduce the number, weight, and height of live fetuses on the 18th day of gestation. Additionally, irradiation had a diminishing effect on fetal weight. Both adverse conditions led to a reduction in birth weight (Nakahira et al., 2021).

To further assess the effects of radiation on fetal growth, this study examined doses of 5, 10, and 50 mGy. The 50 mGy group exhibited the most significant reductions in fetal weight, yet even the 5 mGy group showed measurable effects, reinforcing concerns that even low-dose radiation can trigger biological responses. Dehghan et al. (2016) also showed that elevated radiation and hormone doses reduced the number of surviving fetuses (45 at 2 Gy vs. 29 at 4 Gy). A deceased fetus with a foot abnormality (lack of toes) was seen in the 2 Gy group. In the 4 Gy group, two babies exhibited a short neck and a protrusion at the occipital region, with only fetal weight and birth height showing significant differences among the groups ($p = 0.01$) (Dehghan et al., 2016).

4.3. Effect on fetal length

Gamma irradiation significantly reduced fetal length, as seen in the marked decrease in the 50 mGy group. These findings align with Tokpinar et al. (2024), who investigated melatonin's protective effects on fetal bone development after ionizing radiation exposure. Rats exposed to 0.5 Gy total body irradiation showed statistically significant decreases in fetal and placental weight, and fetal morphometrics compared to controls ($p < .05$). Immunohistochemical analysis revealed significantly lower alkaline phosphatase and tartrate-resistant acid phosphatase levels in the radiation group, along with reduced calcium and sodium concentrations. Melatonin was found to exert a protective effect against radiation-induced bone development damage (Chang et al., 2021; Tokpinar et al., 2024).

This study builds on Tokpinar et al. (2024) by showing that lower doses—10 and 50 mGy—also cause significant skeletal effects, supporting the hypothesis that even moderate prenatal radiation may impair bone integrity. Differences in radiation doses between studies highlight the need to understand specific dose-response relationships in fetal skeletal development. Oxidative stress (OS) negatively impacts osteoblast differentiation, activity, and mineralization, while promoting osteoblast apoptosis and osteoclast differentiation. This leads to an imbalance in bone remodeling, with increased bone turnover and net resorption (Tokpinar et al., 2024).

Recent findings further support melatonin's protective role in fetal skeletal development after radiation exposure. Experimental studies show significantly larger skeletal ossification areas in the melatonin and radiation + melatonin groups (Chang et al., 2021; Tokpinar et al., 2024). As a potent antioxidant, melatonin mitigates oxidative stress by modulating the antioxidant system (Tokpinar et al., 2024). Additionally, radiation-induced skeletal damage may persist into adulthood, increasing susceptibility to osteoporosis and fractures (Dehghan et al., 2016). These long-term consequences highlight the need to understand radiation's effects on skeletal development beyond birth.

4.4. Molecular impact of gamma radiation on fetal development

This study highlights the dose-dependent activation of key regulatory genes, particularly Bax and p53, in response to gamma irradiation, underscoring their roles in apoptosis and DNA damage response mechanisms. The increase in Bax expression, especially in the 50 mGy group, suggests a strong pro-apoptotic response, leading to mitochondrial membrane permeabilization—a crucial step in the intrinsic apoptosis pathway. These results align with Mukherjee et al. (2022), who exposed HepG2 cells to γ-ray doses of 2, 5, and 8 Gy. Compared to controls, bystander cells showed increased H2AX phosphorylation, mitochondrial membrane depolarization, and activation of p53, Bax, caspase-9, caspase-3, and PARP cleavage. PS externalization and scanning electron microscopy confirmed increased bystander cell death. Bcl2 levels and cell viability were reduced in these cells. These findings raise concerns about how localized radiation exposure during pregnancy may affect non-irradiated tissues (Mukherjee et al., 2022).

This study expands on Mukherjee et al. (2022) by showing similar molecular responses in fetal tissues, providing in vivo evidence of systemic apoptotic pathway activation. While their work was based on cell culture, this study demonstrates that Bax translocation, cytochrome c release, and caspase activation occur in a whole-organism model, supporting the role of intrinsic apoptosis in response to radiation.

p53 expression also increased significantly in all irradiated groups, with the highest levels in the 50 mGy group. As a master regulator, p53 maintains genomic stability by initiating cell cycle arrest or apoptosis following DNA damage. These results are consistent with Mukherjee et al. (2022), who reported p53 accumulation due to DNA double-strand breaks and its role in promoting pro-apoptotic mediator expression. They also showed bystander effects in vitro, where irradiated cell media induced mitochondrial-mediated apoptosis in non-irradiated HepG2 cells (Mukherjee et al., 2022).

These findings reinforce Okazaki (2022)'s, work on the broad functionality of p53, which regulates genes involved in DNA repair (e.g., Gadd45, p48), cell cycle arrest (p21, 14-3-3σ), apoptosis (Bax, Noxa, Puma), senescence, ferroptosis, autophagy, and metabolism. Fetal tissues, due to high proliferative activity, are particularly sensitive to these effects. p53 operates through interactions with MDM2, NF-κB, and miRNAs, and is involved in senescence, inflammation, carcinogenesis, and adaptive responses. While p53 plays a critical role in maintaining genome integrity, its loss increases replication stress. Non-canonical functions of p53—such as regulation of DNA replication and NRF2 antioxidant response—may further influence radiation sensitivity. Radiation-induced apoptosis depends on ROS levels and mitochondrial permeability (Castaño et al., 2024).

A key distinction of this study is its focus on moderate radiation doses (10 and 50 mGy), which are more relevant to environmental and medical contexts. Although p53 is essential for tumor suppression, recent evidence suggests that additional copies may not enhance protection against radiation-induced lymphoma (Okazaki, 2022). Understanding these molecular pathways can guide clinical strategies to minimize fetal radiation exposure and its long-term risks

4.5. Histopathological findings in the liver

Gamma radiation exposure during gestation can lead to dose-dependent histopathological changes in fetal liver tissue, particularly when administered during critical developmental windows such as gestational day 15 in mice (Naoe et al., 2023). The liver, essential for metabolism and detoxification, is highly sensitive during fetal development, and even low radiation doses (5, 10, 50 mGy) can cause structural and functional impairments (Thakur et al., 2024).

By gestational day 20, liver tissues showed progressive damage corresponding to radiation dose. At 5 mGy, hepatocytes exhibited mild disorganization and slight vacuolization, indicating early cellular stress. At 10 mGy, these effects intensified, with increased vacuolization, nuclear pyknosis, karyorrhexis, and cytoplasmic disintegration, alongside moderate vascular dilation—signs of necrosis, apoptosis, and vascular stress. At 50 mGy, severe hepatocellular damage was evident, including widespread necrosis and apoptosis, extensive vacuolization, and disruption of liver architecture. Vascular dilation further suggested compromised hepatic circulation and structural integrity.

These pathological alterations are primarily driven by radiation-induced oxidative stress, mediated by the generation of reactive oxygen species (ROS), which damage cellular lipids, proteins, and DNA (Pelcaru et al., 2021). In hepatocytes, this leads to membrane destabilization, mitochondrial dysfunction, and impaired detoxification (Ingawale et al., 2014). Additionally, activation of stress-response pathways, particularly the p53-dependent apoptotic pathway, contributes to cell death and hinders regenerative capacity (Pflaum et al., 2014).

Together, these findings confirm that even low-dose prenatal radiation can significantly impair liver development in a dose-dependent manner, with potential long-term consequences for hepatic function.

4.6. Histopathological findings in the lungs

Exposure to gamma radiation on gestational day 15 induced dose-dependent histopathological changes in fetal lung tissue by day 20. At 5 mGy, minor alterations were noted, such as slight edema and mild bronchiolar hyperplasia, with no significant inflammatory response. At 10 mGy, more evident changes appeared, including thickening of alveolar septa, bronchiolar structural distortion, alveolar congestion, mild inflammatory infiltration, and collapsed alveolar spaces—suggesting early tissue injury and immune activation.

At 50 mGy, lung tissue exhibited severe damage, with pronounced alveolar edema, vascular dilation and congestion, disrupted alveolar architecture, and increased inflammatory infiltrates. Necrosis and early fibrosis were also observed, indicating significant tissue injury and a reparative response.

These findings support earlier research showing that even low-dose gamma radiation can disrupt fetal lung development. Ionizing radiation generates free radicals and induces DNA damage, leading to oxidative stress, vascular injury, and inflammation (Hanania et al., 2019). Edema, an early marker of increased vascular permeability, and inflammatory cell infiltration—particularly by macrophages and neutrophils—are typical responses to such damage (Bouten et al., 2021). These immune cells release cytokines and growth factors that aid in repair but may contribute to further tissue injury if inflammation persists.

Mild hyperplasia observed at lower doses likely reflects an adaptive attempt to maintain tissue integrity. However, features such as alveolar collapse and bronchio-alveolar expansion point to early functional compromise. At 50 mGy, the severity of injury—including necrosis and fibrosis—aligns with previous reports of radiation-induced lung fibrosis, especially in proliferative tissues like the developing lung (Yu et al., 2023). These results emphasize the dose-dependent impact of radiation during critical developmental windows.

4.7. Histopathological findings in the heart

Gamma radiation on gestational day 15 induced dose-dependent cardiac changes by day 20. At 5 mGy, mild vacuolization, cardiomyocyte atrophy, and early nuclear condensation suggested metabolic stress and initial apoptotic signaling, indicating potential long-term cardiac risk.

At 10 and 50 mGy, damage intensified, with pronounced vacuolization, atrophy, nuclear fragmentation, and increased interstitial inflammation, reflecting escalating metabolic disruption and apoptosis.

Cardiomyocytes are highly susceptible to oxidative stress due to limited antioxidant defenses and lipid-rich membranes prone to peroxidation (Rindler et al., 2016). Even low-dose radiation generates reactive oxygen species (ROS) that damage DNA, proteins, and membranes (Schieber & Chandel, 2014), leading to vacuolization, mitochondrial dysfunction, and homeostatic imbalance (Schraps et al., 2024).

Apoptotic features, including nuclear condensation and fragmentation, were evident across all doses. Previous studies report upregulation of apoptotic markers such as cleaved caspase-3 in response to low-dose radiation (Qiu et al., 2021). Sustained apoptosis may reduce cardiomyocyte populations and compromise myocardial integrity.

5. Conclusion

This study demonstrates that even low doses of gamma radiation have a dose-dependent impact on embryonic development, impairing fetal growth, upregulating apoptotic markers (Bax and p53), and disrupting tissue architecture. Histopathology revealed organ-specific damage, including alveo-bronchiolar collapse, vascular dilation in the lungs, myocardial necrosis, and hepatocellular degeneration. These results highlight the sensitivity of embryonic tissues to radiation during critical developmental periods.

The findings underscore the need for strict regulation of radiation exposure during pregnancy and support the relevance of the albino mouse model to human prenatal health. They have implications for imaging practices, radiation safety, and prenatal care guidelines.

Author contributions:

Hagar Rashad: Conceptualization, experimental design, data collection, gene expression analysis, manuscript drafting, and revision.

Mohammed Hussein Awwad: Methodology support, supervision, and critical manuscript review.

Gamal Ahmed: Radiation dosimetry and setup, technical support for irradiation procedures.

Amr M. Abdelhady: Histopathological examination and interpretation.

Mervat K. Iskandar: Project administration, ethical approvals, statistical analysis, and final manuscript editing.

Funding: This work received no external funding.

Acknowledgment: The authors express their sincere gratitude to the Zoology Department at Benha University and the Department of Radiology and Medical Imaging Technology at Misr University for Science and Technology for their valuable support. Special thanks are extended to the staff of the Theodor Bilharz Research Institute for their assistance with animal care and handling. The

authors also acknowledge the technical support provided during the gamma irradiation procedures and histopathological preparations.

Conflict of Interest: The author declares no conflict of interest.

References

Alwood, J. S., Tran, L. H., Schreurs, A. S., Shirazi-Fard, Y., Kumar, A., Hilton, D., Tahimic, C. G. T., & Globus, R. K. (2017). Dose- and ion-dependent effects in the oxidative stress response to space-like radiation exposure in the skeletal system. *International Journal of Molecular Sciences*, *18*(10), 2117. https://doi.org/10.3390/ijms18102117

Bouten, R. M., Young, E. F., Selwyn, R., Iacono, D., W. Bradley Rittase, & Day, R. M. (2021). Effects of radiation on endothelial barrier and vascular integrity. *Tissue Barriers in Disease, Injury and Regeneration*, *1*(1), 43–94. https://doi.org/10.1016/B978-0-12-818561-2.00007-2

Brent, R. L. (2015). Protection of the gametes embryo/fetus from prenatal radiation exposure. *Health Physics*, *108*(2), 242–274. https://doi.org/10.1097/HP.0000000000000235

Castaño, B. A., Schorer, S., Guo, Y., Calzetta, N. L., Gottifredi, V., Wiesmüller, L., & Biber, S. (2024). The levels of p53 govern the hierarchy of DNA damage tolerance pathway usage. *Nucleic Acids Research*, *52*(7), 3740–3760. https://doi.org/10.1093/nar/gkae061

Chang, D. S., Lasley, F. D., Das, I. J., Mendonca, M. S., & Dynlacht, J. R. (2021). Radiation Effects in the Embryo and Fetus. *Basic Radiotherapy Physics and Biology*, *1*(1), 349–353. https://doi.org/10.1007/978-3-030-61899-5_35

Dehghan, T., Mozdarani, H., Khoradmehr, A., & Kalantar, S. M. (2016). Effects of gamma radiation on fetal development in mice. *International Journal of Reproductive BioMedicine*, *14*(4), 247–254. https://doi.org/10.29252/ijrm.14.4.247

Elkady, A. A., & Ibrahim, I. M. (2016). Protective effects of erdosteine against nephrotoxicity caused by gamma radiation in male albino rats. *Human and Experimental Toxicology*, *35*(1), 21–28. https://doi.org/10.1177/0960327115574919

Fujimichi, Y., Sasaki, M., Yoshida, K., & Iwasaki, T. (2023). Effects of irradiation on cumulative mortality in mice: shifting toward a younger age of death. *Journal of Radiation Research*, *64*(2), 412–419. https://doi.org/10.1093/jrr/rrad006

Gomes, M., Matias, A., & Macedo, F. (2015). Risks to the fetus from diagnostic imaging during pregnancy: review and proposal of a clinical protocol. *Pediatric Radiology*, *45*(13), 1916–1929. https://doi.org/10.1007/s00247-015-3403-z

Hanania, A. N., Mainwaring, W., Ghebre, Y. T., Hanania, N. A., & Ludwig, M. (2019). Radiation-Induced Lung Injury: Assessment and Management. *Chest*, *156*(1), 150–162. https://doi.org/10.1016/j.chest.2019.03.033

Hurem, S., Martín, L. M., Brede, D. A., Skjerve, E., Nourizadeh-Lillabadi, R., Lind, O. C., Christensen, T., Berg, V., Teien, H. C., Salbu, B., Oughton, D. H., Aleström, P., & Lyche, J. L. (2017). Dose-dependent effects of gamma radiation on the early zebrafish development and gene expression. *PLoS ONE*, *12*(6), e0179259. https://doi.org/10.1371/journal.pone.0179259

Ingawale, D. K., Mandlik, S. K., & Naik, S. R. (2014). Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism(s): A critical discussion. *Environmental Toxicology and Pharmacology*, *37*(1), 118–133. https://doi.org/10.1016/j.etap.2013.08.015

Jalili, C., Salahshoor, M. R., Moradi, M. T., Ahookhash, M., Taghadosi, M., & Sohrabi, M. (2017). Expression changes of apoptotic genes in tissues from mice exposed to nicotine. *Asian Pacific Journal of Cancer Prevention*, *18*(1), 239–244. https://doi.org/10.22034/APJCP.2017.18.1.239

kareim, A., Shaalan, W., & Iskandar, M. (2022). Possible effects of escitalopram on Hao1, Rspo2 and Runx2 gene expression and bone formation in the foetuses of albino rat. *THE EGYPTIAN JOURNAL OF EXPERIMENTAL BIOLOGY (Zoology)*, *18*(1), 37. https://doi.org/10.5455/egysebz.20220214061458

Mainprize, J. G., Yaffe, M. J., Chawla, T., & Glanc, P. (2023). Effects of ionizing radiation exposure during pregnancy. *Abdominal Radiology*, *48*(5), 1564–1578. https://doi.org/10.1007/s00261-023-03861-w

Matsuya, Y., Tsutsumi, K., Sasaki, K., & Date, H. (2014). Evaluation of the cell survival curve under radiation exposure based on the kinetics of lesions in relation to dose-delivery time. *Journal of Radiation Research*, *56*(1), 90–99. https://doi.org/10.1093/jrr/rru090

Mattsson, S., Leide-Svegborn, S., & Andersson, M. (2021). X-Ray and molecular imaging during pregnancy and breastfeeding - When should we be worried? In *Radiation Protection Dosimetry* (Vol. 195, Issues 3–4, pp. 339–348). Oxford Academic. https://doi.org/10.1093/rpd/ncab041

Mukherjee, S., Dutta, A., & Chakraborty, A. (2022). The cross-talk between Bax, Bcl2, caspases, and DNA damage in bystander HepG2 cells is regulated by γ-radiation dose and time of conditioned media transfer. *Apoptosis*, *27*(3–4), 184–205. https://doi.org/10.1007/s10495-022-01713-4

Nakahira, R., Ayabe, Y., Braga-Tanaka, I., Tanaka, S., & Komura, J. I. (2021). Effects of continuous in utero low- And medium-dose-rate gamma-ray exposure on fetal germ cells. *Radiation Research*, *195*(3), 235–243. https://doi.org/10.1667/RADE-20-00093.1

Naoe, S., Fujimoto, Y., Murakami, K., Yukimine, R., Tanaka, A., Yamaoka, K., & Kataoka, T. (2023). Effects of low-dose/high-dose-rate X-irradiation on oxidative stress in organs following forced swim test and its combined effects on alcohol-induced liver damage in mice. *Journal of Radiation Research*, *64*(4), 635–643. https://doi.org/10.1093/jrr/rrad030

Okazaki, R. (2022). Role of p53 in Regulating Radiation Responses. *Life*, *12*(7), 1099. https://doi.org/10.3390/life12071099

Pathak, R., Kumar, V. P., Hauer-Jensen, M., & Ghosh, S. P. (2019). Enhanced survival in mice exposed to ionizing radiation by combination of gamma-tocotrienol and simvastatin. *Military Medicine*, *184*(1), 644–651. https://doi.org/10.1093/milmed/usy408

Pelcaru, C. F., Ene, M., Petrache, A. M., & Neguţ, D. C. (2021). Low doses of gamma irradiation stimulate synthesis of bioactive compounds with antioxidant activity in Fomes fomentarius living mycelium. *Applied Sciences (Switzerland)*, *11*(9), 4236. https://doi.org/10.3390/app11094236

Pflaum, J., Schlosser, S., & Müller, M. (2014). P53 family and cellular stress responses in cancer. *Frontiers in Oncology*, *4*(OCT), 285. https://doi.org/10.3389/fonc.2014.00285

Ping, Z., Peng, Y., Lang, H., Xinyong, C., Zhiyi, Z., Xiaocheng, W., Hong, Z., & Liang, S. (2020). Oxidative Stress in Radiation-Induced Cardiotoxicity. *Oxidative Medicine and Cellular Longevity*, *2020*(1), 3579143. https://doi.org/10.1155/2020/3579143

Qiu, Z., Wang, Y., Liu, W., Li, C., Zhao, R., Long, X., Rong, J., Deng, W., Shen, C., Yuan, J., Chen, W., & Shi, B. (2021). CircHIPK3 regulates the autophagy and apoptosis of hypoxia/reoxygenation-stimulated cardiomyocytes via the miR-20b-5p/ATG7 axis. *Cell Death Discovery*, *7*(1), 448. https://doi.org/10.1038/s41420-021-00448-6

Rindler, P. M., Cacciola, A., Kinter, M., & Szweda, L. I. (2016). Catalase-dependent H2O2 consumption by cardiac mitochondria and redox-mediated loss in insulin signaling. *American Journal of Physiology - Heart and Circulatory Physiology*, *311*(5), H1091. https://doi.org/10.1152/AJPHEART.00066.2016

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology*, *24*(10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

Schraps, N., Tirre, M., Pyschny, S., Reis, A., Schlierbach, H., Seidl, M., Kehl, H. G., Schänzer, A., Heger, J., Jux, C., & Drenckhahn, J. D. (2024). Cardiomyocyte maturation alters molecular stress response capacities and determines cell survival upon mitochondrial dysfunction. *Free Radical Biology and Medicine*, *213*(1), 248–265. https://doi.org/10.1016/j.freeradbiomed.2024.01.034

Sia, J., Szmyd, R., Hau, E., & Gee, H. E. (2020). Molecular Mechanisms of Radiation-Induced Cancer Cell Death: A Primer. *Frontiers in Cell and Developmental Biology*, *8*(1),

512111. https://doi.org/10.3389/fcell.2020.00041

Sisto, F., Miluzio, A., Leopardi, O., Mirra, M., Boelaert, J. R., & Taramelli, D. (2003). Differential cytokine pattern in the spleens and livers of BALB/c mice infected with Penicillium marneffei: Protective role of gamma interferon. *Infection and Immunity*, *71*(1), 465–473. https://doi.org/10.1128/IAI.71.1.465-473.2003

Suvarna, S. K., Layton, C., & Bancroft, J. D. (2018). Bancroft's Theory and Practice of Histological Techniques, Eighth Edition. *Bancroft's Theory and Practice of Histological Techniques, Eighth Edition*, *1*(1), 1–557. https://doi.org/10.1016/C2015-0-00143-5

Swain, N., Moharana, A. K., Jena, S. R., & Samanta, L. (2022). Impact of Oxidative Stress on Embryogenesis and Fetal Development. *Advances in Experimental Medicine and Biology*, *1391*(1), 221–241. https://doi.org/10.1007/978-3-031-12966-7_13

Thakur, S., Kumar, V., Das, R., Sharma, V., & Mehta, D. K. (2024). Biomarkers of Hepatic Toxicity: An Overview. *Current Therapeutic Research - Clinical and Experimental*, *100*, 100737. https://doi.org/10.1016/j.curtheres.2024.100737

Tharmalingam, S., Sreetharan, S., Kulesza, A. V., Boreham, D. R., & Tai, T. C. (2017). Low-Dose Ionizing Radiation Exposure, Oxidative Stress and Epigenetic Programing of Health and Disease. *Radiation Research*, *188*(4.2), 525–538. https://doi.org/10.1667/RR14587.1

Tohidi, F. Z., Toosi, M. H. B., Azimian, H., Khademi, S., Fardid, R., & Sarab, G. A. (2015). The gene expression level of p53 and p21 in mouse brain exposed to radiofrequency field. *International Journal of Radiation Research*, *13*(4), 337–343. https://doi.org/10.7508/ijrr.2015.04.007

Tokpinar, A., Nisari, M., Yilmaz, S., Yay, A., Yildiz, O. G., Balcioğlu, E., Alişan, P., Bilgici, P., Uçar, S., Ateş, Ş., & Bağci, K. (2024). The effect of ionizing radiation on the fetal bone development in pregnant rats: Role of melatonin. *Microscopy Research and Technique*, *87*(1), 95–104. https://doi.org/10.1002/jemt.24414

Toprani, S. M., Scheibler, C., Mordukhovich, I., McNeely, E., & Nagel, Z. D. (2024). Cosmic Ionizing Radiation: A DNA Damaging Agent That May Underly Excess Cancer in Flight Crews. *International Journal of Molecular Sciences*, *25*(14), 7670. https://doi.org/10.3390/ijms25147670

Yu, Z., Xu, C., Song, B., Zhang, S., Chen, C., Li, C., & Zhang, S. (2023). Tissue fibrosis induced by radiotherapy: current understanding of the molecular mechanisms, diagnosis and therapeutic advances. *Journal of Translational Medicine*, *21*(1), 1–35. https://doi.org/10.1186/s12967-023-04554-0