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γ- Radiation exposure Aggravated High-Fat Diet-induced hepatic injury in rats

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ABSTRACT

Ionizing radiation exposure, whether accidental, occupational, or therapeutic, induces direct and indirect alterations. So, this study was carried out to investigate the effect of γ-radiation (IR) exposure on hepatic injury induced by high-fat diet (HFD). Three groups of thirty female Wister rats were formed. Rats in Group I were fed ordinary chow. HFD was provided to rats in group II. In Group III, HFD-fed animals were subjected to a single dose of γ radiation IR (3.5 Gy). Blood and liver tissues were taken for analysis of biochemical parameters, gene expression, and protein levels, as well as lipid profile and endoplasmic reticulum (ER) stress. The lipid content in serum was dramatically increased after HFD feeding in HFD-fed and/or IR-exposed rats. Furthermore, when lipid buildup increased, ER stress was produced in the HFD group's liver via the IRE1 signaling pathway. Meanwhile, rats given HFD and exposed to IR suffered greater damage. Our findings showed that HFD-fed rats exposed to IR had higher disruptions in lipid metabolism and hepatic ER stress.

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological condition, which is defined as excessive fat accumulation and formation of lipid droplets in the cytoplasm of hepatocytes, accompanied by enlargement of liver and inflammation. It ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) and could eventually lead to cirrhosis and hepato-cellular carcinoma (Angulo 2002 and Rinella, 2015). Currently, it has become clear that NALFD is a multifactorial disease closely related to liver steatosis, insulin resistance, oxidative stress, inflammatory reaction, etc. Persistent endoplasmic reticulum (ER) stress and mitochondrial dysfunction participate in the regulation of the above physiological changes and both play an important role in the progression from NALFD to NASH (Malhi and Kaufman, 2011 and Li *et al.*, 2015).

Protein folding, lipid, and sterol synthesis, and intracellular Ca2+ storage is all mediated by the endoplasmic reticulum (ER), which is essential for the synthesis of membrane and secreted proteins. The accumulation of unfolded protein in the ER lumen, however, is caused by pathogenic events that disturb ER homeostasis and cause ER stress. Typically, the unfolded protein response (UPR) upregulates protein chaperones, inhibits protein translation, and removes unfolded proteins to help cells survive early stress (UPR). However, protracted ER stress can result in cell death and numerous disorders, including ischemia/reperfusion damage, heart disease, and diabetes. According to recent research, metabolic disorders including obesity and diabetes are associated with hepatic ER stress. In non-alcoholic fatty liver disease (NAFLD), ER stress helps to cause hepatic steatosis and insulin resistance (Jang *et al.*, 2016). Here, we evaluated how radiation influences liver function either alone or in combination with high fat diet (HFD)-induced liver steatosis.

Radiation effects on the liver may be influenced by lifestyle, particularly obesity, diet, and alcohol, each of which are also related to various liver diseases. For example, a survey of Japanese nuclear workers suggested relationships between radiation effects and alcohol drinking. Because the by many possible cases. For example, numerous specific environmental factors are encountered in space and extended stays in space are reportedly suggested to increase the risk of non-alcoholic fatty liver disease (NAFLD) in mouse livers. In space, living organisms influenced by environmental factors other than radiation may be exposed to cosmic radiation, including X-rays, gamma rays and particle radiation. Analyses of the mechanisms underlying NAFLD may offer a clue to understand radiation effects in the livers (Jia *et al.*, 2020). Steatosis is the most common phenomenon of lipid metabolism disorder in liver. Various predisposing factors for hepatic steatosis have been reported, such as nutritional imbalance, environmental stress, and physiological dysfunction (Nakajima *et al.*, 2018).

2. MATERIAL AND METHODS

2.1. Chemicals:

Reagents used in research were acquired from Sigma Chemical Company, St. Louis, MO, USA

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2.2. Experimental Animals:
Animals
Thirty female Wister rats (120±10 g) were obtained from The Nile Company. Rats were kept in animal housing fed on normal laboratory feed with free access to water. Rats were cared in agreement with the Clark *et al*. (1997) published Guidelines for the Care and Use of Laboratory animals, and recommendations of animal care committee of National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt.

2.3. Irradiation Process:
Whole-body γ-irradiation (IR) was done using Canadian γ cell-40 (137Cesium) at a dose rate of 0.67 Gy• min-1 for a total dose of 3.5 Gy at NCRRT, Cairo, Egypt, (Ramadan *et al.*, 2021).

2.4. Experimental design:
After a week of acclimation, some of the animals were fed conventional chow, while others were administered a high-fat diet (HFD) for 8 weeks. The HFD received from El-Nasr Co. (Cairo, Egypt) had 50% carbohydrates/starch, 27% fat, 10% protein, 10% sucrose, 1.5% fiber, and 1.5% vitamins (Moustafa et al., 2021). The animals were divided into three groups of ten per each:
Group 1 (control): Rats receiving standard chow.
Group 2 (HFD): Rats were fed with HFD.
Group 3 (HFD+ IR): HFD-fed rats exposed to single dose of γ-radiation IR (3.5 Gy).

2.5. Sampling:
Blood and tissue samples:
By retro-orbital bleeding into tubes, blood samples were obtained. In preparation for further analysis, serum samples were separated, split into aliquots, and kept at – 80 ° C. After cervical dislocation was used to sacrifice all of the rats, the liver was removed, cleaned with saline, dried on filter paper, weighed, and then kept at temperatures up to -80° C for further mRNA extraction.

2.6. Analysis:
2.6.1. Biochemical analysis:
Using an ELISA kit purchased from My Biosource Inc., San Diego, California, USA (Cat Nos. MBS2125216 and MBS263618), the levels of interleukin-1β (IL-1β) and inducible nitric oxide synthase (iNOS) were estimated in liver tissue. A commercial kit from Cairo, Egypt's Biodiagnostic Company was used to assess the serum triglycerides (TG) according to method of Stein (1987) and total cholesterol levels according to method of Young D.S. (2001). Aspartate transaminase (AST, EC 2.6.1.1) and alanine transaminase (ALT, EC 2.6.1.2) serum activities were determined according to the instructions of commercial kinetic assay kits acquired from Spectrum Diagnostic Company, Cairo, Egypt according to method of Young D.S. (1990).

2.6.2. Detection of gene expression by real-time quantitative polymerase chain reaction (PCR):
Isolation of RNA and Reverse Transcription: inositol-requiring enzyme-1 (IRE-1α), C/EBP-homologous protein (CHOP), X-box binding protein 1 (sXBP1) and glucose-regulated protein 78 (GRP78) mRNA expression levels were determined and investigated (see Table-1 for primer sequences used in these investigations). From 30 mg of liver tissues, total RNA was extracted using the TRIzol reagent (Life Technologies, USA) according to the manufacturer's instructions. To ensure the validity of the RNA, ethidium bromide staining, and agarose gel electrophoresis (1% each) were utilized. Reverse transcriptase (Invitrogen) was used according to the manufacturer's instructions to produce the first strand complementary DNA (cDNA) using 1 µg of total RNA as the template. RT-PCRs were carried out in a thermal cycler stage one plus using the Sequence Detection Program (PE Biosystems, CA) (Applied Biosystems, USA). 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer, and 2 µL of cDNA were all combined into a reaction mixture with a total volume of 25 µL. The PCR thermal cycling settings included the first step at 95 °C for 5 min, 40 cycles at 95 °C for 20 s, 60 °C for 30 s, and 72 °C for the 20s. A curve analysis was performed following the reaction. The results were standardized using the β-actin gene that was amplified in each round of PCR tests. Using the comparative Ct approach outlined by Livak and Schmittgen (Livak and Schmittgen, 2001) the relative expression of the target mRNA was assessed.

Table 1 Primer sequences used for RT-PCR.

Primer	Sequence
IRE1α	Forward:5´- TTGACTATGCAGCCTCACTTC -´3 Reverse: 5´- AGTTACCACCAGTCCATCGC -´3
XBP1	Forward:5´- TGAAGCTTTGCGTAGTCTGGAGCTATGG -´3 Reverse: 5´- TGCTCGAGATTGGATCATTCCTTAGACA -´3
GRP78	Forward: 5´- AACCCAGATGAGGCTGTAGCATA -´3 Reverse:5´ - CACAGTGTTCCTCGGAATCAGTT -´3
B-actin	Forward: 5´- AAGTCCCTCACCCTCCCAAAAG -´3 Reverse:5´ - AAGCAATGCTGTCACCTTCCC -´3

2.7. Statistical analysis:
The statistical software SPSS (Statistical Program for Social Science) version 20.0 was used to do statistical analysis on the data and perform tests of significance. A one-way ANOVA test was used, followed by a post hoc test for multiple comparisons. All data are reported as a mean of 8 values, and P< 0.05 is used to determine if the SE and difference between means are significant.

3. RESULTS

Effects of IR exposure on metabolic parameters
At the end of the experiment, serum TC, TG, AST, and ALT levels were measured (Table 2). HFD rats had considerably greater TC, triglyceride (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels compared to normal control rats. However, more disturbances were observed in the activities of liver enzymes and lipid indices levels (TG and total cholesterol) after exposure to γ-radiation (IR) in respect to the HFD group.

Table 2 Effects of IR exposure on metabolic parameters (ALT, AST, TC and TG) in serum of rats' groups.

Groups	Control	HFD	HFD+ IR
ALT U/ml	9.2±0.35 [bc]	18.4±0.29 [ac]	27.2±0.58 [ab]
AST U/ml	7.0± 0.12 [bc]	17.0± 0.17 [ac]	26.0±0.23[ab]
TC (mg/ dl)	64.0±1.73 [bc]	79.0±2.30 [ac]	95.0±2.89 [ab]
TG (mg/ dl)	75.0± 1.15 [bc]	98.0± 2.89 [ac]	125.0±3.46 [ab]

Data were expressed as Mean ± S.E. [a] $P < 0.05$ versus the control group, [b] $P < 0.05$ versus the HFD group, [C] $P < 0.05$ versus the HFD+IR group.

Effects of IR exposure on hepatic expression of inflammatory markers

To determine the effect of IR exposure on the inflammatory status, the hepatic levels of IL-1β and iNOS were determined. It was found that the levels of IL-1β and iNOS significantly increased in the HFD and HFD+IR groups compared to normal control groups. Whereas the increase was significantly higher in the HFD+IR group compared to HFD group (Figure 1).

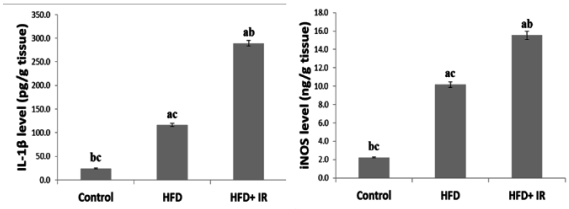

Figure 1 Hepatic levels of inflammatory markers (A) IL-1β and (B) iNOS in all groups. Data are expressed as mean ± SE. $^{a}P < 0.05$ versus control group, $^{b}P < 0.05$ versus HFD group, $^{c}P < 0.05$ versus HFD+IR group.

Effects of IR exposure on hepatic expression of Endoplasmic Reticulum (ER) Stress markers

To determine the status of endoplasmic reticulum, stress the gene expression of GRP78, IRE1α and XBP1, was estimated in the livers of all groups of rats. It was found that the HFD and HFD+IR group's expression of IRE1α and XBP1, was significantly upregulated, and this upregulation was linked to a decrease in the level of GRP78 gene transcript compared to normal control group. However, more alteration was observed in HFD+IR group compared to the HFD group (Figure 2).

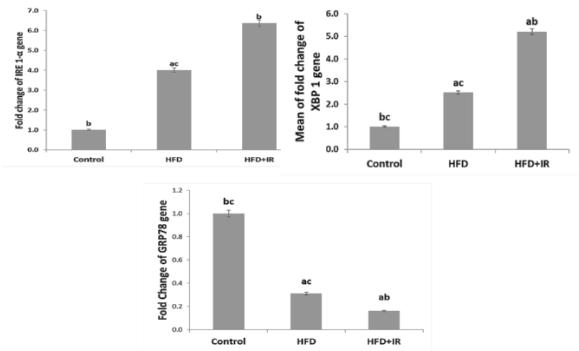

Figure 2 Hepatic ER stress pathway. XBP-1, IRE1α, GRP78 gene expression in all groups. Data were expressed as Mean ± S.E. $^{a}P < 0.05$ versus the control group, $^{b}P < 0.05$ versus the HFD group, $^{C}P < 0.05$ versus the HFD+βSito group.

4. DISCUSSION

Liver is the central organ that regulates the uptake, synthesis, secretion, catabolism, and storage of lipids. The dysregulation of free fatty acids (FAs) and TG is a major reason of steatosis (Bass and Merriman, 2005). This phenomenon was observed in the present study, where HFD feeding induced lipid deposits and steatosis in liver (Bradbury, 2006).

Strategies have been implemented to reduce the risk of liver toxicity due to radiation, opening new prospects to help patients who otherwise have limited options for liver-directed therapies. In the current study, HFD induced inflammation in the liver of rats; however, whole body gamma irradiation of rats with 3.5 Gy provokes more inflammation. This was confirmed by increasing levels of liver enzymes (ALT and AST), lipid indices (TG and TC) and inflammatory markers (IL-1β, and iNOS) when compared with their corresponding values in the control group of rats. In the same line, Al-Khattab *et al.,* (2017) indicated that the stimulation of liver enzymes by gamma irradiation, which is responsible for the biosynthesis of fatty acids and mobilization of fat from adipose tissues to the blood stream, leads to hyperlipidemic state.

The fine structure of hepatic tissue of γ-irradiated rats in this study realized conspicuous hazardous alterations, including degeneration in cytoplasm that is occupied by deteriorated These findings agree with those obtained by (Samy *et al.,* 2016). Kim *et al*., (2019) showed that the main risk of the radiation exposure of cells arises from the formation of (ROS) and the damage of cellular components such as DNA, cell membranes or cell organelles leading to loss function or even cell death. Some cytoplasmic degeneration, as well fragmentation of the rough endoplasmic reticulum after the exposure of accumulated dose of γ-irradiation was also detected. Miller and Zachary, 2017 described that cytoplasmic degeneration as a result of irradiation is due to progressive ischemia or hypoxia. Also, they attributed the fragmentation of endoplasmic reticulum to the ingression of water and solutes into the cell which was referred to dysfunction of the cell membrane permeability. ER, responsible for the protein synthesis, is extremely sensitive to different kinds of endogenous and exogenous harmful stimuli, which can further result in the disturbance of ER lumen called ER stress (Moustafa *et al.,* 2020).

The ER is pivotal organelle with major function in hepatic lipid metabolism including lipid synthesis, storage, and export. Unfolded protein response (UPR), a highly conserved pathway in ER, monitors the status of ER protein assembly and lipid metabolism, and serves to restore ER homeostasis (Gentile et al., 2011). It starts with the activation of three ER-localized proteins (IRE1, ATF6 and PERK), following the release of the chaperone GRP78 (Cnop et al., 2012). IRE1 activation promotes the XBP1s mRNA and subsequent transcription of molecular chaperones. Activation of PERK causes phosphorylation of EIF2a and upregulates ATF4 and molecular chaperones. Activation of ATF6 leads to its release from ER membrane and enters nuclear to target molecular chaperones (Jia *et al*., 2020).

ER stress and their sensors are tightly related to liver metabolism because ER stress has been enhanced in the liver of obese animals and humans (Ozcan *et al.,* 2004, Puri et al., 2008 and Cnop *et al.,* 2012). Also, chronic hepatic ER stress was observed in the steatosis of ob/ob mice as well as mic with genetically modified PERK/eIF2α, IRE1/ XBP1 and ATF6 signaling pathway (Oyadomari *et al.,* 2008 – Kammoun *et al*., 2009). Based on the above associated studies, the present studies especially focused on the applicability of this target in hepatic steatosis.

Notably, in mice and humans, the lipid overload induced ER stress in liver, which led to chronic UPR, and then resulted in oxidative stress, apoptosis, and inflammation (Urra *et al*., 2013). ER stress also promoted lipid accumulation and steatosis via inhibiting FAs β-oxidation in liver (Lebeaupin *et al*., 2018). In HFD-fed rat liver, IRE1-XBP1 pathway-mediated ER stress and ATF6 regulated ER dysfunction were observed, which brought about abnormal lipid metabolism (Dai *et al*., 2015 and Cao *et a*l., 2019). In this study, the upregulated mRNA levels of

IRE1, GRP78 and XBP1s indicated that ER stress was a consequence of lipotoxicity induced by HFD. As such, we surmised that ER stress participated in lipid accumulation and further fatty liver injury *via* activating IRE1 pathway.
Inflammation can result from ER homeostasis disruption (Schroder, 2008). The results of the current study showed that feeding of HFD enhanced the activation of the CHOP, IRE1α, and XBP-1 pathways and triggered the hepatic ER stress pathway. While the exposure to gamma radiation enhances more aggravation in the status of ER stress as shown in the over expression of IRE1α and XBP1 was significantly upregulated, and this upregulated was linked to a decrease in the level of GRP78 gene.

5. CONCLUSION

In conclusion, gamma irradiation worsens the HFD-induced hepatic steatosis through the induction of pro-inflammatory markers and the ER stress pathway. The study suggests that the radioprotector is required for individuals fed on HFD and exposed to gamma radiation.

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