


# Effect of Low-Dose Gamma Radiation and Lipoic Acid on High- Radiation-Dose Induced Rat Brain Injuries

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## Abstract

**Aim:** This work aims to investigate the possible radio-adaptive mechanisms induced by low-dose (LD) whole-body  $\gamma$ -irradiation alone or combined with alpha-lipoic acid (ALA) administration in modulating high-dose (HD) head irradiation-induced brain injury in rats.

**Materials and Methods:** Rats were irradiated with LD (.25 Gy) 24 hours prior HD (20 Gy), and subjected to ALA (100 mg/kg/day) 5 minutes after HD and continued for 10 days. At the end of the experiment, animals were sacrificed and brain samples were dissected for biochemical and histopathological examinations.

**Results:** HD irradiation-induced brain injury as manifested by elevation of oxidative stress, DNA damage, apoptotic, and inflammatory markers in brain tissue. Histological examination of brain sections showed marked alterations. However, LD alone or combined with ALA ameliorated the changes induced by HD.

**Conclusion:** Under the present experimental conditions, LD whole-body irradiation exhibited neuroprotective activity against detrimental effects of a subsequent HD head irradiation. This effect might be due to the adaptive response induced by LD that activated the anti-oxidative, anti-apoptotic, and anti-inflammatory mechanisms in the affected animals making them able to cope with the subsequent high-dose exposure. However, the combined LD exposure and ALA supplementation produced a further modulating effect in the HD-irradiated rats.

## Keywords

$\gamma$ -rays, alpha-lipoic acid, brain, rats

1. High-dose head irradiation induces detrimental effects in brain tissue.
2. Lipoic acid alleviates brain injury.
3. Low-dose whole-body irradiation exhibits neuro-protective activity.
4. Low-dose whole-body irradiation induces radio-adaptive response.

## Introduction

Although radiation is considered a valuable diagnostic and therapeutic tool widely used in medicine, yet if used at a high dose (HD), it is usually harmful to different body organs,

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especially the brain. This harmful effect results from the direct ionization of cellular structures, especially DNA, or from the indirect effect through free radicals produced by radiolysis of water. It was hypothesized that HD radiation affects several targets within the brain including the vasculature and both the inflammatory and stromal cells.<sup>1,2</sup> Rodent studies revealed that whole-body irradiation (7 Gy) induced brain injury through the impairment of brain mitochondrial function<sup>3</sup> and cranial irradiation with 20 Gy (single dose) induced oxidative stress in both brain tissue and blood.<sup>4</sup> Recently, it was observed that head irradiation at a dose level of 10 Gy induced DNA damage associated with a significant increase of the apoptotic and inflammatory markers (Caspase-3, TNF- $\alpha$ , IL-1 $\beta$ , and NF- $\kappa$ B) and a significant decrease in neurotransmitter concentrations of brain tissue.<sup>5</sup> The latter demonstrated that the response of the brain to ionizing radiation is characterized by activation of the NF $\kappa$ B pathway and elevation of TNF- $\alpha$ . The elevated TNF- $\alpha$  contributes to the increase of Caspase-3.

Although there is no doubt about the harmful effects of HDs of ionizing radiation, the biological effects of low doses (LDs) of radiation are often controversial. It was assumed that the response to LDs of ionizing radiation may be loci-specific and have both beneficial and detrimental consequences.<sup>6,7</sup> Indeed, from multiple previous studies, it was suggested that LD irradiation rendered protection against oxidative stress in cells and tissues through different pathways.<sup>8</sup> This effect has been established in both in vitro studies and different animal models. Low doses of radiation (.5 or .7 Gy) have been demonstrated to exert anti-inflammatory effects by suppressing the release of pro-inflammatory cytokines by activated macrophages.<sup>9</sup> Also, it has been shown that .2 Gy enhanced mitochondrial activity to ensure the survival of hippocampal neurons in response to LD radiation.<sup>10</sup> The finding of Yoshimoto et al. (2012) suggested that .5 Gy X-irradiation activated antioxidant function and inhibited cold-induced brain injury.<sup>11</sup> Also, El-Ghazaly et al. (2015) demonstrated that a LD of whole-body irradiation induced neuroprotective effect in a rat model of Parkinson's disease.<sup>12</sup> Recently, Calabrese et al. opined that LD radiation should be regarded as safe and effective for use at almost any stage of COVID-19 infection.<sup>13</sup> In the same context, the phenomena of radio-adaptive response are described as the ability of a biological system exposed to a small dose of ionizing radiation—the preconditioning dose—to reduce the detrimental effects of a subsequent higher dose, the challenge dose.<sup>8</sup> The preconditioning refers to exposing the biological system to a small non-toxic inducing dose of a stressor followed by a larger toxic dose of the same stressor. On the contrary, Abdelrazzak et al. (2019) demonstrated that there is no evidence that low-dose radiation (.1 Gy) protected the liver cells against a subsequent higher dose.<sup>14</sup>

On the other hand, it is well known that low molecular weight antioxidants including alpha-lipoic acid (ALA), glutathione, coenzyme-Q, and vitamins C and E are an important part of the anti-oxidative defense mechanisms of cells and

organisms.<sup>15</sup> Lipoic acid exists in the body in two forms: oxidized and reduced; they both have powerful antioxidant properties. In addition, Molz and Schröder (2017) and Xiao et al. (2018) reported that ALA is a powerful antioxidant that can recycle other endogenous antioxidants such as glutathione and vitamins C and E. Since the level of ALA in the body is low, it is considered as an important candidate for supplementation strategy if the body is subject to oxidative stress.<sup>16,17</sup> It induced a hepatorenal-protective effect against insecticide toxicity in rats.<sup>18,19</sup> Moreover, it was demonstrated that ALA administration decreased the markers of inflammation among patients with metabolic syndrome and related disorders.<sup>20</sup>

Hence, in the present work, it has been emphasized to test whether pre-exposure to whole-body LD  $\gamma$ -radiation (.25 Gy) protects the brain from the damaging effect induced by a subsequent higher dose (20 Gy) head irradiation. Moreover, it has been intended to investigate the possible radio-adaptive mechanisms through which the LD induces its effect and to test whether this LD intensifies the effectiveness of ALA against the brain injury induced by HD head irradiation.

## Materials and Methods

### Experimental Animals

Thirty-five adult male Wistar rats were used (weighing 220–250 g). Animals were obtained from the animal house that belongs to the National Centre for Radiation Research and Technology, Cairo, Egypt. Animals were kept under good ventilation condition, had free access to water and standard pellet concentrated diet, were adapted in specially designed cage with 5 rats per cage and a 12-12 light–dark cycle, and were under normal pressure and temperature conditions. This experiment was carried out according to the international guidelines of animal handling and care (NIH no. 85:23, 1996) and approved by the Research Ethics Committee for animal experimental studies at the NCRRT, EAEA, Cairo (The approval No; 9A/20).

### Radiation Facility

Head irradiation was performed in a ventilated Indian 60Co Cell at the NCRRT, Cairo, Egypt, at a dose rate of 1.132 kGy/h. The rats were anesthetized with an intra-peritoneal injection of pentobarbital 60 mg/kg according to Shekarforoush et al.<sup>21</sup> and then the head of the rat was exposed to a single dose of gamma rays, 20 Gy.<sup>22</sup> The other parts of the body were protected by a lead shield. The lead block is a cylinder with a circular hole in which the rat is placed, except the head that has been exposed to radiation. However, whole-body  $\gamma$ -irradiation was performed at the NCRRT, Cairo, Egypt, using a ventilated Canadian 137Cs Gamma Cell-40 at a dose rate of .387 Gy/min. The rats were exposed to a single dose, .25 Gy, according to Abdel-Rafei et al.<sup>23</sup> and El-Ghazaly et al.<sup>24</sup> The

dose rate of radiation was calculated according to the Radiation Protection and Dosimetry Department in the NCRRT, Cairo, Egypt.

### Drugs and Chemicals

ALA was purchased from the MP Biomedicals, LLC, France (CAT No.: 101138, LOT NO: R 20503). It was suspended in water and each rat was orally administered 100 mg/kg/day for 10 days based on Abdou and Abdel-Daim (2014); Abdel-Fattah et al. (2013); and Andreeva-Gateva et al.<sup>18,25,26</sup> All other chemicals were of analytical grade.

### Experimental Design

Animals were classified to 7 groups: (1) untreated normal control group, (2) animals received via gavages ALA (100 mg/kg/day) for 10 days, (3) animals exposed to single-dose whole-body gamma radiation (.25 Gy), (4) animals exposed to head irradiation at a single dose level of 20 Gy, (5) animals exposed to head irradiation (20 Gy) and received via gavages ALA 5 minutes after irradiation and continued for 10 days, (6) animals exposed to whole-body gamma radiation (.25 Gy) and after 24 hours exposed to head irradiation (20 Gy), and (7) animals exposed to whole-body gamma radiation (.25 Gy) and after 24 hours exposed to head irradiation (20 Gy) and received via gavages ALA as group 4.

### Tissue Sampling

Five animals of each group were sacrificed 10 days' post-irradiation or treatment. Brain samples were immediately excised and divided into 2 hemispheres. One hemisphere was fixed in 10% formalin for histopathological examination. Part of the brain was preserved frozen at  $-80^{\circ}\text{C}$  until used for real-time PCR analysis and DNA damage assay. Another part was homogenized in phosphate-buffered saline (1 g tissue: 10 mL PBS), centrifuged at 3000 rpm for 15 minutes at  $4^{\circ}\text{C}$ , and then the supernatant was collected and preserved frozen at  $-20^{\circ}\text{C}$  until used for biochemical analysis.

### Extraction of RNA and Quantitative RT-PCR Analysis

Brain tissue was homogenized in lysis buffer to assess gene expression of apoptotic (Caspase-3 and Bax) and anti-apoptotic (Bcl-2) markers. The total RNA was isolated from brain tissues' homogenate using Qiagen tissue extraction kit (Qiagen, USA) according to the instructions of the kit. The total RNA was reverse transcribed into cDNA using the high capacity cDNA reverse transcription kit (Fermentas, USA). Quantitative real-time polymerase chain reaction amplification and analysis were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). The reaction contained SYBR Green Master Mix (Applied Biosystems); gene-specific forward and reverse primers were as follows:

Caspase-3 F: 5'-GTGGAAGTACTGACGATGATATGGC-3'  
R: 5'-CGCAAAGTGACTGGATGAACC-3'  
Bax F: 5'-ATGGAGCTGCAGAGGATGA -3'  
R: 5'-CCAGTTTGCTAGCAAAGTAG-3'  
Bcl-2 F: 5'-GAGGATTGTGGCCTTCTTTG-3'  
R: 5'-AGGTACTCAGTCATCCACA-3'  
 $\beta$ -actin F: 5'-TTGTCCCTGTATGCCTCT-3'  
R: 5'-TAATGTCACGCACGATTTC -3'

The relative expression of the studied genes was calculated according to Applied Biosystem software. All values were normalized to the expression of  $\beta$ -actin as an endogenous control (reference gene).

### Biochemical Analysis

In the brain homogenate, malondialdehyde level (MDA) was estimated using Rat Malondialdehyde Quantikine Enzyme-Linked Immunosorbent Assay (ELISA kit, Cat. No. LS-F28018) from LifeSpan BioSciences, Inc. USA, following the manufacturer's guideline. Total nitrate/nitrite (NO(x)) in brain tissue was measured as a stable end product, nitrite, according to the method of Miranda et al.<sup>27</sup> Total antioxidant capacity (TAC) was measured by TAC kit (Cat. No. MBS733414\_48T) from MyBioSource, Inc. USA. Glutathione (GSH) content in brain tissue was determined using Rat Reduced Glutathione ELISA Kit (Cat. No. E02G0367) from Shanghai BlueGene Biotech CO, Ltd, China. Nuclear factor kappa B (NF- $\kappa$ B), tumor necrosis factor-alpha (TNF- $\alpha$ ), and interleukin-1beta (IL-1 $\beta$ ) levels were measured using rat NF- $\kappa$ B, TNF- $\alpha$ , and IL-1 $\beta$  ELISA kits (Cat. No. MBS722386, MBS355371 and MBS825017, respectively), according to the manufacturer's directions. Also, vascular endothelial growth factor (VEGF) and amyloid  $\beta$  protein (A  $\beta$ ) levels in brain tissue were measured using Rat VEGF ELISA Kit (Cat. No. MBS2514825) and Rat A $\beta$ 1-42 ELISA kit (Cat. No. MBS702915), according to manufacturer's recommendations. Oxidative DNA damage was evaluated by measurement of 8-OHdG level in brain tissue using DNA Damage (8-OHdG) ELISA Kit (Cat. No # SKT-120-96S), StressMarq Biosciences Inc, following the manufacturer's instructions.

### Histopathological Study

The brain hemispheres were fixed in formalin solution (10%) for 24 hours followed by dehydration in ascending series of ethyl alcohol, clearing in xylene, and embedding in paraffin wax and then sectioned at 4 microns thickness by sledge microtome and stained routinely with hematoxylin and eosin (H&E) reagent and examined by light microscope.<sup>28</sup>

### Statistical Analysis

The SPSS computer program (version 20) was used in data analysis. Statistical comparison between groups was done by

using one-way analysis of variance (ANOVA) followed by a post hoc test (LSD). The data were presented as mean  $\pm$  standard error (SE).  $P$ -value  $< .05$  was considered to be statistically significant.

## Results

### Oxidative Stress and Inflammatory Markers

The results presented in Figures 1 and 2 and Tables 1 and 2 showed non-significant changes in all studied parameters upon supplementation of ALA to normal rats or exposure to LD as compared to their control counterparts.

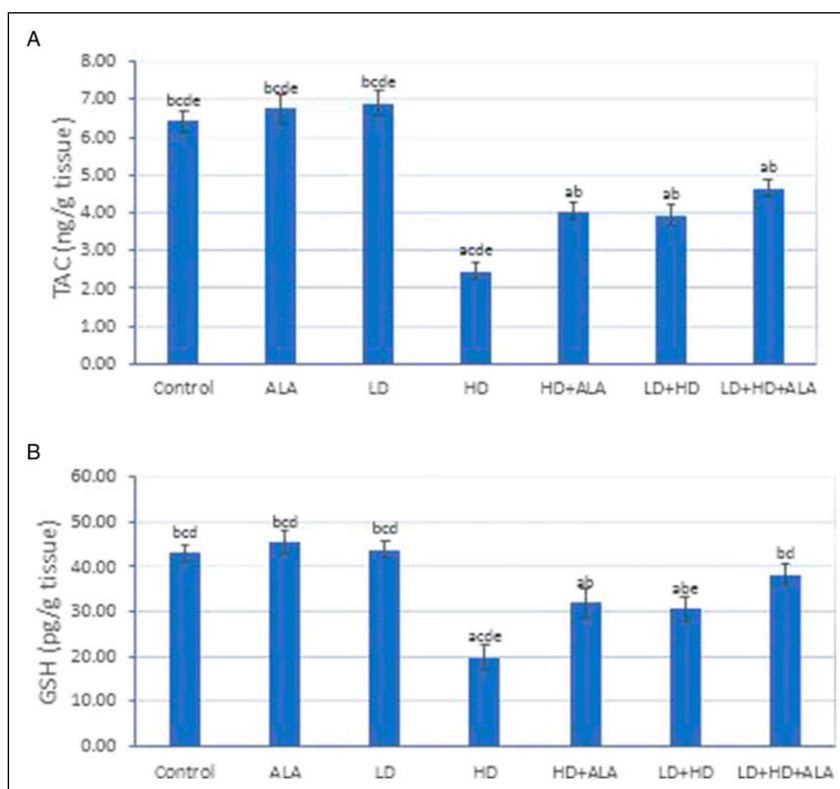
Head irradiation (20 Gy) of rats has provoked the oxidative stress in brain tissue that has been demonstrated by a significant ( $P < .05$ ) elevation in MDA and NO levels together with a significant decrease in GSH level and TAC as compared to their values in the control group. However, ALA supplementation for 10 days post-irradiation or whole-body exposure to LD (.25 Gy) 24 hours prior HD head irradiation or both treatments induced a significant ( $P < .05$ ) decrease in MDA and NO levels and significant elevation in GSH content and TAC as compared with the corresponding values of the irradiated group. Also, the results revealed that the combined

pretreatment of LD whole-body gamma irradiation and ALA supplementation post-HD head irradiation produced a better effect in reducing oxidative stress (Figures 1 and 2).

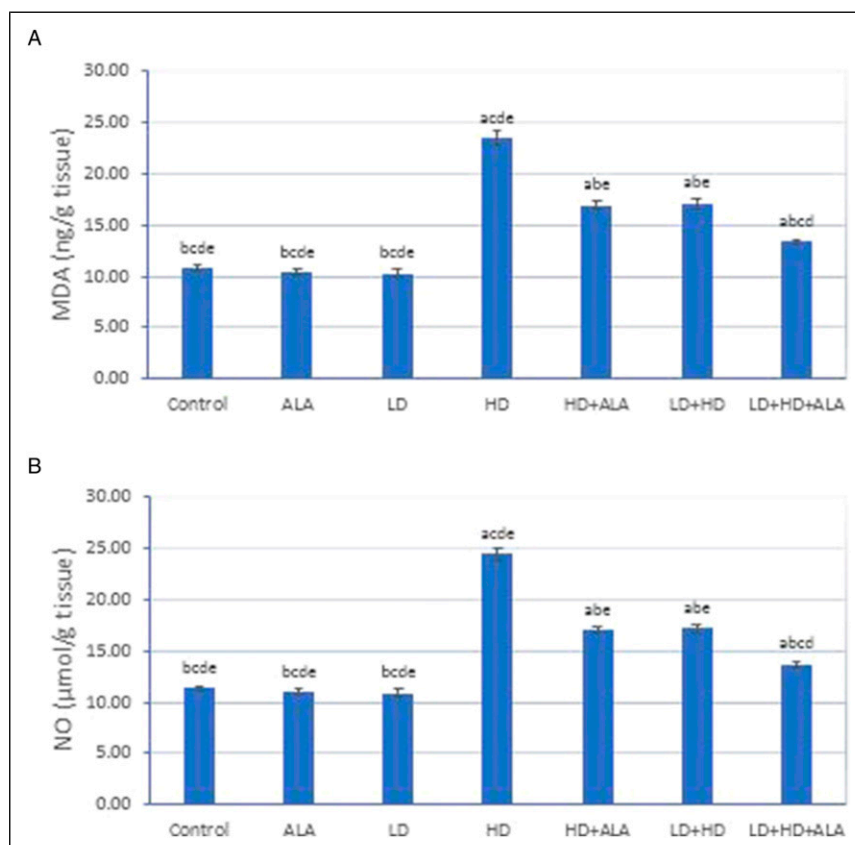
The results in Tables 1 and 2 revealed that exposure to HD ionizing radiation resulted in a significant ( $P < .05$ ) elevation in the levels of brain NF- $\kappa$ B and pro-inflammatory cytokines, TNF- $\alpha$  and IL-1 $\beta$ . As well as a significant ( $P < .05$ ) increase in VEGF and A $\beta$  were observed 10 days after head irradiation as compared to their values in the control group. Oral supplementation with ALA or exposure to LD induced amelioration of the changes induced by exposure to HD radiation. Moreover, the combined treatment of LD and ALA showed a better effect in reducing brain inflammatory markers.

### DNA Damage and mRNA Expression of Apoptotic-Related Markers

The results of the current study (Table 3) showed that the level of 8-OHdG and the expression level of pro-apoptotic molecules (Caspase-3 and Bax) and anti-apoptotic molecule (Bcl-2) were non-significantly changed in the group exposed to LD radiation or supplemented with ALA as compared to the control group. In the head-irradiated group, it was found that



**Figure 1.** Changes in brain total antioxidant capacity (TAC) and glutathione (GSH) levels of adults male rats in different groups. Data are represented as mean  $\pm$  SE. (a): Significantly different from the control group, (b): Significantly different from the HD group, (c): Significantly different from HD + ALA group, (d): Significantly different from LD+HD group, (e): Significantly different from LD + HD + ALA group. the mean different is significant at the .05 level. HD: high dose; LD: low-dose; ALA: alpha-lipoic acid.



**Figure 2.** Changes in malondialdehyde (MDA) and nitric oxide (NO) levels of adults male rats in different groups. Data are represented as mean  $\pm$  SE. a: Significantly different from the control group, b: Significantly different from the HD group, c: Significantly different from HD + ALA group, d: Significantly different from LD+HD group, e: Significantly different from LD + HD + ALA group. the mean different is significant at the .05 level. HD: high dose; LD: low-dose; ALA: alpha-lipoic acid.

**Table 1.** Changes in Brain Nuclear Factor Kappa (NF-Kb), Tumor Necrosis Factor-A (Tnf $\alpha$ ) and Interleukin I Beta (IL-1 $\beta$ ) Levels of Adult Male Albino Rats in Different Groups.

	NF-Kb (pg/mg Tissue)	Tnf $\alpha$ (pg/mg Tissue)	IL-1 $\beta$ (pg/mg Tissue)
Control	16.26 $\pm$ 0.28 <sup>a,b,c,d</sup>	12.56 $\pm$ 0.30 <sup>e</sup>	14.84 $\pm$ 0.31 <sup>a,b,c,d</sup>
ALA	16.20 $\pm$ 0.32 <sup>a,b,c,d</sup>	12.56 $\pm$ .35 <sup>a,b,c,d</sup>	14.44 $\pm$ .42 <sup>a,b,c,d,e</sup>
LD	16.32 $\pm$ 0.28 <sup>a,b,c,d</sup>	12.40 $\pm$ 0.36 <sup>a,b,c,d</sup>	14.14 $\pm$ 0.46 <sup>a,b,c,d,e</sup>
HD	36.52 $\pm$ 1.85 <sup>b,c,d,e</sup>	26.58 $\pm$ 0.57 <sup>b,c,d,e</sup>	25.94 $\pm$ 0.69 <sup>b,c,d,e</sup>
HD+ALA	24.82 $\pm$ 0.55 <sup>a,d,e</sup>	21.62 $\pm$ 0.46 <sup>a,d,e</sup>	19.56 $\pm$ 0.31 <sup>a,d,e</sup>
LD+HD	25.30 $\pm$ 0.55 <sup>a,d,e</sup>	21.02 $\pm$ 0.44 <sup>a,d,e</sup>	19.64 $\pm$ 0.34 <sup>a,d,e</sup>
LD+HD+ALA	20.88 $\pm$ 0.52 <sup>a,b,c,e</sup>	18.32 $\pm$ 0.30 <sup>a,b,c,e</sup>	15.84 $\pm$ 0.34 <sup>a,b,c</sup>

Data are represented as means  $\pm$  SE.

<sup>a</sup>Significantly different from the control group.

<sup>b</sup>Significantly different from the HD group.

<sup>c</sup>Significantly different from HD + ALA group.

<sup>d</sup>Significantly different from LD + HD group.

<sup>e</sup>Significantly different from LD + HD + ALA group. The mean difference is significant at the .05 level.

Abbreviation: NF-Kb, Nuclear Factor Kappa, Tnf $\alpha$ : Tumor Necrosis Factor-A, IL-1 $\beta$ : Interleukin I Beta.

the level of 8-OHdG and the expression levels of Caspase-3 and Bax mRNA were increased significantly while the expression level of Bcl-2 mRNA was decreased significantly in

brain tissue as compared to the control group. However, the results revealed that whole-body exposure to LD (.25 Gy) 24 hours prior HD head irradiation or ALA supplementation

**Table 2.** Changes in Brain Vascular Endothelial Growth Factor (VEGF) and Amyloid Beta-Peptides (A $\beta$ ) Levels of Adult Male Albino Rats in Different Groups.

	A $\beta$ (pg/mg Tissue)	VEGF (pg/mg Tissue)
Control	13.88 $\pm$ 0.38 <sup>a,b,c,d</sup>	12.34 $\pm$ .26 <sup>a,b,c,d</sup>
ALA	13.50 $\pm$ .50 <sup>a,b,c,d</sup>	12.34 $\pm$ 0.32 <sup>a,b,c,d</sup>
LD	13.50 $\pm$ 0.37 <sup>a,b,c,d</sup>	12.12 $\pm$ 0.29 <sup>a,b,c,d</sup>
HD	30.10 $\pm$ 0.82 <sup>b,c,d,e</sup>	24.10 $\pm$ 0.68 <sup>b,c,d,e</sup>
HD+ALA	20.94 $\pm$ 0.35 <sup>a,c,d,e</sup>	17.34 $\pm$ 0.21 <sup>a,d,e</sup>
LD+HD	22.50 $\pm$ 0.74 <sup>a,b,d,e</sup>	18.30 $\pm$ .54 <sup>a,d,e</sup>
LD+HD+ALA	18.96 $\pm$ 0.35 <sup>a,b,c,e</sup>	16.02 $\pm$ 0.38 <sup>a,b,c,e</sup>

Data are represented as means  $\pm$  SE.

<sup>a</sup>Significantly different from the control group.

<sup>b</sup>Significantly different from the HD group.

<sup>c</sup>Significantly different from HD+ALA group.

<sup>d</sup>Significantly different from LD+HD group.

<sup>e</sup>Significantly different from LD+HD+ALA group. The mean difference is significant at the .05 level.

Abbreviations: VEGF: Vascular Endothelial Growth Factor, A $\beta$ : Amyloid Beta-Peptides.

post-irradiation, or both treatments down-regulated 8-OHdG level and mRNA expression of Caspase-3 and Bax and up-regulated mRNA expression of Bcl-2 as compared with the corresponding values of the irradiated group.

### Histopathological Results

The cerebral cortex of control rats is showing normal thick darkly stained gray matter and thin paler white matter (Figures 3A and 4A). In LD irradiated group and ALA group, brain tissues showed normal structure (Figures 3B and 4B). However, in the HD-irradiated rats, the neurophagia and degenerative changes of brain tissue are present in different parts of the brain (e.g., cerebrum, brainstem, and cerebellum), with variable severity in all investigated rats of this group which manifested in gray matters by microglial clustering, pyknotic neurons and satellitosis around neurons (Figure 3C), neuronophagia, severe congestion, perivascular edema, numerous apoptotic neurons, and spongiform degeneration (Figures 3D and 3E). Moreover, white matters in the HD-irradiated group showed hemorrhage with a proliferation of glia cells (Figure 4C) and leukocytic aggregation mainly lymphocytes (Figure 4D). In the LD + HD rat group, the brain lesions are attenuated and characterized by little degenerated pyramidal neurons (Figure 3F). Also, in the case of the HD + ALA rat group, the brain lesions are mild which is demonstrated by focal gliosis around nerve axons in some cases (Figure 4E). Further, the brain lesions in the LD + ALA + HD group are less severe and epitomized by perivascular edema and mild proliferated glia cells with spongiform degeneration (Figures 3G and 4F). Brain lesions in the LD + ALA+ HD group are more improved than in LD + HD-irradiated group or HD-irradiated + ALA group.

### Discussion

Head irradiation is a widely used method in the treatment or management of various types of tumors, but it is associated with negative side effects on brain tissues. Despite the protective measures that are used, these side effects cannot be prevented completely. Previous experimental studies suggested that radiation-induced brain damage can be attenuated by the use of agents that inhibit the action of post-irradiated free radicals and could be effective through the blood-brain barrier.<sup>3,29,30</sup> Another mechanism that can reduce the side effects of HD irradiation is the radio adaptive response to LDs of ionizing radiation. It was reported that LDs of ionizing radiation might induce biological mechanisms that make the cells and tissues better able to cope with the following exposures to HD.<sup>8</sup> So, the present study aimed to investigate the possible protective effect of LD whole-body  $\gamma$ -irradiation alone or combined with ALA administration in modulating HD head irradiation-induced brain injury in rats. Also, to investigate the possible radio adaptive mechanisms through which pre-conditioning low dose induces its effect.

The results of the current study showed that head irradiation (20 Gy) induced a significant decrease in the GSH content as well as the TAC with an obvious increase in MDA, NO and 8-OHdG levels in the brain (Figures 1 and 2 and Table 3). The harmful effect of ionizing radiation has been attributed to the induction of DNA damage following deposition of energy within the nucleus immediately after irradiation.<sup>31</sup> The significant reduction in brain antioxidants could be attributed to its enhanced utilization to cope with radiation-induced ROS and RNS production. Besides, the brain is highly susceptible to oxidative stress due to its high oxygen consumption and lipid-rich content and the relative inadequate of antioxidants.<sup>32</sup> Under these conditions, the endogenous antioxidant defense mechanisms might be insufficient to fully scavenge post-irradiated free radicals. The imbalance between the endogenous antioxidant defense system and free radical production leads to oxidative stress, which in turn leading to an increased membrane lipid peroxidation and elevation of MDA level. Moreover, the free radicals produced by exposure to ionizing radiation can increase the activity of different enzymes, including inducible nitric oxide synthase (iNOS), which is thought to be responsible for high NO production under this circumstance of stress. The produced NO works as a source of toxic oxidants. It reacts with superoxide anion resulting in the formation of peroxynitrite, a more potent oxidant that could participate in GSH depletion and increase MDA level.<sup>33,34</sup> Previous studies indicated that most of the cellular injury attributed to NO is rather due to peroxynitrite, where it reacts as an oxidant and nitrating agent causing more cellular damage thus activating cell death pathways.<sup>35,36</sup>

It is well established that, ionizing radiation at HD-induces structural and functional damage in the brain. The most important target of radiation-induced brain damage is the vasculature; cellular inflammatory pathways have also been

**Table 3.** Changes in Brain Caspase-3, Bax and Bcl-2 Mrna Levels of Adult Male Albino Rats in Different Groups.

	Caspase-3	Bax	Bcl-2
1 Control	1.07 ± 0.03 <sup>a,b,c,d</sup>	1.10 ± 0.05 <sup>a,b,c,d</sup>	1.03 ± 0.02 <sup>a,b,c,d</sup>
2 ALA	0.99 ± 0.05 <sup>a,b,c,d</sup>	1.03 ± 0.07 <sup>a,b,c,d</sup>	1.17 ± 0.08 <sup>a,b,c,d</sup>
3 LD	1.04 ± 0.04 <sup>a,b,c,d</sup>	0.98 ± 0.07 <sup>a,b,c,d</sup>	1.14 ± 0.09 <sup>a,b,c,d</sup>
4 HD	5.28 ± 0.27 <sup>b,c,d,e</sup>	6.58 ± 0.23 <sup>b,c,d,e</sup>	0.28 ± 0.02 <sup>b,c,d,e</sup>
5 HD+ALA	3.02 ± 0.15 <sup>a,c,d</sup>	3.48 ± 0.19 <sup>a,c,d,e</sup>	0.66 ± 0.04 <sup>a,e</sup>
6 LD+HD	4.12 ± 0.26 <sup>a,b,d,e</sup>	5.40 ± 0.17 <sup>a,b,d,e</sup>	0.57 ± 0.03 <sup>a,d,e</sup>
7LD+HD+ALA	2.68 ± 0.17 <sup>a,c,e</sup>	2.96 ± 0.27 <sup>a,b,c,e</sup>	0.78 ± 0.03 <sup>a,c,e</sup>

Data are represented as means ± SE.

<sup>a</sup>Significantly different from the control group.

<sup>b</sup>Significantly different from the HD group.

<sup>c</sup>Significantly different from HD+ALA group.

<sup>d</sup>Significantly different from LD+HD group.

<sup>e</sup>Significantly different from LD+HD+ALA group. The mean difference is significant at the .05 level.

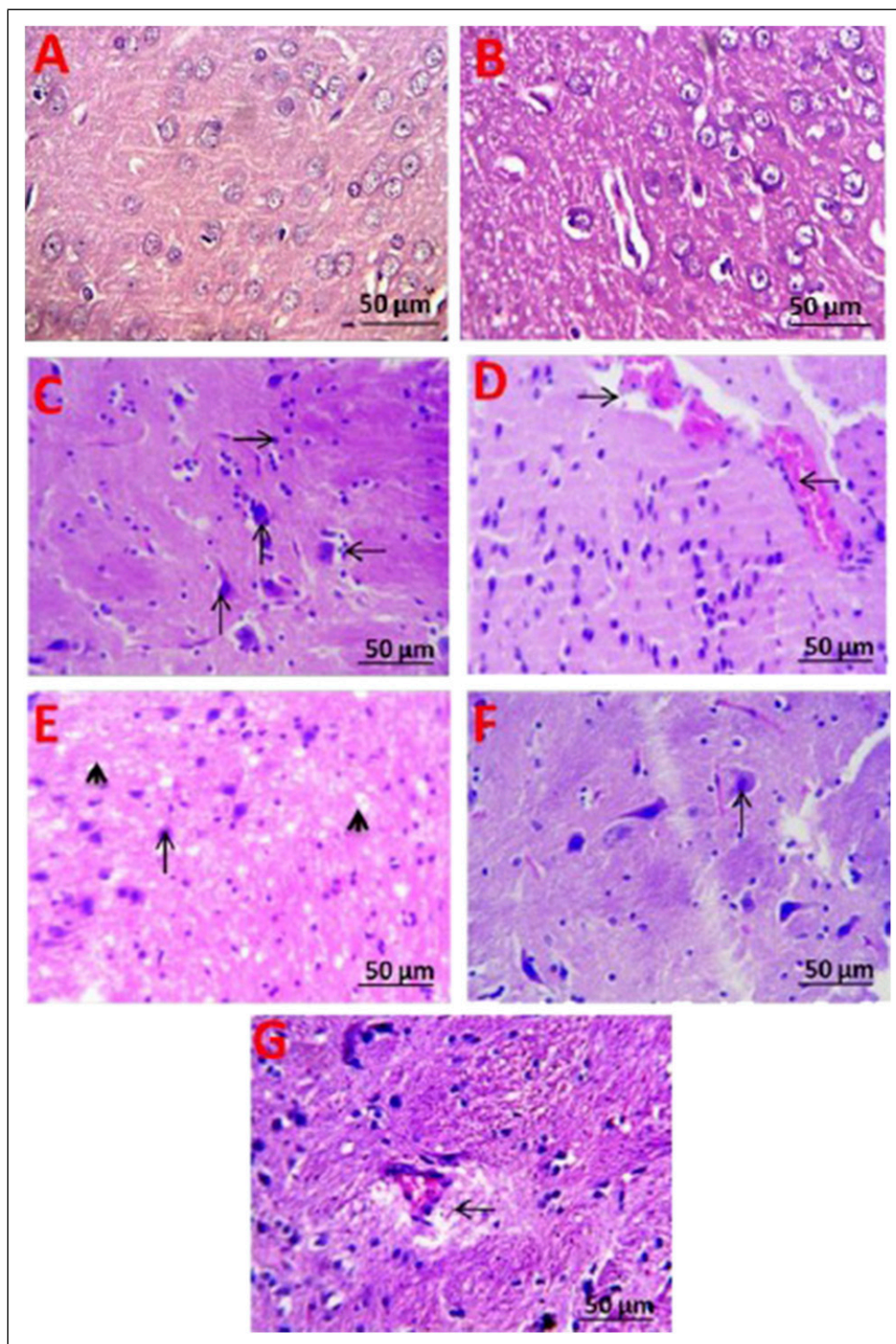
implicated. The injury at the micro-vascular level leads to blood-brain barrier (BBB) leakage, however, at the cellular level; astrocytes and microglia are thought to mediate inflammation within the brain by releasing pro-inflammatory cytokines.

The results of the current study revealed that head irradiation induced a significant increase in NF- $\kappa$ B, TNF- $\alpha$ , VEGF and A $\beta$  levels in the brain (Tables 1 and 2). These results are in accordance with those of Wang et al. (2020) who demonstrated an increase of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 levels and gene expression of NF- $\kappa$ B after whole-brain irradiation.<sup>37</sup> Knowing that NF- $\kappa$ B upregulates pro-inflammatory cytokines production, including that of IL-1 $\beta$  and TNF- $\alpha$ , which in turn increases BBB permeability and evolves demyelination and edema.<sup>38</sup> Previously, it was reported that TNF- $\alpha$  played a critical role in radiation-induced vascular damage, BBB disruption and astrogliosis, where the binding of TNF- $\alpha$  to its receptors on the endothelial cell surface activates NF- $\kappa$ B, which in turn upregulates the synthesis of intercellular adhesion molecule-1 causing increases leukocyte-endothelial cell interactions. Consequently, it increases infiltration of immune and inflammatory cells through the damaged BBB. Further, it has been confirmed that inhibition of TNF- $\alpha$  expression eliminated these effects.<sup>22</sup> Also, VEGF has been involved in radiation-induced brain injury. Although VEGF is necessary for the survival and proliferation of endothelial cells and control vessel permeability, its pathological level results in vessel leakage instead of promoting endothelial proliferation. It was observed a positive correlation between the VEGF expression and the brain injury in mice exposed to 20 Gy whole-brain irradiation.<sup>2</sup> Besides, in vitro study has demonstrated that VEGF expression in astrocytes increased significantly in a dose-dependent manner (Zhou et al. 2020).<sup>39</sup> However, VEGF inhibition may have a potential for treatment of brain injury in irradiated and ischemic experimental animals.<sup>40,41</sup>

Moreover, it was suggested that oxidative stress has an essential role in A $\beta$  peptides generation and accumulation in

the brain tissues. Although A $\beta$ , at a physiological level, serves beneficial roles include maintaining the integrity of the BBB and promoting recovery from brain injury, its pathological deposition results in brain atrophy.<sup>42,43</sup> Previous in vitro study demonstrated that exposure of glioma cells to A $\beta$  could significantly inhibit the proliferation of the cells as well as decreased the GSH content and the ratio of GSH and GSSH. Also, it decreased the expression of both MnSOD protein and mRNA in the mitochondria.<sup>44</sup> Furthermore, the elevated oxidative stress, DNA damage and inflammatory makers elicited cell death including apoptosis demonstrated by increased caspase-3 and Bax mRNA expression and decreased mRNA expression of Bcl-2 (Table 3).

From the above, we confirmed that our histopathological results of HD head-irradiated rats induced microglial clustering, pyknotic neurons and satellitosis around neurons (Figure 3C), severe congestion, perivascular edema, and numerous apoptotic neurons with neuropile microcavitation (spongy degeneration) in gray matters (Figures 3D and E). Moreover, white matters showed hemorrhage with a proliferation of glia cells (Figure 4C) and leukocytic aggregation mainly lymphocytes (Figure 4D). This is in concordance with the previous studies by Sha et al (2014); El-Maraghi et al (2018); Tang et al (2019).<sup>45-47</sup> From these results, it is thought that the oxidative stress, DNA damage and the consequent inflammation and vascular injury are the probable pathogenic mechanisms in radiation-induced brain injury. Since the cellular response to oxidative stress depends on the sensitivity of exposed tissue and the efficiency of its antioxidant defense system,<sup>48</sup> enhancement of tissue resistance to HD radiation and endogenous antioxidant defense system may reduce this pathogenic effect. Previously, it was observed that LD whole-body irradiation (up to .5 Gy) stimulated the resistance of the cells to radicals' toxicity and enhanced the endogenous antioxidants in rats exposed to liver, brain and heart toxicity.<sup>23-49</sup> As well as, supplementation of exogenous antioxidants including ALA has been found to modulate the oxidative stress and prevent or reverse the brain injury in different

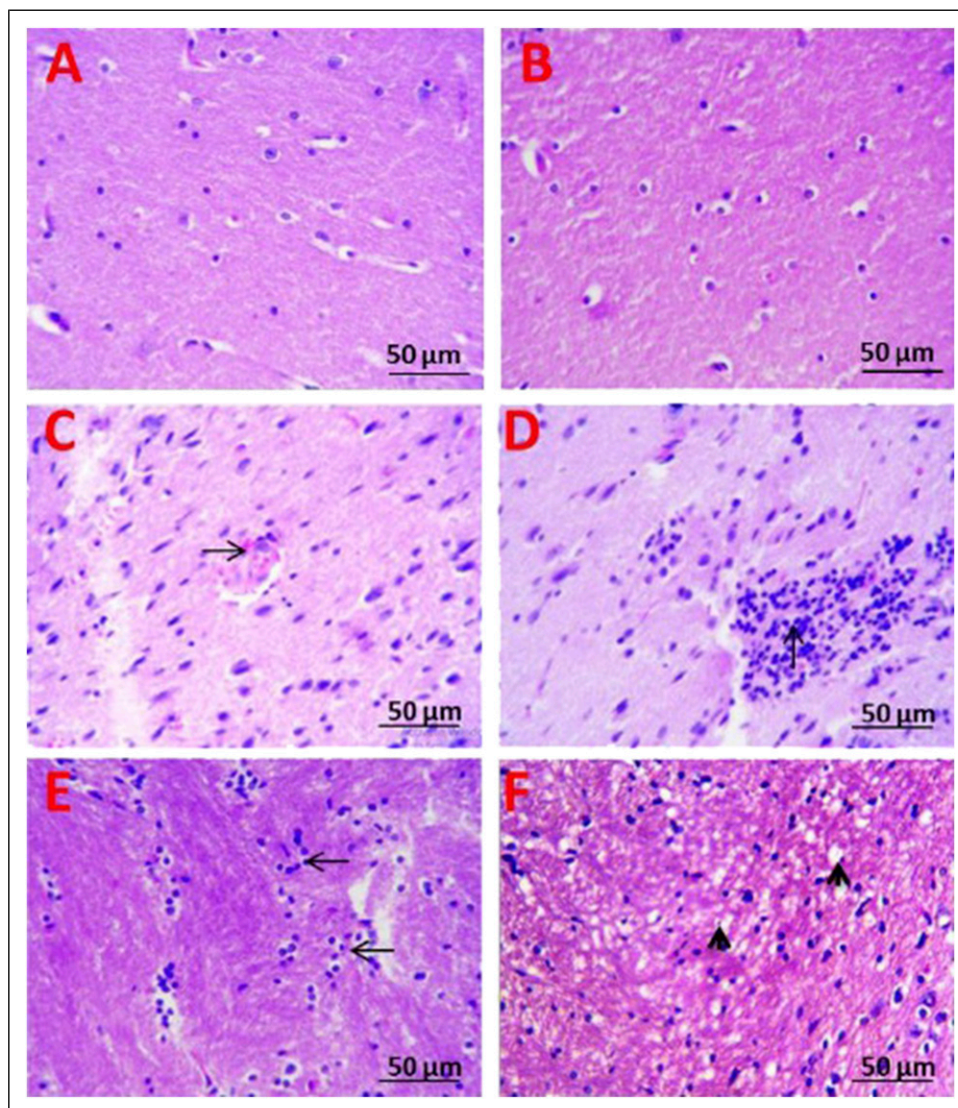


**Figure 3.** Gray matter of rat brain (HE 400 × ;A and B) showing normal structure in control and LD group. (c) showing microglial clustering (→), pyknotic neurons (↑) and satellitosis around a single neuron(←) in HD rat group. (D) showing severe congestion (←) and perivascular edema (→) in HD rat group. (E) showing numerous apoptotic neurons(↑) and microcavitation ( ) in HD rat group. (F) showing little degenerated pyramidal neurons (↑) in LD + HD rat groups. (G) showing mild peri-vassclur edema (←) and mild proliferation of glia cells in LD + ALA + HD rat groups. HD: high dose; LD: low-dose; ALA: alpha-lipoic acid.

experimental models in which ROS are involved.<sup>16,50,51</sup> The results of the current study revealed that pre-conditioning LD gamma radiation or ALA supplementation after head

irradiation or both treatments have significantly increased GSH content and TAC and reduced the levels of MDA and NO (Figures 1 and 2) in the brain as compared to corresponding





**Figure 4.** White matter of rat brain (HE 400 ×; A and B) showing normal structure in control and ALA group. (C) showing hemorrhage (→) with proliferation of glial cells in HD rat group. (D) showing leukocytic aggregation mainly lymphocytes (↑) in HD rat group. (E) showing focal gliosis around nerve axons (←) in HD + ALA rat groups. (F) showing spongiform degeneration (◊) and proliferation of neuroglia cells in LD + ALA + HD rat groups. HD: high dose; LD: low-dose; ALA: alpha-lipoic acid.

values of HD head irradiated group. These results are in agreement with the previous study indicated that ALA could attenuate the oxidative stress-induced neural apoptosis following traumatic brain injury via activation of the Nrf2 signaling pathway and antioxidant enzymes and suppression of MDA production.<sup>51</sup> Also, in vitro study showed that ALA exerts a neuroprotective effect against oxidative stress and iron-dependent damage through inhibition of ROS production, iNOS expression and mitochondria-mediated apoptotic pathway in addition to its direct beneficial effect on astrocyte viability.<sup>52</sup> The neuroprotective effects of ALA are based on the hypothesis that it is a small molecule easily absorbed through the gastrointestinal system and can cross the blood-brain barrier. Following cellular uptake, a substantial part of

ALA is converted to its reduced form, DHLA. Both the oxidized (disulfide) and reduced (dithiol) forms induce antioxidant properties.<sup>53</sup> It has been described that the ALA/DHLA redox couple exerts antioxidant effects by neutralization of ROS and RNS, maintaining the cellular GSH level and regenerates the Vitamin C and Vitamin E, thus maintaining oxidant-antioxidant balance in biological systems. Besides, ALA can restore the reduced/oxidized glutathione ratio (GSH/GSSG) by either transferring electrons directly to the GSSG for reduction or increasing the synthesis of GSH through improving the plasma uptake of cystine to subsequently reduce it to cysteine, which is the precursor of glutathione.<sup>54</sup>

Concerning the radio adaptive effect of LD radiation, the obtained results are in agreement with previous studies<sup>23,53,55</sup>;

the latter indicated that exposure of rats to whole-body LD radiation (.5 Gy as a single dose or 2 fractions) restored GSH and reduced MDA to a significant levels and afforded hepatoprotection and neuroprotection against paracetamol and thioacetamide-induced acute liver damage and hepatic encephalopathy. This effect may be due to simultaneous stimulation of many independent cellular functions as an antioxidant defense mechanism and DNA repair mechanisms through regulation of some gene networks in response to DNA damage (Komova et al. 2018) inducing more radio resistance to a subsequent HD.<sup>56</sup> Previously, Radwan et al. (2012) explained that the LD of radiation might enhance free radical formation with the concomitant enhancement of the antioxidant mechanisms that overwhelmed the formed free radicals and finally predominated.<sup>33</sup> Thus, LD irradiation preconditioned the body to resist the subsequent oxidative injury.

Also, the results revealed that treatment of HD head-irradiated rats with ALA and/or pre-conditioning low-dose irradiation have significantly reduced the marker of DNA damage (8-OHdG) and the levels of studied inflammatory and apoptotic markers (Tables 1–3). This result further confirmed that ALA is a neuroprotective agent and plays a vital role in reducing brain injury by inhibiting inflammatory responses. The results are in agreement with previous studies, showing that ALA suppressed the increase in NF- $\kappa$ B expression in the lacrimal gland of rats exposed to head and neck radiation (18 Gy) by the inhibition of nuclear factor of activated T cells 5 (NFAT5) expression and its dependent signaling pathway.<sup>57</sup> Previously, Ishii et al. (2017) demonstrated that ALA inhibited I $\kappa$ B $\alpha$  phosphorylation and degradation in the NF- $\kappa$ B signaling pathway, NF- $\kappa$ B p65 expression, and translocation were also inhibited, leading to the inhibition of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6) secretion in human gingival fibroblast stimulated by lipopolysaccharide.<sup>58</sup> Moreover, ALA has been shown to preserve BBB integrity and inhibit VEGF release against cigarette smoke-prompted oxidative stress and BBB endothelial damage in Immortalized Human Cerebral Microvascular Endothelial Cell Line (hCMEC/D3) (Kaisar et al. 2015).<sup>59</sup> In addition, Molz and Schröder and colleagues (2017) reported that ALA can alleviate the neurological damage induced by A $\beta$  and inhibit  $\beta$ -amyloid fibrils (FA $\beta$ ) formation from amyloid  $\beta$ -protein.<sup>16</sup>

Furthermore, pre-exposure to a LD of ionizing radiation reduces the detrimental inflammatory effects of a subsequent HD exposure. From the presumable mechanisms of the induction of anti-inflammatory response are the reduced evasion of immune cells from the BBB into the brain tissue and the elimination of damaged cells through apoptosis. Previous studies revealed that LD radiation impacts upon macrophages. Lödermann et al. (2012) observed that distinct low doses [.5 or .7 Gy] of X-rays induce an anti-inflammatory phenotype of activated macrophages by lowering the amount of secreted IL-1  $\beta$  in a NF- $\kappa$  B dependent manner.<sup>9</sup> In vivo study of El-Ghazaly et al. (2011) demonstrated that low doses (.25 or .50 Gy) of gamma rays suppressed the pro-inflammatory

cytokines in the adjuvant-induced arthritis model in rats.<sup>24</sup> Also, it was demonstrated that two fractions of .25 Gy gamma ray reduced serum transforming growth factor-beta and TNF- $\alpha$  and ameliorated the liver injury induced by thioacetamide in female rats.<sup>60</sup> From the above conversation, our pathological brain lesions in LD+ ALA+ HD group are less severe and epitomized by perivascular edema and mild proliferated glia cells with spongiform degeneration.

On the contrary, Abdelrazzak et al. (2019) demonstrated that there is no indication that 10cGy irradiation protected the liver cells against a subsequent higher dose.<sup>14</sup> Indeed, it seems doubtful that radio-adaptive response to low-dose radiation has a consistent impact on the detrimental effects of a subsequent HD exposure.<sup>56</sup> Based on what was mentioned above, it seems that this controversy may be related to the radiation dose, dose rate, type of radiation, time after exposure, type of exposed cells or organs, and also to the time interval between the two treatments.

## Conclusion

Taken together, LD whole-body irradiation exhibited neuroprotective activity against detrimental effects of a subsequent HD head irradiation. This effect might be due to the adaptive response prompted by LD that reduced the DNA damage in brain tissue and activated the anti-oxidative, anti-apoptotic, and anti-inflammatory mechanisms in the affected animals making them able to cope with the subsequent HD exposure. Moreover, the combined LD exposure and ALA supplementation produced a further modulating effect in the HD-irradiated rats.

## Author Contributions

Nahed Abdel-Aziz, Ahmed A. Elkady and Eman M. Elgazzar contributed to the achievement of the research, analysis of the results, and writing of the manuscript.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethical Approval

This experiment was carried out according to the international guidelines of animal handling and care (NIH no. 85:23, 1996) and the NCRRT Independent Committee on the Ethics for the Use and Care of Laboratory Animals Human participants, human data or human tissue are not applicable.

## Informed Consent

Cover letter of author's agreements as attached document.

## Data Availability

The data and materials used in the present study are available from the corresponding author on reasonable request.

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