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The Impact of Di-Indolylmethane on Brain Injury in Rats Exposed to Gamma-Radiation

A.S. Hammad[(1)], H.N. Saada[(1)], N.A. Eltahawy[(1)#], N.Y.S. Morcos[(2)], D.M. Seoudi[(2)]
[(1)]Radiation Biology Department, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt; [(2)]Biochemistry Department, Faculty of Sciences, Ain Shams University, Cairo, Egypt.

THE PRESENT study aims at assessing the role of di-indolylmethane (DIM), a metabolite of indole-3-carbinol, in mitochondrial damage, inflammation, apoptosis, and alteration of serotonin metabolism in the cerebral cortex of young (4± 1month-old) and aged rats (22±1 month-old) exposed to gamma-radiation. The rat's whole body was exposed to 8 Gy administered in 2 doses of 4 Gy at 2weeks interval. DIM (45 mg/kg body- weight) was given to rats by gavage 3 h after the 1st radiation-dose, daily till the 2nd dose and 2 weeks after the 2nd dose. The animals were sacrificed 24 h after the last dose of DIM. In young and aged rats, irradiation induced mitochondrial damage evident by significant decreases of adenosine triphosphate (ATP), and mitochondrial nitric oxide synthase (mtNOS) associated with significant increases of malondialdehyde (MDA), protein carbonyl (CO), and 8-hydroxy-2-deoxyguanosine (8-OHdG), markers of lipids, proteins and DNA oxidation, respectively. Irradiation also induced significant increases in the inflammatory markers, tumor necrosis factor-alpha (TNF-α) and active microglia (CD14) and the apoptotic markers cytochrome c and caspase-3. Alteration in serotonin metabolism was evident by significant decreases of serotonin accompanied by significant increases in the activity of its metabolizing enzyme monoamine oxidase (MAO). Nevertheless, by comparing the biochemical variations occurring in the cerebral cortex of young and aged rats, the changes were generally more pronounced in young rats. DIM significantly reduced the mitochondrial damage, and improved the inflammatory and apoptotic responses and serotonin metabolism in young and aged rats. It could be concluded that age is an important risk factor to consider upon exposure to ionizing radiation during therapy or diagnosis. DIM may exert a beneficial impact on irradiation-induced brain injury in young and aged rats.

Keywords: Aged rats, Apoptosis, Cerebral cortex, Diindolylmethane, Inflammation, Mitochondria, Serotonin, Young rats, γ-ray.

Introduction

Ionizing radiation possesses enough energy to remove tightly bound electrons from their orbits. It may be in the form of rays such as γ- and X-rays or high-speed particles including α- and β- particles and neutrons. Humans are exposed to ionizing radiation encountered in the environment such as cosmic rays, radioactive elements in the earth's crust (uranium, thorium, etc.), radon and its decay products, besides the radioactivity naturally present in the body. In addition, man-made sources as in radio-therapy and radio-diagnosis, research laboratories, and nuclear accidents have increased the risk of exposure to ionizing radiation.

Each year over 200,000 patients are treated with ionizing radiation for primary and metastatic brain tumors. Approximately 50% survive long enough to experience radiation-induced brain injury including progressive impairments

#Corresponding author e-mail: noamaneltahawy@yahoo.com
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in memory, attention, and executive function which have profound effects on the quality of life. The study of the biochemical changes in the brain is particularly difficult because of its own morphological and functional complexity, and the close and intricate relationships between the brain and other systems of the organism. In comparison with other organs, the brain is especially vulnerable to oxidative injury due to its high consumption of oxygen for energy needs. In addition, the neuronal membranes are rich in polyunsaturated fatty acids particularly vulnerable to free radical attack, while the brain is poor in antioxidants. The interference of ion transport activates neuronal nitric oxide synthase (NOS) and thus the generation of nitric oxide, which react with superoxide to form the toxic peroxynitrite (Cobley et al., 2018). It is worthy to mention also that the brain functions depend on the integrity of mitochondria, which are affected by aging (Boveris & Navarro, 2008).

Compelling evidence has demonstrated that 3, 3'di-indolylmethane (DIM), a metabolite of indole-3-carbinol, abundantly found in cruciferous vegetables (e.g., cabbage, broccoli, cauliflower) may reduce the risk of cancer (Thomson et al., 2016; Tian et al., 2019; Isabella & Mirunalini, 2019), and neurodegenerative diseases (Kim et al., 2014). It was also shown to possess radioprotective properties (Lu et al., 2016; Thekkekkara et al., 2018; Jiang et al., 2019).

The current study is designed to investigate the role of DIM in different biochemical changes occurring in the brain of young and aged rats in response to γ-radiation. The level of adenosine triphosphate (ATP), the activity of nitric oxide synthase activity (mtNOS), in parallel with the content of malondialdehyde (MDA), protein carbonyl (CO), and 8-hydroxydeoxyguanosine (8-OHdG) markers of lipid, protein and DNA oxidation, respectively were determined in the mitochondria of the cerebral cortex to follow mitochondrial damage. The cytoplasm was analyzed for active microglia and tumor necrosis factor-alpha (TNF-α) to investigate the inflammatory response.Cytochrome c and caspase-3 were analyzed to follow the dysregulation of apoptosis, and finally, serotonin and its metabolizing enzyme monoamine oxidase (MAO) were measured as markers of alteration in neurotransmitters.

Materials and Methods

Experimental animals: Male albino rats Sprague Dawley purchased from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines (Helwan, Cairo, Egypt) were maintained under standard conditions of ventilation, temperatures (22± 3 ^{o}C), and lighting (light/dark: 13 h/11 h) and kept under observation for one week prior to experimentation. Drinking water and standard pellets diet were provided ad libitum throughout the study. Experimental analyses were performed in the morning at 11:00± 1 h. All the animal procedures are in accordance with the ethical standards of the NCRRT and conform to the "Guide for the care and use of Laboratory Animals" published by the National Institutes of Health (NIH publication No. 85–23, revised 1985).

Gamma irradiation procedure

Gamma irradiation of rats was carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr city, Cairo, Egypt using a Gamma Cell- 40 (Cesium-137) (Atomic Energy of Canada Ltd, Ottawa, Ontario, Canada), which ensured a homogeneous dose distribution all over the irradiation tray. The rat's whole body was exposed to 8 Gy administered in 2 doses of 4 Gy at 2 weeks of interval at a dose rate of 0.5 Gy/min.

3, 3′ Diindolylmethane (DIM) treatment

DIM purchased from Sigma Aldrich (USA) was dissolved in dimethyl sulfoxide (DMSO) and administrated to rats by gavage at a dose of 45 mg/kg body weight/day (Fan et al., 2013) during 28 consecutive days. In the irradiated groups DIM was given to the rats 3hours after the 1st dose of radiation, daily till the 2nd dose and 2 weeks after the 2nd dose.

Animal groups

The present study comprises 40 young rats at full adulthood (4±1 months old; 150 ± 10 g); and 40 aged rats (22±1 months old; 400 ± 10 g), based on the reports of Arivazhagan & Panneerselvam (2000) and Sengupta (2013). Animals were divided into 8 groups (n= 10) and treated in parallel as follows: 1- Young: young rats were administered DMSO daily during 4 weeks. 2- Young +DIM: young rats were administered DIM daily during 4 weeks. 3- Young +R: young were irradiated receiving DMSO treatment. 4- Young +R+DIM: young were irradiated receiving DIM treatment. 5- Aged: Aged rats were administered DMSO daily during 4

weeks. 6- Aged +DIM: Aged rats were administered DIM daily during 4 weeks. 7- Aged +R: Aged were irradiated receiving DMSO treatment. 8- Aged +R+DIM: Aged were irradiated receiving DIM treatment. The animals were sacrificed 24 h after the last dose of DMSO or DIM after a fasting period of 12 h, and the cerebral cortex rapidly excised.

Assessment of mitochondrial damage

The mitochondria were isolated from cerebral cortex according to Rajapakse et al. (2001). Adenosine triphosphate (ATP) level was determined using Rat adenosine triphosphate ELISA Kits (KAMIYA Biomedical Co., Seattle, WA, USA) according to the manufacturer's prescripts. Nitric oxide synthase (mtNOS) activity was determined by measuring spectrophotometrically the rate of nitric oxide (NO) production in the mitochondria according to Boveris et al. (2002). Lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to Yoshioka et al. (1979). The method is based on the determination of malondialdehyde (MDA) an end product of lipid peroxidation, which can react with thiobarbituric acid in acidic medium to yield a pink colored trimethine complex exhibiting an absorption maximum at 532 nm. Protein oxidation was assessed using a colorimetric assay that measured protein carbonyl group (CO) content as described by Levine et al. (1990). DNA oxidation in mitochondria was assessed using a competitive enzyme-linked immunosorbent assay ELISA kit (8-OHdG Check; Japan Institute for the Control of Aging, Fukuroi, Japan) as described by the manufacturer's prescripts.

Determination of inflammatory and apoptotic markers

The inflammatory marker TNF-α and the apoptotic markers cytochrome c and caspase-3 were determined in the cytoplasm using their respective Elisa Kit Quantikine® (R&D Systems, Inc. USA), according to the manufacturer's prescripts. Active microglia were determined utilizing CD14 marker (PE Rat anti-Mouse CD14, Cat.No. 553740) and analyzed by BD Flow cytometry Accuri C_6 (Becton Dickinson, CA, USA).

The percentage of cells marker surface (CD14%) was calculated using the Intuitive Software, BD Accuri C6.

Determination of serotonin metabolism

In the cytoplasm of the cerebral cortex, the neurotransmitter serotonin was determined according to Ciarlone (1978). The method is based on the markedly fluorescence behavior when heated with o-phthalaldehyde in the presence of strong acid. The intensity of fluorescence is proportional to the concentrations. The concentration of serotonin is expressed in ng/g wet tissue. The activity of monoamine oxidase (MAO) was determined according to the modified method of Ozaki et al. (1960). The enzyme extract is incubated with a serotonin substrate for a specified time. The reading of serotonin is assayed spectrophotometrically at 540 nm.

Statistical analysis

All values are presented as mean ± S.D. All groups were compared by one- way analyses of variance (ANOVA) and post hoc multiple comparisons were conducted using the LSD test in SPSS/PC software program (version 17.0; SPSS Inc., Chicago, IL, USA) to determine the differences between the groups studied. The difference was considered significant at P-value < 0.05.

Results

In the aged rats, the level of ATP and the activity of mtNOS were significantly lower than their corresponding values in the young rats (Table 1). The content of MDA, CO, and 8-OHdG markers of lipids, protein and DNA oxidation were significantly higher in aged animals (Table 2). The mean values of the inflammatory markers, TNF-α and active microglia and the apoptotic markers cytochrome c and active caspase-3 were significantly higher in the aged rats (Table 3). In the aged rats, the level of serotonin was significantly lower while the activity of its metabolizing enzyme MAO was significantly higher than their values in the young rats (Table 4).

The administration of DIM treatment to the young rats has not induced significant effects on the mitochondrial (Tables 1, 2), apoptotic and inflammatory markers (Table 3), and serotonin and MAO (Table 4). On the contrary, in the aged rats, the administration of DIM has significantly improved the apparent age-related decrease of ATP; boosted the activity of mtNOS, and reduced the level of MDA, CO, and 8-OHdG (Tables 1&2). The administration of DIM treatment has also alleviated the apparent age-related increase of the inflammatory and apoptotic markers (Table 3),

TABLE 1. Effect of 3, 3′ Diindolylmethane (DIM) on adenosine triphosphate (ATP), and nitric oxide synthase (mtNOS in the mitochondria of the cerebral cortex of young rats at full adulthood (4±1 months old; 150 ± 10 g); and aged rats (22± 1 months old; 400 ± 10 g), exposed to γ-radiation (R)

	ATP (Pg/mg mitochondria)	mtNOS (nmol NO/ min/mg protein)
Young	40.95 ± 6.87 **cdegh**	2.35 ± 0.39 **ceg**
Young-DIM	41.35 ± 4.34 **cdegh**	2.31 ± 0.54 **ceg**
Young R	24.96 ± 3.40 **abdefg**	0.74 ± 0.17 **abdefgh**
Young R-DIM	34.08 ± 5.29 **abcfgh**	2.19 ± 0.34 **ceg**
Aged	34.08 ± 3.55 **abcfgh**	2.01 ± 0.48 **abcdfgh**
Aged-DIM	38.35 ± 4.00 **cdegh**	2.41 ± 0.37 **ceg**
Aged R	18.92 ± 4.61 **abcdefh**	1.02 ± 0.17 **abcdefh**
Aged R-DIM	27.74 ± 4.25 **abdefg**	2.13 ± 0.29 **ceg**

The results are expressed as Mean ± SD (n = 10). Values are significantly different at $P < 0.05$.

a: Significantly different from Young
b: Significantly different from Young-DIM
c: Significantly different from Young R
d: Significantly different from Young R-DIM
e: Significantly different from Aged
f: Significantly different from Aged-DIM
g: Significantly different from Aged R
h: Significantly different from Aged R-DIM

TABLE 2. Effect of 3, 3′ Diindolylmethane (DIM) on malondialdehyde (MDA), protein carbonyl (CO), 8-hydroxyl-deoxyguanosine (8-OHdG) in the mitochondria of the cerebral cortex of young rats at full adulthood (4±1months old; 150 ± 10 g); and aged rats (22± 1 months old; 400 ± 10 g), exposed to γ-radiation (R)

	MDA (nmol/ g fresh tissue)	CO (nmol/g fresh tissue)	8-OHdG (ng/ mg DNA)
Young	95 ± 8.7 **cegh**	69.6 ± 13.4 **cegh**	1.09 ± 0.23 **cegh**
Young-DIM	93 ± 13.9 **cegh**	70.4 ± 15.6 **cegh**	0.99 ± 0.23 **cegh**
Young R	141 ± 22.9 **abdfg**	82.6 ± 7.7 **abdfg**	2.32 ± 0.32 **abdefh**
Young R-DIM	104 ± 22.9 **cegh**	70.3 ± 7.7 **cegh**	1.13 ± 0.28 **cegh**
Aged	136 ± 21.8 **abdfg**	85.7 ± 16.3 **abdfg**	1.34 ± 0.14 **abcdfg**
Aged-DIM	113 ± 26.4 **cegh**	74.9 ± 7.3 **cegh**	1.09 ± 0.26 **cegh**
Aged R	160 ± 31.3 **abcdefh**	96.0 ± 8.4 **abcdefh**	2.30 ± 0.27 **abdefh**
Aged R-DIM	133 ± 18.1 **abdfg**	80.6 ± 6.2 **abdfg**	1.31 ± 0.22 **abcdfg**

The results are expressed as Mean ± SD (n = 10). Values are significantly different at $P < 0.05$.

a: Significantly different from Young
b: Significantly different from Young-DIM
c: Significantly different from Young R
d: Significantly different from Young R-DIM
e: Significantly different from Aged
f: Significantly different from Aged-DIM
g: Significantly different from Aged R
h: Significantly different from Aged R-DIM

TABLE 3. Effect of 3, 3′ Diindolylmethane (DIM) on the inflammatory markers, tumor necrosis factor (TNF-α) and active microglia (CD14%) and the apoptotic markers, cytochrome c and caspase-3 in the cytoplasm of the cerebral cortex of young rats at full adulthood (4±1 months old; 150 ± 10g); and aged rats (22±1 months old; 400 ± 10g), exposed to γ-radiation (R)

	TNF-α (Pg/mg fresh tissue)	Active microglia (CD14%)	Cytochrome c (ng/mg protein)	Caspase- 3 (ng/mg protein)
Young	10.24 ±3.07 **cdefgh**	11.93 ± 2.23 **cefgh**	2.51 ± 0.33 **cegh**	3.00 ± 0.56 **cegh**
Young-DIM	11.18 ±2.58 **cdefgh**	12.95 ± 2.61 **cefgh**	2.58 ± 0.27 **cegh**	3.26 ± 0.55 **cegh**
Young R	19.95 ±4.09 **abdfg**	14.77 ±1.34 **abdefgh**	4.68 ± 0.43 **abdefgh**	5.50 ± 0.50 **abdefg**
Young R-DIM	14.35 ±3.88 **abcegh**	10.72 ± 2.62 **cefgh**	2.54 ± 0.35 **cegh**	3.06 ± 0.69 **cegh**
Aged	18.32 ±4.38 **abdfg**	24.41 ±3.38 **abcdfg**	3.82 ± 0.53 **abcdfg**	4.51 ± 0.73 **abcdfgh**
Aged-DIM	13.95 ±3.26 **abcegh**	18.98±3.29 **abcdegh**	2.51 ± 0.32 **cegh**	3.35 ± 0.53 **cegh**
Aged R	25.47 ±4.97 **abcdefh**	27.78 ±4.44 **abcdefh**	7.20 ± 1.79 **abcdefh**	7.70 ± 0.79 **abcdefh**
Aged R-DIM	18.48 ±3.74 **abdfg**	24.17 ±3.82 **abcdfg**	3.71 ± 0.53 **abcdfg**	5.51 ± 1.33 **abdefg**

The results are expressed as Mean ± SD (n = 10). Values are significantly different at $P < 0.05$.

a: Significantly different from Young
b: Significantly different from Young-DIM
c: Significantly different from Young R
d: Significantly different from Young R-DIM
e: Significantly different from Aged
f: Significantly different from Aged-DIM
g: Significantly different from Aged R
h: Significantly different from Aged R-DIM

TABLE 4. Effect of 3, 3′ Diindolylmethane (DIM) on serotonin, and monoaminoxidase in the cytoplasm of the cerebral cortex of young rats at full adulthood (4±1months old; 150 ± 10 g); and aged rats (22 ±1 months old; 400 ± 10 g), exposed to γ-radiation (R)

	Serotonin (ng/ g tissue)	Monoaminoxidase (mg consumed 5-HT/g tissue/h)
Young	36.95 ± 5.54 **cdefgh**	2.94 ± 0.74 **cegh**
Young-DIM	38.22±3.20 **cdefgh**	2.90±0.47 **cegh**
Young R	17.52±1.89 **abdefg**	5.47±1.34 **abdefg**
Young R-DIM	32.76±2.49 **abcefgh**	2.97±0.69 **cegh**
Aged	24.43 ± 3.20 **abcdfgh**	3.70 ± 0.12 **abcdfgh**
Aged-DIM	11.06±1.47 **abcdegh**	2.97±0.19 **cegh**
Aged R	11.06±1.47 **abcdegh**	9.23±1.20 **abcdefh**
Aged R-DIM	19.77±2.11 **abdefg**	5.22±0.93 **abdefg**

The results are expressed as Mean ± SD (n = 10). Values are significantly different at $P < 0.05$.

a: Significantly different from Young
b: Significantly different from Young-DIM
c: Significantly different from Young R
d: Significantly different from Young R-DIM
e: Significantly different from Aged
f: Significantly different from Aged-DIM
g: Significantly different from Aged R
h: Significantly different from Aged R-DIM

elevated the level of serotonin, and decreased the activity of MAO (Table 4).

The exposure of rats to γ-ray has induced significant decreases in ATP levels and mtNOS activities accompanied by significant increases in the content of MDA, CO and 8-OHdG in the young as well as in the aged rats (Tables 1&2). Irradiation has also elevated significantly the level of TNF-α, CD14%, cytochrome c, and active caspase-3 in the young and aged rats (Table 3). The results revealed also that exposure to γ-ray significantly reduced the content of serotonin and increased the activity of MAO in the young and aged rats (Table 4). Nevertheless, the results pointed out that the changes were more pronounced in the case of young rats, except for the depletion of ATP,and the increase of cytochrome c and MAO which were more pronounced in the aged rats (Fig. 1). The decrease of serotonin was nearly similar in the young and aged rats.

The administration of DIM has significantly improved the irradiation-induced mitochondrial damage in the cerebral cortex of young and aged rats, the content of MDA, CO and 8-OHdG and the activity of mtNOS reached their normal values (Tables 1, 2). The decrease of ATP was -17% in the young and -19% in the aged rats instead of -39% and -45% in the irradiated rats respectively (Fig. 1). Furthermore, active microglia (CD14%), and the level of cytochrome c showed approximately their respective normal values in the young and aged rats (Table 3). The only difference was that in the young rats, caspase-3 activity showed normal values while TNF-α was still +40% higher than its respective control value instead of +95% while in the aged rats, the level of TNF-α showed normal values and the activity of caspase-3 was still 22% higher than the normal value instead of +71% (Fig. 2). The decrease of serotonin was -11% in the young and -19% in the aged rats as compared to -53% and -55%, respectively in the irradiated rats not treated with DIM. The increase of MAO was 1% in the young rats and 41% in the aged rats as compared to +86% and 149% in the irradiated rats not treated with DIM (Fig. 3).

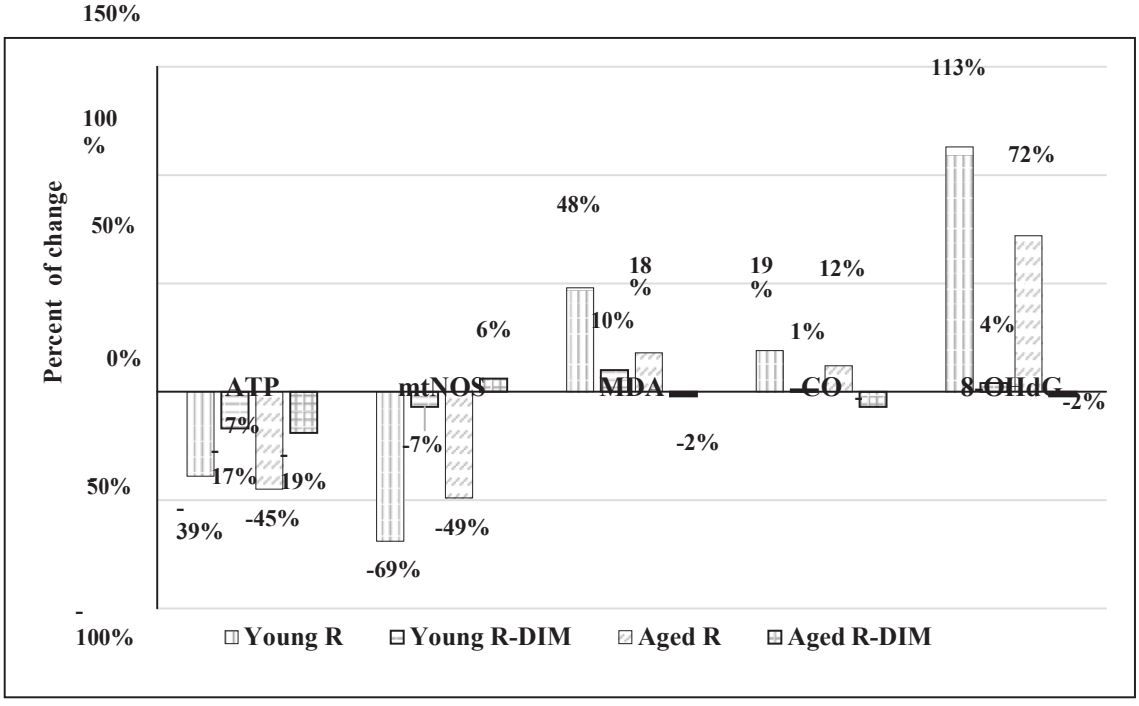

Fig. 1. Effect of 3, 3′ Diindolylmethane (DIM) on adenosine triphosphate (ATP), mitochondrial nitric oxide synthase (mtNOS), malondialdehyde (MDA), protein carbonyl (CO), 8-hydroxyl- deoxyguanosine (8-OHdG) in the mitochondria of the cerebral cortex of young rats at full adulthood (4± 1, months old; 150 ± 10 g); and aged rats (22± 1, months old; 400 ± 10 g), exposed to γ-radiation (R) calculated in percentage from their respective values in irradiated rats not treatedwith DIM

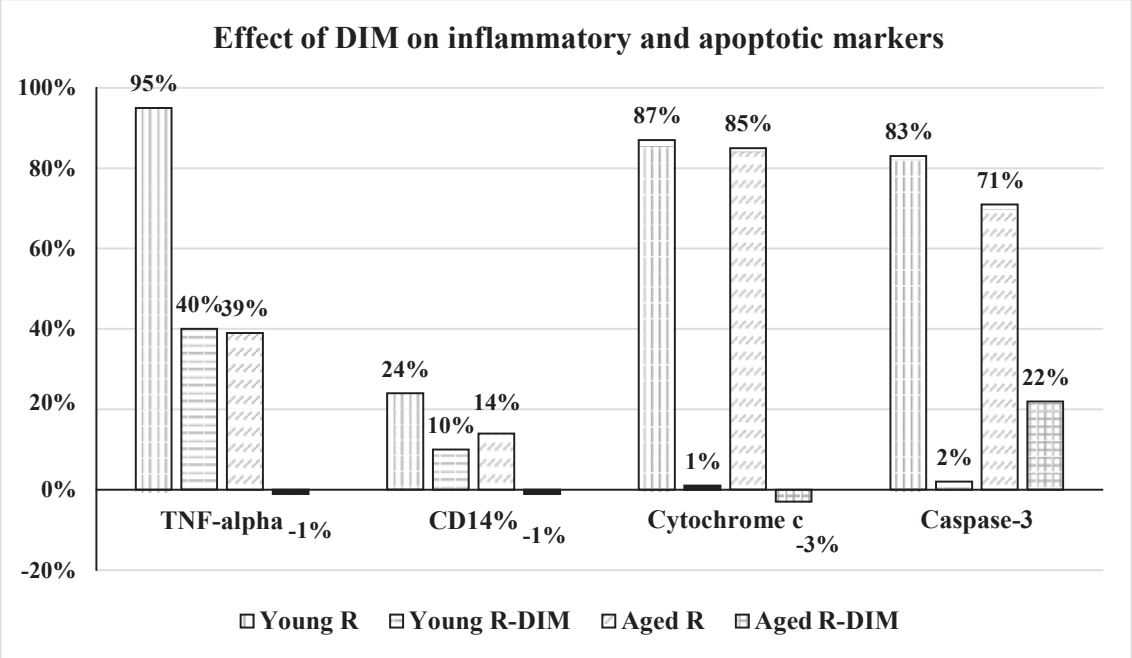

Fig. 2 . The effect of 3, 3′ Diindolylmethane (DIM) on the level of the inflammatory and apoptotic markers in the cytoplasm of the cerebral cortex of young rats at full adulthood (4±1, months old; 150 ± 10g); and aged rats (22±1, months old; 400 ± 10g), exposed to γ-radiation (R), calculated in percentage from their respective values in irradiated rats not treated with DIM.

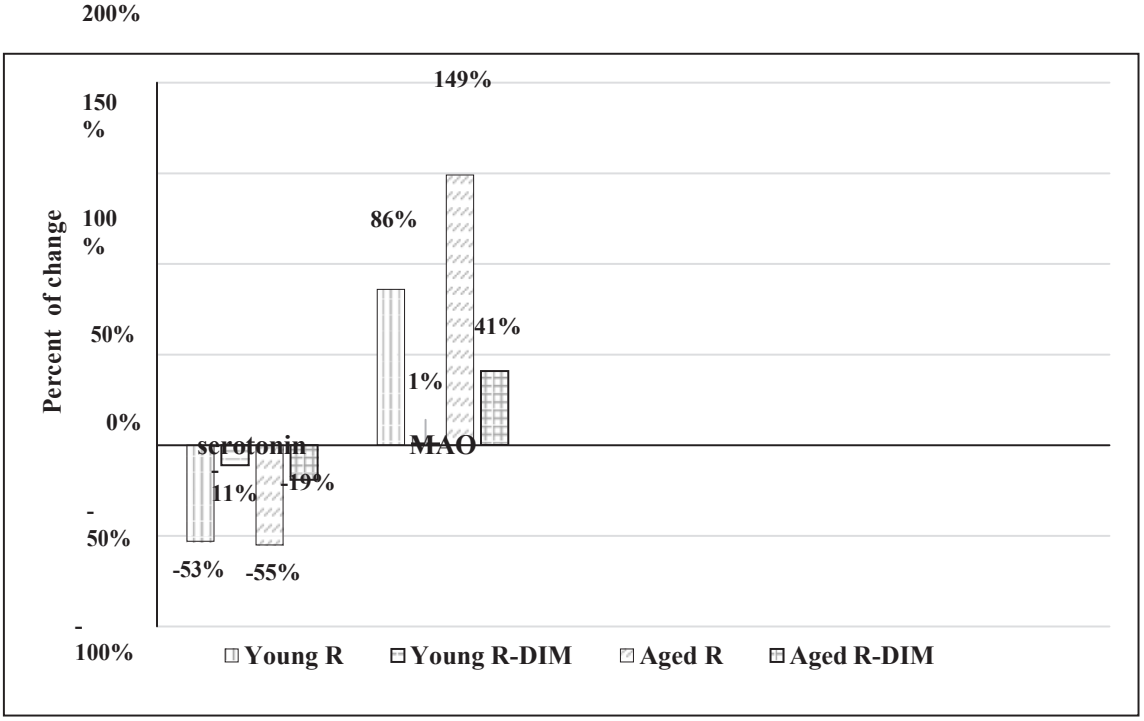

Fig. 3. The effect of 3, 3′ Diindolylmethane (DIM) on the level of serotonin and the activity of monoamine oxidase (MAO) in the cytoplasm of the cerebral cortex of young rats at full adulthood (4± 1 months old; 150 ± 10 g); and aged rats (22± 1 months old; 400 ± 10 g), exposed to γ-radiation (R), calculated in percentage from their respective values in irradiated rats not treated with DIM

Discussion

Ageing is a complex process associated with a progressive decline in the physiological functions. Despite significant research, the process of aging remains largely elusive and, unfortunately, inevitable. Although it is undeniable that free radicals play a role in several pathologies, their exact influence in mammalian aging is still undetermined (Jackson & McArdle, 2016).

In the current study by comparing the results obtained for the aged rats (22± 1, months old; 400 ± 10 g) with those recorded for the young rats, (4±1 , months old; 150 ± 10 g) it was noted that the levels of the mitochondrial MDA, CO, and 8-OHdG markers of lipids, proteins and DNA oxidation were higher in the cerebral cortex of the aged rats, and were associated with lower levels of ATP and mtNOS activity. The results indicate that in ageing the progressive depletion of energy may be related to an imbalance between the generation of free radicals and the radical scavenging system. Furthermore, the results indicate that the increase of the oxidative products in the mitochondria inhibits the activity of mtNOS, an enzyme responsible for the production of nitric oxide, a key signaling molecule in the regulation of cerebral blood flow (Garrya et al., 2015). Thus, the progressive alteration of the brain functions in ageing may be attributed to an increase of free radicals.

In the cerebral cortex of the aged rats, there was an increase of active microglia, and TNF-α accompanied by an elevation in the level of the apoptotic markers, cytochrome c and caspase-3. The increase of active microglia indicates that the microglia undergo several age-related changes that cause overproduction of free radicals and TNF-α. TNF-α connects to TNF-α receptors on the mitochondrial membrane, liberating cytochrome c which through the activation of caspase-3 initiates apoptosis (Orrenius & Zhivotovsky, 2005). This may provide an explanation for the increased neuronal apoptosis, a phenomenon that has been implicated in a variety of neurodegenerative diseases (Torres et al., 2014; Probert, 2015; Donat et al., 2017). Moreover, in the current study, the decrease of serotonin accompanied by an increase in the activity of its metabolizing enzyme MAO suggests that the detrimental effect of the free radicals is enhanced in old age. Finally, aging is associated with a series of biochemical alterations that may be attributed to increased production of free radicals and mitochondrial damage.

The damage of ionizing radiation may be direct, when the radiation energy is absorbed directly by critical cellular components such as nucleic acids, lipids, and proteins, or indirect, through the radiolysis of water and the generation of free radicals including hydrogen radicals, hydroxyl radicals, singlet oxygen, and peroxyl radicals, in a cascade pathway. Because free radicals are more able to diffuse, damage from the indirect action is much more common than damage from the direct action (Panganiban et al., 2013; Sharma et al., 2018).

In the current study, the exposure of young and aged rats to γ-rays has significantly increased the level of mitochondrial MDA, CO and 8-OHdG, concomitant with significant decreases in the level of ATP, and the activity of mtNOS. Irradiation has also increased active microglia, TNF-α,cytochrome c and caspase-3. A significant decrease of serotonin associated with a significant increase of MAO activities was also recorded. Nevertheless, when compared to their respective control levels, the changes induced by irradiation appears to be more pronounced in the young than in the aged rats. The results indicate that radiation can induce changes in the brain and accelerate and/or aggravate the onset of chronic degenerative disorders characteristic of the elderly (Lumniczky et al., 2017). Free radicals trigger oxidative stress, mitochondrial damage with impairment of the electron transport chain and decrease of ATP, induce DNA oxidation, dysfunction of cellular membrane caused by lipid and protein oxidation, inflammatory responses, and dysregulation of apoptosis.

The results are in agreement with a previous report that mitochondrial oxidative stress causes depletion of ATP. Considering that mitochondrial DNA encodes essential components of oxidative phosphorylation, consequently its damage will impair the respiratory chain, leading to energy depletion (Goldberg et al., 2018). The decrease of mtNOS activities indicates that mitochondrial enzymes are negatively correlated to the mitochondrial content of lipid and protein oxidation products.

The significant increase of MDA, CO, and 8-OHdG, are possibly the result of the interaction of the hydroxyl radical ($^{\bullet}$OH) generated in the body upon irradiation, with polyunsaturated fatty acids

in phospholipids, amino acids in proteins and guanine in DNA, respectively. In addition, MDA is able to react with primary amines on proteins to form crosslinks and to interact with nucleic acid bases to form several different adducts (Gaschler & Stockwell, 2017). The elevation of CD14% and TNFα might be attributed to the correlation between oxidative stress and inflammation, one of which can be easily induced by another (Sandoval et al., 2018). The decrease of serotonin suggests the possibility that exposure to γ-radiation provokes the degradation of neurotransmitters.

The results of the experimental studies on rats have shown that the radiation-induced biochemical changes in the brain can be improved by diet supplements (Saada et al., 2014; Shedid et al., 2017). DIM (3, 3′ Diindolylmethane) is a small molecule with no toxicity, and wide tissue distribution (Anderton et al., 2004; Fan et al., 2013). Another outstanding feature of DIM is that it is active when delivered by oral, subcutaneous and intra-peritoneal routes (Kim et al., 2014).

The modulator role of DIM may be attributed to its antioxidant and free radicals scavenging activities which go in lines with the previous findings of Hajra et al. (2017). DIM was found to control the activity of NADPH oxidase and accordingly contributes to the decrease of free radical production (Fan et al., 2013; Lu et al., 2016). In addition, the modulator effect of DIM may be in part ascribed to its capacity to activate a nuclear kinase involved in the repair of the damaged DNA (Stagni et al., 2018). The anti-inflammatory effect of DIM can be attributed to its efficiency in suppressing the expression of inflammatory mediators and to inhibit microglial hyperactivation (Kim et al., 2014; Luo et al., 2018). The anti-apoptotic effect of DIM could be attributed to its free radical scavenging capacity, which prevents the oxidation of cardiolipin, thus lowering the release of cytochrome c from the mitochondria followed by a consequent decrease of caspase 3 activity. Notably, the anti-apoptotic potential of DIM was correlated with increased expression of the anti-apoptotic protein Bcl-2 and decreased expression of the pro-apoptotic protein Bax (Lu et al., 2016), and down regulation of caspase-3 expression (Hajra et al. 2017).

Conclusion

Age is an important risk factor to consider upon exposure to ionizing radiation during therapy or diagnosis. DIM possesses antioxidant, anti-inflammatory, and anti-apoptotic activities and may offer protection against the different biological variations in the brain of rats exposed to ionizing radiation.

Recommendation: It could be recommended to take DIM found in commonly consumed crucifers. However, clinical trials must be completed to determine the evidence-driven basis for a dietary recommendation.

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