Original Article

The possible radioprotective effects of vitamin E, Nigella sativa oil, and melatonin against X-ray induced early acute changes in cerebral and cerebellar cortices in Albino rats: Histological and Immunohistochemical

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ABSTRACT

Background: Radiation induced brain and cerebellum injury is common in patients receiving radiotherapy for head and neck tumors. Administration of antioxidants before radiation as vitamin E, nigella sativa oil and melatonin could be valuable in limiting this injury.

Material and Methods: 40 rats were divided into five different groups. Group I: served as the control group and subdivided into 4subgroups. subroup Ia: negative control, subgroup Ib: animals received vitamin E only (300 mg/kg orally once, subgroup Ic: animals received nigella sativa oil only (1ml/kg orally once and subgroup Id: animals received melatonin only 100 mg/kg intraperitoneally once: Group II: animals were exposed to x ray irradiation (XRI) (8 Gy whole body). Group III: animals were pretreated with vitamin E (300 mg/kg orally once 1h before XRI. Group IV: animals were pretreated with nigella sativa oil(1ml/kg orally once1h before XRI). Group V: animals were pretreated with melatonin (100 mg/kg intraperitoneally once 1h before XRI). Animals were sacrificed 48h after irradiation. Brain and cerebellum were dissected, formalin fixed and processed for histological and glial fibrillary acidic protein (GFAP) immunostainig. The number of GFAP positive astrocytes were counted and statistically analyzed using SPSS program.

Results: Radiation induced loss of lamellar pattern in the cerebral cortex with degeneration and necrosis of cortical nerve cells. In the cerebellum, purkinje cell degeneration was observed. Radiation induced a significant increase in the number of GFAP positive astrocytes in both cerebral and cererbellar cortices compared to the control group. In groups treated with vitamin E, nigella sativa oil and melatonin, there was partial improvement in cerebral and cerebellar changes with a significant decrease in the number of GFAP positive astrocytes.

Conclusion: The use of antioxidants such as vitamin E, nigella and melatonin could limit radiation induced injury in brain and cerebellum.

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Key Words: GFAP, melatonin, nigella, radiation.

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INTRODUCTION

Cranial radiation is an essential therapeutic tool in the treatment of primary and secondary brain tumors. It has adverse effects including cognitive dysfunction which can affect the quality of life [1,2].

The response of the normal tissues to therapeutic radiation can range from mild discomfort to life threatening effects. The rate of these responses depends on the amount and distribution of the dose of radiation received by tissues[3,4]. Radiation toxicity was reported to be mediated by the mechanism of free radical injury of tissues thereby causing oxidative damage. Antioxidants can reduce the toxicity associated with radiation damage as free radical scavengers[5,6].

Nigella sativa is a plant of the Ranunculaceae spp. family that grows spontaneously and widely in several southern Mediterranean and Middle Eastern countries. Its seed has over 100 different chemical constituents, including abundant sources of all the essential fatty acids[7].

It has many pharmacological effects such as antioxidative[8], immunomodulation[9], anti-inflammatory, neuroprotective, anti-ischemic, antiepileptic and anxiolytic[10,11]. Many of the pharmacological activities mentioned above have been attributed to quinone constituents in the seed, especially thymoquinone[12]. Ahlatci *et al.* 2014 reported the significant radiation-modifying abilities of Nigella sativa and thymoquinone on the brain tissue[13].

Vitamin E is a natural component of cell membranes and is considered the main defense against membrane lipid peroxidation. There are several types of tocopherol, α-tocopherol being more reactive and with stronger antioxidant power. It reacts quickly with peroxyl free

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radicals interrupting the free radical chain reaction and consequently protecting cells from damage. Many researches upon the radioprotective effects of vitamin E have been reported, such as preservation of the small bowel crypt, increase in the rate of DNA repair process, salivary dysfunction[14], against mutagenic and/or carcinogenic agents in animals and cell cultures, and a reduction in the number of micronuclei in human lymphocytes in vitro[15].

Melatonin is an endogenous hormone secreted by the pineal gland characterized by its small size and high lipophilicity. It can cross the blood brain barrier and distributes throughout the cells[16]. It is reported that melatonin decreased the radiation-induced parotid and submandibular histological damage as well as decreased the oxidative stress markers[17]. Elmissiry *et al* reported the ameliorative effect of melatonin in radiation induced liver damage by decreasing the liver enzymes levels and the oxidative stress markers[18].

AIM OF OUR WORK

To study the possibility of the protective effect of vitamin E, Nigella sativa and melatonin on radiation induced brain and cerebellar damage in acute exposure if they are taken before irradiation and to assess the role of astrocyte in these changes.

MATERIAL AND METHODS

Materials

Vitamin E capsules (400 mg) and nigella sativa oil were obtained from PHARCO Pharmaceuticals (Cairo, Egypt).

Melatonin (sigma, St. Louis, MO, USA).

Animals

Three month old Albino rats were obtained from Assuit University Animal Facility, Faculty of Medicine, Egypt. They were housed in Animal Facility at Faculty of Medicine, Sohag University, Egypt. All rats were given access for rodent chow diet and water. This study was carried out in accordance with the guidelines of the University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals.

Experimental design

After a 7-day acclimatization period, 40 rats were divided into 5 different groups as following.

Group I: included 20 rats subdivided into the following 4 subgroups 5 rats each as following:

subgroup Ia: served as the negative control group.

subgroup Ib: animals were treated with vitamin E only 300 mg/kg once orally via gastric tube.

subgroup Ic: animals were treated with nigella sativa oil only 1ml/kg once orally via gastric tube

subgroup Id: animals were treated with melatonin 100 mg/kg once intraperitoneally.

Group II: included 5 animals that were exposed to x ray irradiation (XRI) (8 Gy whole body)[19].

Group III: included 5animals that were pretreated with vitamin E (300 mg/kg orally once 1h before XRI)[20].

Group IV: included 5 animals that were pretreated with nigella sativa oil (1ml/kg orally once 1h before XRI)[21].

Group V: included 5 animals that were pretreated with melatonin (100 mg/kg intraperitoneally once 1h before irradiation)[19]. Freshly prepared in 1000 ml of 5% ethanol (made with phosphate-buffered saline).

X-ray irradiation

X-ray irradiation was carried out at The Department of Radiology and Oncology, Sohag University Hospitals, Egypt using a linear accelerator (Philips SL75.5). This device was adjusted to provide X-ray but not gamma irradiation, and therefore, no filters were used in these experiments. Each animal was placed separately in a special small box with adjustable width that can fairly accommodate the animal without allowing any movements. Each animal was exposed to a whole-body XRI dose of 8 Grays (Gy). The dose was delivered at a rate of 400 motor unit/minute. The XRI dose for the brain was measured using special equation, and it was 8 Gy/brain.

Methods

The animals were sacrificed 48 h after XRI[19]. Brain and cerebellum were dissected, formalin fixed and processed for the following:

1. Histological examination by H and E
2. Immunohistochemical study:

Paraffin sections of 4um thickness were immunostained using peroxidase-labelled streptavidin-biotin technique to detect glial fibrillary acidic protein (GFAP) in astrocytes. This stain is considered specific for the intermediate filaments fibrillary acidic protein which is found in astrocytes and not found in nerve cells and even other types of glial cells as oligodendroglia or microglia.

Staining procedure: Formalin-fixed, paraffin-embedded tissue sections were done and mounted on coated glass slides. Sections were deparaffinized and rehydrated through descending grades of alcohols (100%, 90%, 80% and 70%) then put in distilled water for 5 min. Endogenous peroxidase activity was blocked with 0.6% hydrogen peroxide for 10 minutes using peroxidase blocking reagent. Antigenretrieval was done by boiling slides in citrate buffer solution (pH 6.0). The slides were microwaved at a high medium for 10 min. The sections were incubated with 1/50 of anti GFAP antibody at 4°C for 18–20h, washed and incubated with biotinylated secondary antibodies, and then with the avidin–biotin complex. Finally, sections were counterstained with hematoxylin, dehydrated, cleared, and mounted. GFAP-positive cells appeared brown and nuclei appeared blue. Negative control sections was done with omission of the 1ry (anti GFAP) antibody. Universal

kits and primary antibody (anti GFAP antibody) were purchased from Thermo fisher scientific company.

Morphometric and Statistical Analysis

The number of GFAP-positive cells was counted per 5 high power fields (x400) in slides from each animal in each group using (CX21, Japan. Light microscope) in Histology Department, Faculty of Medicine, Sohag University. For statistical analysis ANOVA test was performed to compare the mean number of GFAP positive cells between the different groups followed by posthoc test. *P value* was considered significant when it is ≤ 0.05.

RESULTS

Cerebral Cortex

1. Histological Structure

Examination of H&E stained sections form positive and negative control groups revealed no morphological differences. The cerebral hemisphere of the control rat consisted of an outer cortex of gray matter and a sub-cortical region of white matter. The cortex contained neurons, glial cells and nerve fibers. The organization of neurons in the cerebral cortex appeared as six layers; the molecular layer (fibrous with few nerve cell bodies), the external granular layer, the external pyramidal cell layer, the internal granular layer, the internal pyramidal cell layer and the multiform cell layer (Figure 1). The neuropil contained neuroglia, nerve fibers and blood vessels with a narrow perivascular space. Cortical neurons had rounded vesicular nuclei with prominent nucleoli, slight basophilic cytoplasm and peripheral processes (Figure 2). Examination of cerebral cortex of irradiated animals revealed dilated perivascular space with loss of lamellar pattern and damaged meninges. They are patches of pale areas in between nerve cells (cerebral necrosis), with degenerated nerve cells which appeared shrunken with pyknotic nuclei, deeply stained cytoplasm and increased perineural space (Figures 3& 4).

Examination of cerebral cortex of irradiated animals pretreated with Vitamin E revealed, return of the lamellar pattern as control group with intact meninges. Nerve cells appeared more or less as control group. others showed degeneration. cells appeared shrunken with pyknotic nuclei, deeply stained cytoplasm and increased perineural space (Figures 5&6).

Examination of sections from irradiated rat's cerebral cortex pretreated with Nigella revealed, some nerve cells appeared more or less as the control. Others appeared degenerated with pyknotic nuclei and deeply stained cytoplasm (Figures 7&8).

Examination of Melatonin pretreated irradiated group revealed that some nerve cells are degenerated leaving empty spaces while others were similar to the control. There was mild blood vessels dilatation under meninges (Figures 9 &10).

2. Immunohistochemical and Morphometric Study

Immunohistochemical staining for GFAP revealed positive staining in the form of brown coloration in the cytoplasm of astrocytes and their processes. They appeared small with few short, thin processes. (Figure 11). Examination of irradiated rat's cerebral cortex revealed a significant increase in the number of astrocytes compared to the control group with thick long processes (Figure 12, Table 1, Histogram 1). Examination of irradiated rat's cerebral cortex pretreated with vitamin E revealed a a significant decrease in the number of astrocytes compared to the previous group with abundant many process (Figure 13, Table 1, Histogram 2). Examination of the Nigella pretreated irradiated group showed a significant decrease in the number of astrocyte with localized distribution compared to the irradiated group (Figure 14, Table 1, Histogram 1). With examination of irradiated rat's cerebral cortex pretreated with melatonin immunostained with GFAP, showed a significant decrease in the number of astrocytes compared to the irradiated group (Figure 15, Table 1, Histogram 1).

Cerebellar cortex

1-Histological structure

Examination of the control rat cerebellar cortex revealed that the cortex formed of three layers;molecular layer which contained few cells. Purkinje cell layer with their rounded vesicular nucleus and basophilic cytoplasm and basophilic granules (Nissel bodies) and granular cell layer which had numerous densely packed cells (Figure 16).

With examination of irradiated rat cerebellar cortex, It showed mild dissociation of Purkinje cell layer and molecular layer. Some Purkinje cells had vacuolated cytoplasm other cells were shrunken with deeply stained cytoplasm. The granular cell layer and molecular layer were more or less as the control group (Figure 17). An examination of irradiated rat cerebellar cortex pretreated with Vitamin E revealed degenerated Purkinje cells with vacuolated cytoplasm, other cells were shrunken with deeply stained cytoplasm. The granular cell layer and molecular layer were more or less as the control group (Figure 18). The irradiated rat cerebellar cortex pretreated with Nigella, examination showed some Purkinje cells were degenerated, others were more or less as the control group. The granular cell layer and molecular layer were more or less as the control group (Figure 19). An examination of cerebellar cortex of rats treated with melatonin demonstrates that some Purkinje cells were degenerated, other cells had deeply stained small nuclei. Granular cell layer and molecular layer were more or less as the control group (Figure 20).

2-Immunohistochemical and Morphometric Study

Examination of control rat cerebellar cortex demonstrates that immunoreactive astrocytes had brown

coloration of the bodies and their long thick processes in molecular layer. Few astrocytes with few and thick processes in the granular and pukinje layer also had brown positive reaction(Figure 21).

With examination of irradiated rat's cerebellar cortex, it showed a significant increase in the number of astrocytes with apparent increase their intensity for staining in the molecular layer compared to the control groupin molecular layer. There was a significant increase in the number of astrocytes with increase their intensity for staining in the granular cell layer compared to the control group (Figure 22, Table 2, Histogram 2).

The irradiated rat's cerebellar cortex pretreated with vitamin E, on examination revealed that, the astrocyte number was significantly decreased compared to the irradiated one with apparent decrease in the intensity of staining (Figure 23, Table 2, Histogram 2).

Examination of irradiated rat's cerebellar cortex pretreated with Nigella demonstrated a significant decrease in the number of astrocytes compared to the irradiated group (Figure 24, Table 2, Histogram 2).

Examination of irradiated rat's cerebellar cortex pretreated with Melatonin revealed a significant decrease in the number of astrocytes in molecular, purkinje cell and granular cell layers intensity and number are less than the irradiated one (Figure 25, Table 2, Histogram 2).

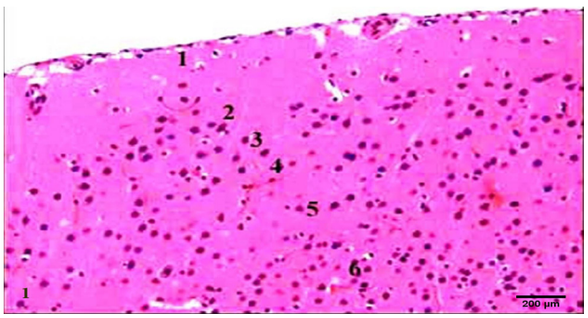


Fig. 1: A photomicrograph of a section from a control rat's cerebral cortex showing the six layers, which are clearly defined as: molecular (1), external granular (2), external pyramidal (3), internal granular (4), internal pyramidal (5), and multiform, which is the deepest layer (6). (Group I H&Ex200)

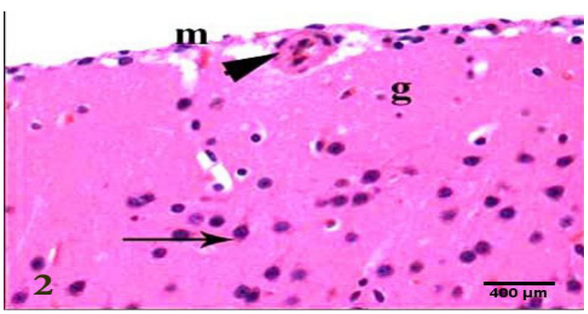


Fig. 2: A magnified part of previous section showing; cortical neurons (arrow) with rounded vesicular nuclei, basophilic cytoplasm and peripheral dendrites. Intercellular neuropils shows different types of neuroglia (g) and nerve fibers. Note: blood vessels (arrow head) are seen under meninges (m). (Group I H&E x400)

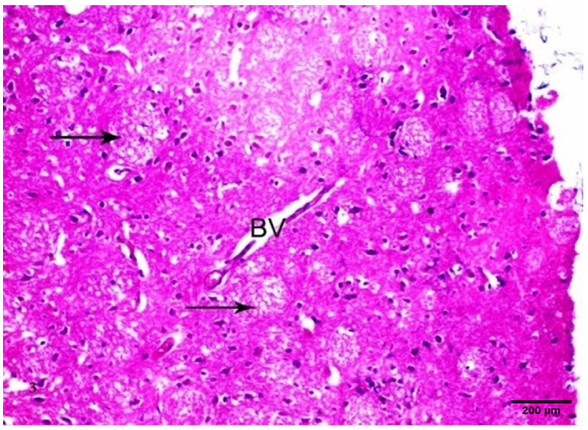


Fig. 3: A photomicrograph of a section from irradiated rat's cerebral cortex showing ., patches of pale areas in between nerve cells (arrow) ,widened perivascular space (BV) with loss of lamellar pattern (Group II H&Ex200)

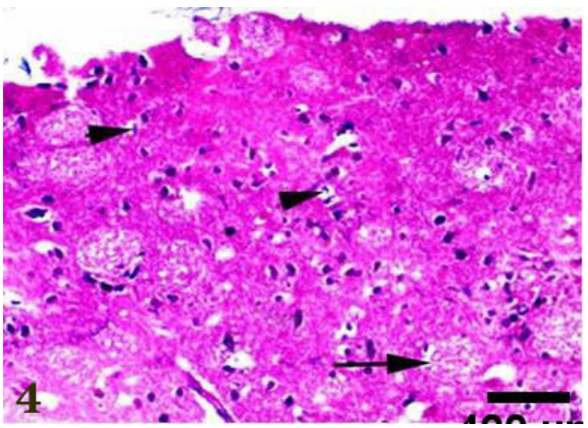


Fig. 4: A magnified image of the previous section showing., areas of cerebral necrosis (arrow) with degenerated nerve cells (arrow head) (Group II H& E X 400)

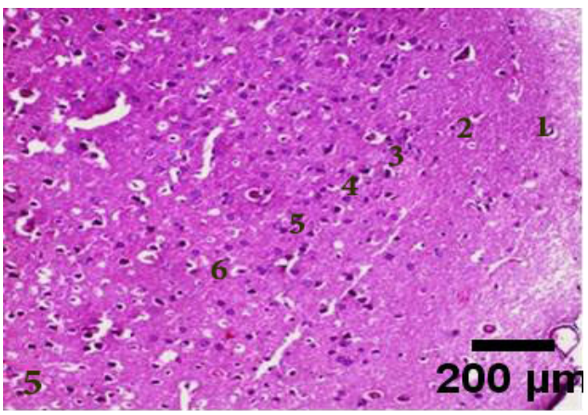


Fig. 5: A photomicrograph of a section of irradiated rat's cerebral cortex pretreated with vitamin E showing., return of the lamellar pattern (Group III H&E x200)

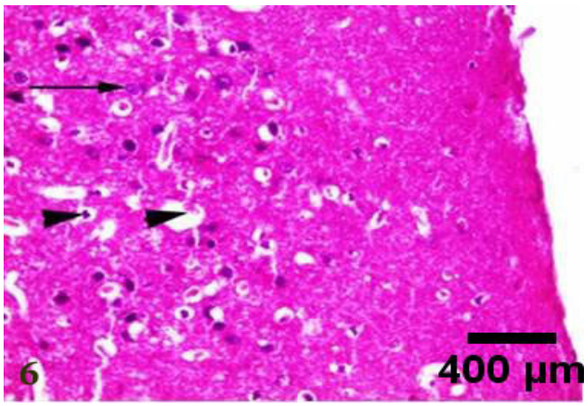


Fig. 6: A magnified image of the previous section showing, most of the nerve cells appear more or less as the control (arrow). Other nerve cells are degenerated.(arrow head) (Group III H&E x400)

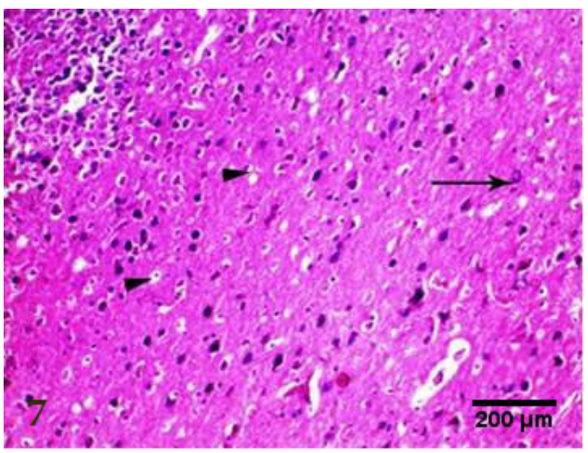


Fig. 7: A photomicrograph of a section from irradiated rat's cerebral cortex pretreated with Nigella showing ., Some nerve cells appear more or less as the control (arrow). others appear degenerated (arrow head). (Group IV H&E X200)

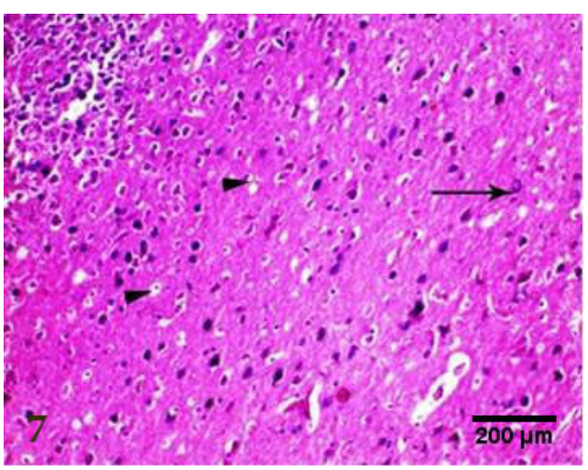


Fig. 8: A magnified image of the previous section showing ., Some nerve cells appear more or less as the control (arrow). Others are degenerated (arrow head). (Group IV H&E X400)

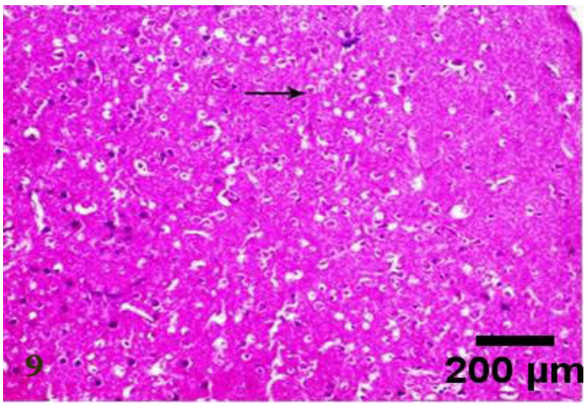


Fig. 9: A photomicrograph of a section from irradiated rat's cerebral cortex pretreated with Melatonin showing some of nerve cells are degenerated leaving empty spaces (arrow),others are similar to control(arrow head). (Group V H&E X200)

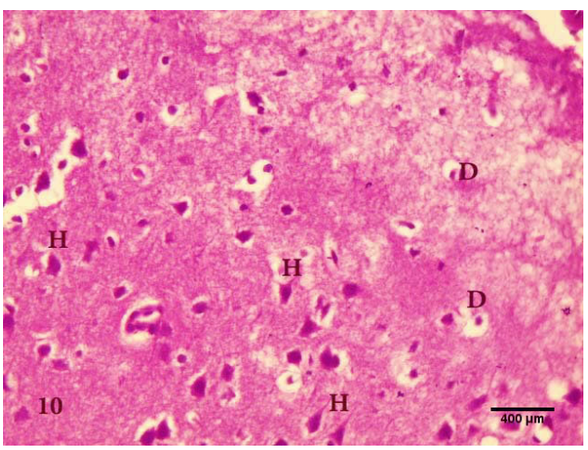


Fig. 10: A photomicrograph from irradiated rat's cerebral cortex pretreated with Melatonin showing some of nerve cells are degenerated leaving empty spaces (D). Others are similar to control (H) (Group V H&E X400)

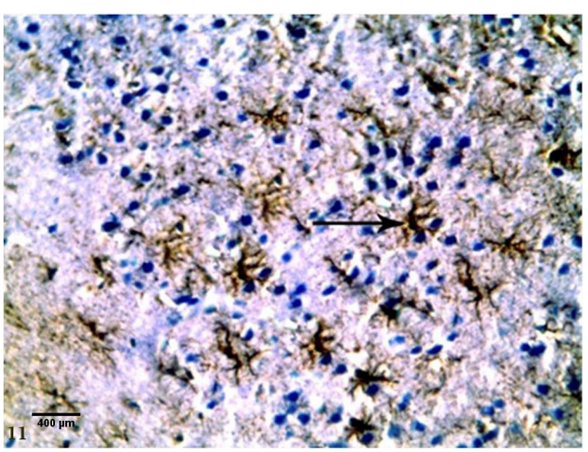


Fig. 11: A photomicrograph of a section from a control rat's cerebral cortex showing astrocytes with few and thin processes (arrow). Note: the brown coloration in the cytoplasm of astrocytes and their processes.). (Group I GFAP immunostainedx400)

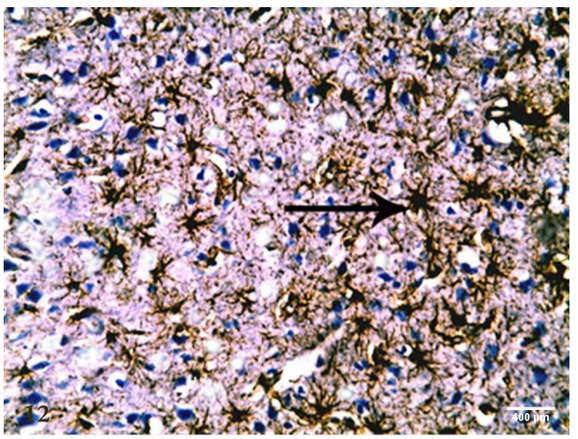


Fig. 12: A photomicrograph of a section from irradiated rat's cerebral cortex showing., proliferation of astrocytes (arrow). (Group II GFAP immunostainedx400)

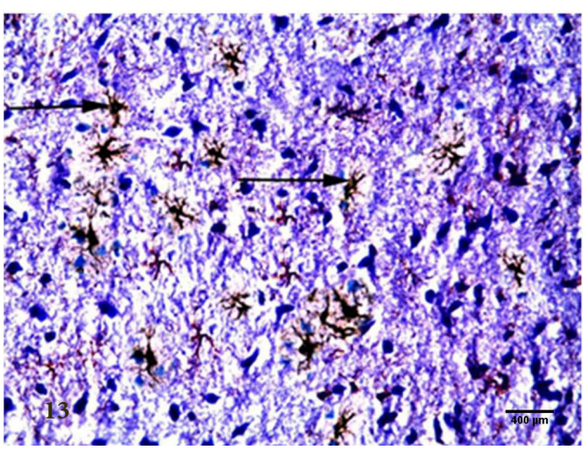


Fig. 13: A photomicrograph of a section from irradiated rat's cerebral cortex pretreated with vitamin E showing., astrocyte number is less than the irradiated one. (Group III GFAP immunostainedx400)

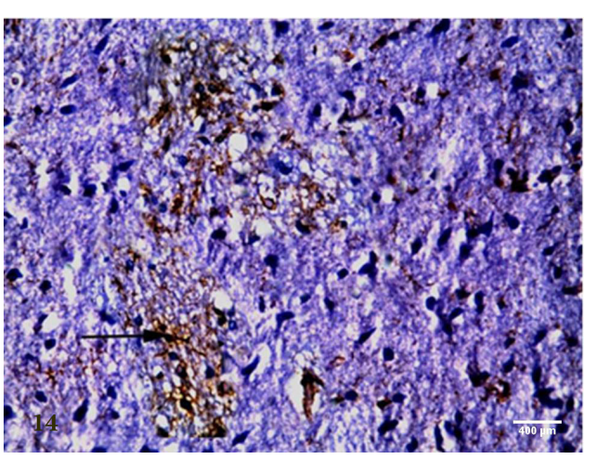


Fig. 14: A photomicrograph of a section from irradiated rat's cerebral cortex pretreated with Nigella showing., astrocyte number is less than irradiated one with localized distribution (arrow) (Group IV GFAP immunostainedx400).

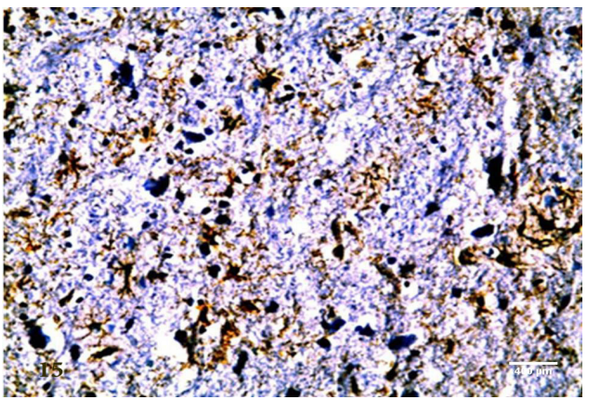


Fig. 15: A photomicrograph of a section from irradiated rat's cerebral cortex pretreated with melatonin showing., astrocyte number is less than irradiated one (arrow) (Group V GFAP immunostainedx400)

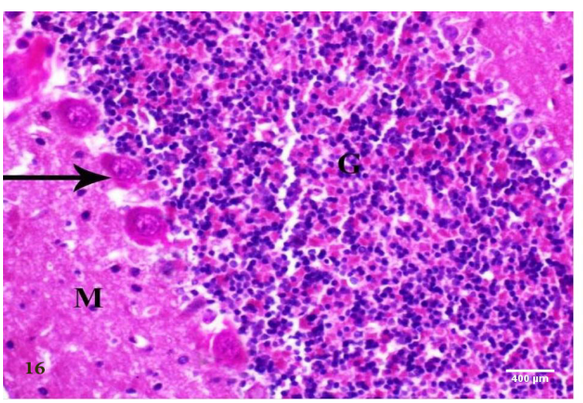


Fig. 16: A photomicrograph of a section from control rat cerebellar cortex showing, molecular layer(M) . Purkinje cell layer with their rounded vesicular nucleus (arrow) and granular cell layer (G) (Group I H&EX400)

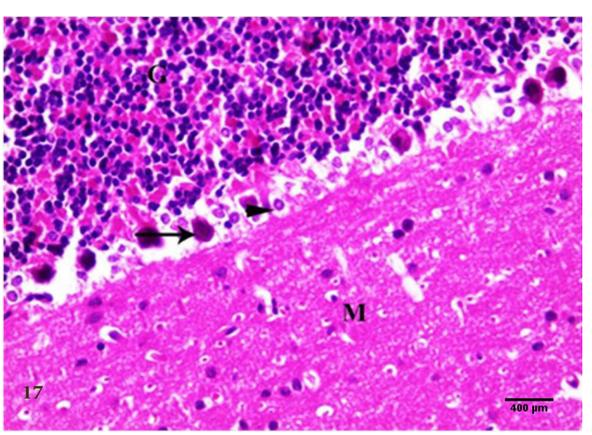


Fig. 17: A photomicrograph of a section from irradiated rat's cerebellar cortex showing., degenerated Purkinje cells with vacuolated cytoplasm (arrow head) other cells are shrunken with deeply stained cytoplasm in granular cell layer(G) and molecular layer are more or less as the control group . There are empty spaces in the molecular layer (arrow). (Group II H&E X400)

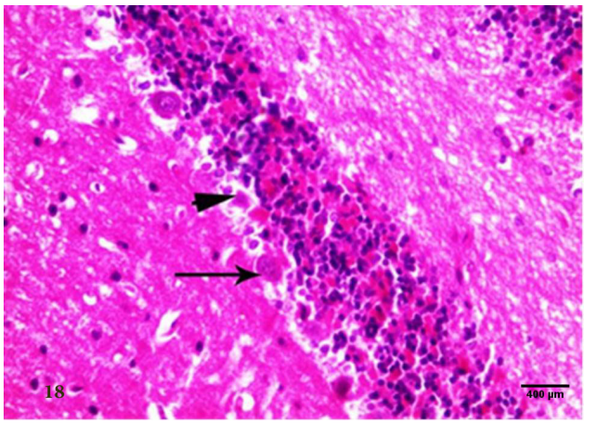


Fig. 18: A photomicrograph of a section from irradiated rat's cerebellar cortex pretreated with Vitamin E showing., some purkinje cells appear more or less as control group (arrow), other cells are shrunken with deeply stained cytoplasm. (arrow head).Granular layer(G) and molecular layer are more or less as the control group (Group III H&EX400)

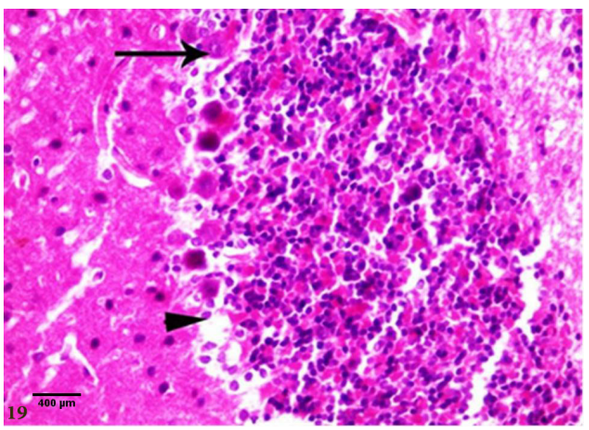


Fig. 19: A photomicrograph of a section from irradiated rat's cerebellar cortex pretreated with Nigella showing., Some Purkinje cells are degenerated other cells are more or less as the control group granular cell layer (G) and molecular layer are more or less as the control group. (Group IV H&EX400)

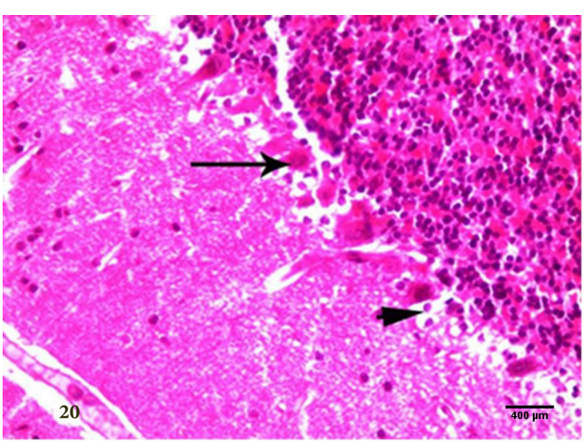


Fig. 20: A photomicrograph of a section from irradiated rat 's cerebellar cortex pretreated with melatonin showing., Some Purkinje cells are degenerated (arrow head) other cells has deeply stained small nuclei. Granular cell layer(G) and molecular layer are more or less as the control group (Group V H&EX400)

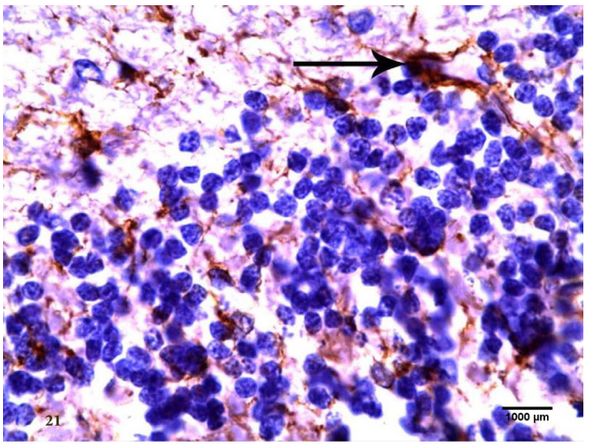


Fig. 21: A photomicrograph of a section from control rat's cerebellar cortex showing, positive immun-reaction for GFAP in the cytoplasm of astrocytes that appear with few, thick processes (arrow) in the granular and pukinje layer . Note: the brown coloration in the cytoplasm of astrocytes and their processes. (Group I GFAP immunostainedx1000)

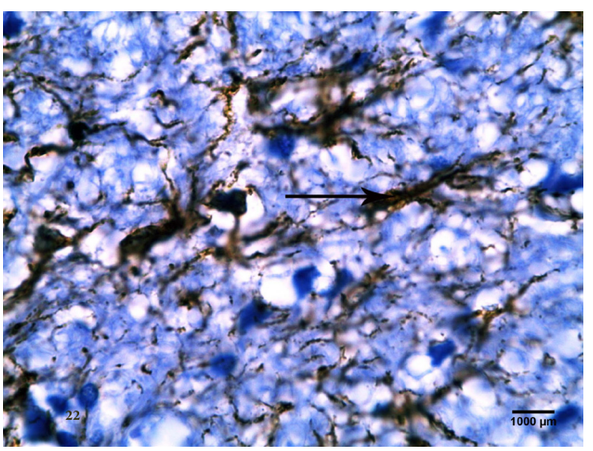


Fig. 22: A photomicrograph of a section from irradiated rat's cerebellar cortex, showing the number of astrocytes as well as intensity of their reaction for GFAP staining increased compared to the control group (Group II GFAPimmunostainedx1000)

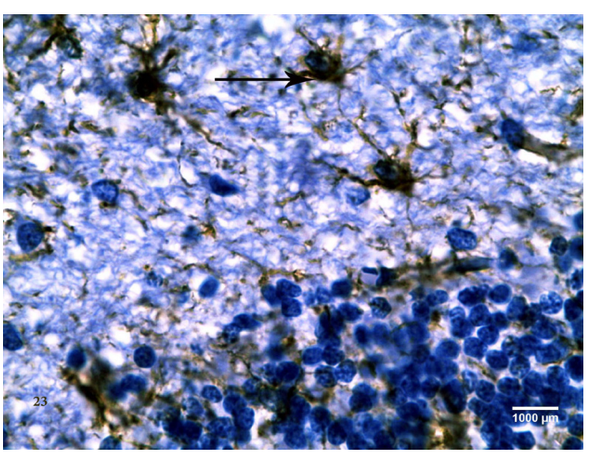


Fig. 23: A photomicrograph of a section from irradiated rat's cerebellar cortex pretreated with vitamin E showing, the number of the astrocyte as well as their intensity of the reaction are less than irradiated one . (Group III GFAP immunostainedx1000)

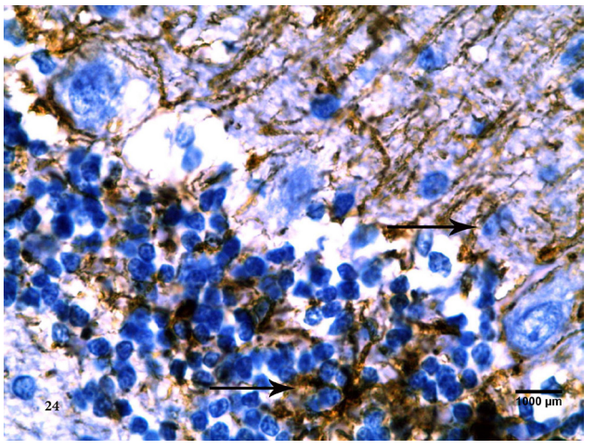


Fig. 24: A photomicrograph of a section from irradiated rat's cerebellar cortex pretreated with Nigella showing, the astrocyte number and intensity of staining are less than irradiated group (Group IV GFAP immunostainedx1000)

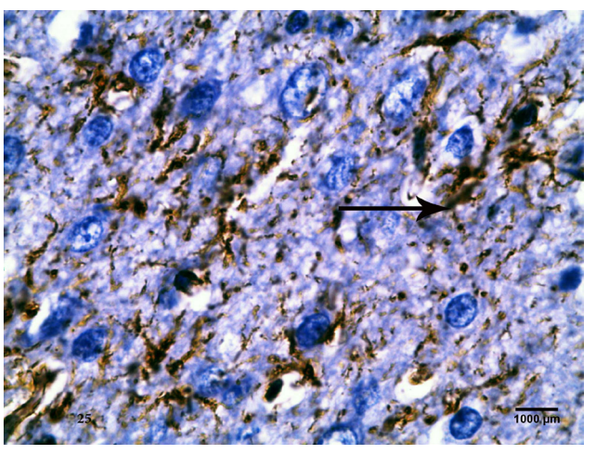


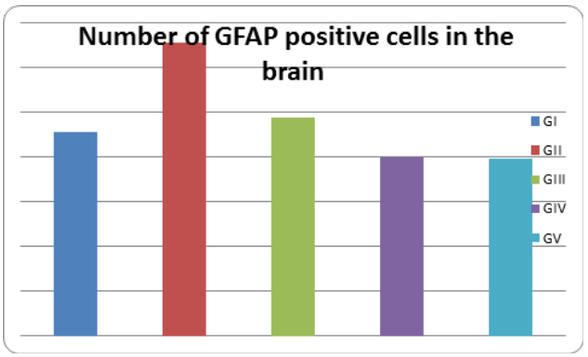
Fig. 25: A photomicrograph of a section from irradiated rat's cerebellar cortex pretreated with Melatonin showing, the astrocyte number is less than the irradiated one (Group V GFAP immunostainedx1000)

Table 1: Mean of GFAP positive cells in the brain

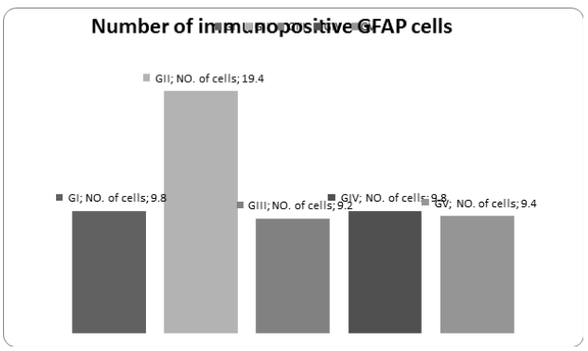
Groups	mean±SD	*P value*
GI	22.8±5.44	
GII	32.2±6.68	
GIII	24.4±4.33	0.001*
GIV	20±8.33	
GV	19.8±7.75	

Table 2: Mean of GFAP positive cells in the cerebellum

Groups	mean±SD	*P value*
GI	9.8±0.83	
GII	19.4±1.1	
GIII	9.2±0.83	0.000*
GIV	9.8±1.3	
GV	9.4±0.89	



Histogram 1: Showing the difference between GFAP positive cells in the brain



Histogram 2: Showing the difference in GFAP positive cells in the cerebellar cortex between the different groups.

DISCUSSION

Cranial radiation is an essential therapeutic tool in the treatment of primary and secondary brain tumors. It has adverse effects including cognitive dysfunction which can affect the quality of life[1,2].

Radiation affect normal tissues a well as the tumor mass. The effect on normal tissue can range from mild discomfort to life threatening effects. The rates of these responses depend on the amount and distribution of the dose of radiation received by tissues[3,4].

Although x-ray is widely used for both imaging and therapeutic purposes, their possible early and acute morphological changes on the cerebral and cerebellum cortices are few in literature.

In the present study, irradiation resulted in necrosis and degenerative changes in the cerebral cortex. These results are in accordance with Erol *et al*., 2004 who reported that 7.2 Gy whole body induced brain necrosis and neuronal degeneration with an increase in brain malonaldehyde level which is an oxidative stress marker[22]. Other studies reported that exposure of male rabbits to radiation showed increase in the oxidative stress markers resulting in brain necrosis and congestion[23].

Brain tissues are highly sensitive to abnormal levels of reactive oxygen species and their defensive mechanisms are limited. Moreover, the free radical are involved in the development of the inflammatory response, which is an important element of the pathogenesis of neurodegenerative diseases such as Alzheimer's disease[24] or Parkinson's disease[25].

A significant increase in the number of GFAP positive astrocytes was observed in the present study in the XRI group compared to the control. This is in accordance with Chan and his colleagues (2009) who reported, the hypertrophy and hyperplasia of GFAP positive astrocytes at the site of irradiation in the ipsilateral brain relative to the contralateral side[26].

Reactive gliosis is a reaction to brain injury to restrict inflammation and neuronal death. Astrocytes react to injury by hypertrophy and up-regulation GFAP. It is reported that ,some astrocytes acquire stem cell properties in adult mouse cerebral cortex after injury and hence may provide a promising cell type to initiate repair after brain injury[27,28,29].

The cross-link between astrocyte and neuron occurs through the release of several neurotrophic factors that maintain CNS homeostasis[30,31]. Activated astrocytes secrete different neurotrophic factors to improve the neuronal survival. On the other hand, it was found that rapid and severe activation led to an inflammatory response and neuronal death[32,33].

Some studies suggest that intrinsic recovery and repair responses induced by specific cytokines released by astrocytes may initiate secondary reactive processes, resulting in the progressive development of a persistent oxidative stress on the CNS months after irradiation and that this oxidative stress may induce the development of radiation injury in the more sensitive white matter of the brain[34]. Some studies demonstrated that vascular/ inflammatory morphologic changes predominate the earliest stages of the post-radiation response and are followed by morphologic cellular and interstitial changes in the CNS white matter[35]. Some explanations reported by other authors , impaired perfusion due to cerebral vessel occlusion resulting from luminal constriction because of fibrinoid necrosis, endothelial proliferation, and periadventitial fibroblastic proliferation with coexistent thrombus plays an important role in the development of late radionecrosis[36]. The development and extent of all these pathologies depend on radiation dose, duration of administration, and frequency[37].

In the present study, Pre-irradiation use of vitamin E resulted in return of the lamellar pattern in the cerebral cortex with some nerve cells appeared degenerated. Immunohistochemical and morphometric study revealed a significant decrease in number of astrocytes compared to the previous group. Some studies revealed that vitamin E therapy reduced radiation injury via its antioxidant effects by combating free radicals, whether given before or immediately after irradiation[38]. Additionally, vitamin E supports immunity and protects bone marrow cells from the harmful effects of gamma radiation by not only scavenging free radicals but also stimulating the repairing factors of DNA[39]. Some authors found that vitamin E reduced only necrosis but did not prevent vasodilatation[40].

Previous studies on rats have shown that vitamin E and other antioxidant vitamins, by decreasing oxidative stress, could have protective effect against radiation-induced injuries such as oral mucositis, myelosuppression[41], intestinal injuries[42], lipid and DNA damage to the liver[43], and cataract[44]. Protection against radiation-induced damage in small bowel crypts of rats[45], modification of micronucleus induction by g rays in mice[46], and in-vitro reduction in the number of micronuclei in human lymphocytes before and after gamma-ray irradiation have also been reported by others[15]. The same results were found in salivary gland[47]. On the other hand, some authors found no changes resulted in pre-irradiation treatment with vitamin E[48].

In a similar study, Siu *et al*. investigated lipid antilipid peroxidation effects of melatonin and vitamin E on retinal homogenates in rats and found that melatonin's prevention of lipid peroxidation was 7.2-fold greater than that of vitamin E[49].

Brain tissue is highly sensitive to free-radical damage because of its low level of endogenous antioxidants, notably vitamin E and superoxide dismutase. Vitamin E protects the integrity of acetyl choline receptors in normal neurons. it prevents toxicity and apoptosis induced by ROS producing amyloid _ peptides that increase in the brain with age and in dementia[50].

In the current study, examination of the rat's cerebral cortex treated with Nigella revealed nerve cells appeared more or less as the control group. There was a highly significant decrease in the number of astrocytes in nigella treated group compared to the XRI one. Similar results were reported by others in the liver tissue. It was found that nigella sativa oil reduced oxidative stress and has antioxidant effects when administered 1 hour before irradiation[21,51]. Some authors found that extract of Nigella sativa seeds, could be used in combination with radiation to protect against oxidative stress in normal tissues and improve the quality of life of cancer patients by mitigating unwanted side effects of radiation in normal tissues[52].

In the present study ,melatonin pretreated group showed partial improvement with a significant decrease in the number of GFAP positive astrocytes. Erol and his colleagues (2004) reported that melatonin may be useful in preventing the pathological changes of secondary brain damage as a result of free oxygen radicals generated by irradiation[22].

Also, melatonin reduces oxidative stress markers and augments anti-oxidant capacity in the rat lens[53] and testis[19]. It also provide a significant decrease in the DNA

strand breakage and lipid peroxidation hence protect brain cells from oxidative damage induced by radiation[54].

These results are in accordance with the studies of pretreatment with melatonin prevented radiation induced damage on peripheral blood cells[55]. Irradiation with 6 and 8 Gy associated with increased malonaldehyde, nitric oxide levels and were reduced with melatonin[56]. Therefore, melatonin by its antioxidant properties and free radical scavenging ameliorates irradiation induced cell damage[57]. Babaee reported that melatonin could protect neurons and glial cells following traumatic brain injury in rat. Its administration decreasing GFAP positive astrocyte number (astrogliosis), as well as the number of apoptotic neurons in brain cortex of traumatic brain injury[58].

Experimental and clinical data confirmed that melatonin has anti-inflammatory effect and exerts important role in the reduction of adhesion molecules and pro-inflammatory cytokines. In addition to its direct free radicals scavenger and antioxidant enzymes stimulating effect[59].

In the current study, with examination of irradiated rat cerebellar cortex, mild dissociation of Purkinje cell layer and molecular layer was observed. Purkinje cells showed degenerative changes. Win *et al.,* 2000 reported that, although Purkinje cells were resistant to X-ray induced mortality, they exhibited disturbed alignment with abnormal dendritic arborization[60]. Many authors suggested that susceptibility of the cerebellum to irradiation should be taken into consideration for future protective strategies[61]. This is in accordance with the study of Li Cui and his colleagues[62] which reported that total body irradiation significantly increased vacuolization of the molecular layer. At high magnification, deformed fiber-like structures were found along with the empty matrix space. Necrotic Purkinje cells were observed. Substantial damage to the cerebellum can be detectable as early as 1–3.5 days in adult animals following sublethal total body irradiation. Oxidative stress, inflammatory response and calcium neurotoxicity-associated mechanisms are involved in radiation-induced neuronal damage. Some authors explained the oxidative stress that irradiation induces radiolysis of water. It generates reactive radicals, which initiate oxidative damage of intracellular target molecules including RNA, DNA and membrane lipid[63]. Similar findings were reported in irradiation-induced chronic neuronal damage: Oxidative stress, inflammatory response and calcium neurotoxicity-associated mechanisms are involved in radiation-induced neuronal damage[64].

In our results, there was proliferation of the astrocytes with increase their intensity for staining in examination of irradiated rat's cerebellar cortex. These results in accordance with some authors who explained this due to astrocytes preserve neuronal survival through inactivation of ROS[65]. Glial cells exhibit one of the earliest and most obvious cellular responses, reactive gliosis, following a variety of insults to the CNS[66].

Examination of irradiated rats pretreated with vitamin E showed partial improvement of Purkinje cells changes. It is known that the brain tissue is highly sensitive to free-radical damage because of its low level of endogenous antioxidants, notably vitamin E and superoxide dismutase. Vitamin E protects the integrity of acetyl choline receptors in normal neurons and prevents toxicity and apoptosis induced by ROS-producing amyloid β peptides that increase in the brain[50]. The role of vitamin E is to scavenge free radicals thereby preventing radiation-induced damage to the cell membrane[67]. The action of free radicals leads to oxidative stress and lipid peroxidation, which may result in cell death[68]. Further investigation is required to assess other radiation doses and evaluation times and mainly for application in humans[20]. Farombi and Onyemamentioned that, dietary antioxidants as vitamin E had protective potential against oxidative stress induced by some toxins[69]. Some studies reported that vitamin E reduced only necrosis in rat exposed to gamma irradiation[40], while others reported that, protective effects of vitamin E were not observed in their study[70].

In the present study, The rat cerebellar cortex, treated with Nigella, was similar to control group. Few showed some degenerated Purkinje cells. These results are in accordance with previous study of Ahamed and Siddiqui who found that Nigella sativa has reduced the damage done to the cerebral cortex and this agrees to the fact that natural compounds are rich in antioxidants, can reduce oxidative stress and alleviate the effect of oxidative agents[71]. Kanter in 2008 obtained similar results when he evaluated the effects of N. sativa on induced neuronal injury by chronic toluene exposure in the frontal cortex and brain stem in rats. He reported no histopathological lesions after treatment with nigella sativa for 12 weeks[72]. Nigella sativa also provided protection to hippocampal cells exposed to lead and the frontal cortex[73]. The findings of this study also indicate a dose dependant relationship.

The same results obtained by Jagetia and Ravikiran[51]. They explained this radioprotective effect may be due to free radical scavenging and increased antioxidant status. They said that administration of NSE protected mice against the radiation-induced sickness and mortality. The principle mechanism of radioprotection seems to be scavenging of various radiation-induced free radicals and increase in the activity of antioxidant enzymes. The molecular mechanisms that may have played important role in radioprotection by NSE include inhibition of radiation-induced transactivation of NF-κB, suppression of radiation-induced elevation in COX-II expression. They suggested that its radioprotective action may be mediated by the presence of various phytochemicals including thymoquinone, nigellidine, and sesquiterpenelongifolene. Other authors proved the radioprotective effect of Nigella on peripheral blood[74].

In our study, an examination of cerebellar cortex of rats treated with melatonin demonstrates that most Purkinje cells are similar to control group. Granular cell layer and molecular layer are more or less as the control group. This is in accordance with Sisodia and his colleagues supported the idea that melatonin may be used as an anti-irradiation drug due to its potent free radical scavenging and antioxidative efficacy[75].

The same results obtained by Cakman and his colleagues on salivary gland[17] bone[71] and radisensitive organs as skin, GIT and bone marrow[76]. Some authors explain mechanism of melatonin radioprotective effect by many theories. Melatonin is antioxidant and free radicle scavengers[78&79]. So it has neuroprotective mechanism[80]. Melatonin is able to reduce the incidence of necrosis and subsequent inflammatory responses after total body irradiation.

Melatonin administration to rats has shown amelioration of neuronal necrosis and degeneration, leading to reduction of edema and histopathological changes in the brain[40]. Melatonin has shown ability to change genes involved in apoptosis. The most common regulatory genes involved in apoptosis following exposure to radiation are Bcl-2 and Bax[81]. After exposure to IR, down regulation of Bcl-2 and upregulation of Bax stimulate apoptosis via stimulation of caspase-3 and release of cytochrome C from the mitochondria. Mohseni and his colleagues. evaluated the anti-apoptosis role of melatonin on rat's peripheral blood lymphocytes. Their results showed that melatonin reduces apoptosis via reduction of bax/bcl-2 ratio. This was more obvious for higher doses of melatonin[81]. Similar results were obtained in an in vitro study by Jang *et al*.[82]. Melatonin is a potent stimulator of DNA repair responses such as BER pathway genes. These properties of melatonin could alleviate acute reactions during radiotherapy[76]. Melatonin counteract any potential oxidative DNA damage in lung tissue after partial body irradiation. Melatonin could modulate the indirect destructive effect of radiation and reduce DNA damage in non-targeted cells[83]. In our study. Cerebellum pretreated with melatonin showed decrease in astrocytes than irradiated ones. This is explained by Baydas and his colleagues (2003). Their results suggest that elevated oxidative stress causes increased glial reactivity and administration of melatonin represents an achievable adjunct therapy for preventing gliosis[84].

CONCLUSION

Vitamin E, Nigella sativa and Melatonin are strong antioxidant and neuroprotective agents against brain and cerebellar damage induced by radiation.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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الملخص العربى

التاثير الوقائى المحتمل لفيتامين هـ وزيت حبة البركة والميلاتونين ضد التغيرات المبكرة والحادة المحدثة بالاشعة السينية على قشرة المخ والمخيخ فى الجرذان البيض: دراسة نسيجية وكيمائية نسيجية مناعية

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مقدمة: الأضرار الدماغية الناجمة عن الإشعاع شائعة في المرضى الذين يتلقون العلاج الإشعاعي لأورام الرأس والعنق. يمكن أن يكون إعطاء مضادات الأكسدة قبل التعرض للاشعاع مثل فيتامين هـ وزيت حبة البركة والميلاتونين له دور في الحد من هذه الأضرار.

المواد والطرق المستخدمة: تم استخدام ٤٠ جرذًا وقسمت إلى خمس مجموعات مختلفة. المجموعة الأولى وهى المجموعة الضابطة والتى تم تقسيمها الى ٤ مجموعات فرعية وتشمل كل منها خمس حيوانات. المجموعة الفرعية الأولى، المجموعة الفرعية الثانية: تمت معالجة الحيوانات باستخدام فيتامين هـ ٣٠٠ مجم/كغم عن طريق الفم مرة واحدة، المجموعة الفرعية الثالثة تم معالجة الحيوانات باستخدام زيت حبة البركة ١ مل / كجم عن طريق الفم مرة واحدة، المجموعة الفرعية الرابعة: تم معالجة الحيوانات بالميلاتونين ١٠٠ ملغم / كغم من وزن الجسم داخل الصفاق مرة واحدة).

المجموعة الثانية: تعرض الفئران إلى الأشعة السينية (8 Gy لكل الجسم). المجموعة الثالثة: تمت معالجة الحيوانات باستخدام فيتامين هـ ٣٠٠ مجم/كغم من وزن الجسم عن طريق الفم مرة واحدة ساعة قبل التعرض للاشعاع. المجموعة الرابعة: تم معالجة الحيوانات باستخدام زيت حبة البركة ١ مل / كجم عن طريق الفم مرة واحدة ساعة قبل التعرض للاشعاع. المجموعة الخامسة: تم معالجة الحيوانات بالميلاتونين ١٠٠ ملغم / كغم من وزن الجسم (داخل الصفاق مرة واحدة ساعة قبل التعرض للاشعاع).

النتائج: تسبب الإشعاع فى فقدان للنمط الرقائقي في القشرة الدماغية مع تغيرات تحللية فى الخلايا العصبية. في المخيخ، لوحظ تغيرات تحللية فى خلايا بركنجى. كما تسبب الإشعاع في زيادة ذات دلالة احصائية في عدد الخلايا النجمية الايجابية للبروتين الحامضى الليفى الدبقى في كل من القشر ة الدماغية و وقشرة المخيخ الذي انخفض في جميع المجموعات المعالجة.

الاستنتاج: إن استخدام مضادات الأكسدة مثل فيتامين هـ، وحبة البركة والميلاتونين يمكن أن يحد من الاثارالضارة للإشعاع على الدماغ والمخيخ.