RESEARCH PAPER | Zoology | Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Prophylactic Effect of Sesame Oil Against Radiotoxicity With Special Reference to Mice Brain

KEYWORDS: Whole brain, cerebrum, cerebellum, oxidative stress, lipid peroxidation, glutathione, nucleic acids, purkinje cells.

*** Shikha Patni**
Department of Zoology, University of Rajasthan, Jaipur, India * Corresponding Author

Rashmi Sisodia
Department of Zoology, University of Rajasthan, Jaipur, India

ABSTRACT *Study was done to evaluate the biochemical, qualitative and quantitative histopathological changes after 5 Gy whole body irradiation and its modulation by supplementation of Sesame oil on male Swiss albino mice brain up to 30 days. For this, healthy mice from an inbred colony were divided into four groups: (i) Control-vehicle treated (ii) Sesame oil treated-mice in this group were orally supplemented with Sesame oil (150 µl/mice/day) once daily for 15 consecutive days; (iii) Irradiated mice (iv) SO + irradiated-mice in this group were orally supplemented with SO for 15 days (once a day) prior to irradiation. Marked radiation induced changes in the amount of whole brain lipid peroxidation (LPO), Glutathione (GSH), protein, nucleic acids and histopathological changes could be significantly ($p < 0.001$) ameliorated specially at later intervals by supplementation of SO prior to irradiation. The results of present study showed that prior supplementation of SO has radioprotective potential as well as neuroprotective properties against the radiation.*

INTRODUCTION

Humans have always been exposed to natural background radiation. During oxidative stress and exposure to radiation, excessive free radicals are produced [1,2]. The free radicals disturb cellular homeostasis by enhancing the peroxidation of membrane lipids, oxidation of proteins, base damage and adduct formation in DNA which ultimately leads to cell death if the damage is beyond repair. The inherent antioxidant defense systems of the cell competitively counteract the oxidative stress[3]. However, free radical insult surpassing the inherent cellular defense mechanism warrants external antioxidant supplementation. Naturally occurring antioxidants may also provide an extended window of protection against low dose-rate irradiation, and may have therapeutic potential when administered after irradiation. Several plant constituents have been proven to possess considerable free radical scavenging or antioxidant activity [4-14].

For the present investigation, sesame oil (til oil), a very ancient ingredient, has been selected. The Assyrians, more than 600 years BC, used it as a vegetable oil. Still used as a medicine in India, oil pressed from the raw seed is used as massage oil in ayurvedic medicine. Sesame and its lignans boost antioxidant levels, reduce inflammation, normalize blood pressure, improve lipid levels, and promote fat burning. They act synergistically with other nutrients such as gamma tocopherol, fish oil, and conjugated linoleic acid, thereby enhancing the bioavailability and effectiveness of those nutrients. Through its many biochemical actions, sesame may help in managing some of today's most pressing health concerns, including heart disease, high blood pressure, obesity, and inflammatory disorders [15, 16]. Several pharmaceutical uses have been identified from sesame. The high antioxidative properties of sesame appear to be related to lignans, such as sesamol [17], sesamolinol, pinoresinol, p1[18, 19] and sesaminol[20, 21]. These compounds also have inhibitory effect on membrane lipid peroxidation, the microsome peroxidation induced by ADP-Fe3+/NADPH [21] and the oxidation of LDL induced by copper ions [22] and show synergistic effects in elevating the levels of vitamin E in rat liver and plasma [23, 24].

It was found that dietary sesame oils inhibits iron-induced oxidative stress in rats[25]. The nicotine-induced oxidative and genotoxic damages on the tissues can be effectively attenuated by sesame lignans supplemented diet[26]. Protective effect of sesamol was found by Kumar[27] against 3-Nitropropionic Acid-induced cognitive dysfunction and altered glutathione redox balance in rats. In another study it has been observed that sesame lignans ameliorates lipid profile and lipid peroxidation in induced diabetic rats[28]. It was recently found that sesaminol acts as an antioxidant by scavenging peroxyl radicals[20]. Myristic acid has cancer preventive capability and is found in sesame seed[29]. Shimizu et al.[30] suggest that sesamolin may contribute to the antioxidative properties of sesame seeds and oil and support the hypothesis that sesame lignans reduce susceptibility to oxidative stress.

In order to provide a novel insight into radioprotection, we tried to evaluate the effect of oral administration of sesame oil before irradiation, on brain dysfunction with reference to biochemical estimations in whole brain, qualitative and quantitative histopathological changes in the cerebellum and cerebrum.

Materials and methods

Animals

The Departmental Animal Ethical Committee (DAEC) approved the study. Animal care and handling was done according the guidelines of INSA (Indian National Science Academy, New Delhi, India). Swiss albino mice (Mus musculus) (6-8 weeks old, weighing 25±2 gms) from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature (25±2 °C) and light (light: dark, 14 : 10 hrs). Four animals were housed in a poly propylene cage containing sterile paddy husk (procured locally) as bedding through the experiment. They were provided standard mice feed (procured from Hindustan Lever Ltd. Mumbai) and water ad libitum.

Chemicals

Thiobarbituric acid was purchased from Sigma, USA and Sesame oil was procured from standard general store. All other chemicals used were of analytical grade.

Source of Irradiation

The cobalt teletherapy unit (ATC-C9) at the Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajsthan, India, was used for irradiation. Unanesethesized animals were restrained in well-ventilated Perspex box and whole body exposed to gamma radiation.

Dose Selection of Sesame Oil

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Dose selection was performed on the basis of a drug tolerance study. Various doses of sesame oil 100µl, 150µl, 200µl, 250µl/mice /day) were tested against 10 Gy of gamma radiation. Percentage survivability at 150µl / mice /day for sesame oil was higher than the other, hence 150 µl/ mice /day was obtained as optimum dose based on survivability of mice. This dose was used for further experiments (Fig. 1).

Experimental Design
For studying modification of radiation induced oxidative stress by sesame oil animals were divided into four groups having twelve animals in each group:

Group I. Control (vehicle treated)): Mice of this group did not receive any treatment.

Group II. SO treated-mice: In this group mice were orally supplemented with sesame oil (150 µl/mice/day) once daily for 15 consecutive days.

Group III. Irradiated mice (IR): The animals were exposed whole body to gamma radiation (5Gy) at a distance (SSD) of 77.5 cm. from the source to deliver the dose rate 1.07 Gy/ min.

Group IV. SO + irradiation**:** Mice were orally administered SO (150 µl/mice/day) for fifteen consecutive days then exposed to a single dose of whole body to gamma radiation (5Gy with the same dose rate) one hour after last treatment with SO.

Removal of Brain Tissue
Animals of each group were humanly sacrificed by cervical dislocation and autopsied at various post irradiation intervals viz,1-30. To remove brain an incision was given at the sides of the jaws to separate the upper and the lower palates. The upper palate was cut in the middle and after having cleared the surrounding tissues; brain was excised and separated from the spinal cord at the decussation of pyramids. Whole brain was used to estimate various changes in biochemical parameters and cerebellum was fixed in bouins for paraffin sectioning and stained with hematoxyin, eosin for histopathological studies.

Biochemical assay
Lipid peroxidation (LPO) assay. LPO was measured by the method of Buege and Aust [31]. Briefly, 1.2 ml solution of TCA-TBA-HCl prepared in 1:1:1 was added to tissue homogenate (0.8 ml). This final mixture was heated on a water bath for 30 min at 80°C and cooled. After centrifugation the absorbance was recorded at 532 nm using a UV (ultraviolet)-Visible double beam spectrophotometer. The LPO has been expressed as malondialdehyde (MDA) in n mole/gm tissue.

Reduced glutathione (GSH) assay. The reduced glutathione (GSH) content of tissue samples was determined by the method of Moron et al. [32]. Tissue sample was homogenised in the sodium phosphate-EDTA (ethylenediaminetetraacetic acid) buffer then 0.6 M DTNB [5, 5_dithiobis-(2-nitrobenzoic acid)] was added. The optical density of the yellow coloured complex developed by the reaction of GSH and DTNB was measured at 412 nm using a ultraviolet (UV)-vis spectrophotometer. The results were expressed as nano mol/100 mg of tissue.

Protein assay. Estimation of protein was based on the method proposed by Bradford [33] and 10% homogenate was prepared (1 gm of tissue in 9 ml of NaCl) and 0.1 ml of the sample was taken for the Bradford assay. Three repeats of the assay from each animal were carried out. The absorbance was read at 595 nm.

Deoxyribonucleic acid (DNA) estimation assay. DNA was measured by the method of Ceriotti[34]. The brain was dissected out and homogenate was prepared in glacial distilled water (10 mg/ml). 2 ml homogenate was taken in a centrifuge tube and 1 ml indole reagent was added, followed by 1 ml concentrated HCl. The tubes were kept in a water bath for 10 min for boiling and then cooled under running tap water; 4 ml of this solution was taken out and extracted three times with 4 ml of chloroform. The water layer from the organic layer was separated out by centrifugation. The intensity of the yellow colour lift in H_2O phore at green filter against blank was measured out. The volume of DNA from graph was calculated and then the quantity of DNA/gm of tissue was calculated. Blank: 2 ml H_2O + 1 ml indole +1 ml concentrated HCl + 4 ml chloroform.

Ribonucleic acid (RNA) estimation assay. RNA was measured by the method of Ceriotti[35]. The brain was dissected out and homogenate was prepared in glacial distilled water (10 mg/ml). 5 ml homogenate was taken in a centrifuge tube and mixed with 5 ml orcinol reagent. The tubes were kept in a water bath for 40 min for boiling and then cooled under running tap water; 5 ml Isoamyl alcohol was added to each tube and centrifuged for 5 min. The upper coloured layer was separated with the help of a vaccumiser and the intensity was measured against red filter. The concentration of RNA from standard graph was calculated and then the amount of RNA/ gm of tissue was calculated. Blank: 5 ml H_2O + 5 ml orcinol + 5 ml Isoamyl alcohol.

Histopathological study. Blind examination of the stained sections of cerebrum (diencephalon region) and cerebellum, were done using a light microscope, at a magnification of 40x. Counts of Purkinje cells were made for a total length of 2500 µm in the lobule simplex VI B[36] in ansiform lobule using an occularmeter in the eyepiece, in mice cerebellum.

Volumes of Purkinje cells were measured using the formula $4/3\pi r^3$ where 'r' is the radius of the cell. In order to determine the radii of the Purkinje cells, measurement at their major and minor axes was taken and the average obtained by dividing them by '2'.

Thickness of molecular and granular layers were also observed at a magnification of 40x and expressed in terms of µm.

Statistical analysis
The results obtained in the present study were expressed as mean ± Standard error of mean. Statistical difference between various groups were analyzed by student t-test and the significance was observed at $P<0.001$, $P<0.005$ levels.

Results
Irradiation raised the LPO levels at all the autopsy intervals. Initially sharp increase was noted (approximately 57%) in comparison to later autopsy intervals. Oral supplementation of sesame oil prior to irradiation succeeded to bring the LPO level near normalcy (100.95 %) (Table-1). Oral supplementation of only sesame oil (Group-II) was also able to reduce the base line values approximately by 20 % (79.42%).

Glutathione (GSH) content decreased sharply after radiation exposure (Group-III) till 7th day, thereafter slight recovery was noted. In Group-IV GSH estimated was raised at all the corresponding intervals compared to Group-III. Magnitude of such a recovery from oxidative damage was significantly higher ($p< 0.001$, $p<0.005$) in SO+IR group as compared to irradiated mice. Only SO treated mice also showed significant increase ($p< 0.001$) in GSH content as compared to control (Table-1).

Protein estimated also showed statistically significant increase ($p< 0.001$) in SO+IR treated mice brain in comparison to irradiated group at all the autopsy intervals. Only SO treated (Group-II) also showed significant increase ($p<0.001$) in protein content as compared to the control (Table-2).

DNA content declined continuously up to 15th day after irradiation (Group-III), but on day 30, a slight recovery was noticed as evident by increased DNA content. In the

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

SO+IR group values of DNA content increased significantly ($p<0.001$), continuously after day 3 and attained the normal level of DNA by 30th days post-irradiation (Table-2).

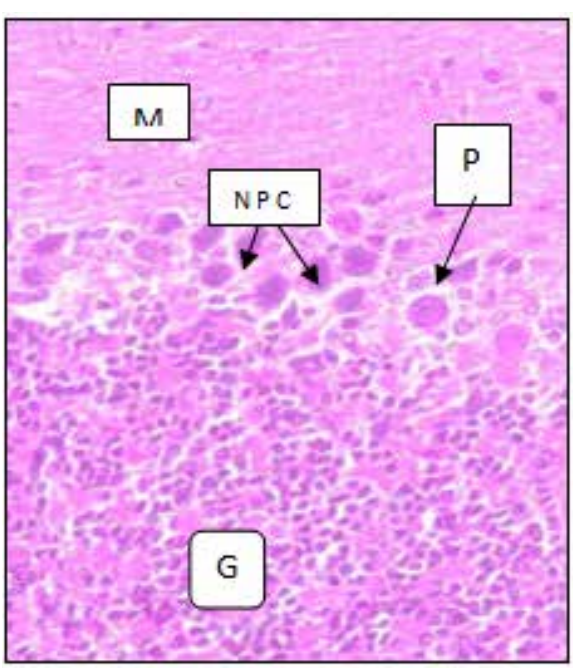
Radiation induced continuous deficit ($p<0.001$) in whole brain RNA was noted in comparison to Group I (Control). In SO pre-treated irradiated group (Group-IV) at all the corresponding intervals, RNA levels were noted at higher concentration compared to only irradiated (Group-III) but the values were still lower by approximately 3.38% from the control at the end of the experiment. Non- significant differences existed between group I and II (Table-2).

Quantitative and histopathological observations

Quantitative histopathological observations in cerebellar folia in both irradiated and experimental groups in terms of alteration of volume and number of purkinje cells indicates a statistically significant ($p<0.001$) deficit, which showed slight recovery after 15 days in Group-III and after 7 days in Group-IV, indicating early recovery. In mice pre-treated with sesame oil, however, the deficit was less than irradiated group mice (Group-III), indicating protection offered by sesame against irradiation induced oxidative stress which resulted in cyto-architectural damage.

Alterations in the thickness of molecular and granular layer in mice cerebellum, control and SO treated mice depicted similar pattern of changes, though molecular layer showed more damage till 7th day but on 15 and 30th day granular layer showed more damage. Maximum reduction (68.41% and 63.96%) in the thickness of molecular layer and granular layer respectively occurred at day 15th from the control. The thickness of molecular and granular layer was more at all the post irradiation intervals in SO+ IR group compared to irradiated group (Table-3).

Histopathological observations in the irradiated mice indicated an extensive damage in the cerebellar folia. The damage included necrosis, pyknosis of Purkinje cells and granular cells together with cytoarchitectural disturbances. Migration of Purkinje cells into granular layer leading to the formation of empty baskets and dissolution of connective tissue could be seen. Reduction in number of Purkinje cells was also noted (Plate 1 and 2). Similar pattern of damage was noticed in SO pretreated irradiated group, though it was of relatively lesser degree. The results indicate protective efficacy of sesame oil against radiation damage (Plate 1 and 2).



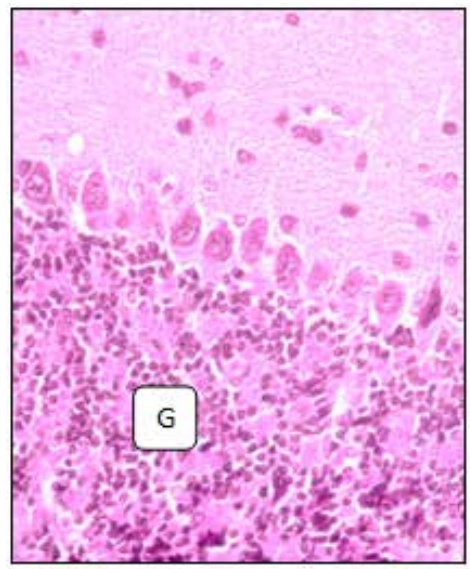


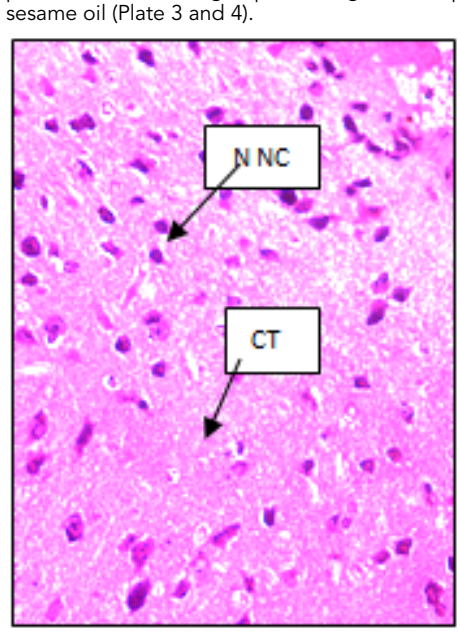
Plate:1 and 2

A. Photomicrograph of cerebellar folia of Swiss albino mice at 3rd day, after irradiation to 5 Gy of gamma radiation (Group-3) (20X).

B. Photomicrograph of cerebellar folia of sesame oil pre-treated mice (SO+IR) at 3rd day post irradiation (Group-4) (20X).

Abbreviations: G= granular layer, M=molecular layer, P= Purkinje cell, NPC=necrotic Purkinje cells

The histological observations of cerebral cortex of irradiated mice showed necrotic and pyknotic neuroglial (astrocytes) cells and pyramidal cells. Axons of pyramidal cells were damaged. Large numbers of necrotic neuroglial cells were seen throughout the cortex with sharp reduction in their number. Mild degree of spongy degeneration of connective tissue was evident. Increased number of glial cells has been noticed with cytoarchitectural disturbance. Relatively lesser degree of damage was noticed in SO pretreated mice. A better cytoarchitecture was seen as compared to irradiated group. Number of cells (cellular density) and number of undamaged cells was relatively higher in sesame oil treated group in comparison to irradiated group, showing a better protection by sesame oil (Plate 3 and 4).



Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

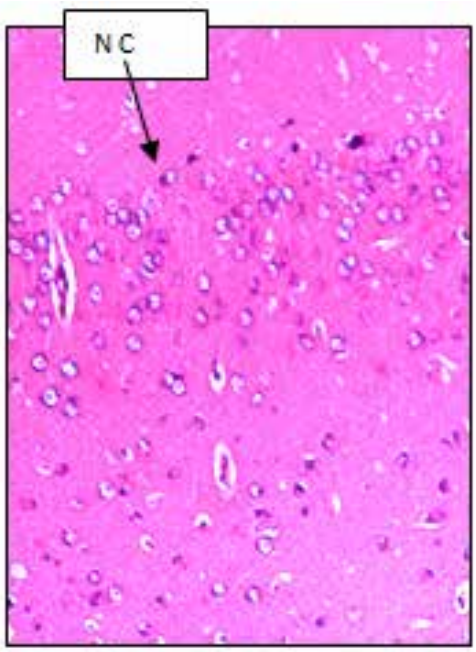


Plate: 3 and 4
A. Photomicrograph of cerebral cortex from the diencephalon region of Swiss albino mice at 3rd day, after irradiation to 5 Gy of gamma radiation (Group-3) (20X).

B. Photomicrograph of cerebral cortex from the diencephalon region of sesame oil pretreated mice (SO+IR) at 3rd day post irradiation (20X).

Abbreviations: NNC= necrotic neuroglial cells, ct=connective tissue, NC= neuroglial cells

Discussion
Results obtained from this study indicate that SO may act as a prophylactic agent and render protection against radiation induced oxidative stress. Oxidative stress leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders [37, 38]. The product of LPO such as MDA (malondialdehyde) and 4-hydroxynonenal are toxic to cells [39]. LPO within the membrane has a devastating effect on the functional state of the membrane because it alters membrane fluidity, typically decreasing it and thereby allowing ions such as Ca^{+2} to leak into the cell. The peroxyl radical formed through lipid peroxidation attacks the protein membrane and enzymes and reinitiates lipid peroxidation. The preservation of cellular integrity of the cellular membrane depends on protection or repair mechanisms capable of neutralizing oxidative reactions.

Inhibition of LPO in cell membrane can be caused by antioxidants[40]. Shimoi et al.[41] concluded that plant flavonoids, which show antioxidants activity in vitro also, function as antioxidants in vivo, and their radioprotective effect may be attributed to their radical scavenging activity. Several reports have suggested that tocopherol and sesame lignans act synergistically as antioxidants [23, 24]. Our results are in accordance with Yamashita et al.[42], who observed that sesame seed and its lignan induced higher gamma tocopherol and lowers TBARS concentration.

In the present study the reduction in the amount of TBARS or MDA equivalents and elevation in the GSH level in the SO treated animals suggests that SO may scavenge the free radicals generated during oxidative stress. GSH, with its sulfhydryl group, functions in the maintenance of the sulfhydryl group of the other molecule (especially protein), acts as a catalyst for disulfide exchange reactions and in the detoxification of foreign compounds like hydrogen peroxide and free radicals. The lesser depletion of brain GSH content in the SO +IR treated group compared to the irradiated group may be an indication of higher availability of GSH, which increases the ability to cope with the free radicals produced by radiation. Decreased brain GSH levels have been reported in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease, in which oxidative processes contribute to the pathology [43, 44]. In the present study, supplementation of only SO has also resulted in statistically significant ($p<0.001$) increase in protein content in comparison to control mice. Decrease in the protein content after exposure to irradiation might be due to either decline in the rate of protein synthesis or increase in the consumption of protein. It may also be the result of the depression of enzyme involved in the activation of amino acid and transferring to t-RNA or by the inhibition of release of synthesised polypeptides from polysomes [45]. Some studies have indicated that oxidative stress diminishes and the levels of some proteins vary during the progression of Alzheimer's disease (AD) [46, 47]. Increased protein concentration in the present study may be due to improved ribosomal activities, which enhance protein synthesis.

DNA damage induced by ROS is involved in mutagenesis and generation of mutation related diseases, including cancer. In the present investigation nucleic acids (DNA+ RNA) concentration showed depletion after radiation exposure which could be elevated by SO supplementation at all intervals indicating a significant protection. Reduced DNA noticed post-irradiation may be due to acute cell death leading to loss of DNA in excess than is normally eliminated from the tissue. The prolonged interphase or delayed onset of DNA synthesis after irradiation also could lead to decreased content of DNA. Egana et al. [48] corroborated present finding that $_{60}Co$ gamma irradiation had more effect in DNA, RNA and protein function (in developing CNS). A correlation seems to exist between DNA degradation and cell pyknosis. The RNA in the transcription of genetic information (mRNA) as components of ribosomes and as an intermediate in the activation of amino acids (acy1 t-RNA) is responsible for active synthesis of new proteins. Although new protein synthesis ceases at maturation, RNA synthesis continues at a diminished rate and a rapid turnover persists throughout the life span of the organisms. The maximum activity of RNA polymerase at birth decreased upto the age of 30 days and remained at the minimal level in adult brain. This is the enzyme responsible for protein synthesis.

Results indicated continuous decline in the number and volume of Purkinje cells up to 15 days post irradiation, then recovery starts in both SO +IR and irradiated group. The reduction in volume and number of Purkinje cells may be due to the radiosensitive nature of the cells. The potent antioxidative action of SO may make it a significant factor on reducing damage to the brain from oxidative stress. Gueneau et al.[49] also reported that granular cells were the most radiosensitive. Sharma[50] also reported that granular layer was more radiosensitive compared to molecular layer. Similar results have been obtained in our study at later intervals.

Mostly neural proliferation ends soon after birth, in some regions of the brain the cell proliferation continues, leaving the brain radiosensitive. The most common histological changes induced by X-rays were dilation of vascular endothelial cells, perivasculitis and edema. The permeability of blood barrier significantly increased within few hours after exposure[51]. Qualitative analysis in our study revealed damage present in the neuroglial cells of cerebral cortex and granular cells and Purkinje cells of brain cerebellum, which could be modulated by sesame oil.

If SO is to produce an antioxidative effect, its antioxidant constituents must be absorbed by the body and available to the tissue exposed to oxidative stress. It was reported in a study that sesame is a rich source of mammalian lignan

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

precursors. The lignans present in sesame are converted to mammalian lignans enterolactone and enterodiol, which are produced by the microflora in the colon[52]. When sesame oil is administered for a period of 15 days of time just before irradiation it confronts the oxidative load generated by irradiation. The damage is prevented by the pre-existing load of antioxidants. The antioxidative load combats and balances by lignan sesamin and sesamolin. Sesamolin and its metabolites enterolactone (EL) and enterodiol (ED) have a number of antioxidant activities, including inhibition of lipid peroxidation and scavenging of hydroxyl radicals. The free radicals are not only quenched and scavenged by lignans but also it suppresses the oxidant load. In this manner the ultimate mode of action becomes prophylactic.

The results of the present investigation demonstrate that SO treatment protects the mice whole brain and cerebellum against radiation-induced damage by inhibiting the glutathione, protein depletion and nucleic acids and ameliorating lipid peroxidation levels and it also reduced radiation induced histopathological damage in terms of quality and quantity. The metabolites of sesamolin may contribute to the antioxidative properties of sesame lignans and reduce susceptibility to some forms of oxidative stress.

Conclusion

The radioprotective activity of SO may be mediated through several mechanisms. The presence of lignans and synergistic effect with gamma tocopherol elevates the cellular antioxidants and enables it to scavenge free radicals in the irradiated system, which could be a leading mechanism for radioprotection. Reduction in lipid peroxidation and elevation in non-protein sulphydryl groups may also contribute to some extent to their radioprotective activity. Supplementation with sesame may be a useful adjuvant treatment mitigating adverse effects of radiation.

Acknowledgements

We thankfully acknowledge Department of Zoology, University of Rajasthan, Jaipur, for the liberal use of facilities. The authors are also thankful to Dr A. Chougle, Radiotherapy Unit, SMS Medical College and Hospital, Jaipur (India) for providing us with an irradiation facility and for help in radiation dosimetry.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

I. Variations in lipid peroxidation (LPO) and glutathione level (GSH) in whole brain at various post-treatment days, in the presence or absence of Sesame Oil (+SEM).

Parameter	Group	Days post treatment					Control No treatment	SO treated (150 µl / mice /day)
		1	3	7	15	30		
LPO (nmol/gm)	Irradiated	222.50±2.06 (157.07 %) b ##	184.50±1.64 (130.25 %) b ##	170.68±1.90 (120.49 %) b ##	167.20±1.21 (118.03 %) b ##	160.34±1.13 (113.19 %) b ##	141.65 ± 2.118 (100 %)	112.50 ±2.76 (79.42%) a# #
	SO+IR	143.75 ±1.25 (100.95 %) c## d##	138.75 ±1.23 (97.95 %) c## d#	124.89 ±0.93 (88.16 %) c## d##	116.25±1.11 (82.06 %) c## d##	130.36 ±1.13 (92.02 %) c## d##		
GSH (nmol/ 100mg)	Irradiated	8.25 ±0.509 (45.05%) b # #	7.382±0.994 (40.31%) b # #	7.49 ±0.658 (40.90%) b # #	12.08 ±0.721 (65.97%) b # #	13.246±0.745 (72.34%) b # #	18.31 ±0.987 (100%)	19.76±0.934 (107.91%) a ##
	SO+IR	12.553±0.282 (68.54%) c## d ##	12.09±0.681 (66.02%) c## d ##	12.982±0.669 (70.90%) c## d ##	14.524±0.675 (79.32%) c## d ##	17.254±0.882 (94.23%) c## d ##		

Normal vs. Drug alone = a, Normal vs. Control = b, Normal vs. Exp. = c, Control vs. Exp. = d.
Significance levels: # # p<0.001, # p<0.005

Table 2. Variations in Protein, DNA and RNA in whole brain at various post-treatment days, in the presence or absence of Sesame Oil (+SEM).

Parameter	Group	Days post treatment					Control No treatment	SO treated (150 µl / mice /day)
		1	3	7	15	30		
Protein (mg/gm)	Irradiated	76.19±2.919 (72.38%) b # #	85.00 ±3.44 (80.75%) b # #	73.33 ±3.059 (67.66%) b # #	58.00 ± 2.497 (55.10%) b # #	48.90 ± 3.27 (46.45%) b # #	105.263 ±1.8439 (100%)	108.00± 3.00 (102.60%) a ##
	SO+IR	83.33 ± 2.39 (79.16%) c## d##	102.69±2.24 (97.55 %) c## d##	106.66 ±1.33 (101.32 %) c## d##	96.00 ±1.78 (91.20 %) c## d##	105.00 ±2.76 (100.70 %) c## d##		
DNA (mg/gm)	Irradiated	1.325 ±0.465 (73.36%) b # #	1.292 ±0.078 (71.53%) b # #	1.107±0.065 (61.29%) b # #	0.994 ±0.032 (55.03%) b # #	1.128±0.125 (62.45%) b # #	1.806 ± 0.031 (100%)	1.816±0.754 (100.59%) a NS
	SO+IR	1.4533±0.034 (80.47%) c## d ##	1.4350±0.035 (79.45%) c## d ##	1.572±0.165 (87.04%) c## d ##	1.802±0.097 (99.77%) c## d ##	1.811±0.022 (100.27%) c NS d ##		

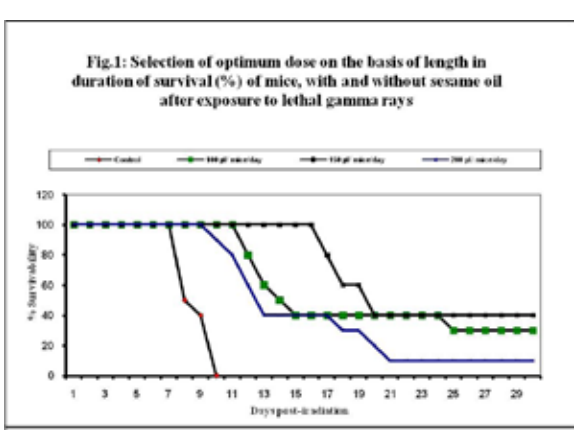
RNA (µg/mg)	Irradiated	1.59 ±0.171 (89.32%) b # #	1.42 ±0.078 (79.77%) b # #	1.36±0.165 (76.40%) b # #	1.28 ±0.028 (71.91%) b # #	1.19±0.125 (66.85%) b # #	1.78 ± 0.18 (100%)	1.74±0.382 (97.75%) a^{NS}
	SO+IR	1.64±0.22 (92.13%) $c^{\#\#}$ $d^{\#\#}$	1.55±0.035 (87.07%) $c^{\#\#}$ $d^{\#\#}$	1.58±0.186 (88.76%) $c^{\#\#}$ $d^{\#\#}$	1.65±0.099 (92.69%) $c^{\#\#}$ $d^{\#\#}$	1.72±0.12 (96.62%) $c^{\#\#}$ $d^{\#\#}$		

Normal vs. Drug alone = a, Normal vs. Control = b, Normal vs. Exp. = c, Control vs. Exp. = d.
Significance levels: # # p<0.001, # p<0.005

Table-3 Variations in thickness of granular and molecular layer and counts and volume of Purkinje Cells in cerebellum at various post-treatment days, in the presence or absence of Sesame Oil (±SEM).

Parameter	Group	Days post treatment					Control No treatment	SO treated (150 µl / mice /day)
		1	3	7	15	30		
Thickness of granular layer (mm)	Irradiated	88.45±1.77 (93.43%) b # #	80.12±1.48 (84.63%) b # #	75.28±1.98 (79.52%) b # #	60.55±0.924 (63.96%) b # #	62.92±0.865 (66.46%) b # #	94.66± 0.787 (100%)	91.45±2.39 (96.60%) $a^{\#\#}$
	SO+IR	88.76±2.26 (93.76%) $c^{\#\#}$ $d^{\#\#}$	86.34±1.93 (91.21%) $c^{\#\#}$ $d^{\#\#}$	83.08±2.59 (87.76%) $c^{\#\#}$ $d^{\#\#}$	89.59±2.86 (94.64%) $c^{\#\#}$ $d^{\#\#}$	92.02±1.71 (97.21%) $c^{\#\#}$ $d^{\#\#}$		
Thickness of molecular layer (mm)	Irradiated	88.54±0.739 (81.79%) b # #	85.09±1.56 (78.60%) b # #	82.75±0.875 (76.44%) b # #	74.06 ±0.370 (68.41%) b # #	77.82 ±0.647 (71.88%) b # #	108.25± 1.76 (100%)	106.97±1.494 (98.81%) $a^{\#\#}$
	SO+IR	100.89±0.59 (93.20%) $c^{\#\#}$ $d^{\#\#}$	103.37±1.109 (95.39%) $c^{\#\#}$ $d^{\#\#}$	100.29±1.17 (92.64%) $c^{\#\#}$ $d^{\#\#}$	104.77±1.42 (96.78%) $c^{\#\#}$ $d^{\#\#}$	106.24±2.66 (98.14%) $c^{\#\#}$ $d^{\#\#}$		
Count of Purkinje cells/2500 mm lobule	Irradiated	84.09 ±2.556 (85.80%) b # #	77.24 ±4.20 (78.81%) b # #	70.00 ±4.20 (71.42%) b # #	66.08 ±3.57 (67.42%) b # #	69.11 ±4.01 (70.52%) b # #	98.00 ± 1.109 (100%)	112.00±4.222 (114.28%) $a^{\#\#}$
	SO+IR	92.44 ±2.333 (94.32%) $c^{\#\#}$ $d^{\#\#}$	89.59 ±3.40 (91.417%) $c^{\#\#}$ $d^{\#\#}$	87.88 ±4.39 (89.67%) $c^{\#\#}$ $d^{\#\#}$	90.04 ±2.358 (91.87%) $c^{\#\#}$ $d^{\#\#}$	91.89 ±3.66 (93.76%) c^{NS} $d^{\#\#}$		
Volume of Purkinje Cells (mm^3)	Irradiated	408.23 ± 1.337 (87.08%) b # #	399.01 ± 2.324 (85.11%) b # #	382.89±2.449 (81.67%) b # #	392.91±2.741 (83.81%) b # #	409.80±2.333 (87.41%) b # #	468.79 ± 3.224 (100%)	472.58 ± 2.483 (100.80%) a^{NS}
	SO+IR	413.99 ± 3.224 (88.31%) $c^{\#\#}$ $d^{\#\#}$	411.50 ± 2.256 (78.77%) $c^{\#\#}$ $d^{\#\#}$	419.20±2.222 (89.42%) $c^{\#\#}$ $d^{\#\#}$	433.55±4.022 (92.48%) $c^{\#\#}$ $d^{\#\#}$	457.84±3.991 (97.66%) $c^{\#\#}$ $d^{\#\#}$		

Normal vs. Drug alone = a, Normal vs. Control = b, Normal vs. Exp. = c, Control vs. Exp. = d.
Significance levels: # # p<0.001, # p<0.005



Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

REFERENCE

1. Sonntag C V, The chemical basis of radiation biology (London: Taylor and Francis), (1987) 65-84. | 2. Halliwell B and Gutteridge J M C, Free Radicals in Biology and Medicine (Oxford: Clarendon) (1993) 22-81. | 3. Szumiel I, Ionizing radiation induced cell death, Int. J. Radiat. Biol., 66 (1994) 329-41. | 4. Weiss J F and Landauer M R, Protection against ionizing radiation by antioxidant nutrients and phytochemicals, Toxicology, 189 (2003) 1-20. | 5. Bhatia A L, Sisodia R, Manda K and Sharma M, Dose dependent study on the effectiveness of -carotene on the survivability of mice against lethal gamma irradiation. Radiation Protection and Environment, 24 (1&2) (2001) 96-101. | 6. Bhatia A L, Manda K, Patni S and Sharma A L, Prophylactic Action of flaxseed (Linum usitatissimum) oil against cyclophosphamide-induced oxidative stress in mouse brain, J. Med. Food, 9 (2), 2006, 261-264. | 7. Sharma M K, Sisodia R, ß-carotene against Radiation-induced Oxidative Stress in Mice Brain, Asian J.Exp.Sci., 14, (1&2) (2000) 43-44. | 8. Bhatia A L and Jain M, Spinacia oleracea L. protects against gamma radiations: a study on glutathione and lipid peroxidation in mouse liver, Phytomedicine, 11 (2004) 607-615. | 9. Yadav R K, Bhatia A L and Sisodia R, Modulation of radiation induced biochemical changes in testis of swiss albino mice by Amaranthus paniculatus Linn. Asian J. Exp. Sci. 18 (1&2) (2004) 63-74. | 10. Verma R K, Sisodia R and Bhatia A L, Radioprotective role of Amaranthus gangeticus Linn.: A Biochemical Study on Mouse Brain, J Med Food, 5 (2002)189-195. | 11. Sisodia R, Singh S, Mundotiya C, Meghnani E and Srivastava P, Radioprotection of swiss albino mice by Prunus avium with special reference to the hematopoietic system, J. Envt. Path., Toxicol. and Onco., 30 (1) (2011) 53-68. | 12. Sisodia R and Singh S, Biochemical, behavioral and quantitative alterations in cerebellum of Swiss albino mice following irradiation and its modulation by Grewia asiatica, Int. J.Rad. Bio. (2009) 1- 9. | 13. Sharma K V and Sisodia R, Hepatoprotective efficacy of Grewia asiatica fruit against oxidative stress in Swiss albino mice, Iran. J. Radiat. Res., 8 (2) (2010) 75-85. | 14. Jagetia G C, Baliga M S and Venkatesh P. Influence of seed extract of Syzygium cumini (Jamun) on mice exposed to different doses of radiation, J. Radiat. Res., 46 (2005) 59-65. | 15. Oidwai W, Alim S R and Dhanani R H, Use of folk remedies among patients in Karachi Pakistan, J Ayub Med Coll Abbottabad, 5(2) (2003) 31-3. | 16. Minamiyama Y, Takcmura S, Yoshikawa T and Okada S, Fermented grain products, produt-lion. properlies and benefits lo health, Pathophysiology, 4 (2003) 221-7. | 17. Budovski P, Sesame oil. #3. Antioxidant properties of sesamol, J.Am. Oil Chem. Soc., 27 (1950) 264-267. | 18. Fukuda Y, Osawa T, Namiki M, Ozaki T, Studies on the antioxidative substances in sesame seed, Agric. Biol. Chem., 49 (1985) 301-306. | 19. Fukuda Y, Nagata T, Osawa T, Namiki M, Contribution of lignan analogues to antioxidative activity of refined unroasted sesame seed oil, J.Am. Oil Chem. Soc., 63 (1986) 1027-1031. | 20. Kang, M.-H., Katsuzaki, H., Osawa, T, Inhibition of 2,2'-azobis (2,4-dimethylvaeronitrile)-induced lipid peroxidation by sesaminols, Lipids, 33 (1998a)1031-1036. | 21. Kang M-H, Naito M, Tsujihara N, Osawa T, Sesamolin inhibits lipid peroxidation in rat liver and kidney, J. Nutr., 128 (1998b) 1081-1022. | 22. Kang M-H, Naito M, Sakai K, Uchita K and Osawa T, Action of mode of sesame lignans in protecting low-density lipoprotein against oxidative damage in vitro, Life Sci., (1999). | 23. Kamal-Eldin A, Pettersson D, Appelqvist L-A, Sesamin (a compound from sesame oil) increases tocopherol levels in rats fed ad libitum, Lipids, 30 (1995) 499-505. | 24. Yamashita K, Nohara Y, Katayama K, Sesame seed lignans and gamma-tocopherol act synergistically to produce vitamin E activity in rats, J. Nutr, 122 (12) (1992) 2440-6. | 25. Hemalatha S, Raghunath M, Ghafoorunissa Dietary sesame oils inhibits iron-induced oxidative stress in rats, Br. J Nutr, 92(4) (2004) 581-7. | 26. Chattopadhyay K, Mondal S, Chattopadhyay B, Ghosh S, Ameliorative effect of sesame lignans on nicotine toxicity in rats, Food Chem Toxicol, 48(11) (2010) 3215-20. | 27. Kumar P, Kalonia H, Kumar A, Protective effect of sesamol against 3-Nitropropionic Acid-induced cognitive dysfunction and altered glutathione redox balance in rats, Basic Clin Pharmacol Toxicol, 107(1) (2010) 577-82. | 28. Dhar P, Chattopadhya K, Bhattacharyya D, Biswas A, Roy B, and Ghosh S, Ameliorative influence of sesame lignans on lipid profile and lipid peroxidation in induced diabetic rats, J. Agric. Food Chem, 55 (14) (2007) 5875-5880. | 29. Beckstrom-Sternberg S M and Duke J A, The phytochemical database." Arsgenome. Cornell. Edu/cgi-bin/webAce/ webace?db= phytochemdb (1994). | 30. Shimizu S, Akimoto K and Sugano M. Studies on desaturase inhibitors of polyunsaturated fatty acid biosynthesis, in Essential Fatty Acids and Eicosanoids. Sinclair, A. and Gibson, R., editors. American Oil Chemist Society, Champaign, (1992) 37-41. | 31. Buege J A, Aust S D, In: Methods in Enzymology, (New York) Academic Press, 52 (1978) 302-314. | 32. Moron M S, Depierre J W, Mannervik B, Levels of GSH, GR and GST activities in rat lung and liver, Biochimica et Biophysica Acta, 582 (1979) 67-78. | 33. Bradford M M, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein-By binding. Analytical Biochemistry, 72 (1976) 248-254. | 34. Ceriotti G, A microchemical determination of desoxyribonucleic acid, J. Biol Chem, 198 (1952) 297-303. | 35. Ceriotti G, Determination of nucleic acids in animal tissue. J. Bio.Chem., 214 (1955) 59-70. | 36. Larsell O, The Morphogenesis and adult pattern of the lobules and fissures of the cerebellum of white rat, J. Comp. Neurol., 97 (1952) 281-356. | 37. Sies H, Oxidative stress, Academic Press, London, (1985) 1-8. | 38. Pryor W A and Godber S S, Noninvasive measures of oxidative stress status in humans, Free Rad Biol Med, 10 (1991) 177-84. | 39. Raleigh J A, Radioprotection of membranes, Pharmacology and Therapeutics, 39 (1985) 109-113. | 40. Konings A W T and Osterloo S K, Radiation effects on membranes: A comparison of the effects of X- irradiation and ozone exposure with respect to the relation of antioxidant concentration and the capacity for lipid peroxidation, Radiat. Res., 81(1980) 200-207. | 41. Shimoi K, Masuda S, Shen B, Furugori B and Kinae N, Radioprotective effect of antioxidative plant flavonoids in mice, Mutat. Res, 350 (1996) 153. | 42. Yamashita K, Ikada S and Obayashi M, Comparative effect of flaxseed and sesame seed on vitamin E and cholesterol level in rats, Lipids, 38(12) (2003) 1249-1255. | 43. Reiter R J, Oxidative properties and antioxidative defanse mechanisms in the aging brain, FASEB J, 9 (1995) 526-533. | 44. Wu G, Fang Y Z, Yang S, Lupton J R, Turner N D, Glutathione metabolism and its implications for health, Nutrition, 134 (2004) 489-492. | 45. Bhatia A L, Sharma A, Patni S, Sharma A L, Prophylactic effect of flaxseed oil against radiation-induced hepatotoxicity in mice, Phytotherapy Res, 21(2007) 852-859. | 46. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj E K, Oxidative damage is the earliest event in Alzheimer disease, Journal of Neuropathology & Experimental Neurology, 60 (2001) 759-767. | 47. Lee H G, Perry G, Moreira P I, Garrett M R, Liu Q, Zhu X, Takeda A, Tau phosphorylation in Alzheimer's disease: Pathogen or protector? Trends in Molecular Medicine, 11(2005)164- 169. | 48. Egana E, Liona I, Raimirej M T, Effect of whole body gamma radiation in DNA, RNA and protein function in developing CNS. In: Proc. VII Int. Cong. Radiation Research, (1983) 1-7. | 49. Gueneau G, Drouet J, Privat A, Court L, Differential radio sensitivity of neurons and neuroglia of the hippocampus in adult rabbit, Acta Neuropath, 48(1979)199-209. | 50. Sharma M, Investigation on the beta-carotene vs radiation effects on mice cerebellum. Ph.D Thesis submitted to the University of Rajasthan, India. 2001. | 51. Fogarty K, Volonino V, Caul J, Acute leukemia. learning disabilities following CNS irradiation, Clin Pediatr (Phila), 27 (1988) 524-528. | 52. Karen D C, Zhen L, Winston Q H, John M, and Lilian U, Whole sesame seed is as richa Source of mammalian lignan precursors as whole flaxseed, Nutrition and Cancer, 52(2) (2005) 156-165. |