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Modulation of Radiation Induced Biochemical Changes in Brain of Swiss Albino Mice by *Grewia asiatica*

Muktika Ahaskar and Rashmi Sisodia*
Radiation Biology Laboratory,
Department of Zoology,
University of Rajasthan, Jaipur, India.

Abstract : Increasing use of nuclear radiation for human welfare necessitates a new, safe and cost effective radioprotector not only for personnels charged with responsibility of testing or working with radiations in laboratories, but also for the general public. Keeping this in view, the study has been undertaken to find out the possible radioprotective potential of the *Grewia asiatica* fruit extract *(*GAE), having high content of antioxidants like vitamin C, anthocyanin and folate that may play a possible role in radioprotection. For the sexperimental study, healthy Swiss albino mice were selected from an inbred colony and divided into four groups. Group I (normal) did not receive any treatment. Group II was orally supplemented (GAE), once daily, at the dose of 700 mg/Kg.b.wt/day for fifteen consecutive days. Group III (control) received distilled water orally equivalent to GAE for fifteen days than exposed to 5 Gy of gamma radiation. Group IV (experimental) was administered orally (GAE) for 15 consecutive days, once daily, and exposed to single dose of 5Gy of gamma radiation. Mice were sacrificed at different post irradiation intervals viz. 1, 3, 7, 15 and 30 days. Brain was removed for various biochemical estimations *viz*. glutathione (GSH) and lipid peroxidation (LPO). GAE supplementation checked the augmentation levels of LPO due to radiation, approximately by 5% at day 30^{th} post irradiation whereas radiation induced depleted levels of GSH could be raised by 14.57% by the day 30^{th} post exposure.

Key words: *Grewia asiatica*, Antioxidant, Brain, Radioprotection

Introduction :

A large number of compounds from various plant sources have been shown to posses antioxidant properties (Bhattacharya *et al*., 1996; Yen *et al*., 1996 and Bhatia, 1998). Antioxidants of plant origin include vitamin E, C, selenium, phenolic compounds, flavonoids and others (Chandha, 1997). Nutritional intervention to increase intake of phyto-antioxidants may reduce the threat of free radicals. Recent researches have indicated that the people who eat higher amounts of fruits and vegetables have about one half the risk of cancer and less mortality from cancer (Steinmetz and Potter, 1991; Ziegler, 1991).

India has a rich heritage of medicinal plants many of which have been explored for the various bioactivities since ages, but the radioprotective potential of the plants have been hardly explored. In this context *Grewia asiatica* (Phalsa in Hindi) cultivated on a commercial scale mainly in the northern and western states of India (Hays, 1953; Sastri, 1956), is known for its medicinal properties. The fruit is astringent and stomachic. Morton (1987) reported that unripe phalsa fruit alleviates inflammation and is administered in respiratory, cardiac and blood disorders, as well as in fever reduction. Furthermore, infusion of the bark is given as a demulcent, febrifuge, and treatment for diarrhea. *Grewia asiatica* contains anthocyanin type cyanidin 3- glucoside (Nair *et.al*., 2005), vitamin C, minerals and dietary fibers etc. (Yadav, 1999). The antioxidant properties of vitamin C are well known and anthocyanin has recently emerged as a powerful antioxidant.

* **Corresponding author :** Rashmi Sisodia, e-mail - rashsisodia@yahoo.co.in

Brain tissue is highly susceptible to oxidative damage due to its high utilization of oxygen and its poorly developed antioxidative defense mechanism. The present study is hence an attempt to evaluate the protective effect of *Grewia asiatica* fruit extract in mice brain against radiation induced oxidative stress.

Materials and Methods :

Animals : Adult male Swiss albino mice, 6 – 8 weeks old, weighing 25±2 gm were used for the present study. These animals were maintained in the animal house as an inbred colony as per norms laid down by the Institutional Ethical Committee. They were maintained on standard mouse feed (Hindustan Lever, Delhi, India) and provided tap water *ad libitum* at constant temperature (22 ± 1.5^{0}C) and light (12L: 12D).

Extract preparation (Drug) : Fresh fruits of *Grewia asiatica* collected locally in summer season were washed, shade dried and powdered after removal of seeds. Methanolic extract was then prepared by refluxing for 36 hours (3 × 12) at 40^o C. The extract thus obtained was vacuum evaporated so as to get in the powdered form. The extract was redissolved in doubled-distilled water (DDW) just before the oral administration. For the various concen-trations, a known amount of GAE was suspended in DDW and GAE suspension was given to each mouse by oral gavage.

Source of irradiation : The cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajasthan, India was used for irradiation. Unanaesthestized animals were restrained in well-ventilated Perspex boxes and whole body exposed to gamma radiation at a distance (SSD) of 77.5 cm from the source to deliver the dose rate of 1.07 Gy/ min.

***Experimental design* :** Mice selected from an inbred colony were divided into 4 groups (30 animals in each Group).

Group I (Normal) : Mice of this group did not receive any treatment.

Group II (Drug) : Mice of this group were administered with GAE (700 mg/kg of b.wt./ day) for 15 consecutive days once daily. The dose was selected on the basis of drug tolerance study conducted in our laboratory.

Group III (Control) : Mice received DDW (volume equal to *Grewia asiatica* solution) for 15 days and were then exposed whole body to a single dose of 5 Gy of gamma-radiation.

Group IV (Experimental) : In this group oral administration of GAE (700 mg/kg b.wt./ day) was made once daily for 15 consecutive days. One hour after administration of last dose of GAE, mice were whole body exposed to single dose of 5 Gy gamma-radiation as in group third.

Treatment in all the groups was carried out 10 AM. Six mice from each groups were necropsied at the same time (10 AM.) and intervals, *i.e.* 1, 3, 7, 15 and 30 days *post irradiation.*

***Removal of brain tissue* :** The mice were weighed and sacrificed by cervical dislocation. 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 tissue, the brain was excised and separated from the spinal cord at the decussation of the pyramids. .

Biochemical Assay : Lipid peroxidation was measured using Thiobarbituric Acid Reactive Substances (TBARS) according to the method of Ohkhawa *et al.* (1979). The brain was removed and immediately placed in cold 0.9% NaCl and washed in the same. 10% homogenate was prepared (1 gm of tissue in 9 ml of 1.15 KCl) and 0.2 ml of the sample was taken for the assay. Two repeats of the assay from each animal were carried out. The absorbance was read at 532 nm.

Spectrophotometric quantification of reduced glutathione (GSH) has been carried out

using 5, 5_dithiobis- (2-nitrobenzoic acid) (DTNB) reagent according to the method proposed by Moron *et al.* (1979). Briefly, 200 µl of tissue homogenate (20%) was added to 800 µl distilled water and then 2 ml of sodium phosphate–EDTA buffer (0.1 M sodium phosphate, 0.005 M EDTA buffer, pH 8.0), containing 0.6 mol l-1 DTNB were added. The optical density of the yellow coloured complex developed by the reaction of GSH and DTNB was measured at 412 nm using a UV–vis spectrophotometer.

Statistical Analysis : The results obtained in the present study were expressed as mean ± SEM. The statistical difference between various groups were analysed by the Student's *t*-test and the significance was observed at the $p < 0.4$, $p < 0.02$, $p < 0.01$and $p < 0.001$ level.

Results :

Brain shows gradual augmentation in the level of TBARS content after gamma irradiation till day 7^{th} in control as well as in the experimental group (Table1). Thereafter, depletion in TBARS content was noticed indicating recovery. In experimental group, TBARS content reached near normalcy (101.52%) by day 30^{th} post irradiation but in the control group, it was higher by 5.82% compared to the normal. At all the post irradiation intervals, the LPO values remained significantly lower in experimental group in comparison to the control.

GAE supplementation for 15 days continuously raised the GSH level (Table 2) by 12.62% in comparison to the normal. In both the control and experimental groups initially the GSH level declined upto day 7^{th}, thereafter GSH level increased continuously upto the last interval. The maximum decrease noted at day 7^{th} was 30.1% and 15.53% in control and experimental group, respectively, as compared to the normal. At day 30 in the experimental group, GSH level reached near normalcy but was still deficient by 3.88%. GAE supplementation prior to irradiation raised the GSH level at all the autopsy intervals compared to the control group.

The present study therefore indicates that supplementation of GAE prior to irradiation helps to lower the LPO level and raise the GSH concentration in whole brain of mice.

Discussion :

Results obtained from this study indicate that *Grewia asiatica* extract may act as radioprotective agent and render protection against radiation induced oxidative stress. Oxidative stress refers to the cytotoxic consequence of reactive oxygen by products: superoxide anions and hydroxyl radicals which are generated as metabolites of normal and aberrant metabolic processes that utilize molecular oxygen (Sies and Stahl, 1995). Oxidative stress leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders (Sies, 1985; Pryor and Godber, 1991; Helliwell, 1994). The products of LPO such as MDA (malondialdehyde) and 4-hydroxynonenal are toxic to cells (Raleigh, 1985). 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 the integrity of the cellular membrane depends on protection or repair mechanisms capable of neutralizing oxidative reactions. In the present study the reduction in the amount of TBARS or MDA equivalents and elevation in the GSH level in the GAE treated animals suggest that GAE may scavenge the free radicals generated during oxidative stress. GSH, with its sulfhydryl group, functions in the maintenance of the sulfhydryl group of other molecules (especially proteins), acts as a catalyst for disulfide exchange reactions and in

Table 1 : Variation in the LPO ± SEM (n mole MDA/gm protein) in brain at various post irradiation intervals after 5 Gy radiation exposure.

Normal	103.551±0.633 (100%)				
Only drug	99.808±0.588[a] (96.38%)				
	1 Day	3 Day	7 Day	15 Day	30 Day
Control	199.215±1.057 (192.38%)	142.111 ± 1.223 (137.23%)	147.034 ±1.454 (141.99%)	121.118±0.528 (116.96%)	109.576±0.927 (105.82%)
Experimental	150.972±0.839[a] (145.79%)	138.248±0.916[d] (133.51%)	144.951±0.743[h] (139.98%)	114.466±0.805[a] (110.54%)	105.131±0.821[b] (101.52%)

Table 2 : Variation in the GSH ± SEM (n mole /100mg tissue) in brain at various post irradiation intervals after 5 Gy radiation exposure.

Normal	30.403±1.769 (100%)				
Only drug	34.240±1.007[c] (112.62%)				
	1 Day	3 Day	7 Day	15 Day	30 Day
Control	24.168±1.579 (79.49%)	22.138±1.480 (72.82%)	21.253±2.265 (69.90%)	23.909±2.312 (78.64%)	24.795±1.510 (81.55%)
Experimental	29.042±1.422[e] (95.52%)	26.566±1.602[f] (87.38%)	25.974±1.479[f] (84.47%)	28.632±1.913[g] (94.17%)	29.223±1.387[e] (96.12%)

Each value represents mean ±SEM (n=12).

Statistical comparisons between following groups : Normal vs. Only drug; Control vs. Experimental

Significance level : [a]p=<0.001; [b]p=<0.01; [c]p=<0.1; [d]p=<0.02; [e]p = <0.05; [f]p = <0.1; [g]p = <0.2; [h]p = <0.4

the detoxification of foreign compounds like hydrogen peroxide and free radicals. When GSH acts as a reducing agent, its SH becomes oxidised and forms a disulfide link with other molecules of GSH. The lesser depletion of whole brain GSH content in the experimental group as compared to the control 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 and Alzheimer's diseases in which oxidative processes contribute to the pathology (Reiter *et al.*, 2001).

Earlier studies in our laboratory demonstrated that oral administration of 700 mg/k.g. b.wt/day dose of GAE, prior to radiation exposure (10 Gy), was found to be effective in terms of survivality then other higher and lower doses of GAE. The radioprotective effect of GAE was also determined by calculating the dose reduction factor (DRF), which was 1.53.

Sharma and Sisodia (2000) observed the â-carotene against radiation-induced oxidative stress in mice brain. The possible radioprotective potential of the *Amaranthus paniculatus* (AE) has been investigated by Yadav *et al*. (2004), as its leaves have high content of carotenoids, proteins, minerals, vitamin C and high level of nutritionally critical amino acids lysine and methionine. AE (amaranthus extract) for 15 days prior to exposure to 5 Gy of gamma radiation at different autopsy intervals viz. 1,3,7,15 and 30

days in mice for various biochemical parameters viz. LPO, protein, cholesterol and glycogen. Radiation induced augmentation in lipid peroxidation, cholesterol was significantly ameliorated by AE extract and deficit produced in protein content by radiation was checked. Amaranthus extract pre-treatment hence renders protection against various biochemical changes in mice testis. In the same manner Verma et al. (2003) investigated the radioprotective efficacy of spinach against radiation induced oxidative stress, since its leaves are rich in antioxidants like carotenoids (lutein, b-carotene, zeaxanthin), p-Coumaric acid, ascorbic acid, proteins, vitamins etc. Healthy Swiss albino male mice of 6-week-old mice. Radiation induced significant elevation in the LPO values in brain was lowered by supplementation of spinach prior to irradiation at all the intervals studied. At day 30th LPO values attained normalcy in the experimental group, but in the control group LPO values was still higher by approximately 12%. The levels of LPO products in brain of SE supplemented mice activates antioxidant enzymes in brain suggesting that spinach leaf extract reduces LPO values by quenching free radicals. The protection rendered with SE in LPO value of brain in the present study indicates the possible role *Spinacia* as radioprotector to some extent if taken continuously which might be due to synergistic effect of antioxidant constituents present in the spinach. Sharma and Jaimala (2003) observed the alteration of acid phosphatase activity in the liver of gamma irradiated mouse by *Centella Asiatic*. *Centella asiatica* extract, which increased the survival of gamma irradiated Swiss albino mouse when given prior to irradiation was tested to observe changes in the acid phosphatase activity in the liver of irradiated mouse.

Fruits like ber, phalsa, apple and strawberry have been shown to possess moderate antioxidant activity ranging from 12-64 mM FRAP (Kaur and Kapoor, 2005). Matsumoto *et al*., 2002 have shown that the antioxidative activity of plasma lasted longer than the presence of anthocyanin glycosides in the plasma. They assumed that anthocyanins were converted into some metabolites having antioxidant activity. Like other flavonoids, anthocyanins and anthocyanidins (the aglycone form) have antioxidant properties (Wang *et al*., 1997). The antioxidant potency of anthocyanin extracts is concentration dependent (Gabrielska *et al*., 1999). The positive effects of anthocyanin pigments could be related to their potent antioxidant activity demonstrated in various in vitro and in vivo studies (Tsuda *et al.*, 1994; Wang *et al.*, 1997; Wang *et al.*, 1999; Ramirez-Tortosa *et al.*, 2001; Matsumoto *et al*., 2002). So, prophylactic action of *Grewia asiatica* against radiation-induced metabolic disorders may be due to presence of antioxidant like anthocyanin, vitamin c etc.

The results of our study on mice brain indicate that radiation induced disturbances in biochemical indices may be checked by oral supplementation of GAE for 15 consecutive days in the body, suggesting thereby that nutritional intervention might prove prophylactic to radiation induced alterations.

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