

Modulation of radiation-induced biochemical changes in cerebrum of Swiss albino mice by *Grewia asiatica*

Rashmi Sisodia*, Muktika Ahaskar, K.V. Sharma, and Smita Singh

Radiation Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur, 302004 India,

*Email: rashsisodia@yahoo.co.in

The present study evaluates the possible radioprotective effect of *Grewia asiatica* fruit (rich in anthocyanin, carotenes, vitamin C, etc.) pulp extract (GAE) on cerebrum of Swiss albino mice exposed to 5 Gy gamma radiation. For this, healthy mice from an inbred colony were divided into four groups: (1) Control (vehicle treated), (2) GAE treated – mice in this group were orally supplemented with GAE (700 mg/kg. b.w. /day) once daily for fifteen consecutive days, (3) Vehicle treated irradiated mice, and (4) GAE + Irradiated – Mice in this group received distilled water orally equivalent to GAE (700 mg/kg. b.w./day) for fifteen days consecutively. Mice were sacrificed at various intervals viz. 1–30 days. Radiation-induced augmentation in the levels of lipid peroxidation of mice cerebrum was significantly ameliorated by GAE pretreatment. Radiation-induced depletion in the level of glutathione and protein was prevented significantly by GAE administration.

Key words: *Grewia Asiatica*, antioxidants, cerebrum, radioprotection

INTRODUCTION

Synthetic protectors have toxicity, which limits their value in the clinical field. Therefore, now the search is on for some natural compounds that can quench the reactive energy of free radicals and eliminate singlet oxygen, one of the major participants in lipid peroxidation (LPO). A large number of compounds from various plant sources have been shown to possess antioxidant properties (Bhattacharya et al. 1996, Yen et al. 1996, Bhatia 1998). Antioxidants of plant origin are vitamin E, C, selenium, phenolic compounds, flavonoids, etc. (Chandha 1997). It has been assumed that nutritional intervention to increase intake of phyto-antioxidants may reduce the threat of free radicals. India has a rich heritage of medicinal plants, many of which have been explored for various bioactivities for ages, but the radioprotective potential of the plants have been hardly explored. In this context *Grewia asiatica*

(Phalsa) 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* has been reported to contain anthocyanin type cyanidin 3-glucoside (Nair et al. 2004), vitamin C, carotenoids, minerals and dietary fibers, etc. (Yadav 1999). The antioxidant properties of carotenoids and vitamin C are well known and anthocyanin has recently emerged as a powerful antioxidant. Therefore, *Grewia asiatica* may prove to be an efficient antioxidant.

Brain tissue is highly susceptible to oxidative damage due to its high utilization of oxygen (20% of the total oxygen inhaled by the body) that accounts for the increased generation of oxygen free radicals and reactive oxygen substrates. Reactive oxygen species (ROS) are capable of oxidation of proteins, lipids and DNA leading to cellular damage. Free radicals are potentially

Correspondence should be addressed to R. Sisodia,
Email: rashsisodia@yahoo.co.in

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dangerous for cells (Hochstein and Atollah 1988). LPO is a good biomarker of damage occurring due to radiation and the inhibition of lipid peroxidation is suggestive of radioprotective action. Brain has a poorly developed antioxidative defense mechanism. The concentration of various antioxidative enzymes is low in brain. The glutathione (GSH) concentration is also very much reduced in the brain when compared to other organs in the body (Zhang et al. 1993). Brain is also enriched with polyunsaturated fatty acid (PUFA) that renders it susceptible to oxidative attack. The present study is therefore an attempt to evaluate the possible protective effects of *Grewia asiatica* fruit extract in mice cerebrum against radiation-induced oxidative stress.

METHODS

Animal care and handling

The animal care and handling was done according to the guidelines set by World Health Organization, Geneva, Switzerland and INSA (Indian National Science Academy, New Delhi, India). The Departmental Animal Ethical Committee approved this study. Swiss albino mice, 6–8 weeks old, weighing 23 ± 2 g, from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature and light (light/dark, 10 h/ 14 h). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mice feed (procured from Hindustan Levers Ltd., India) and water *ad libitum*. Tetracycline water was given once a fortnight as a preventive measure against infection.

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°C . The extract thus obtained was vacuum evaporated to produce a powdered form. The extract was redissolved in doubled-distilled water (DDW) just before the oral administration. For various concentrations, a known amount of GAE was suspended in DDW and 50 μl of GAE suspension was given to each mouse by oral gavage as given by Ahaskar and colleagues (2007).

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. Unanesthetized animals were restrained in well-ventilated Perspex boxes and the whole body was exposed to gamma radiation at a distance (SSD) of 77.5 cm from the source to deliver a dose rate of 1.07 Gy/min.

Chemicals

Thiobarbituric acid (TBA), glutathione (GSH), and DTNB (5,5 dithio-bis 2-Nitrobenzoic acid) were purchased from Sigma Co. USA. 1,1,3,3, tetramethoxy propane and other chemicals used were of analytical grade and were procured from Central Drug House (Pvt.) Ltd., Mumbai.

Dose selection

Dose selection of *Grewia asiatica* was done on the basis of a drug tolerance study in our laboratory by Ahaskar and others (2007). Various doses of *Grewia asiatica* (100, 400, 700, 1000, 1300 mg/kg b.wt.) were tested against gamma irradiation (10 Gy) and 700 mg/kg b.wt./day was obtained as the optimum dose based on survivability of mice. This dose was used for further experiments.

Experimental design

Mice selected from an inbred colony were divided into 4 groups (30 animals in each group).

(1) Control vehicle treated – mice of this group received only DDW water for 15 days; (2) GAE treated – mice of this group were administered only once with only GAE (700 mg/kg of b.w./day) for 15 consecutive days; (3) Irradiated – mice received DDW (volume equal to *Grewia asiatica* solution) for 15 days and were whole-body exposed to 5 Gy of gamma-radiation; (4) GAE treated + Irradiated – in this group oral administration of GAE (700 mg/kg of b.w./day) was made once daily for 15 consecutive days as done in GAE treated group. One hour after administration of the last dose of GAE, mice were whole-body exposed to a single dose of 5 Gy gamma-radiation as in group three.

Six mice from each group were necropsied at various intervals viz. 1, 3, 7, 15, and 30 days post irradiation.

Removal of brain tissue

The mice were sacrificed by cervical dislocation. An incision was made 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. The intact cerebrum was then removed carefully from the brain and homogenate was prepared and used for quantitative estimation for various biochemical changes.

Biochemical assay

Lipid peroxidation (LPO) assay: LPO was measured by the method of Buege and Aust (1978). Briefly, to tissue homogenate (0.8 ml), 1.2 ml solution of TCA-TBA-HCl prepared in 1/1/1 was added. 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-Vis double beam spectrophotometer. The LPO has been expressed as MDA in nm/g tissue.

Reduced glutathione (GSH) assay: The reduced glutathione (GSH) content of tissue samples was determined by the method of Moron and coauthors (1979). Tissue sample was homogenized in the sodium phosphate-EDTA buffer then 0.6 M DTNB [5,5-dithiobis-(2-nitrobenzoic acid)] was added. The optical density of the yellow colored complex developed by the reaction of GSH and DTNB was measured at 412 nm using a UV-vis spectrophotometer. The results were expressed as nm/100 mg of tissue.

Protein assay: Estimation of protein was based on the method proposed by Bradford (1976) and 10% homogenate was prepared (1 g 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.

STATISTICAL ANALYSIS

The results obtained in the present study were expressed as mean \pm SEM. The statistical differences between various groups were analyzed by the Student's *t*-test and the significance was observed at the $P < 0.05$, $P < 0.01$, and $P < 0.001$ level.

RESULTS

Lipid peroxidation product as reflected by TBARS equivalent content was augmented after radiation exposure in both GAE-treated and vehicle treated-irradiated mice cerebrum. This increase in lipid peroxidation product was not stable up to day 7 post-exposure, as there was a slight decline on day 3 post-exposure. After day 7, a continuous decrease in TBARS content was observed in both groups up to day 30 post-irradiation. The magnitude of such a recovery from oxidative damage in terms of TBARS content was significantly higher ($P < 0.001$) in GAE treated-irradiated mice as compared to vehicle treated-irradiated mice. Moreover, GAE treated-irradiated group has attended the normal level of TBARS at 30 day post-irradiation. Only GAE treated mice didn't show any significant deviation in the level of TBARS equivalent as compared to control (Table I).

Glutathione (GSH) content was decreased after radiation exposure in the GAE treated and vehicle treated-irradiated mice cerebrum. Such a decrease in GSH content was noted continuously up to the seventh day post-exposure. After day 7, a continuous increase in GSH content was observed in both groups up to day 30 post-irradiation. The magnitude of such a recovery from oxidative damage was significantly higher ($P < 0.001$) in GAE treated + irradiated mice as compared to vehicle treated-irradiated mice. GAE treated + irradiated group showed a higher degree of recovery at day 30 post-exposure by attaining control level. Only GAE treated mice also showed a significant increase ($P < 0.01$) in GSH content as compared to control (Table II).

Protein estimates also showed a statistically significant decrease after radiation exposure in both GAE treated and vehicle treated-irradiated mice cerebrum. Such a decrease in protein content was noted continuously till day 7 post-exposure. After day 7, a continuous increase in protein content was observed in both groups up to day 30 post-irradiation. The magnitude of such a recovery from oxidative damage was significantly higher ($P < 0.001$) in GAE treated + irradiated mice as compared to vehicle treated-irradiated mice. Moreover, GAE treated + irradiated group attained the normal level of protein at 30 days post-irradiation. Only GAE treated mice also showed a significant increase ($P < 0.001$) in protein content as compared to control (Table III).

Table I

Radiomodulatory influence of <i>Grewia asiatica</i> fruit extract on cerebrum LPO \pm SEM ($n=12$) (nm MDA/g protein) of Swiss albino mice at various post irradiation interval after 5 Gy radiation exposure							
Group	Post Irradiation Intervals (in days)					Control	GEA treated
	1	3	7	15	30		
Irradiated	137.19 \pm 1.33 $P<0.001^b$	131.28 \pm 1.11 $P<0.001^b$	139.16 \pm 0.84 $P<0.001^b$	134.24 \pm 1.76 $P<0.001^b$	128.65 \pm 2.36 $P<0.001^b$	104.41 \pm 0.821	100.79 \pm 0.628
GEA treated + Irradiated	124.71 \pm 1.77 $P<0.001^c$	121.43 \pm 1.57 $P<0.001^c$	126.69 \pm 1.52 $P<0.001^c$	118.88 \pm 1.48 $P<0.001^c$	107.19 \pm 1.27 $P<0.001^c$		$P<0.01^a$

(a) Control (vehicle treated) vs. GAE treated; (b) Control (vehicle treated) vs. Irradiated; (c) Irradiated vs. GAE treated + Irradiated

DISCUSSION

One of the basic mechanisms of radiation damage is production of free radicals leading to the formation of peroxides and oxidative reactive species. The peroxides via lipid peroxidation damage the cell membrane and other components of the cell. In the present study, there was a considerable increase in TBARS content after radiation exposure. The magnitude of such a recovery from oxidative damage in term of TBARS content was higher in GAE pretreated irradiated animals (Table I).

Similar results against 5 Gy gamma radiation on the whole brain of mice were noted by Ahaskar and

Sisodia (2006) after oral administration of GAE. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. The presence of antioxidants in the GAE suppresses the formation of free lipid radicals and thus prevents the formation of endoperoxidation. Riveron and colleagues (2007) reported that MDA levels were significantly higher in Alzheimer's disease (AD) patients compared with normal controls, which means that these patients were exposed to oxidative stress via lipid peroxidation. Similar results were reported by Marcus and colleagues (1998), supporting other findings in brain tissues and cere-

Table II

Radiomodulatory influence of <i>Grewia asiatica</i> fruit extract on cerebrum GSH \pm SEM ($n=12$) (nm/100 mg tissue) of Swiss albino mice at various post irradiation interval after 5 Gy radiation exposure							
Group	Post Irradiation Intervals (in days)					Control	GEA treated
	1	3	7	15	30		
Irradiated	23.82 \pm 0.29 $P<0.001^b$	22.22 \pm 0.31 $P<0.001^b$	20.98 \pm 0.23 $P<0.001^b$	21.78 \pm 0.49 $P<0.001^b$	22.13 \pm 0.34 $P<0.001^b$	26.86 \pm 0.172	27.70 \pm 0.21
GEA treated + Irradiated	24.97 \pm 0.42 $P<0.05^c$	23.64 \pm 0.25 $P<0.01^c$	22.32 \pm 0.41 $P<0.01^c$	23.21 \pm 0.24 $P<0.05^c$	26.66 \pm 0.63 $P<0.001^c$		$P<0.01^a$

(a) Control (vehicle treated) vs. GAE treated; (b) Control (vehicle treated) vs. Irradiated; (c) Irradiated vs. GAE treated + Irradiated

brospinal fluid of AD patients (Rinaldi et al. 2003, Galbusera et al. 2004, Kawamoto et al. 2005, Migliore et al. 2005).

Glutathione plays an important role in antioxidant defense, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases, including Alzheimer's disease (AD) (Wu et al. 2004). The present study showed that in GAE pretreated irradiated group, the glutathione level reached the control level on day 30th p.i. and was also significantly higher than the corresponding irradiated group at all the post irradiation intervals studied (Table II). The increased glutathione levels by GAE pretreatment in irradiated mice may facilitate the reduction of oxidative free radicals by H⁺ donation. This allows the restoration of glutathione by glutathione reductase activity.

Most of the researchers have observed that glutathione levels decreased with ageing (Zemlan et al. 1989, Villa and Gorini 1993, Mo et al. 1995). Results presented by Riveron and coauthors (2007) showed that GSH levels were quite low in AD patients compared to normal controls. Similar results for LPO and GSH in whole brain, liver and blood of mice with GAE treatment were also noted (Ahaskar and Sisodia 2006, Sharma et al. 2007). Dose reduction factor (DRF) was

calculated to be 1.53 for GAE in earlier studies in our laboratory (Ahaskar et al. 2007).

In the present study, supplementation of only GAE has also resulted in a statistically significant ($P<0.001$) increase in protein content in comparison to control mice. There was considerable decrease in protein content after radiation exposure at all the post irradiation intervals, whereas, in the GAE pretreated irradiated group, it seems that GAE provides protection as evident by higher values at all the intervals reaching normalcy at day 30 (Table III).

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 synthesized polypeptides from polysomes (Kim et al. 1970). Some studies have indicated that oxidative stress diminishes and the levels of some proteins vary during the progression of AD (Nunomura et al. 2001, Lee et al. 2005). Increased protein concentration in the present study may be due to improved ribosomal activities, which enhance protein synthesis.

The results of the present investigation demonstrate that GAE pretreatment protects the mice cerebrum against radiation-induced damage by inhibiting the glutathione and protein depletion and ameliorating lipid peroxidation levels. GAE contains anthocyanin type cyanidin 3- glucoside (Nair et al. 2005), vitamin C, and carotenoids, etc. (Yadav 1999). Anthocyanins are

Table III

Radiomodulatory influence of <i>Grewia asiatica</i> fruit extract on cerebrum protein \pm SEM ($n=12$) (mg/g) of Swiss albino mice at various post irradiation interval after 5 Gy radiation exposure							
Group	Post Irradiation Intervals (in days)					Control	GEA treated
	1	3	7	15	30		
Irradiated	85.87 \pm 1.44 $P<0.001^b$	82.68 \pm 1.24 $P<0.001^b$	78.99 \pm 1.71 $P<0.001^b$	80.16 \pm 1.61 $P<0.001^b$	83.35 \pm 1.49 $P<0.001^b$	92.64 \pm 0.58	97.77 \pm 0.57
GEA treated + Irradiated	90.78 \pm 0.26 $P<0.01^c$	86.47 \pm 1.41 $P<0.05^c$	84.54 \pm 1.19 $P<0.05^c$	87.90 \pm 0.79 $P<0.001^c$	95.57 \pm 1.09 $P<0.001^c$		$P<0.001^a$

(a) Control (vehicle treated) vs. GAE treated; (b) Control (vehicle treated) vs. Irradiated; (c) Irradiated vs. GAE treated + Irradiated

known for their antioxidant properties (Wang et al. 1997). Delgado-Vargas and others (2000) demonstrated that anthocyanins have scavenging properties against OH and O₂ and are better agents against lipid peroxidation than α -tocopherol (up to seven times). It has been reported that fruits that are richest in anthocyanins (>20 mg/100 g FW) are very strongly colored (deep purple or black) berries (Macheix et al. 1990). *Grewia asiatica* is also strongly colored. Moreover, following consumption of an anthocyanin-rich diet, anthocyanins enter the brain and can exert protective activities against the oxidative damages responsible for numerous neurological disorders (Joseph et al. 2000). In the brain, total anthocyanin content from black berries reached 0.25 ± 0.05 nm/g of tissue (Talavera et al. 2005). The biological benefits of certain carotenoids may be due to their potent antioxidant properties attributed to specific physicochemical interactions with membranes (McNulty et al. 2007). Therefore, the protection afforded by GAE treatment may be due to the synergistic effect of these antioxidants present in GAE.

CONCLUSION

Results obtained from the present study indicate that the natural medicines found in *Grewia asiatica*, including antioxidants and other phytonutrients, substantially protect the cerebrum from radiation damage. However, further research is needed especially regarding the mechanistic aspect of this protection.

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