

POST TREATMENT EFFECT OF *GREWIA ASIATICA* AGAINST RADIATION
INDUCED BIOCHEMICAL CHANGES IN CEREBRUM OF SWISS ALBINO MICE

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Summary

The aim of the present study was to evaluate the radioprotective effect of *Grewia asiatica* fruit pulp extract (GAE) on Swiss albino mice exposed to gamma radiation. In the present study radioprotective efficacy of *Grewia asiatica* (rich in anthocyanin, carotenes, Vit.C etc.) was studied against radiation induced biochemical alterations in mice cerebrum. For experimental 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 (IR+Drug) was administered orally (GAE) for 15 consecutive days once daily after exposed to single dose of 5Gy of gamma radiation respectively. Mice were sacrificed at different autopsy intervals viz. 1, 3, 7, 15 and 30 days and cerebrum were removed for various biochemical estimations viz. glutathione (GSH), lipid peroxidation (LPO) and protein. GAE post treatment renders protection against various biochemical changes in mice cerebrum. Radiation induced augmentation in the levels of LPO was significantly ameliorated by GAE post-treatment. Radiation-induced depletion in the level of GSH, protein was checked significantly by GAE administration.

Key words: *Grewia asiatica*, Antioxidant, Radioprotection, Cerebrum, reduced glutathione, lipid peroxidation.

Introduction

Radiation is known to produce various reactive oxygen species (ROS) in biological systems such as superoxide, hydrogen peroxide and hydroxyl radical and various types of tissue damage due to free radical reactions.¹⁾ The range of antioxidant defenses available within the cell and in the extracellular fluid should be adequate to protect against oxidative damage. Radiation therapy (RT) is considered to be one of the most popular and important tools to cure cancer.²⁾ The radiosensitivity of normal tissues particularly organs away from the tumor sites are suggested to limit the therapeutic gain.³⁾ Detrimental effect of ionizing radiation occurs mainly due to free radicals generated through the decomposition of cellular water.⁴⁾ However, organisms have protective systems against free radical reactions, for example, endogenous antioxidants and antioxidative enzymes. All aerobic organisms are susceptible to oxidative stress simply because semireduced oxygen species are produced by mitochondria during respiration.⁵⁾ Brain is considered abnormally sensitive to oxidative damage⁶⁾ and in fact early studies demonstrating the ease of peroxidation of brain membranes. Brain is enriched in the more easily oxidizable polyunsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid as it has a limited ability to perform aerobic glycolysis; it is unusually vulnerable to hypoxia⁷⁾. On the other hand, brain is not enriched in antioxidant defenses; it contains relatively low levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)⁸⁾.

Diets high in antioxidant properties are known to reverse some deficits in neuronal and cognitive function that occur in aging animals. Antioxidants are also known to reduce levels of pro-inflammatory factors in the CNS. A large numbers of compounds from various plant sources have been shown to possess antioxidant properties.⁹⁻¹¹⁾ 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 the various bioactivities since 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 India¹²⁻¹³⁾, is known for its medicinal properties. The fruit is astringent and stomachic. Morton¹⁴⁾ reported that unripe phalsa fruit alleviates inflammation and is administered in respiratory, cardiac and blood disorders, as well as in fever reduction. Furthermore and infusion of the bark is given as a demulcent, febrifuge, and treatment for diarrhea. *Grewia asiatica* contains anthocyanin type cyanidin 3- glucoside¹⁵⁾, vitamin C, minerals and dietary fibers etc.¹⁶⁾ The antioxidant properties of vitamin C are well known and anthocyanin has recently emerged as a powerful antioxidant.

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 look for the protective effect of alcoholic extract of *Grewia asiatica* fruit in mice cerebrum against radiation induced oxidative stress.

Materials and 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 gm, from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). 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 once a fortnight was given as preventive measures against infections.

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 48 hours (4x12) at 40°C. The extract thus obtained was vacuum evaporated so as to get in powdered form. The extract was redissolved in doubled-distilled water (DDW) just before the oral administration. For the 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 et al.¹⁷⁾

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

Chemicals

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

Dose selection

The dose selection of *Grewia asiatica* was done on the basis of drug tolerance study in our laboratory. Various dose of *Grewia asiatica* (100, 400, 700, 1000, 1300 mg/kg b.wt. of animal) were tested for their effects on the tolerance to 10 Gy gamma irradiation in Swiss albino mice. Thus 700 mg/kg b.wt. /day was obtained as optimum dose and used for further experimentation.

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 (700mg/kg of b.wt. /day) for 15 consecutive days once daily.

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

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

Six mice from each group were necropsied at various intervals, i.e. 1,3,7,15,30 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. The intact cerebrum was then removed carefully from the brain and homogenate was prepared and used for quantitative estimation of various biochemical changes.

Biochemical Assay

Reduced glutathione (GSH) assay

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.*¹⁸⁾ Briefly, 200 μ l of tissue homogenate (20%) was added to 800 μ l 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 M 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.

Lipid peroxidation (LPO) assay

LPO was measured by the method of Buege and Aust.¹⁹⁾ Briefly, tissue homogenate was mixed with TCA-TBA-HCl and was heated for 15 min in a boiling water bath. After centrifugation the absorbance was recorded at 535 nm using a UV-Vis double beam spectrophotometer. The LPO has been expressed as MDA in n mole/ gm tissue.

Protein assay

Estimation of protein was based on the method proposed by Bradford.²⁰⁾ 10% homogenate was prepared (1 gm of tissue in 9 ml of NaCl) and 0.1ml of the sample was taken for the 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 difference between various groups were analysed by the Student's *t*-test and the significance was observed at the $p < 0.02$, $p < 0.01$ and $p < 0.001$ level.

Results

In cerebrum gradual augmentation in the level of TBARS content after gamma irradiation till day 7th in control as well as in experimental group was noticed. Thereafter, depletion in TBARS content was noticed i.e. recovery starts. In experimental group, TBARS content became almost normal by the day 30th post treatment but in the control group, it was higher (23.22%) than the normal value. At all the post irradiation intervals, the LPO values remained significantly lower in experimental group as compared to the control group. (Table_1)

Table 1. Radiomodulatory influence of *Grewia asiatica* fruit extract on cerebrum LPO \pm SEM (n mole MDA/gm protein) of Swiss albino mice at various post irradiation interval after 5 Gy radiation exposures.

Normal	104.41 \pm 0.82				
Only drug	100.79 \pm 0.62 ^b				
	1 Day	3 Day	7 Day	15 Day	30 Day
Control	137.19 \pm 1.32	131.28 \pm 1.11	139.16 \pm 0.839	134.24 \pm 1.75	128.65 \pm 2.36
Experimental	123.89 \pm 1.98 ^a	116.48 \pm 1.89 ^a	120.16 \pm 1.927 ^a	113.94 \pm 1.46 ^a	103.97 \pm 0.89 ^a

(a = P<0.001; b= p<0.01; d= p<0.05; n= not significant)

GAE supplementation for 15 days continuously raised the GSH level by 3.13% (P<0.01) in comparison to the normal. In both the control and experimental groups initially the GSH level declined upto day 7th and day 3rd respectively, thereafter GSH level increased continuously upto the last interval. The maximum decrease noted at day 7th was 21.87% in control group with compare to normal. Whereas, in experimental group, maximum decrease of 12.64% was noted at day 3rd with compare to normal. At day 30 in the experimental group GSH level crosses the normal level. GAE supplementation after irradiation raised the GSH level at all the autopsy intervals compared to the control group. (Table_2)

Significant rise ($p < 0.001$) in cerebrum protein levels was observed in GAE alone treated animals (Group II) as compared to normal (Group I). In group III, protein level decreases upto day 7 post treatment following 5 Gy irradiation, but at later post treatment intervals it increased. A significant declined ($p < 0.001$) in protein content was noted in irradiated animals (Group III) as compared to normal. In the group IV (IR +GAE) protein level decreased upto 3rd day *p.i.* there after it increased continuously upto the last interval studied, where it attained higher value than the normal level. Post treatment of GAE in Group IV significantly increases the protein levels at all the post treatment interval studied compared to control. (Table_3)

Table 2. Radiomodulatory influence of *Grewia asiatica* fruit extract on cerebrum GSH \pm SEM (n mole /100mg tissue) of Swiss albino mice at various post irradiation interval after 5 Gy radiation exposures.

Normal	26.862 \pm 0.172				
Only drug	27.702 \pm 0.218 ^b				
	1 Day	3 Day	7 Day	15 Day	30 Day
Control	23.821 \pm 0.297	22.227 \pm 0.314	20.987 \pm 0.231	21.784 \pm 0.498	22.138 \pm 0.341
Experimental	24.529 \pm 0.189 ^d	23.466 \pm 0.291 ^b	24.706 \pm 0.628 ^a	25.415 \pm 1.02 ^b	26.920 \pm 0.720 ^a

(a = $P < 0.001$; b= $p < 0.01$; d= $p < 0.05$; n= not significant)

Table 3. Radiomodulatory influence of *Grewia asiatica* fruit extract on cerebrum Protein \pm SEM (mg/gm) of Swiss albino mice at various post irradiation interval after 5 Gy radiation exposures.

Normal	92.64 \pm 0.581				
Only drug	97.77 \pm 0.571 ^a				
	1 Day	3 Day	7 Day	15 Day	30 Day
Control	85.87 \pm 1.447	82.68 \pm 1.241	78.99 \pm 1.706	80.16 \pm 1.606	83.35 \pm 1.498
Experimental	89.73 \pm 1.771 ⁿ	85.43 \pm 0.372 ^d	88.65 \pm 1.833 ^a	91.27 \pm 1.499 ^a	97.77 \pm 1.705 ^a

(a = $P < 0.001$; b= $p < 0.01$; d= $p < 0.05$; n= not significant)

Discussion

Results obtained from this study indicate that *Grewia asiatica* extract may act as radioprotective agent and render protection against radiation induced oxidative stress. Similar result of GAE against 5Gy gamma radiation in whole brain of mice was obtained by Ahaskar et al.¹⁷⁾

Oxidative stress leads to lipid peroxidation, which is a highly destructive process and cellular organelles and whole organism, lose biochemical function and/or structural and architecture,²¹⁾ which may lead to damage or death of cell. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing

oxidative reactions. The presence of antioxidants in the plants suppresses the formation of free lipid radical and thus prevents the formation of endoperoxidation.

Under normal conditions, the inherent defense system including glutathione and antioxidant enzymes protects against the oxidative damage. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation.²²⁾ GSH is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of the damaged molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state.²³⁾ A significant decrease in GSH content in whole brain was observed following gamma irradiation (5Gy) by Ahaskar *et al.*¹⁷⁾ In the present study the oral administration of GAE protects the GSH depletion due to irradiation. These results suggest that endogenous non-protein sulfhydryl content (GSH) is maintained by the extracts in the experimental group. GSH might be reacting with the peroxide intermediates; since peroxide intermediates cannot stimulate further lipid peroxidation by autocatalysis and enhance the damage.

Reduction in rate of the protein synthesis may be due to unfavorable condition like unavailability of one or more essential enzymes and/or reduction in sites of protein synthesis.²⁴⁾ Decrease in the protein content after exposure to irradiation might be due to either decline in the rate of protein synthesis or an 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 tRNA or by the inhibition of release of synthesized polypeptides from polysomes.²⁵⁾ Increased protein concentration recorded in our study, shows that GAE supplemented irradiated mice are a beneficial effect. This process showed the improvement in the ribosomal activities, which enhance protein synthesis. This can be treated as an antiradiation effect.

Earlier studies in our laboratory demonstrated the radioprotective effect of GAE was also determined by calculating the dose reduction factor (DRF), which was 1.53 by Ahaskar *et al.*²⁶⁾ Protective role of GAE in liver and blood of mice against 5Gy gamma radiation was also studied by Sharma *et al.*²⁷⁾ they found that pretreatment of GAE significantly reduced the LPO level in Liver and blood where as it increase the GSH level. Singh *et al.*²⁸⁾ demonstrate that GAE pretreatment protects the hematopoietic system of mice against radiation-induced damage by inhibiting the glutathione depletion, decreasing lipid peroxidation level, and increasing hematological constituents in peripheral blood.

Fruits like ber, phalsa, apple and strawberry have been shown to possess moderate antioxidant activity ranging from 12-64 mM FRAP.²⁹⁾ Matsumoto *et al.*³⁰⁾ 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.³¹⁾ The antioxidant potency of anthocyanin extracts is concentration dependent.³²⁾ The positive effects of anthocyanin pigments could be related to their potent antioxidant activity demonstrated in various *in vitro* and *in vivo* studies.³¹⁻³⁵⁾ All evaluated anthocyanins were better agents against lipid peroxidation than α -tocopherol (up to

seven times). Also, it was demonstrated that anthocyanins have scavenging properties against $\cdot\text{OH}$ and O_2^{\cdot} .³⁶⁾

Anthocyanins detected in the brain homogenate were a reflection of their presence in the tissue. These molecules could permeate the blood-brain barrier, in accordance with a recent *in vitro* study showing that brain endothelial cell lines took up cyanidin 3-rutinoside and pelargonidin 3-glucoside.³⁷⁾ At this level, anthocyanins, known for their antioxidant properties,³¹⁾ could exert protective activities against the oxidative damages responsible for numerous neurological disorders.³⁸⁾ Cyanidin 3-glucoside, which was the predominant form (84%) in the brain, has been shown to possess a high antioxidant capacity.^{31,39)} Its presence at the cellular level could compensate the oxidative vulnerability of neuronal cells in aging or neurodegenerative diseases.⁴⁰⁻⁴²⁾

Total intake of fruits, vegetables and fruit juices was positively associated with plasma levels of several carotenoids and vitamin C. Mechanisms of antioxidative action of vitamin C are direct scavenging and blocking of ROS, as well as regeneration of other antioxidative systems.⁴³⁾

The biological benefits of certain carotenoids may be due to their potent antioxidant properties attributed to specific physico-chemical interactions with membranes. McNulty *et al*⁴⁴⁾ test this by measuring the effects of various carotenoids on rates of lipid peroxidation and correlated these findings with their membrane interactions, as determined by small angle X-ray diffraction approaches. The findings indicate distinct effects of carotenoids on lipid peroxidation due to membrane structure changes. These contrasting effects of carotenoids on lipid peroxidation may explain differences in their biological activity.

The protection afforded by GAE might be due to the synergistic effect of antioxidants present in it. From the present study it can be concluded that regular supplementation of GAE may exert an antiradiation influence in the body.

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