Asian J. Exp. Sci., Vol. 21, No. 2, 2007, 00-00

Radioprotective Effect of Fruit Extract of *Grewia asiatica* in Swiss Albino Mice Against Lethal Dose of γ-irradiation.

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Abstract : The radioprotective efficacy of methanolic extract of *Grewia asiatica* (Phalsa) fruit (GAE) against whole body gamma radiation was studied in Swiss albino mice. After drug toxicity test, the oral administration of 700 mg//kg body weight /day of GAE for 15 consecutive days before exposure to 10 Gy of ? radiation was found to afford maximum protection as evidenced by the highest number of survivors after 30 days post irradiation. At this dose level GAE was found to be effective against different levels of radiation doses. $LD_{50/30}$ value of 6.21 for irradiation alone (control) and 9.53 for *Grewia asiatica* + irradiation group (experimental) was obtained; a dose reduction factor (DRF) 1.53 was calculated. The mice of experimental group exhibited significant modulation of radiation- induced decreases of reduced glutathione (GSH) and radiation- induced increase in lipid peroxidation (LPO) in the whole brain and liver at 24 hours after radiation exposure.

Key words : Radioprotection, *Grewia asiatica*, $LD_{50/30}$, LPO, GSH, Gamma radiation, Survival, Toxicity.

Introduction :

Search for the chemical agents that are able to protect human beings from the ionizing radiation is a key issue in radiation biology (Nair *et al*, 2001). Radiation produces various pathological changes in living systems like lipid peroxidation (LPO) (Fitchett *et al*, 1985; Yagi 1988) and damages cellular macromolecules. A number of synthetic compounds of diverse structure and presumed mechanism of action have displayed significant protection against radiation, which include thiole, interlukin 1, cysteine (Patt *et al* 1949) cysteamine (Luning *et al*, 1961), 2-MPG (Dev *et al*, 1981), WR-2721 (Bogo *et al*, 1985), lipoic acid (Ramakrishnan *et al*, 1992) and deoxyspergualin (Nemato *et al* 1995), including some vitamins (Sies and Stahl, 1995; Ramesh *et al*, 1997) and pro-vitamins (Benova, 1992), However clinical applications of these compounds are very few owing to their high toxicity at optimum dose level. The plants have been the companion of man since times immemorial and formed the basis of several useful drugs for treatment of various ailments. The use of plants and natural products may be beneficial in protecting against the radiation-induced damage, as they are less toxic or practically non-toxic compared to the synthetic compounds at their optimum protective dose levels. Therefore, the interest has been increased in development of potential drug

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of plant origin for the modification of radiation effect. Recent studies have indicated that some commonly used medicinal plants may be good sources of potent but non-toxic radioprotectors. Antioxidants of plant origin include vitamin E, C, selenium, phenolic compounds, carotenoids and flavonoids (Chandha, 1996) Earlier studies in our laboratory indicated that oral administration of -carotene (Bhatia *et al*, 2001; Sharma and Sisodia, 2000) and plant extract of spinach (Bhatia and Jain, 2004), amaranths (Yadav *et al*, 2004; Verma *et al* , 2002), and linseed (Bhatia *et al*, 2006), to Swiss albino mice protects various tissues against oxidative stress induced by radiation. *Grewia asiatica*, which is commonly called Phalsa, is cultivated on a commercial scale mainly in the northern and western states of India (Hays, 1953; Sastri, 1956). It is known for its medicinal properties. The fruit is astringent and stomachic. It is reported that unripe phalsa fruit alleviates inflammation and is administered in respiratory, cardiac and blood disorders, as well as in fever reduction (Morton, 1987). 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. But the possible role of *Grewia asiatica* in radioprotection has not been explored. Therefore, the present study is an attempt to investigate the dose dependent variation of the effectiveness of *Grewia asiatica*, on the survival of mice after lethal gamma radiation. Investigation on the changes in LPO, GSH at 24 hour after irradiation and its modulation by GAE in liver and brain of Swiss albino mice has been attempted.

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 an Institutional ethical committee. They were maintained at constant temperature (22 ± 1.50C) and light (12L: 12D) on standard mouse feed (Hindustan Lever, Delhi, India) and provided tap water ad libitum.

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 40oC. 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.

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 77.5cm from the source to deliver the dose rate of 1.07 Gy/ min.

Experiment designs

Experiment 1 : Determination of acute drug toxicity of GAE:

To determine the acute toxicity of GAE according to Jasper *et al* (2003). The animals were divided into 5 groups of 10 each and GAE was given orally to them at the concentration of 100, 400, 700, 1000, 1300 mg/kg body weight/day for 15 consecutive days. The mice were observed continuously for 30 days to determine the toxicity of GAE in the form of mortality or any other symptoms, if present.

Experiment 2: Selection of optimum dose of GAE against radiation:

For the selection of optimum dose of GAE against radiation, animals were given 100, 400, 700,1000,1300 mg/kg body weight/day for 15 consecutive days. One hour after the final administration, mice were exposed whole body to 10 Gy gamma radiations. All the mice were observed for 30 days for any sign of radiation sickness, behavioral change, toxicity and mortality. Changes in body weight were recorded. Usually 10 animals were used for each drug dose and control group. The optimum dose thus obtained was used for further investigation.

Experiment 3 : The radioprotective effect of GAE:

The protective efficacy of any agent (chemical or plant extract) is expressed as dose reduction factor (DRF). It can be calculated by the formula DRF = The $LD_{50/30}$ of experimental group/ The $LD_{50/30}$ of control group.

For this, animals were divided into two groups *viz* **control group (irradiation alone):** Animals of this group were administered DDW of equivalent amount GAE for 15 consecutive days. Second was **experimental group (GAE + irradiation):** the animals of which were given GAE orally at the dose level 700 mg/ kg body weight/ day for 15 consecutive days. One hour after of last administration of DDW or GAE, the animals were exposed to 5, 7, 10, 12 Gy of gamma radiation. The animals of both the groups were observed daily for upto 30 days post irradiation for signs of radiation sickness and mortality.

Body weight: In experiments 2 and 3, the general condition of the mice was observed daily and recorded through measurement of body weights. The percent change in each group was recorded daily by dividing the average weight of mice surviving on a given day by the average weight of the same mice treated on the first day.

Experiment 4 : Biochemical Assays:

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.

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

Group IV (Experimental) : In this group oral administration of GAE (700 mg/ kg of 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 at 10 a.m. Six mice from each groups were necropsied at the same time (10 a.m.)

and intervals, i.e. 1, 3, 7, 15, 30 days post irradiation; brain and liver were immediately removed for biochemical assay.

Removal of Tissue

Brain and liver was excised and used for various biochemical assays. To remove the brain, 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 following parameters were studied to assess the radioprotective effect of a fruit extract of *Grewia asiatica*.

Reduced glutathione (GSH)

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 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

Lipid peroxidation was measured using thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa *et al* (1979). Once removed the brain and liver were immediately placed in cold 0.9% NaCl and washed in the same.

Ten percent homogenate were prepared (1 g of tissue in 9 ml of 1.15 KCl) and 0.2 ml of the sample was taken for the assay. The absorbance was read at 532 nm.

Two repeats of the each assay from each animal were carried out.

Statistical analysis :

The results obtained in the present study were expressed as mean ± SEM. The statistical difference between various groups was analysed by the Student's t-test and the significance was observed at the $p < 0.02$, $p < 0.01$and $p < 0.001$ levels. Regression analysis was done to obtain $LD_{50/30}$ values and to determine the DRF.

Results

Experiment 1: Effect of GAE on acute toxicity

The administration of 100 to 1000 mg/kg b.wt. of GAE to mice did not induce drugrelated toxicity in the mice as evident by 100% survival of treated animals. In 1300 mg/kg b.wt, only one animal died out of 10 at 27 day of observation. No toxic effects were observed in terms of behavior, sickness, urination, and defecation pattern and body weight in all groups. Therefore, it was concluded that GAE as such did not induce any toxic manifestations up to a dose of 1000 mg/kg b.wt.

Experiment 2 : Selection of optimum GAE dose:

The animals of DDW+ irradiation group exhibited signs of radiation sickness with in 2-3 days after exposure to 10 Gy of gamma radiation. The irradiated animals exhibited reduction in the food and water intake, ruffling of hair, diarrhea, watering of eyes and irritability. A few animals showed paralysis and difficulty in locomotion during the second week of after exposure. Maximum increase in body weight was noticed in 700 mg/kg

Figure 1 : Curves of body weight of surviving mice after irradiation (10 Gy) with or without different doses of GAE treatment

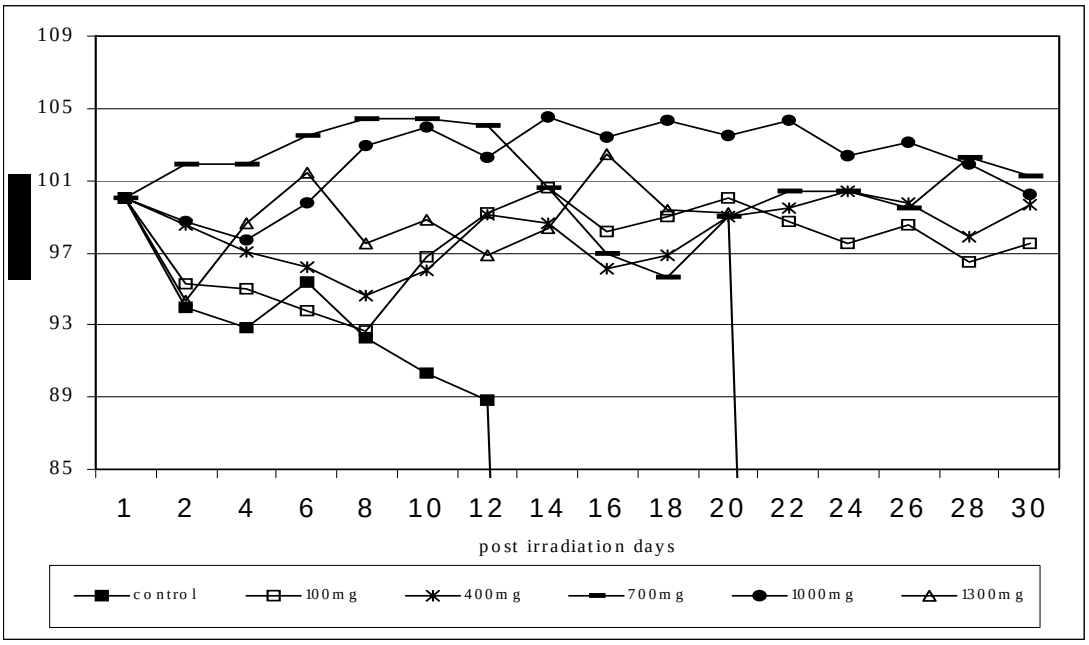
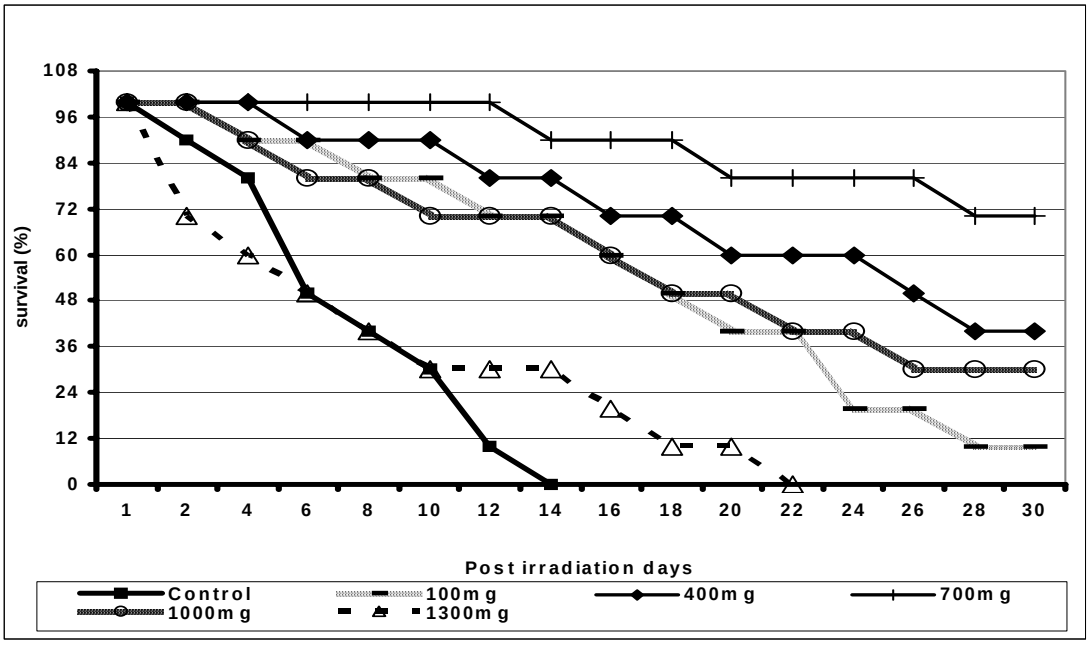


Figure 2 : The survival curves after irradiation (10 Gy) of mice (n=10) with or without administration of GAE (Experiment Number 2)



b.wt. GAE dose at 30 day compared to all other groups which showed lesser body weight (Figure 1). The optimal effectiveness of different *Grewia asiatica* extract doses in the term of survivability (Figure 2) is as follows:

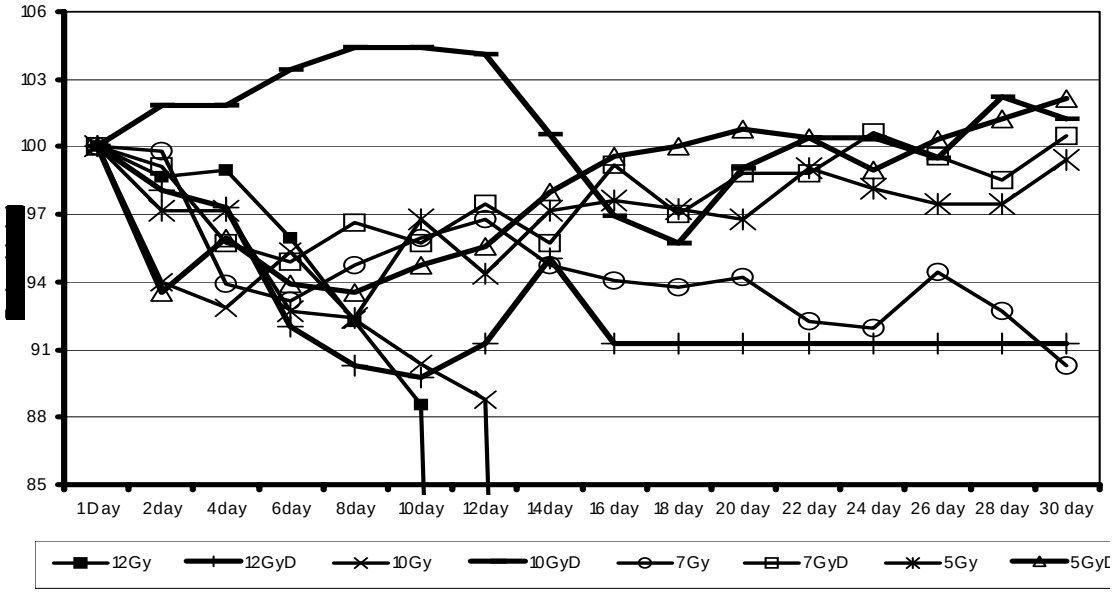
700mg >400mg>1000mg>100mg> 1300mg> control

Further, the following pertinent information can also be gathered from this Figure 2. Amongst all the groups, the first or early mortality has been observed in higher dose group (1300 mg) as well as in control group on day 2nd. Maximum mortality (i.e.100%) with in minimum time (14 days) observed in control group and long survivability or minimum mortality (i.e. 30%) was observed in 700 mg dose group within maximum observation time (30 days). Death of all animals occurred within 22 days in 1300 mg dose group. In 100 mg, 400 mg, 1000 mg dose group survival was 10%, 40%, and 30% till the last day of observation, respectively. In 700mg dose group, the deaths occurred slowly from 1st to 18th days post irradiation and 70% animals survived up to 45 days post irradiation. Therefore, 700 mg/kg b.wt. /day GAE has been used for the detailed investigation.

Experiment 3 : In the present investigation, it was observed that the pretreatment of GAE enhanced the body weights of mice exposed to different doses of gamma radiation. (Figure 3).

A radiation dose dependent survival of mice has been observed in both, the control and the experimental groups as shown in Figure 4. In control group 40 & 50% of total animals died within 30 days when exposed to 5 and 7 Gy gamma radiation, respectively. The first death, was recorded on day 2 after irradiation with 10 and 12 Gy. With 7 and 5 Gy, first deaths were recorded on 16 and

Figure 3 : Curves of body weight of mice surviving after different doses of radiations with or without of GAE treatment



18th day, respectively. Exposure to 12 and 10 Gy resulted in 100% mortality within 12 and 14 days, respectively. First deaths recorded were delayed after exposure to irradiation at all the intervals in the experimental group. They were recorded on 8, 14, 16 and 20 days after radiation exposure with 12, 10, 7 and 5 Gy, respectively. GAE pre treatment reduced 90% mortality at 5 Gy (Figure 5). $LD_{50/30}$ for control and experimental groups was calculated as 6.21 and 9.53 Gy, respectively (Table1). GAE treatment produced a dose reduction factor of 1.53.

Experiment 4 : Reduced glutathione (GSH) measured as acid soluble sulfhydryl group (-SH) in liver homogenate showed a significant decline after exposure to 5.0 Gy gamma radiations. However, GAE pre-treated groups caused a significant elevation. Administration of only GAE (Group II) for 15 consecutive days elevated the levels of GSH by 3.96 % from normal, which is statistically significant ($p<0.001$). In the experimental group pre treatment with GAE for 15 days increased the GSH level by 19.47 % from the control ($p<0.001$). Lipid peroxidation estimated from MDA production in the microsomal fraction of liver homogenate could be significantly decreased by 6.8% ($p<0.001$) from the normal group by the supplementation of only GAE. Irradiation (control group) significantly raised the MDA level by 10.8% ($p<0.001$) as compared to normal group. But oral supplementation of GAE prior to irradiation for 15 consecutive days did not significantly change the LPO compared to normal. However, statistically significant ($p<0.001$) reduction in LPO by 8.19 % was noted in the experimental group from the control in the liver (Table 2).

Figure 4 : Survival percentage of mice (n=10) at different dose of gamma radiation with or without GAE treatment (Experiment Number 3)

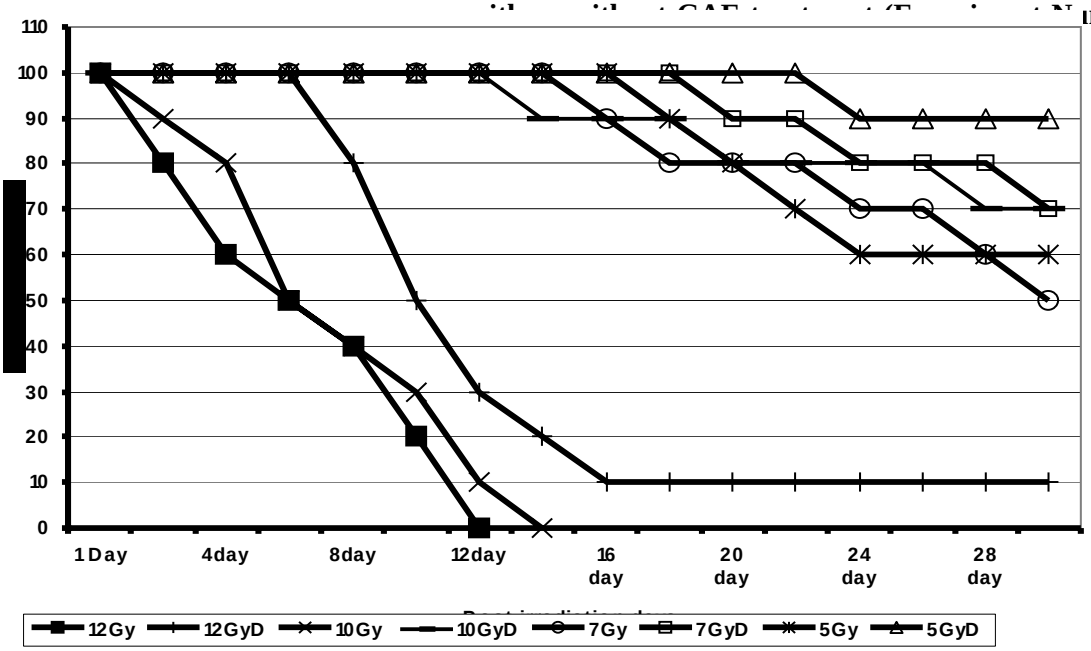


Figure 5 : Percentage mortality within 30 days post-irradiation, with and without GAE (LD50/30 estimation)

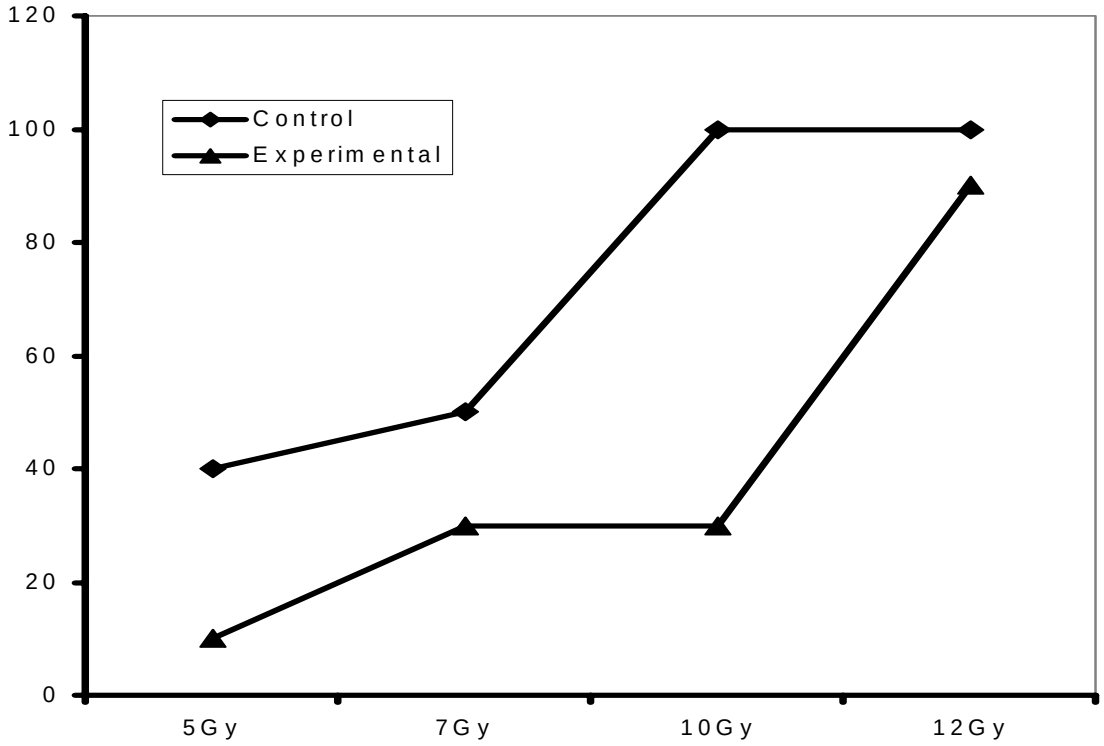


Table 1. Regression analysis of percentage mortality ($LD_{50/30}$)

Group	Intercept (b)	Slope (m)	Y = mx +b	$LD_{50/30}$	DRF
Control	-11.04	9.83	50= -11.04+ 9.83x	6.21	1.53
Experimental	-42.07	9.66	50= -42.07+ 9.66x	9.53	

Table 2. Radiomodulatory influence of *Grewia asiatica* fruit extract on hepatic GSH and LPO ± SEM of Swiss albino mice after 24 hrs of 5 Gy radiation exposures.

Group	GSH (n mole /100mg tissue)	LPO (n mole MDA/gm
I (Normal)	55.966± 0.799	347.393± 5.366
II (Only drug)	58.181± 0.706[a]	323.763±4.903[b]
III (Control)	40.026±0.495 [a]	384.837±2.645[a]
IV(Experimental)	47.819± 0.434[a]	353.301±1.698[a]

Significance level :
[a]p=<0.001; [b]p=<0.01; [c]p=<0.05; [d]p=<0.02;
Each value represent mean ±SE (n=12).
Statistical comparisons between following group:
Group I vs. Group II; Group I vs. Group III; Group III vs. Group IV.

Table 3 : Radiomodulatory influence of *Grewia asiatica* fruit extract on GSH and LPO ± SEM in whole brain of Swiss albino mice after 24 hrs of 5 Gy radiation exposures

Group	GSH (n mole /100mg tissue)	LPO (nmole MDA/gm protein)
I(Normal)	30.403±1.769	103.551±0.633
II(Only drug)	34.240±1.007	99.808±0.588[a]
III(Control)	24.168±1.579 [d]	199.215±1.057[a]
IV(Experimental)	29.042±1.422[c]	150.972±0.839[a]

Significance level :
[a]p=<0.001; [b]p=<0.01; [c]p=<0.05; [d]p =<0.02;
Each value represent mean ±SE (n=12).
Statistical comparisons between following group:
Group I vs. Group II; Group I vs. Group III; Group III vs. Group IV.

In brain, GSH increased by 12.62% in GAE pretreated group as compared to the normal. In the experimental group GSH raised by 20.17% in comparison to the control. Administration of only GAE could significantly ($p<0.001$) reduce the LPO by 3.61% in comparison to the normal group. In the experimental group LPO levels reduced significantly ($p<0.001$) by 24.22% with respect to the control group.

Discussion:

The animals of the control group exhibited signs of radiation sickness within 2-3 days after exposure to 10 Gy which is in good agreement with our earlier findings and those of other researchers where a similar observation has been made (Jagetia and Baliga 2002). The death of animals is due to hematopoetic syndrome characterized by symptoms like, irritability, epilation, weight loss, lacremation and ruffling of hairs. Pretreatment of mice with different doses of GAE resulted in a dose dependent reduction in radiation induced mortality upto 700 mg/kg b.wt/day, whereas higher doses declined the survival of mice as compared to 700 mg/kg b.wt/day. Similarly, -carotene (30 mg/kg b.wt) has been reported to provide maximum protection against the 30-days mortality in the whole body irradiated mice; a further increase in drug dose did not increase the survival significantly, moreover higher doses were found to be toxic (Bhatia *et al*, 2001).

In vivo studies in experimental animals have included protection against radiation induced lethality due to GI injury, a specific tissue damage, and carcinogensis. The most reliable procedures involve determination of a dose reduction factor (DRF) or dose-modifying factor (DMF). In animal studies, irradiating mice with and without administered agent at a range of radiation doses and then comparing the endpoint of interest typically determine DRFs. Unfortunately, DRFs have not been reported often for naturally occurring compounds. In mice, high DRFs (>2.0) may be obtained with synthetic drugs whereas those for naturally occurring compounds are not likely to be greater than 1.3. (Weiss *et al*, 2003). In our present studies the radioprotective effect of GAE was demonstrated by the $LD_{50/30}$ values and (DRF=1.53), which is seemingly quite high and proves the efficacy of the extract.

The pattern of survivals after GAE treatment was similar to that of the irradiated control group except that mortality was delayed. This may be due to the effectiveness of GAE in arresting GI death, as indicated by increased number of survival days in all the treatment groups, compared to the control. The administrations of 100, 400, 700, 1000 mg/kg b.wt/day of GAE were more effective than these of other higher doses of GAE in reducing the GI death. A similar effect has also been observed earlier for amaranthus (Yadav *et al*, 2004;Verma *et al*, 2002), seeds of Jamun (Jagetia *et al*, 2005), and -carotene (Bhatia *et al*, 2001; Sharma and Sisodia, 2000). This reduction in GI death may also be due to the protection of intestinal epithelium, which would have allowed proper absorption of the nutrition as also noted by metabolic activity in liver in the present study.

The mechanism of action of herbal drugs and their extract preparations differ in many respect from those of the synthetic drugs or single substances (Wagner, 1999). The exact mechanism of action of GAE is not known. However, it is possible that scavenging of free radicals by GAE may play an important role in providing protection against the radiation induced damage. Prophylactic action of

Grewia asiatica against radiation-induced metabolic disorders may be due to presence of antioxidants like anthocyanin, vitamin C etc. The positive effects of anthocyanin pigments could be related to their potent antioxidant activity demonstrated in various in vitro and in vivo studies (Wang *et al*, 1997,1999; Tsuda *et al*, 1994; Matsumoto *et al*, 2002; Ramirez-Tortosa *et al*, 2001).

Fruits like 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 in 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 extract is concentration dependent (Gabrielska, 1999).

The free radicals generated during the radiolysis of water play the most important role in the biological damage induced by ionizing radiation (Hall, 1978). 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 (Biaglow *et al*, 1987). 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 (Bump and Brown, 1990). A significant decrease in GSH content in brain was observed following gamma irradiation (5 Gy). 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 can not stimulate further lipid peroxidation by autocatalysis and enhance the damage.

The basic effects of radiation on cellular membrane are believed to be the peroxidation of membrane lipids. Radiolytic products can initiate LPO, including hydroxyl and hydroperoxy radicals (Konings and Drijver, 1979). Lipid peroxidation 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. It is a highly destructive process causing cellular organelles and the whole organism to lose biochemical function and/or structural architecture (Kale and Sitaswad, 1990) which may lead to damage or death of cells. Above results show that GAE renders protection against radiation induced oxidative stress by altering the lipid peroxidation level in term of malanodialdhyde production. The measurement of lipid peroxidation is thus the convenient method to monitor oxidative cell damage (Girotti 1985). The products of lipid peroxidation such as malanodialdhyde and 4-hydroxynonenal are to the toxic to the cell (Esterbauer *et al*, 1988). The preservation of cellular membrane integrity depends on protection and repair mechanism capable of neutralizing oxidative reactions.

The protection afforded with *Grewia asiatica*, in the biochemical activity of liver and brain in the present study may prove to

be beneficial for the clinical use of such dietary compounds as radioprotectors.

Acknowledgements :

We are grateful to Prof. A.L. Bhatia for his critical guidance and support. Authors gratefully acknowledge Prof. D.P. Agrawal, Dr. A.A. Chougle, and the Department of Radiobiology, SMS Medical College and Hospital, Jaipur, India, for the irradiation facilities and dosimetry. Facilities from Center for Advanced Studies (CAS), of Department of Zoology, University of Rajasthan, Jaipur is thankfully acknowledged.

References :

Benova D. K. (1992) : Anticlastiogenic effects of a polyvitamin product 'Pharmavit', on ? -ray induction of somatic and germ cell chromosome aberrations in the mouse. *Mutation Res*., **269**, 251-8.

Bhatia A.L., Sisodia R., Manda K., and Sharma M. (2001): Dose dependent study on the effectiveness of -carotene on the survivability of mice against lethal gamma irradiation. *Radiation Protection and Environment*., **24** (1&2), 96-101.

Bhatia A.L. and Jain M. (2004): Spinacia oleracea L. protects against gamma radiations: a study on glutathione and lipid peroxidation in mouse liver. *Phytomedicine*., **11**, 607-615.

Bhatia A.L., Manda K., Patni S. and Sharma A.L. (2006): Prophylactic Action of Linseed (Linum usitatissimum) Oil Against Cyclophosphamide-Induced Oxidative Stress in Mouse Brain. J *Med Food*., **9 (2)**, 261-264.

Biaglow J. E., Varnes M. E., Epp E. R. and Clark E. P. (1987) : In: Anticarcinogenesis and Radiation Protection, Eds. P. A. Cerrutti, O. F. Nygaard and M.G. Simic, Plennum Press, New York, p. 387.

Bogo V., Jacobs A. J. and Weiss J. F. (1985): Behavioral toxicity and efficacy of WR-2721 as a radioprotectant. *Radiat. Res*., **104**, 182-190.

Bump E. A. and Brown J. M. (1990) : Role of glutathione in the radiation response of mammalian cells in vitro and in vivo. *Pharmacol. Ther*., **47**, 117-136.

Chandha S. L. (1996): Natural sources of antioxidants and their adequacy in diet to prevent atherosclerosis. *Mediquest*, **143**, 337-351.

Dev P. K., Gupta S. M., Goyal P. K., Mehta. G. and Pareek B. P. (1981): Protection from body weight loss by 2-mercaptopropionylglycine (MPG) in growing mice irradiated in uterus with gamma radiation. *Experientia*., **38**, 962-963.

Esterbauer H., Zollner H. and Schaur R. J. (1988): Hydroxyalkkenals: cytotoxic products of lipid peroxidation. ISI Atlas of Science Biochem., 1, 311-317.

Fitchett M., Gilbert B.C., and Jeff M. (1985): Chemical models and radiation damage. *Pilots Trans R Soc Lond B Biol Sci*., **311(1152)**, 517-529.

Gabrielska J., Oszmiansk, J., Komorowska M. and Langner M. (1999): Anthocyanin extracts with antioxidant and radical scavenging effect. *Z. Naturforsch*., **54c**, 319-324.

Girotti A. W. (1985): Mechanisms of lipid peroxidation. J. *Free Rad. Biol. Med*,. **1**, 87-95.

Hall E. J. (1978): In: Radiobiology for the Radiologists, 2nd edition, Harper and Row Publishers, Philadelphia.

Hays W. B. (1953): Fruit growing in India. 2nd Revised edition. Kitabistan, Allahabad, India.

Jagetia G. C., Baliga M. S. and Venkatesh P. (2005): Influence of seed extract of Syzygium Cumini (Jamun) on mice exposed to different doses of radiation, J. *Radiat. Res*., **46**, 59-65.

Jasper W., Ogwal-Okeng, Celestino Obua and William W. A. (2003): Acute toxicity effects of the methanolic extract of Fagara zanthoxyloides (Lam.) root-bark. *African Health Sciences*.,**3 (3)**, 124-126.

Kale R. K. and Sitaswad S. L. (1990): Radiation induced lipid peroxidation in liposomes. *Radiat.Phys.Chem*., **36** , 361-364.

Kaur C. and Kapoor H. C. (2005): Antioxidant activity of some fruits in Indian diet. In ISHS Acta Horticulturae 696; VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics - Part Two..

Konings A. W. T. and Drijver E. B. (1979): Radiation effects on membranes. I. Vitamin E deficiency and lipid peroxidation, *Radiat. Res*., **80**, 494-501.

Luning K. G., Frelon H. and Nelson A. (1961): A protective effect of cysteamine against genetic damages by X-rays in spermatozoa from mice, *Radiat. Res*., **14**, 813.

Matsumoto H., Nakamura Y., Hirayama M., Yoshiki Y. and Okubo K. (2002): Antioxidant activity of black currant anthocyanin aglycons and their glycosides measured by chemiluminescence in a neutral pH region and in human plasma. J. *Agric. Food Chem*., **50**,5034-5037.

Moron M. S., Depierre J. W. and Mannervik B. (1979): Levels of GSH, GR and GST activities in rat lung and liver. *Biochim. Biophys. Acta*., **582**, 67-78.

Morton J. F. (1987): Phalsa, Fruits of warm climate. Julia Morton, Miami, F.L. 276-277.

Nair C.K., Parida D. K. and Nomura T. (2001): Radioprotectors in radiotherapy. J. *Radiat. Res*.,**42**, 21-37.

Nair M. G., Wang H., Mody D. K. and Deueitt D. L. (2005): Dietary food supplement containing natural cyclooxygenase inhibitors and methods for inhibiting pain and inflammation. United states patent application.

Nemato K., Horiuohi R. and Miyamoto T. (1995) Deoxyspergualin is a new radioprotector in mice. *Radiat. Res*., **141**, 223-225.

Ohkhawa H., Ohishi N. and Yogi K. (1979): Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. *Anal. Biochem*., **95**, 351-358.

Patt H. M., Tyree E. B., Straube R. L. and Smith D. E. (1949): Cysteine against *X-irradiation*. *Science*., **110**, 213.

Ramakrishnan N., Wolfe W. W. and Catravas G. N. (1992): Radioprotection hemopoietic tissues in mice by lipoic acid. *Radiat. Res*., **130**, 360-365.

Ramesh S., Manda K. and Bhatia A. L. (1997): Protective effect of ß-carotene on radiation induced lipid peroxidation. *Curr. Sci*., **73**, 470-471.

Ramirez-Tortosa C., Andersen O. M., Gardner P. T., Morrice P. C., Wood S. G., Duthie S. J., Collins A. R. and Duthie G. G. (2001): Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radic. *Biol. Med*., **31**,1033-1037.

Sastri B. N. (1956): The wealth of India: Raw materials#4.Grewia linn. Tilliaceae. In: Council of Scientific and Industrial Research, New Delhi, India, 260-266.

Sharma M.K., Sisodia R. (2000): ß-carotene against Radiation-induced Oxidative Stress in Mice Brain. Asian J.Exp.Sci, 14, No.1&2, 2000,43-44.

Sies H. and Stahl W. (1995): Vitamin E and C, ß-carotene P < 0.001 and other carotenoids as antioxidants. *Am. J. Clin.Nutr*., **62**, 1315S-21S.

Tsuda T., Watanabe M., Ohshima K., Norinobu S., Choi S. W., Kawakishi S. and Osawa T. (1994): Antioxidative activity of the anthocyanin pigments cyanidin 3-O-ß-D-glucoside and cyanidin. *J. Agric. Food Chem*., **42**, 2407-2410.

Verma R .K., Sisodia R. and Bhatia A.L. (2002): Radioprotective Role of Amaranthus gangeticus Linn.: A Biochemical Study on Mouse Brain. J *Med Food*., **5** ,189-195.

Wagner H. (1999): Phytomedicine research in Germany. Environ. *Health. Perspect*.,**107** ,779-781.

Wang H., Cao G. and Prior R. L. (1997): Oxygen radical absorbing capacity of anthocyanins. J. *Agric. Food Chem*., **45**, 304-309.

Wang H., Nair M. G., Strasburg G. M., Chang Y. C., Booren A. M., Gray J. I. and DeWitt D. L. (1999): Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J. *Nat. Prod*., **62**, 294-296.

Weiss J. F. and Landauer M.R.(2003): Protection against ionizing radiation by antioxidant nutrients and phytochemicals .*Toxicology*, **189**, 1-20.

Yadav A. K. (1999): Phalsa: A Potential New Small Fruit for Georgia. J. anick, 348-352.

Yadav R. K., Bhatia A.L. and Sisodia R. (2004): 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.

Yagi K. (1988): The Biological role of reactive oxygen species in skin. Eds: by Hyashi, D., Imamura, S.and Miyachi, V.Tokyo: Tokyo Press. 109-116.