MODULATION OF RADIATION INDUCED BIOCHEMICAL ALTERATIONS IN SWISS ALBINO MICE BY *GREWIA ASIATICA* (PHALSA)

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

Radiation & Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur, India-302004
Tel.: +91-141-2370880; fax: +91-141-2701137.
*Corresponding author: E-mail – rashsisodia@yahoo.co.in

Summary

Increasing use of nuclear radiation for human welfare necessitates a new, safe and cost effective radio protector not only for personnel's charged with responsibility of testing or with radiations in laboratories, but also for the general public. Keeping this view, this study has been undertaken to find out the possible radio protective potential of the *Grewia asiatica* fruit pulp extract *(*GAE), *Grewia asiatica* has a high content of antioxidants like Vitamin C, anthocyanin and folate that may play a possible role in radioprotection .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 (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 autopsy intervals viz. 1, 3, 7, 15 and 30 days and blood and liver were removed for various biochemical estimations viz. glutathione (GSH), lipid peroxidation (LPO). Irradiation resulted an elevation in lipid peroxidation (LPO) and a decline in glutathione (GSH) level in liver as well as blood. On the other hand, treatment of animals with GAE extract before irradiation caused a significant decrease in LPO and a marked elevation in GSH.

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

Introduction

Synthetic protectors against oxidative damage to tissue have toxicity. This limits their value in the clinical field. The search is on for some natural compounds, which 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 posses antioxidant properties.[1-3)] Antioxidants of plant origin include vitamin E, C, selenium, phenolic compounds, flavonoids and others.[4)] Nutritional intervention to increase intake of phyto-antioxidants may reduce the threat of free radicals. Recent research has 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.[5-6)] 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 India, [7-8)] is known for its medicinal properties. The fruit is astringent and stomachic. Morton[9)] 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-glucoside,[10)] vitamin C, minerals and dietary fibers etc.[11)] The antioxidant properties of vitamin C are well known and anthocyanin has recently emerged as a powerful antioxidant. The present study is therefore an attempt to investigate the possible protective role of *Grewia asiatica* fruit against radiation induced oxidative stress in mice with special reference to blood and liver.

Materials and Methods

Animal care and handling:

Adult male Swiss albino mice (6-8 weeks old) weighing 23±2 g from inbred colony were used for the present study. The animals were maintained on the standard mice feed (procured from Hindustan Lever Ltd., India) and water *ad libitum.* Four animals were housed in polypropylene cage containing paddy husk (procured locally) as bedding throughout the experiment. Animal care and handling were performed according to guidelines issued by the World Health Organization (Geneva, Switzerland) and the Indian National Science Academy (New Delhi, India). The Department Ethical Committee has approved the present study.

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 36hours (3x12) at 40^{o}C. The extract thus obtained was vacuum evaporated so as to get in powdered form. The extract was redisolved 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* .[12)]

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.

Dose selection

Dose selection of *Grewia asiatica* was done on the basis of drug tolerance study (Jain *et al*., 2002b). Various dose of *Grewia asiatica* (100,400,700,1000,1300 mg/kg b.wt.) were tested against gamma irradiation (10Gy). Thus 700 mg/kg b.wt. /day was used as optimum dose for further experimentation as obtained by Ahaskar *et al* . [12]

.

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 oral administration of GAE (700mg/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. Six mice from each groups were necropsied at various intervals, i.e. 1,3,7,15,30 days post irradiation

These animals were killed by cervical dislocation, and their blood was collected from orbital sinus by heparinised needle. Also, their livers were profuse transcardially with ice-cold saline and Blood ad liver were used to estimated for various biochemical changes viz. glutathione, protein and Lipid peroxidation.

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* [13]. 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: LPO was measured by the method of Buege and Aust[14]. 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.

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 $p < 0.02$, $p < 0.01$and $p < 0.001$ level.

Results

Lipid Peroxides: - It is evident from graph_1 that control group (untreated irradiated) has a significantly higher value of MDA content in blood serum at all autopsy intervals then compare to normal or experimental group. MDA increases after irradiation up to day 15th in control group and at day 3rd in experimental group. Thereafter decrease in LPO values were observed. In control group the value were higher (220.45%) then normal at day 15th. Mice exposed to radiation have a significantly ($P<0.001$) higher values of MDA content, to normal mice. Such radiation induced changes in LPO level appears to be was ameliorated by the administration of GAE.

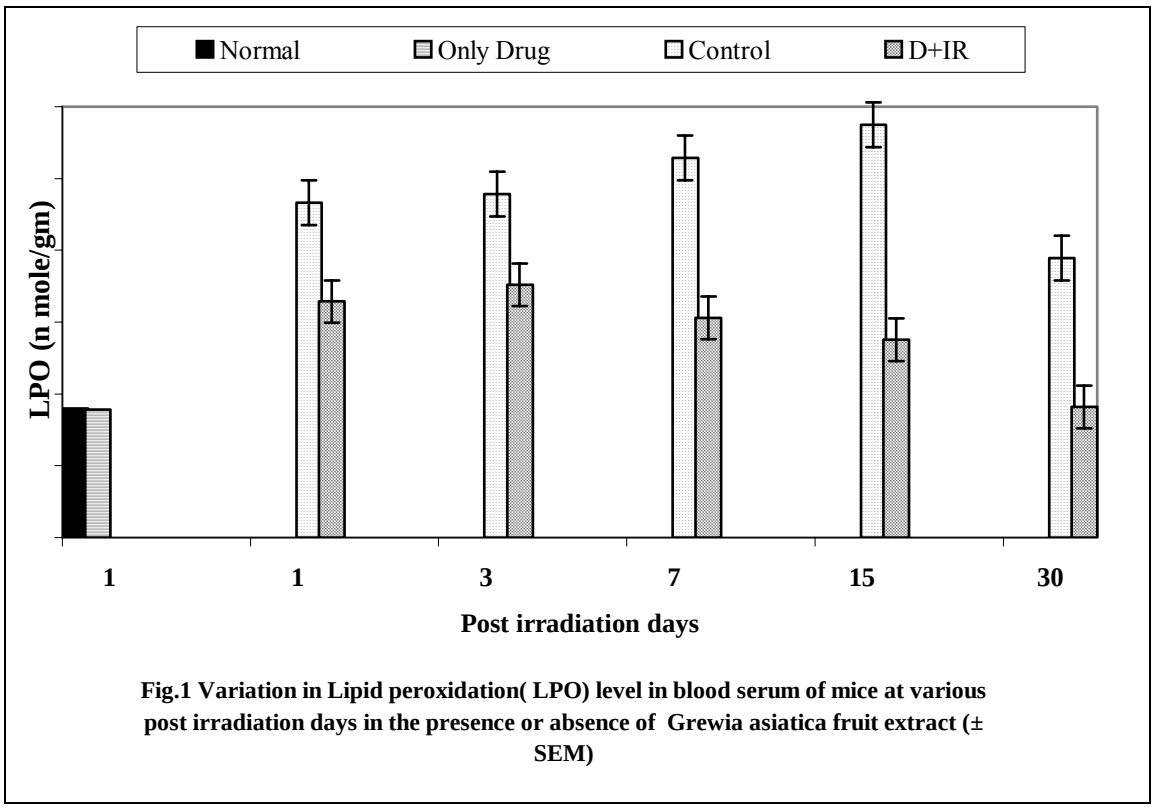

Fig.1 Variation in Lipid peroxidation(LPO) level in blood serum of mice at various post irradiation days in the presence or absence of Grewia asiatica fruit extract (± SEM)

The value represents mean ± S.E.M. The statistical significance was obtained between normal V/s only drug and Control V/s Experimental ($^{b}p < 0.01$; $^{a}p < 0.001$: ^{n}p= no significant)

Liver shows (graph_3) continuous augmentation in the level of TBARS content after gamma irradiation till day 15th in control where as in experimental group it shows till 7th day. Thereafter, depletion in TBARS content was noticed i.e. recovery starts. In experimental group, TBARS content became almost normal by the day 30th post irradiation but in the control group, it was higher (10.35%) 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.

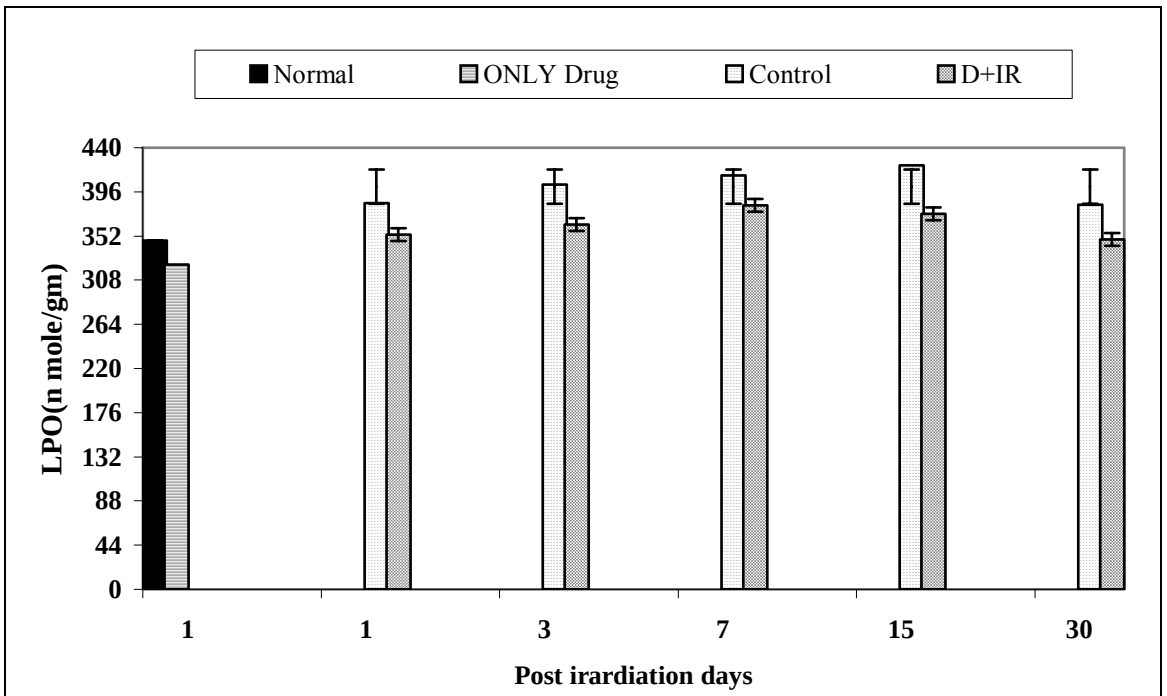

Fig.3 Variation in Lipid peroxidation(LPO) level in liver of mice at various post irradiation days in the presence or absence of Grewia asiatica fruit extract (± SEM)

The value represents mean ± S.E.M. The statistical significance was obtained between normal V/s only drug and Control V/s Experimental ($^{d}p < 0.05$; $^{b}p < 0.01$; $^{a}p < 0.001$: ^{n}p= no significant)

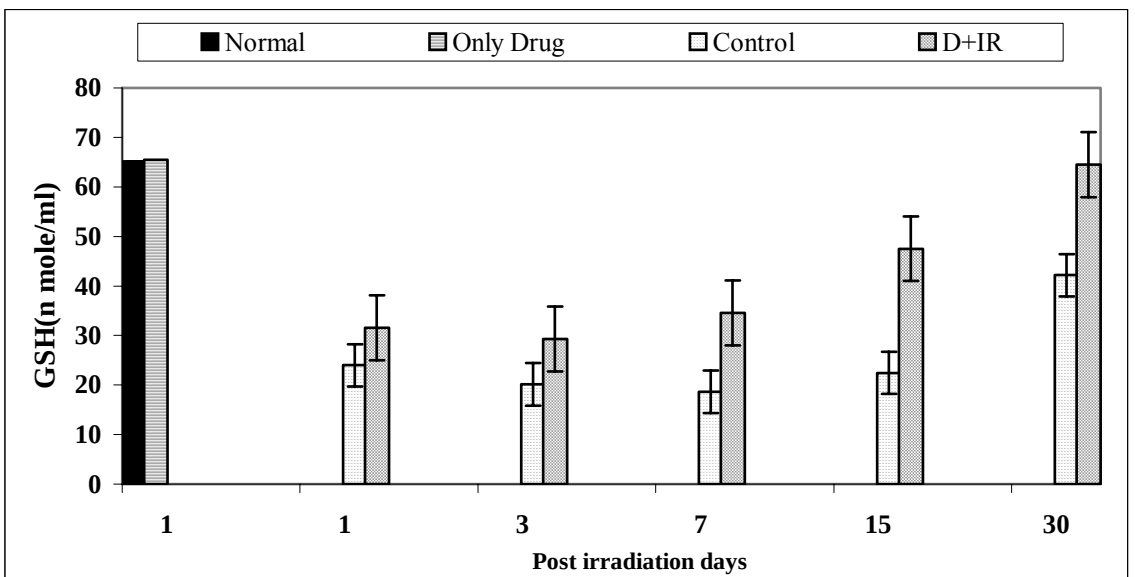

Fig.2 Variation in Glutathion(GSH) level in blood serum of mice at various post irradiation days in the presence or absence of Grewia asiatica fruit extract (± SEM)

The value represents mean ± S.E.M. The statistical significance was obtained between normal V/s only drug and Control V/s Experimental ($^{d}p < 0.05$; $^{b}p < 0.01$; $^{a}p < 0.001$: ^{n}p= no significant)

Glutathione

There was an insignificant variation in blood glutathione values from 1 to 30 days post-treatment in GAE treated unirradiated and normal mice; therefore a single value was taken for statistical comparison. It is evident from garph_2 that the values of reduced Glutathione were significantly not different in GAE treated group from normal group. Glutathione decreased in control group till 7th day and then increased upto last autopsy interval, however it was remained lower at all autopsy intervals with respect to normal. In GAE pretreated irradiated group, it decreased upto day 3rd and afterwards it increased until it reached normal values at the last autopsy intervals. Percentage protection observed in experimental group was 11.7%, 14.04%, 24.45%, 38.54%, and 34.24% respectively higher then control at 1, 3, 7, 15, and 30 days post exposure, with respect to normal group.

GAE supplementation for 15 days continuously raised the GSH level by 3.96 % in comparison to the normal. In both the control and experimental groups initially the GSH level declined upto day 15th and 7th day respectively, thereafter GSH level increased continuously upto the last interval. The maximum decrease noted at day 7th and 15th was 50.79 % and 29.75% in control and experimental group respectively with compare to normal. At day 30 in the experimental group GSH level reached near normalcy but was still deficient by 3.01%. GAE supplementation prior to irradiation raised the GSH level at all the autopsy intervals compared to the control group as shown in graph_4.

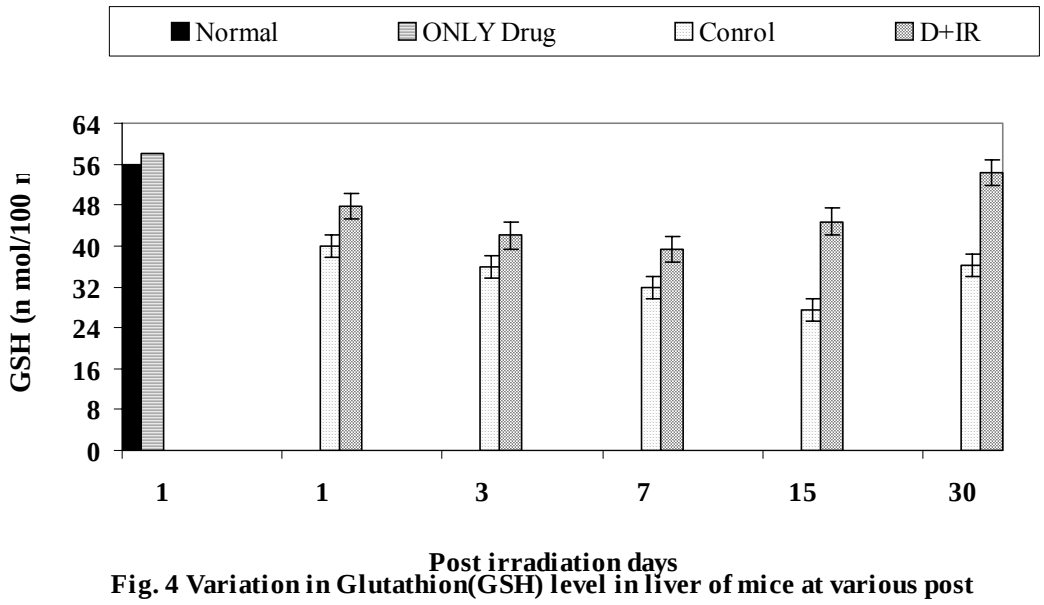

Fig. 4 Variation in Glutathion(GSH) level in liver of mice at various post irradiation days in the presence or absence of Grewia asiatica fruit extract (± SEM)

The value represents mean ± S.E.M. The statistical significance was obtained between normal V/s only drug and Control V/s Experimental ($^{d}p < 0.05$; $^{b}p < 0.01$; $^{a}p < 0.001$: ^{n}p= no 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. 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.[15] Oxidative stress leads to lipid peroxidation and metabolic disorders.[16-18] Lipid peroxidation is a highly destructive process and cellular organelles and whole organism, lose biochemical function and/or structural and architecture, [19] 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. [20] 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. [21]

The present study demonstrates a significant reduction in liver and blood GSH following exposure. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Oral administration of GAE did not significantly influence the endogenous GSH level either in liver or blood, but its presence during radiation exposure protects the endogenous GSH depletion due to irradiation. The lower depletion of liver and blood GSH in the GAE pre-treated irradiated animals could be due to the higher availability of GSH, which increases the ability to cope up with the free radicals produced by irradiation. The increased GSH level suggests that protection by GAE may be mediated through the modulation of cellular antioxidant levels. The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Radiolytic products, including hydroxyl and hydroperoxyl radicals, can initiate lipid peroxidation[22]. In the present study, however, GAE treatment did not significantly alter the lipid peroxidation level in unirradiated animals, but it significantly lowered the radiation-induced lipid peroxidation in terms of malondialdehyde.The Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants [23-24].

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 survivability 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.Protective role of GAE in mice brain against 5Gy gamma radiation was also studied by Ahaskar et al[12].

Fruits like ber, phalsa, apple and strawberry have been shown to possess moderate antioxidant activity ranging from 12-64 mM FRAP. [25] Matsumoto *et al* [26] 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.[27)] The antioxidant potency of anthocyanin extracts is concentration dependent. [28)] The positive effects of anthocyanin pigments could be related to their potent antioxidant activity demonstrated in various in vitro and in vivo studies. [26-27,29-31)] So, prophylactic action of *Grewia asiatica* against radiation-induced metabolic disorders may be due to presence of antioxidant like anthocyanin, vitamin c etc.

The protection afforded with GAE in biochemical activity of blood and liver in the present study may prove to be beneficial for the clinical use of such dietary compound as radio protector.

References

1.Bhattacharya SK, Satyam K, Ghoshal S.Antioxidant activity of glycowithanolides from Withenia somnifera .ind J of expl Biol 1996; **35**:236.
2.Yen, Grow-Chin, She, Chig W, Pin-der D. Extraction and identification of components from the levels of Mulberry. J of Agri and Food Chem 1996; **44**: 1687-1690.
3.Bhatia AL. The anti-aging role of vitamin A and β-carotene. Indian J Gerontology 1998.
4.Chandha SL Natural source of antioxidant and their adequacy in diet to prevent atherosclerosis. Mediquest 1997; **14** (3): 337-351.
5.Steinmetz K, Potter J. Vegetables, fruit and cancer, I. Epidemiology. Cancer Causes, Control 1991; 2 (suppl): 325-57.
6.Ziegler RG. Vegetables, fruits, and carotenoids and the risk of cancer. Am J Clin Nutr 1991; 53(suppl): 25 1S-59S.
7.Hays WB. Fruit growing in India. 2nd Revised edition. Kitabistan, Allahabad, India. 1953.
8. Sastri BN. The wealth of India: Raw materials#4.*Grewia* Linn. Tilliaceae. In Council of Scientific and Industrial Research, New Delhi, India. 1956; 260-266
9.Morton JF. Phalsa, Fruits of warm climate. Julia Morton, Miami, F.L 1987: 276-277.
10.Nair MG *et.al.* Dietary food supplement containing natural cyclooxygenase inhibitors and methods for inhibiting pain and inflammation. United states patent application. 2005.
11.Yadav AK. Phalsa: A Potential New Small Fruit for Georgia.J Janick(ed) 1999: 348-352.
12. Ahaskar M, Sisodia R. Modulation of Radiation induced Biochemical Changes in brain of Swiss Albino Mice by *Grewia asiatica*. Asian J Exp Sci 2006; 20(2): 399-404.
13.Moron MS, Depierre JW, Mannervik B. Levels of GSH, GR and GST activities in rat lung and liver. Biochim Biophys Acta 1979; 582: 67-78.
14.Buege JA, Aust SD. In: Methods in Enzymology, Academic Press, New York, 1978; 52: 302–314.
15.Sies H, Stahl W. Vitamin E and C, □-carotene and other carotenoids as antioxidants. Am J Clin Nutr 1995; 62: 315 S-215.
16.Sies H. Oxidative Stress: Introductory remarks. London Academic press 1985; 1-8.
17.Pryor WA, Godber SS. Noninvasive measures of oxidative stress status in humans. Free Radic Biol Med 1991; 10:177-184.
18.Helliwell B. Reactive oxygen species in living system Source, biochemistry, and role in human disease. Am J Med 1994; 91(3c Suppl): 14-22.
19.Kale RK, Sitaswad SL. Radiation induced lipid Peroxidation in liposomes. Radiat Phys Chem 1990; 36: 361-364.
20.Biaglow JE, Varnes ME, Epp ER, Clark EP. In: Anticarcinogenesis and Radiation Protection, Eds. P. A. Cerrutti, O. F. Nygaard and M.G. Simic, Plennum Press, New York; 1987:387

21.Bump EA, Brown JM. Role of glutathione in the radiation response of mammalian cells in vitro and in vivo. Pharmacol Ther 1990; 47: 117-136.
22.Raleigh JA. In: Prostaglandin and lipid metabolism in radiation injury, edited by Walden Jr T. C. & Huges, H. N. Plenum Press, New York 1989: 3.
23.Konings AWT, Drijver EB. Radiation effect on membranes. I. Vitamin E deficiency and lipid peroxidation. Radia Res 1979; 80: 494.
24.Konings AWT, Osterloo SK. Radiation effect on membranes. II. A comparison of the effect of X- irradiation and ozone exposure with respect to the relation of antioxidant concentration and the capacity for lipid peroxidation. Radiat Res 1979; 81: 200.
25Kaur C, Kapoor HC. Antioxidant activity of some fruits in Indian diet. In ISHS Acta Horticulture; VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics - Part Two 2005: 696.
26.Matsumoto H, Nakamura Y, Hirayama M, Yoshiki Y, Okubo K. 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 2002; 50:5034-5037.
27.Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem 1997; 45:304-309.
28.Gabrielska J, Oszmiański J, Komorowska M, Langner M. Anthocyanin extracts with antioxidant and radical scavenging effect. Z Naturforsch 1999; 54c: 319-324.
29.Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JI, DeWitt DL. Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. J Nat Prod 1999; 62:294-296.
30. Tsuda T, Watanabe M, Ohshima K, Norinobu S, Choi SW, Kawakishi S, Osawa T. Antioxidative activity of the anthocyanin pigments cyanidin 3-O-ß-D-glucoside and cyanidin. J Agric Food Chem 1994; 42:2407-2410.
31.Ramirez-Tortosa C, Andersen OM, Gardner PT, Morrice PC, Wood SG, Duthie SJ, Collins AR, Duthie GG. Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. Free Radic Biol Med 2001; 31:1033-1037.