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Modulation of Radiation Induced Biochemical Changes in Testis of Swiss Albino Mice by *Amaranthus paniculatus* Linn

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Increasing use of nuclear radiations for human welfare necessitates a new, safe and cost effective radioprotector not only for personnels charged with the responsibility of testing or working with radiations in laboratories, but also for the general public inhabiting in the uricirity of nuclear reactors.. Keeping this view, this study has been undertaken to find out the possible radioprotective potential of the *Amaranthus paniculatus*(AE) as its leaves have high content of carotenoids, proteins, minerals, vitamin C and high level of nutritionally critical amino acids lysine and methionine. For experimental study, healthy Swiss albino male mice were selected from an inbred colony and divided in four groups. Group first (normal) did not receive any treatment. Group second was orally supplemented AE once daily at the dose of 600 mg/kg.b.wt for 15 consecutive days. Group third (control) received distilled water orally equivalent to AE for 15 days then exposed to 5 Gy of gamma radiation. Group fourth (experimental) was administered orally AE for 15 consecutive days once daily and exposed to single dose of 5 Gy of gamma radiation. Mice were sacrificed at different autopsy intervals viz. 1,3,7,15 and 30 days and testis was removed for various biochemical estimations viz. LPO, protein, cholesterol and glycogen. Radiation induced augmentation in lipid peroxidation, cholesterol was significantly ameliorated by AE extract and deficit produced in protein content by radiation was checked. Amaranthus extract pre-treatment hence renders protection against various biochemical changes in mice testis.

Key Words : *Amaranthus paniculatus*, Antioxidant, Testis, Radioprotection.

Introduction :

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. Synthetic protectors have toxicity which limit their value in the clinical field. Therefore, now search is on for some natural compounds, which can quench the reactive energy of free radicals and eliminate oxygen which is one of major participants in lipid peroxidation. 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 phytoantioxidants may reduce threat by free radicals.

Present investigation has been undertaken to search out common nutritional plants as prophylactic agent which may prove efficient antioxidants and could be easily recommended in nutritional dietary course for the population residing in areas where they are continuously exposed to radiation relieving the people from psychological stress of affordability of tablets.

In this context, *Amaranthus paniculatus* is chosen which has been reported to contain good natural source of carotenoids (14,190, μg/100g of edible portion) vitamin C, folate folic acid, high level of nutritional critical lysine and methionine amino acids, protein content (22g/100g of edible portion) and promising oil composition with regard to polyunsaturated fatty acid (Prakash *et al.*, 1995, 2000; Guil *et al*, 1997; Koch *et al.*, 1965 and special series No 42 ICMR, Rajyalakshmi and Geervani, 1994) Amaranthus are broad, brilliantly coloured leafy plants represented by 17 genera and 50 species. In the tropics, amaranths can be produced year round for little effort. *Amaranthus* rich in β-carotene as well as ascorbic acid may prove efficient antioxidant. Free radicals are potentially dangerous for cell (Hochstein, *et.al*.,1988). The LPO is a good biomarker of damage occurring due to radiation and the inhibition of lipid peroxidation is suggestive of radioprotective action. Previous preliminary studies in our laboratory shows that β-carotene renders protection against radiation induced LPO content in mice brain, liver, spleen and testis (Ramesh *et.al*., 1997, Bhatia and Manda, 2000). The present study looks for the protective effect of alcoholic extract of *Amaranthus* in mice testis against radiation-induced oxidative stress, since testicular tissues are rich in poly-unsaturated fatty acid content and is poor in antioxidant defence, therefore, they are prone to attack by Reactive Oxygen Species (ROS), which are capable of oxidation of proteins lipids and DNA leading to cellular damage.

Material and Methods :

Animals : Swiss albino male mice 6-8 weeks (*Mus musculus*) were selected from an inbred colony weighing 23±2 gms. They were maintained under controlled conditions of temperature and light (light: dark, 10h: 14h), and were fed with balanced food in the form of pellets manufactured by Hindustan Lever Ltd. Bombay and water was provided *ad libitum*. Tetracycline water, was given as prevention against infection once a fornight.

Source of Radiation : The cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Department of Radiotherapy, SMS Medical College and Hospital, Jaipur was used for irradiation. Unanaesthestized mice were restrained in well ventilated boxes and exposed whole-body to gamma radiation (5 Gy) at the dose rate of 1.071 Gy/min from the source to surface distance (SSD) 77.5 cm.

Preparation of Plant Extract : *Amaranth* leaves (*Amaranthus paniculatus)* collected locally and air dried,were powdered and extracted with methanol. The *Amaranthus* extract (AE) thus obtained was vacuum evaporated so as to get in powder form. For the experiment, AE was dissolved in the double distilled water (DDW) and dose of required concentration was prepared.

Dose Selection : Dose selection of *Amaranthus paniculatus* was done on the basis of drug tolerance study (Jain *et al*. 2002b) . Various doses of *Amaranthus paniculatus* (200, 400, 600, 800 mg/kg.b.wt.) were tested against gamma irradiation (9Gy) from which 600 mg/kg. b.wt./day was found as an optimum dose for further experimentation.

Design of Experiment : Mice were divided into four groups. Group I (normal) did not receive any treatment. Group II (drug treated) was supplemented amaranths extract (AE) dissolved in double distilled water was provided, once a day at the dose of 600 mg/kg.b.wt. /day for 15 consecutive days. Group III (control) received distilled water orally equivalent to amaranths extract for 15 days, thereafter, it was exposed to single dose of 5 Gy of gamma radiation. Group IV (experimental) was also administered orally amaranths extract at the dose of 600 mg/kg.b.wt./day for 15 consecutive days; thereafter this was exposed to single dose of 5 Gy

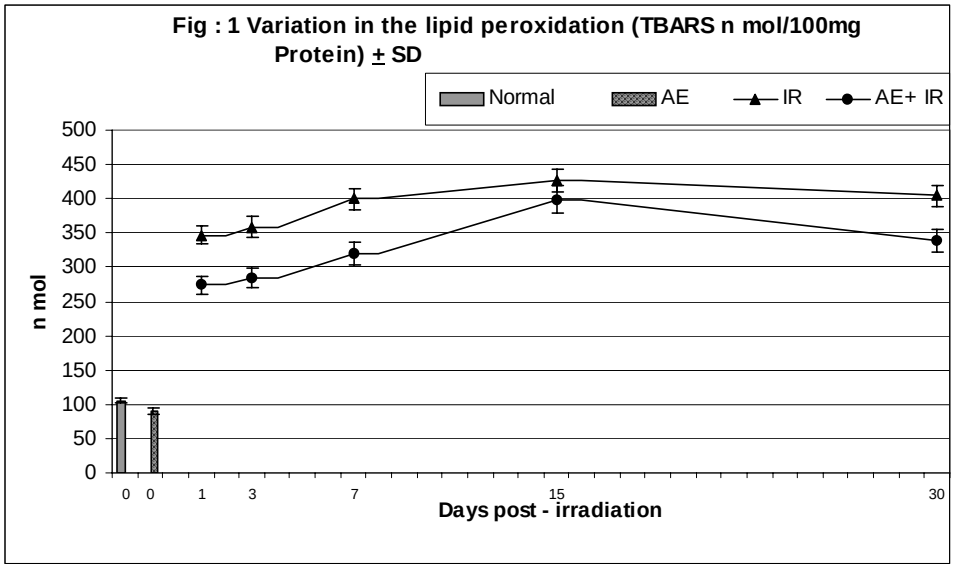
of gamma radiation at the dose rate of 1.07 Gy/min. Mice were sacrificed at different post irradiation intervals viz. 1, 3, 7, 15 and 30 days. Testis was removed and homogenate was prepared and estimated for various biochemical changes viz. protein (Bradford, 1976), Glycogen (Montgomery, 1957), Cholesterol (Burchard, 1959) and Lipid peroxidation (Okhawa, 1979).

Results and Discussion : The role of β-carotene and vitamin A in radiation protection with their antioxidant properties have been indicated by Seifter and Collaborators (1984). A beneficial influence of β-carotene on the conditions of patients under going radiotherapy was also observed by Mills (1988). Cozzi *et al.,* (1997) suggested that ascorbic acid and β-carotene are effective in reducing H_2O_2 induced sister chromatid exchange. They concluded that both vitamins act as scavengers of endogenous and H_2O_2 induced reactive oxygen species. There is a growing body of evidence regarding the beneficial properties of β-carotene in human and animal diets (Gerster,1993 and Mathews-Roth1991).

The observed beneficial effects of supplemented vegetable intake may be contributed by the carotenoids, folate and vitamin C. Currently, knowledge on the bioavailability of these compounds from vegetables is limited. (Van het hof *et al* 1999) reported the β-carotene supplemented meal increased plasma concentration of β-carotene effectively. All vegetable meals increased the plasma concentration of lutein and vitamin C significantly.

Lipid Peroxidation (LPO) : Fig-1 indicates the effect of AE against radiation induced lipid peroxidation. Testis showed gradual and continuous augmentation in the level of TBARS content after gamma irradiation till 15^{th} day post irradiation in both the control and experimental group. Thereafter, depletion in TBARS content was noticed which was indicative of recovery. In experimental group TBARS content was lowered by 66.28% compared to the control on day 30 *post irradiation*. At all post irradiation intervals the LPO value was significantly lower in experimental group compared to the control group. However, it did not attain the normal values. AE treatment for 15 days could bring down the LPO by14% compared to normal. Increase in TBARS is correlated with a decrease in body weight, organ weight and protein value in irradiated group. Since lipid peroxidation

is a good biomarker of damage that occurs due to radiation and so the inhibition of lipid peroxidation is suggestive of radioprotective action of the AE supplementation prior to irradiation.



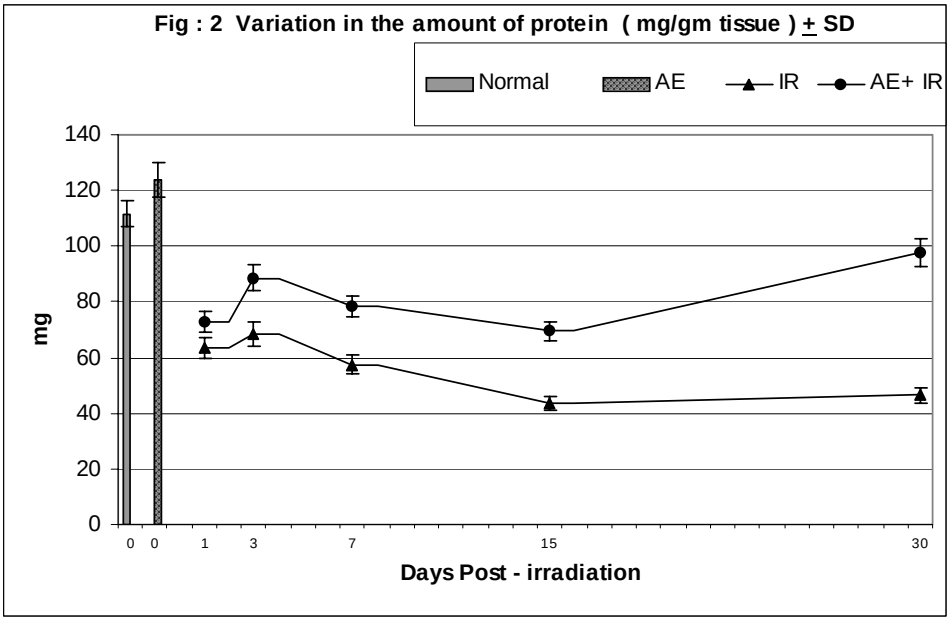
β-carotene, ascorbic acid, folate are well proved antioxidant present in large quantities in the *Amaranthus* and the beneficial effect noticed in relation to TBARS content, may be related to antioxidant properties of these nutrients. Radiation induced lipid peroxidation is a free radical process. It brings about several changes in biological membrane (Leyko *et al* 1986). It is a highly destructive process and cellular organelles and whole organism, lose biochemical function and/or structural architecture (Kale *et al* 1990), which may lead to damage or death of cell.The presence of antioxidants in the plants suppress the formation of free lipid radical and thus prevents the formation of endoperoxidation.

It is well known that exposure of biological membrane to oxidative stress result in the progressive degeneration of membran structure and loss of activity. The measurement of TBARS thus gives on index of free radical activity. Radical scavenging by protectors results in inhibition of TBARS lipid peroxidation can which be initiated by hydrogen abstraction from lipid

molecules by lipid radiolytic products, including hydroxyl and hydorperoxyl radicals (Raleigh 1987). The superoxide anion (O_2^-), $H_2O^{\bullet}_2$ and (OH.) are the major ROS which induce cell degeneration by increasing LPO of cell membrane lipid. The product of lipid proxidation such as malonaldehyde and 4-hydroxynonenal are toxic to the cell (Ester baver *et al* 1988; El-Habit *et al* 2000) also reported that administration of AE pre radiation significantly reduced the levels of TBARS in plasma and liver of male rats. Significant protection of the radiation induced changes in the activities of SOD and catalase were also reported in β–carotene treated and irradiated rats. These results indicated that β–carotene could suppress lipid peroxidation in mouse tissues. The protection afforded with AE in biochemical activity of testis in the present study may prove to be beneficial for the clinical use of such dietary compound as radioprotector.

Protein : The amount of total protein (mg/gm tissue) in testis decreased after irradiation in both the groups. A slight increase in the concentration of protein observed at day 3 post irradiation in both groups, failed to sustain on later intervab and again resulted in protein deficit till 15 days post exposure. Recovery was noticed thereafter, on day 30. Percentage protection offered by administration of AE, prior to irradiation at day 30 was 45.57%. (Fig-2). Reduction in rate of the protein synthesis may be due to unfavourable condition like unavailability of one or more essential enzymes and/or reduction in the sites of protein synthesis. (Bacq. *et al*., 1961). Grant (1969) suggested that protection of protein is due to the hydrogen atom donation by the protector. Zhang and Omaye (2000) reported that high concentration of beta-carotene products produces more protein oxidation in the presence of high O radicals tension by proxidant mechanism. Verma *et al*., (2002) reported that MDA content level in brain due to radiation was depleted after AE supplementation prior to radiation. The decrease of protein noted may be due to its lysis by X-irradition or may be at the synthesis level, or also may be the depression of enzyme involved in the activation of amino acids and transferring to t-RNA (Wender and Zgorzalewiez 1970), or by the inhibition of release of synthesized polypeptides from polysomes (Kim *et al.*, 1970). Increased protein concentration recorded in our study at day 30 post exposure in AE supplemented irradiated mice appears a protective effect. This proves an

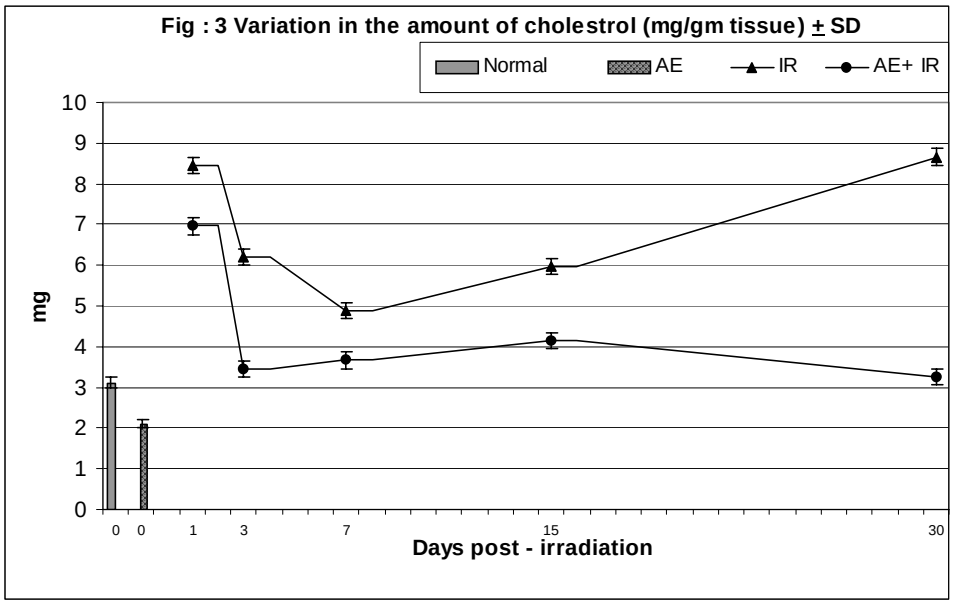
improvement in the ribosomal activities, which enhance the protein synthesis and if can be treated as antiradiation effect. Increase in the protein concentration at day 30 post irradiation may also be due to the elimination of the most of the degenerated cells from the tissue and thus rosulting into the testicular weight loss or it may be due to the increased demand of proteins in repair process as recovery is evident at day 30 post irradiation (Wills and Willkinson, 1967).



Cholesterol : Administration of AE prior to irradiation brought down highly augmented levels of cholesterol at all *post irradiation* intervals by approximately 49% at day 1, 88% at day 3, 40% at day 7, 58% at day 15 and 173% on day 30, respectively. As a result cholesterol level reached near normalcy on day 30 (Fig-3).

This lowered concentration of cholesterol might be due to the higher activity of steroid synthesis, which is corresponding with the higher counts of spermatogenic cell population in the drug exposed group. Irradiated animals exhibited an initial decline in cholesterol concentration in the testis upto day 7 post irradiatiation which increased upto 30 days, post irradiation. Maini (1992) also observed the decrease in cholesterol concentration of the

testis after whole body exposure of mice to 5 Gy gamma radiations. The increase in levels of cholesterol may be attributed to its decreased utilization for steroidogenesis which may be due to pituitary inhibition or a direct inhibitory action of the target tissue (Nair *et al*., 1987). Cholesterol is present in sertoli cells, spermatogonia and spermatocytes. Impaired spermatogenesis results in a marked increase in cholesterol content regardless of the functional status of interstitial cells (Singh *et al*., 1971). It has also been suggested that the lipid fraction tends to increase when the testicular spermatozoal population depletes (Johnson, 1970).



Glycogen : The presence of glycogen in the testis has been documented by Nicander (1957) who postulated its presence in the sertoli cells and germinal tissue indicating that it serves as an energy substrate for the developing spermatids. A sharp increase in the amount of glycogen by 4 times compared to the normal is observed in the irradiated group initially 24 hrs. after irradiation in our study (Fig-4). Thereafter, it decreases continuously upto 15 days post irradiation which is then maintained almost at steady state at all the intervals. Administration of AE extract prior to irradiation lowers the glycogen level of the testis at all the intervals. Maximum protection provided by AE extract was 77.8% at day 15. Initial

increase in glycogen concentration suggested the increased energy requirement of degenerating and aberrant spermatogenic cell population and further decrease in glycogen concentration may be due to the recovery in cell population at late post irradiation intervals. It is also possible that decreased concentration of glycogen on days 3 and 7 post irradiation might have led to decrease in energy reserves which resulted in the degeneration of spermatogenic cell population. Similar results have been obtained by Gupta and Bawa (1977) who studied the effect of radiation on enzymes of carbohydrate metabolism. They observed that testicular hexokinase is highly sensitive to radiation damage. The reduced hexokinase activity seems to be related to those parts of the testis (spermatocytes and spermatids) which depend on glucose for their functioning. Radiation induced atrophic testis is rich in glycogen content because of the inhibition of glucose-6-phosphatase as a phosphorylase. This may explain the high levels of polysaccharide although the possibility of enhanced glycogenolysis due to activation of glycogen synthetase has also been suggested. Changmma and Reddanna (1985) however suggested that the decrease in glycogen content could also be due to the increased glycogenolysis.

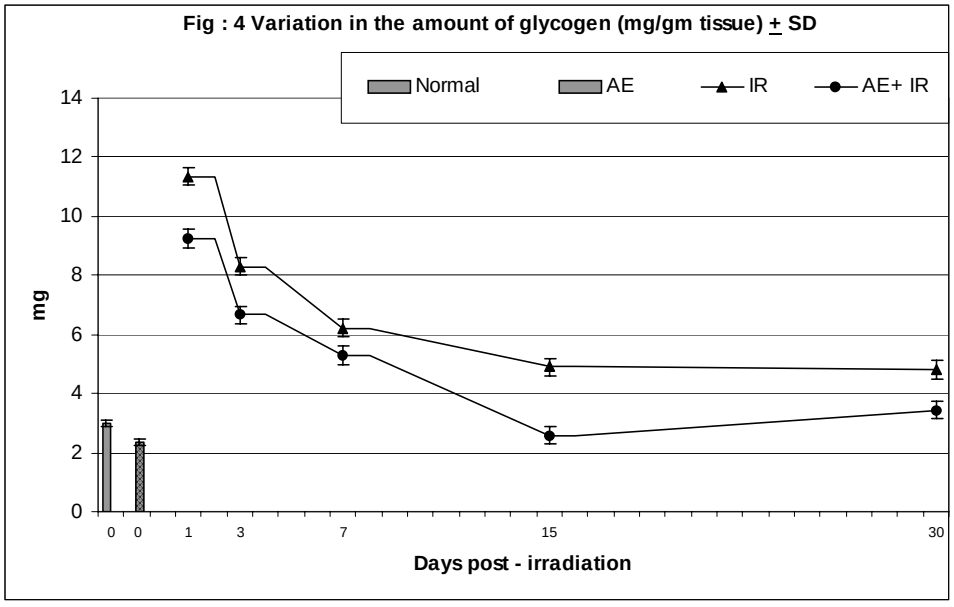


Fig : 4 Variation in the amount of glycogen (mg/gm tissue) ± SD

The results of the present investigation support the postulate that increased ROS induced by radiation exposure may be involved in some of the aversive effects of stress. The available antioxidants in the amaranthus extract are able to cope up with the radiation induced oxidative stress to an extent . This may be due to the synergistic effects of the evaluable constituents present in the herb.

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