Studies of Methanolic extract of Amaranthus paniculatus L. on Mice Liver against Gamma Radiation

Manish Jain, Rashmi Sisodia and A.L. Bhatia*
Radiation Biology Laboratory,
Department of Zoology,
University of Rajasthan,
Jaipur-302004

Abstract: 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. Since Amaranthus, a common weed and very often eaten as vegetable by rural population, has been used as emollient, astringent, diuretic, blood purifier, hemorrhagic diathesis and biliousness from time immemorial. Hence the present study aims to judge whether *Amaranthus paniculatus* (Linn.) has the antiradiation efficacy against radiation induced histopathological and biochemical alterations in mice liver. *Amaranthus paniculatus* (Linn.) belongs to family Amaranthaceae and commonly called as "Amaranth", has good natural sources of carotenoids (beta carotene- 14190 µg/100 gm of edible portion), vitamin C and high level of critical lysine and methionine, protein content (22 gm/100 gm of edible portion). Swiss albino mice of 6-8 weeks weighing 22 ±3 gm were selected from an inbred colony and divided into four groups. One group served as normal and two groups were administered with alcoholic extract at a dose of 600 mg /Kg-body weight/ day dissolved in distilled water for fifteen days. Fourth group was given distilled water, orally and *ad libitum*. Then two groups, one with drug treated and another with distilled water treated, were exposed to 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min with a source to surface distance (SSD) of 77.5 cm. The animals were autopsied at 1, 3, 7, 15 and 30 days post exposure. The optimum dose was calculated to be **600 mg/ kg b.wt./day** after treating mice with AE for fifteen consecutive days prior to irradiation (9 Gy) to get maximum protection against radiation injury. By the survival assay, DRF 1.43 was calculated with different doses of gamma radiation (6, 9, 12 Gy).

The radiation induced augmentation in MDA, protein, glycogen, alkaline and acid phosphatase content of liver is significantly ameliorated by the drug. The radiation induced depletion in cholesterol and glutathione is also significantly checked. It is suggestive that radiation induced alteration in the histology and biochemistry of liver can be ameliorated or checked by *Amaranthus paniculatus*. Mechanism of radioprotection may be by reducing the radiation induced augmentation in lipid peroxidation or by reduced depletion of glutathione after irradiation, by decreasing acid phosphatase activity in mice liver. The histopathological studies reveal that the increase in abnormal cell and biphasic changes in binucleated cell was not evident in Amaranthus pretreated irradiated group instead the greater number of normal hepatocytes was recorded. After irradiation the distortion in hepatic architecture, wider sinusoids, increase in number of Kupffer's cells, giant hepatocytes (mononucleated and multinucleated) were observed in only irradiated group which were either absent or lesser degree in Amaranthus pretreated groups. The present studies show that extract exerts its radioprotective effect in two ways: (i) it is able to curb the initial damage caused due to radiation (by antioxidant activity), and (ii) it stimulates the cellular regeneration in the post-irradiation period (particularly hematopoietic regeneration, liver recovery, gastrointestinal system recovery). The protection may be attributed to the synergistic effects of its constituents rather than any single factor, as all the constituents present in Amaranthus are well known antioxidants. Amaranthus thus showing protection in liver may also prove promising rich source of antioxidants for common people.

*** Corresponding author: E-mail: armbha@sancharnet.in, Fax. No. 0141-2701137**
Telephone no. 0141- 2 711304(R), 2711158.

1. Introduction

Developments in the use of radioactive materials during the past few decades have increased amazingly. At present, there is hardly any aspect of human welfare in which nuclear radiation does not play an important role. Hence, preventive methods to protect not only human but also for animals and plants are necessary. It is well established that radiation or pro-oxidants interact with cells and tissues through secondary ionization like peroxidation. It is also known that peroxidation can be inhibited by antioxidants. Recently, increased interest has developed on search for potential drugs of plant origin which can quench the reactive energy of free radicals and eliminate oxygen, one of major participants in lipid peroxidation and are capable of modifying radiation responses (radioprotectors/sensitizers) with minimum side effects. Antioxidants of plant origin are vitamin E, C, selenium, phenolic compounds, carotenoids, flavonoids etc. [1]. Plant products appear to have an advantage over synthetic compounds in terms of low/no toxicity at the effective dose.

It has been assumed that nutritional intervention to increase intake of phyto-antioxidants may reduce threat of free radicals. Antioxidant free radical scavenging compounds such as β-carotene, vitamin C,

can protect DNA from oxidizing radical reactions. Out of these, β-carotene have excellent antioxidant property [2]. It is a potent free radical quencher, singlet oxygen scavenger and lipid antioxidant [3, 4, 5]. β-carotene supplemented meals, increased plasma concentration of β-carotene effectively [6]. We are to search out efficient natural radioprotectors, which could be well in reach of common people. Thus it could be recommended in the nutritional dietary course without causing psychological stress related to medicinal availability and affordability problems. The present study hence had been aimed to investigate the possible antioxidative efficacy of *Amaranthus paniculatus* which has good quantity of carotenoid content, vitamin C, high level of nutritionally critical lysine and methionine amino acids [7, 8, 9] so its protective effect must due to combined effects of constituents rather than to a one single factor. *Amaranthus paniculatus* (Linn.) (English name Amaranth; Hindi name – Lal Choulai), which is full of antioxidant constituent (Vitamin C, folate, folic acid, β-carotene) is used as emollient, astringent, diuretic, blood purifier, hemorrhagic diathesis and biliousness [10].

Livers of mammals have been reported as highly radiosensitive organ [11]. Hepatic injury can be life threatened complications when the entire or most of liver is exposed to ionizing radiation. Most instances of radiation hepatitis occur in human within 90 days after irradiation [12, 13]. The present study looks for the protective effect of alcoholic extract of *Amaranthus paniculatus* (Linn.) in Swiss albino mice liver against radiation induced oxidative stress.

2. Materials and Methods

2.1 Animals

Male Swiss albino mice (Mus musculus), 6-8 weeks old, weighing 22 ± 3 gm from an inbred colony were selected and maintained under controlled conditions of temperature and light (light: dark, 10h : 14h). They were provided standard mice feed (procured from Hindustan Liver Ltd., Mumbai) and water ad libitum.

2.2 Irradiation

The cobalt teletherapy unit (ATC-C9) at Cancer Treatment Centre, Radiotherapy Department, S.M.S. Medical College and Hospital, Jaipur was used for irradiation. Unanaesthesized animals were restrained in well ventilated boxes and exposed whole-body to gamma radiation (5 GY), the dose rate being, 1.07 Gy/min (107.10 rad/min) at the source to surface distance (SSD) 77.5 cm.

Fresh *Amaranthus* leaves [*Amaranthus paniculatus* (Linn.) RUBL-19866] collected locally were air dried, powdered and extracted with methanol by refluxing for 48 hr. (16 hr. x 3). The extract thus obtained was vacuum evaporated so as to get in powder form and was dissolved in DDW just before oral administration. Fixed weight/volume of the extract to solvent after complete dissolution of the extract was used for administration.

2.3 Experimental Design

2.3.1. Determination of optimum dose of A.E. against radiation

Mice were divided into 5 groups of 10 animals each and were administered AE orally (200, 400, 600, 800 mg/kg b.wt./day) for fifteen days. Thirty minutes after the last administration, animals were exposed to whole body 9.0 Gy gamma radiation (Fig. 1). All such animals were observed till 30 day for any sign of radiation sickness, mortality, behavioural toxicity and morbidity. The optimum dose (600 mg) thus obtained was used for experimentation in detail (Fig. 2). By the survival assay, DRF 1.43 was calculated with different doses of gamma radiation (6, 9, 12 Gy).

Fig. 1: Thirty (30) days survival of mice with or without Amaranthus paniculatus Linn. extract (AE) after exposure to different doses of gamma radiation.

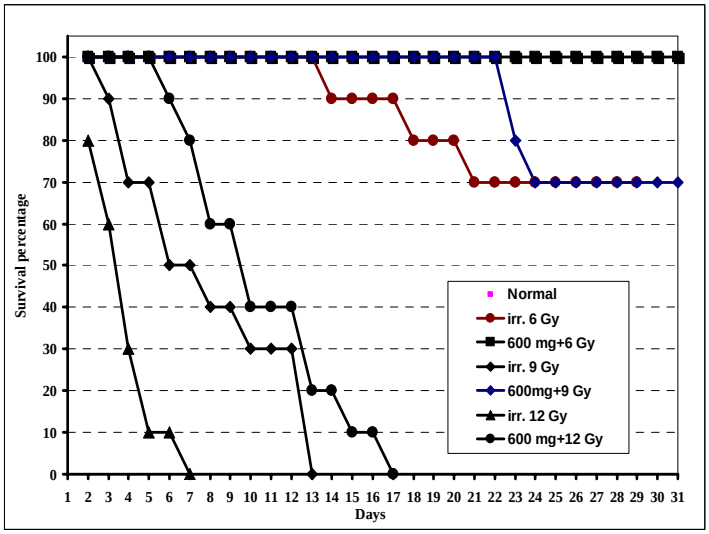
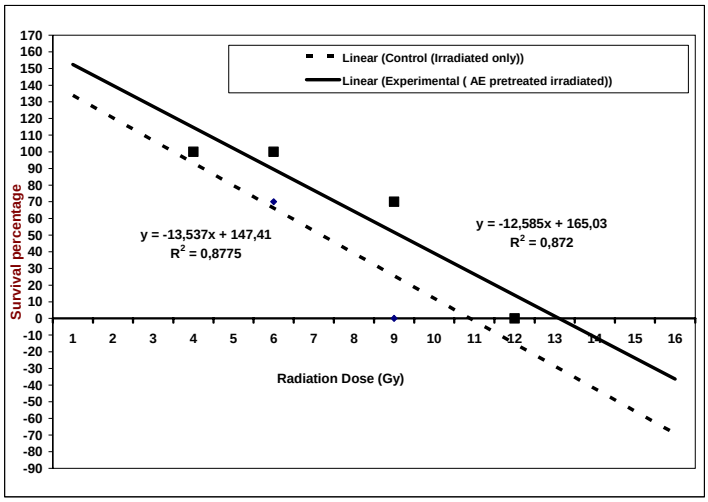


Fig.2:Survival dose-response curve for determination of DRF from LD50/30 for Amaranthus paniculatus Linn. extract



2.3.1.1. Modification of radiation response

Mice selected from an inbred colony were divided into four groups. First group did not receive any treatment, served as normal group. Second group was fed with 600 mg/kg b.wt./day of AE extract for fifteen days. This group served as determination of drug tolerance. Third group animals were fed orally 600 mg/kg body weight/day AE in double distilled water (DDW) for 15 days to serve as experimental group. Mice in fourth received equal volume of DDW served as control group. After 1 hour of the last administration, animals of both the groups (III and IV) were whole-body exposed to 5 Gy gamma radiations at the dose rate of 1.07 Gy/min (107 rad/min). The animals were autopsied at 1, 3, 7, 15 and 30 days post-irradiation. Liver was taken out for estimation of various biochemical parameters viz., as Protein [14], Cholesterol [15], Glycogen [16], Lipid peroxidation [17] and Glutathione [18]. Acid phosphatases (AcP) and Alkaline phosphatases (ALP) in liver were assayed by using method of Fiske and Subbarow.

2.3.1.2. Statistical Analysis

The results obtained in the present study were expressed as mean ± SEM. Student's "t" test was used to make statistical comparison between the studied groups and the significance was observed at $P<0.05$, $P<0.005$ and $P<0.001$ level.

Table-1: Variations in biochemical parameters in liver of Swiss albino mice after 5 Gy gamma irradiation in the presence or absence of *Amaranthus paniculatus* (Linn.) Extract.

Biochemical Parameter's	Group	DAYS POST IRRADIATION				
		1	3	7	15	30
Protein (mg/gm tissue)	Control	213.01 ± 4.21 (158.277%)	227.30 ± 4.32 (168.895%)	241.28 ± 3.89 (179.280%)	180.43 ± 3.98 (134.071%)	155.41 ± (115.4
	Experimental	170.46 ± 3.98** (126.66%)	174.21 ± 3.95** (129.447%)	170.07 ± 3.80** (126.37%)	140.30 ± 3.84** (104.25%)	135.251 ± (100.4
Cholesterol (mg/gm tissue)	Control	3.83 ± 0.03 (87.02%)	3.77 ± 0.04 (85.66%)	3.51 ± 0.02 (79.75%)	3.81 ± 0.04 (86.57%)	3.94 ± (89.7
	Experimental	4.01 ± 0.01** (91.11%)	3.92 ± 0.02* (89.07%)	3.7 ± 0.03** (84.07%)	4.306 ± 0.02** (97.84%)	4.396 ± ((99.8
Glycogen (mg/gm tissue)	Control	7.823 ± 0.05 (186.26%)	8.358 ± 0.06 (199%)	8.353 ± 0.04 (198.88%)	6.428 ± 0.03 (153.05%)	5.39 ± (128.1
	Experimental	7.021 ± 0.03** (167.17%)	7.441 ± 0.02** (177.17%)	6.9 ± 0.01** (164.29%)	5.92 ± 0.03** (140.95%)	4.205 ± ((100.1
Lipid-Peroxidation (TBARS) (n mol/gm tissue)	Control	395.21 ± 3.48 (125.36%)	420.31 ± 3.45 (133.32%)	455.21 ± 3.60 (144.40%)	470.19 ± 3.21 (149.15%)	400.18 ± (126.9
	Experimental	335.18 ± 2.89** (106.32%)	355.17 ± 3.01** (122.66)	370.21 ± 3.10** (117.43%)	345.25 ± 3.0** (109.52%)	320.16 ± (101.5
Glutathione (n mol/gm tissue)	Control	4.97 ± 0.03 (64.80%)	3.83 ± 0.02 (49.84%)	3.19 ± 0.04 (41.54%)	3.07 ± 0.03 (40.01%)	4.65 ± (60.54
	Experimental	7.02 ± 0.02** (91.49%)	6.91 ± 0.01** (90.04%)	6.01 ± 0.03** (78.36%)	6.23 ± 0.02** (81.20%)	7.17 ± 0 (93.47

Each value represents Mean ± SEM
Statistical Comparison Exp. Vs control
P < 0.05 - *, P < 0.001 - **
Control = Untreated irradiated, AE = Amaranthus treated unirradiated,
Experimental = AE pretreated irradiated, Normal = No treatment.
Values in parenthesis indicate percentage

3. Results

3.1. Group I and II – [Normal and Treated animals (AE only)] –

Various biochemical parameters like glycogen, protein, cholesterol, LPO and glutathione in liver exhibited an insignificant variation in treated mice from 1 to 30 days of post-treatment time. It indicates that *Amaranthus* treatment did not cause any significant changes in these biochemical parameters in 30 days.

2.3.2.Group III (Control, Irradiated only) and IV (Experimental, AE + Irradiated group).

2.3.2.1. Glycogen

There is a rise in glycogen content in liver after irradiation, the values increased till day 7, being significantly higher (P<0.001) than respective experimental group. However it decreased at later intervals being significantly lowered in experimental. The values in experimental mice tends to be normalized on day 30, which were not restored even at last interval, values were higher in control (28%) than normal.

2.3.2.2 Protein

There is a rise in protein content in liver after irradiation, which increased upto day 7 in both experimental and control groups being significantly higher (P<0.001) in control group than respective experimental group. At later intervals these values were lowered in both the groups being significantly lower (P<0.001) in experimental than control. The values in experimental animals tend to be normalized on day 30 but not restored in control even at last interval, (15.47% higher).

2.3.2.3. Cholesterol

Cholesterol content decreases after irradiation in liver upto day 7 in both groups, these values being significantly higher (P<0.001) in experimental than control group. At later intervals, cholesterol content increase in both the groups being significantly higher (P< 0.001) in experimental group from their respective control. The values in experimental animals tended to be normalized upto day 30. However the values did not restore even up to day 30, (10.25% lower in control).

2.3.2.4 Lipid Peroxidation

Lipid peroxidation increases after irradiation in liver upto day 15 of control and up to day 7 in experimental mice. At later intervals, a decrease in lipid peroxidation in on day 30 in control and day 15 in experimental. It lowers down at later intervals. LPO values were significantly lower in experimental group from their respective control at all intervals, which compensated to day 30. In control the values were higher (26.94%) than normal.

2.3.2.5. Glutathione

Glutathione (GSH) decreased after irradiation upto day 15 in liver. Thereafter it tended to increase in liver in both control and experimental groups being significant higher than their respective controls at all autopsy intervals. The values were lowered by 39.46% in control and 6.53% in liver of control and experimental mice, respectively at day 30.

4. Discussion

Methanolic extract of *Amaranthus paniculatus* increased survivability in Swiss albino mice against lethal dose of gamma radiation (9 Gy) and Dose Reduction Factor (DRF) was calculated as 1. *Amaranthus paniculatus* (Linn.) extract provides protection by exhibiting a significant decrease in liver alkaline and acid phosphatases activity in experimental animals. *Amaranthus paniculatus* (Linn.) contains good natural sources of carotenoids (14190 µg/ 100gm of edible portion), vitamin C, folate, folic acid, high level of nutritional critical lysine and methionine amino acid, protein content (22 gm/100 gm of edible portion) and promising oil composition with regard to polyunsaturated fatty acid.

Mechanism of radioprotection may be by checking the radiation induced augmentation in lipid peroxidation or checking the depletion of glutathione after irradiation, as well as by decreasing acid phosphatase activity in mice liver. The histopathological studies reveal that the increase in abnormal cell and biphasic changes in binucleated cell was not evident in Amaranthus pretreated irradiated group instead the greater number of normal hepatocytes was recorded. After irradiation the distortion in hepatic architecture, wider sinusoids, increase in number of Kupffer's cells, giant hepatocytes (mononucleated and multinucleated) were observed in only irradiated group which were either absent or of lesser degree in Amaranthus pretreated groups.

4.1 Protein

Increase in protein synthesis in liver of mice noticed has also been reported by others which may be attributed due to higher amino acid precursor pool in X-irradiated liver. This may be due to the increase of amino acid through the plasma membrane as a consequence of permeability change in irradiated cell membrane, which might be due to significant increase in the number of ribosome due to

their increased mobilization from endoplasmic reticulum [19]. Maximum level of protein was observed on day 7 after 5 Gy irradiation, which tended to go down to the normal at later intervals.

4.2. Cholesterol

Liver is the chief organ concerned with regulation of total body contents of cholesterol and plasma cholesterol. Vitamin C efficacy in protecting lipids from oxidation has been reported by Simon [20] The decreased cholesterol content of liver in present study may also be due to the increased catabolism in the tissues, the decreased activity of HMG Co. A reductase might have been compensated by the decreased metabolism in the liver maintaining liver cholesterol [21]. Experimental group showed a significant compensatory concentration of cholesterol than their corresponding control groups. β-carotene inhibits the oxidative modification of low-density lipoproteins when added in near-physiological concentrations to both cell free and cellular *in vitro* system [22]. It also protects lipids from peroxidation. Vitamin C decreases lipid peroxidation either directly or indirectly by regenerating vitamin E [23]. Grant (24] suggested that protection is due to hydrogen atom donation by the protector. Ascorbic acid and α-tocopherol and β-carotene reported to be present in *Amaranthus* are H atom donors therefore, chemical repair of the radiation induced changes in DNA radicals by hydrogen atom may be an important mechanism of protection against radiation-induced protein denaturation in liver of mice. Reduction during early intervals might due to the stress response caused by irradiation to stimulate synthesis of steroid hormones via hypothalamic-pituitary system. Wexler *et al.*, [25] has also suggested that the decreased concentration of cholesterol might be due to increased demand for cortical secretion or increased ACTH secretion by pituitary leading to decreased cholesterol concentration.

4.3. Glycogen

Glycogen content in liver increased significantly in control group than their respective experimental group upto day 7. After this elevated level of glycogen decreased and returned almost to normal level in experimental group, in liver. Others also reported an increase in liver glycogen after irradiation. An elevated level of glycogen contents in liver in present study might be due to the increasing energy requirement of degenerating and aberrant hepatic cells. The elevation in glycogen concentration decreased at later autopsy intervals, which may be due to the recovery in cell population at such post-irradiation intervals.

4.4. Lipid Peroxidation

A.E. supplementation prevented the radiation induced lipid peroxidation in liver, as statistically there is a significant difference between control (irradiated) and experimental (AE + radiation) animals. A statistically significant difference was also noted between only AE treated and normal animals. The basic effect of radiation on cellular membrane is believed to be peroxidation of membrane lipids. Above results showed that the A.E. renders protection against radiation-induced oxidative stress. The measurement of lipid peroxidation is thus a convenient method to monitor oxidative cell damage [26]. Reactive oxygen species (ROS) causes LPO, which within the membrane has devastating effect on functional state. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions. Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants [27, 28]. AE has β-carotene whose antioxidative mechanism has been suggested to be singlet oxygen quenching free radical scavenging and chain breaking during lipid peroxidation [2]. Jain *et al.* [29] also reported the MDA level in liver and brain due to radiation was also depleted after supplementation of *Amaranthus* and *Spinacia oleraceae* prior to irradiation.

4.5. Glutathione

Under normal conditions the inherent defense system including glutathione and the antioxidant enzymes, protects against the oxidative damage. The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. GSH offers protection against oxygen

derived free radicals and cellular lethality following exposure to ionizing radiation [30] (Biaglow *et al.*, 1987). The present study denotes a significant reduction in liver GSH, following exposure. This could be due to enhanced utilization of antioxidant system as an attempt to detoxify the free radicals generated by radiation. Oral administration of AE to Swiss albino mice did not significantly influences the endogenous GSH levels in liver, but its mere presence while radiation exposure protects the endogenous GSH depletion due to irradiation, lower depletion of liver in AE pretreated irradiated animals could be due to higher availability of GSH, which increases the ability to cope with the free radicals produced by radiation or it might be due to less utilization of GSH in AE pretreated animals low level of TBARS equivalent are found means less lipid peroxidation and hence less utilization of antioxidant defense system. So the increased GSH level suggests that protection by AE may be mediated through the modulation of cellular antioxidant level.

4.6. Alkaline and Acid Phosphatases

Liver being as radiosensitive organ lead to any kind of Hepatic injury which can be life threatened complications when the entire or most of the liver is exposed to ionizing radiation. An increase in acid phosphatases activity after radiation exposure in the present experiment could be ascribed either to a direct effect of radiation which results in enhanced Golgi activity and peroxidation of lysosomal membranes causing lysis of membranes and oozing out of enzyme causing increase acid phosphatase level. The increased activity of acid phosphatase may also be due to the lesions produced in membrane lipids by peroxidation leading to activation of latent acid hydrolyases, which could result in digestion of membrane itself with consequent activation and release of other lysosomal enzymes.

Hepatic alkaline phosphatase is a zinc-containing enzyme of 154,000 mol. wt. Partial hepatectomy causes a large increase in its activity that takes place exclusively in the plasma membrane. In the present investigation, liver alkaline phosphatases activity was found to increase after irradiation at all the autopsy intervals studied. Increase in phosphatases activity in liver might be due to impairment of tissues due to irradiation. Radiation induced cell death may be another reason for the increased activity of acid and alkaline phosphatases. Acid and alkaline phosphatases are the enzymes concerned with biosynthesis of fibrous proteins and mucopolysaccharides. These also may act as the hydrolytic enzymes, which play an important role in dissolution of dead cells of the body.

The observed beneficial effects of supplemented vegetable intake may be contributed by the carotenoids, folate and vitamin C. Guil *et al.* [7] studied the nutritional (ascorbic acid, dehydroascorbic acid and carotenes), antinutritional and toxic compounds (oxalic acid, nitrate and uric acid) determined in sixteen popular species of wild edible plants which were collected for human consumption in South East Spain. Carotenoid content was 15.4 mg/100 gm and nitrate content was 597 mg/100 gm in *Amranthus*. The foliage of 62 specimens of *Amaranthus* belonging to ten species of grain and four of vegetable type were analyzed by Prakash *et al.* [8] for vitamin C content. Most of the specimens had promising oil composition with regard to unsaturated fatty acids. Devadas Rajammal *et al.*, [31] reported that entire day's requirement of β-carotene (2400 micrograms) could be obtained in the form of amaranth throughout the year.

Protection afforded after supplementation of *Amaranthus* might be due to β-carotene. β-carotene has been reported to quench not only singlet oxygen, but also to scavenge a variety of free radical species [32]. β -carotene renders protection against radiation induced lipid-peroxidation [33, 34, 35]. Antiaging role of vitamin A and β-carotene was also discussed earlier [5]. Epidemiological studies have clearly demonstrated a link between dietary carotenoids and reduced incidence of certain disease including some cancers [36].

- AE pretreatment renders protection against various biochemical changes in mice liver.
- Radiation induced augmentation in MDA, protein and glycogen content of liver is significantly ameliorated by amaranth extract (AE).
- In the same manner, radiation induced depletion in glutathione and cholesterol is also significantly checked by this herbal treatment of amaranth extract (AE).

- ❖ It is suggestive that radiation induced injury in liver can be subsidized or prophylactic by *Amaranthus paniculatus* (Linn.).
- ❖ Protection afforded by AE may be attributed to carotenoid content, vitamin C and high level of nutritionally critical lysine and methionine amino acid.
- ❖ Protective efficacy may be due to combined/synergistic impact of its constituents rather than to a one single factor.

Amaranthus in carotene rich food remains available round the year. Entire day requirement of β-carotene (2400 microgram) may be obtained in form of amaranth throughout the year and low-cost high carotene foods can be selected and used for increasing β -carotene intake in the community [31]. So *Amaranthus* can be recommended in nutritional dietary course easily without causing psychological stress due to its availability and affordability of beta carotene tablets.

5. Conclusion

The present studies show that extract exerts its radioprotective effect in two ways: (i) it is able to curb the initial damage caused due to radiation (by antioxidant activity), and (ii) it stimulates the cellular regeneration in the post-irradiation period (particularly hematopoietic regeneration, liver recovery, gastrointestinal system recovery). The protection may be attributed to the synergistic effects of its constituents rather than any single factor, as all the constituents present in Amaranthus are well known antioxidants. Amaranthus thus showing protection in liver may also prove promising rich source of antioxidants for common people.

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