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Evaluation of Antioxidant Activities of Spinacia Oleracea Extract on the Sensitivity of Spermatogonia Against Radiation Induced Oxidative Stress in Swiss Albino Mice

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ABSTRACT

: The present work was undertaken with a view to study the effect of oral feeding 1100 mg/kg/wt/day Spinacia oleracia extract for 15 day on testis of Swiss albino mice after whole body exposure to dose 5 Gy of gamma radiation . Radiosensitivity of various kind of spermatogonia (A,I,B) cells was noticed at post irradiation intervals of 1,3,7,15 and 30 days in both the drug treated and irradiated mice group. It was observed that Spinacia oleracea administrated values remained significantly higher than the irradiated at all the intervals studied . Hence, the result indicate that the Spinacia oleracea extract render protection against radiation –induced stress evaluated in terms of histopathology and biochemical changes.

KEYWORDS Spinacia oleracea extract, Testis, Spermatogonia, Radiosensitivity.

Introduction :

In recent years there is a tremendous increase in the interest in areas related to the possible role of nutrition or dietary supplements in prevention of disease or protection from environmental pollutants .In this context antioxidant especially from dietary constituents or herbal products require special attention. A potent radioprotector neutralizes free radicals and mitigates oxidant defense system . Antioxidant are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged . The biological effects of the radiation cause damage to DNAs , lipid and proteins . Exposure to high amounts of ionizing radiation cause damages to the hematopoietic ,reproductive, gastrointestinal or central nervous systems depending on radiation dose (K. Yamini and V. Gopal, 2000). Testis is the radiosensitive organ due to the spermatogenic elements derived from the earliest spermatogonia to the mature spermatozoa exhibit different sensitivity to the same irradiation (De Rooij 1988) .

Testicular tissues are rich in polyunsaturated fatty acid content and poor in antioxidant defense and therefore, they are prone to attack by ROS. ROS are capable of oxidation of proteins, lipids and DNA leading to celluer damage. Current evidence implicates ROS induced peroxidative damage as one of the major cause of defective sperm function exhibit different sensitivity to the same irradiation (De Rooij 1988).

Therefore, in the present investigation, the extracts of *Spinacia oleracea* was further evaluated for their radioprotective activity on the sensitivity of mouse spermatogenesis against gamma radiation

The our study hence is an attempt to investigate the possible radioprotective efficacy of *Spinacia oleracea* which is reported to be a good source of minerals, Vitamin B-Complex, Vitamin K, ascorbic acid and Carotene, besides these, spinach also comes loaded with two flavonoids lutein and zeaxathin (Bhatia 1998). Present study is a search for an anti-radiation drug through nutritional intake, which protects the general public. Therefore the present quest is undertaken on the nutritional supplementation with *Spinacia oleracea* against the damage from radiation exposure in the testis of Swiss albino mice.

Material and Methods:

Animals: For the experimental purpose healthy Swiss albino male mice (6 weeks) were selected from inbred colony and were maintained in air-cooled laboratory. They were fed with balanced food in the form of pellets manufactured by Hindustan Lever Ltd., Mumbai and water provided *ad libitum*.

Source of Radiation and Dosimetry : 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 single dose of gamma radiation (5 Gy) at the dose rate of 1.071 Gy/min from the source to surface distance of (SSD) of 77.5 cm.

Preparation of Plant Extract: Fresh *Spinacia oleracea* leaves collected locally were air dried, powdered and extracted with methanol by refluxing for 48 hours (16hr. x 3). The Spinach extract (SE) thus obtained was vacuum evaporated so as to get in powder form and was dissolved in DDW just before oral administration.

Selection of Optimum Dose of Drug (SE): Mice were divided into four groups of 6 animals each and were administered SE orally (200, 400, 600, 800,1100 and 1400 mg/kg b.wt./day) for fifteen days. Thirty minutes after the last administration, mice were exposed to whole body single dose of 9.0 Gy gamma radiation. All such mice were observed till 30 day for any sign of radiation sickness, mortality, behavioural toxicity and morbidity. The optimum dose (1100 mg/kg.b.wt./day) thus obtained was used for experimentation in detail.

Design of Experiment:

Radiation Response with Optimum Dose of Drug (SE): Selected mice were divided into four groups. **Group I** (normal) it did not received any treatment. **Group II** (drug treated) was orally supplemented Spinach extract, once daily at the dose of 1100 mg/kg.b.wt./day for 15 consecutive days dissolved in double distilled water. **Group III** (control) received distilled water orally equivalent to Spinach extract for 15 days there after it was exposed to 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min with a source to surface distance of 77.5 cm. **Group IV** (experimental) was also administered orally Spinach extract at the dose of 1100 mg/kg.b.wt./day for 15 consecutive days thereafter exposed to single dose of 5 Gy of gamma radiation at the dose rate of 1.07 Gy/min. The mice were autopsied at 1, 3, 7, 15 and 30 days *post-irradiation*.

Qualitative Observations: Testis was removed and fixed in Bouin`s fluids was dehydrated and embedded in to paraffin. Transverse selections of 5mm thickness were cut from the middle part of testis and stained with Harris Hsematox-

ylin-Eosin stain. For detailed examination of the Spermatogenic stages within the seminiferous tubules. Histological examination was carried out from sections of each testis.

Spermatogonia Cell Counting:
Spermatogonia 'A', 'I' and 'B' were counted from the cross sections of approximately circular tubules at stage XII, II and V respectively. The cells were counted only when the greater part of the nucleus was included in the sections. The counts were corrected by Aberchrombie`s method (1946). Identification and selection of tubular cross sections for counting the various cell types in the testis were based on the description given by Oakberg (1956) and De Rooij (1988).

About 10 tubules cross-sections scored for counting cell types per-interval per-testis. Identification and classification of Spermatogonia is the most controversial issue. Criteria used is given below:

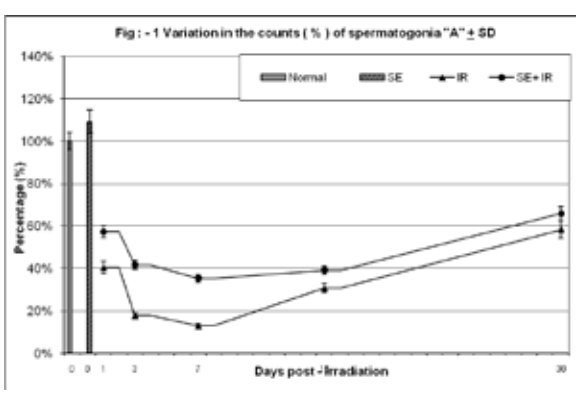
Type A : These are the largest among Spermatogonia with ovoid pale nucleus and a little chromatin, distributed as fine dust like granules.

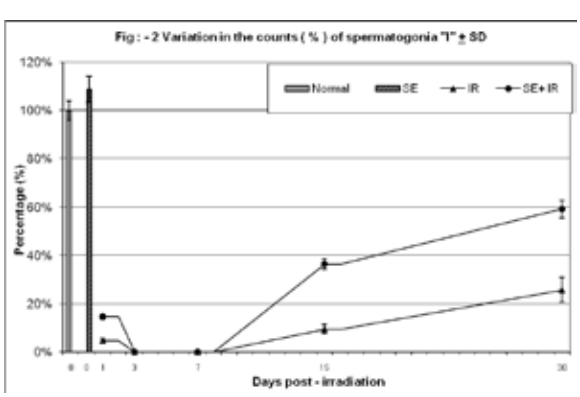
Type I : These are the smallest among Spermatogonia with dark spherical nucleus having 'crust' like chromatin.

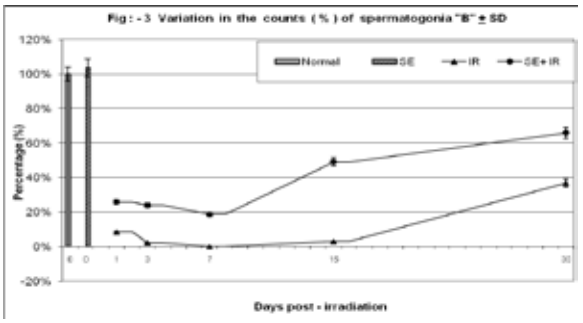
Type B: These are similar to type 'A', but with more chromatin, some what ovoid in shape with a thin layer of cytoplasm surrounding a large nucleus.

Statistical analysis:
The results obtained were expressed, as Mean±SEM. Student "t" test was used to make a statistical comparison between the groups. Significance levels were set at P< 0.001 , P<0.002, P<0.01, P<0.05.

Results and Discussions:
Figure-1, 2 and 3 indicate the effect of SE against radiation induced Spermatogonia. Spermatogonia 'A', 'I' and 'B' counts are significantly higher in drug supplementation group prior to irradiation compared to only irradiate group.







Drug supplemented exposed animals show a statistically highly significant protection in the terms of Spermatogenic cell population at all of the post irradiation intervals. This is attributed by the antioxidative property of b-carotene and other constituents present in both the pre-treated groups (SE). Protective effect of Spinach as evaluated in present study is due to the antioxidative mechanism of b-carotene and synergistic effect of other constituents which has been suggested to be singlet oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation (Gerster, 1993).

The values of all the type of spermatogonia ('A', 'I' and ' B') in SE group are significantly higher than their corresponding irradiated value at all the intervals, which show the protective potential of drugs SE against radiation.

Spermatogonia 'A' were found to be highly radio-resistant followed by Spermatogonia 'B' and 'I'. Intermediate Spermatogonia is found to be more radiosensitive than the spermatogonia 'B'. Thus the present study indicates that more radiosensitive cell types are protected to a greater extent than the radio-resistant ones .Type 'B spermatogonia and I spermatogonia are depleted more rapidly .This may be assigned to average DNA synthesis time which is highly variable in type B and less variable in type 'I'. The values ,of all type of spermatogonia A , I and B in SE+IR groups are significantly higher than their corresponding irradiated value , at all the intervals which show the protective potential of drugs SE against radiation .This may be attributed to the antioxidative property of a carotene and other constituents present in both the pre –treated groups SE ,which has been suggested to be singlet oxygen quenching , free radical scavenging and chain breaking during lipid peroxidation Bolt *et al*.(1995).

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