

Radio Protective Effect of Brassica Compestris (Variety Sarason) Seed Extract *in Swiss Albino Mice*



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Abstract

Plant products have an advantage over the synthetic compounds because of low toxicity at the effective dose with minimum side effects several medicinal and dietary plant and fruits possess very good antioxidant properties. Many of them are rich in antioxidant chemicals like flavones, flavonoids and other polyphenolic compounds, vitamins A, C and reducing sugars, such plants can be good natural sources of effective radioprotectors. Continuous search for cheaper and non-toxic radioprotectors has led to the discovery of many natural antioxidant nutrients and plant products with promise. Present work shows that the methanolic extract of *Brassica campestris* seed renders protection against radiation induced sickness, mortality and hematopoietic system of Swiss albino mice due to its antioxidative property. Oral administration of MBE to Swiss albino mice before radiation exposure was effective in increasing the frequency of radiation induced endogenous spleen colony forming units (CFU-S). A significant weight loss in spleen was observed following irradiation on day 10, however, it was found to be considerably higher in MBE pretreated animals. The weight of spleen in sham irradiated (normal) animals did not show any noticeable change at various autopsy intervals from 6 hr to day 30. No significant change in the weight of spleen was observed in MBE treated animals as compared to normal. A significant decrease in the weight of spleen was evident from day 10th in control animal (8 Gy). However, increase in the weight of spleen was observed on day 10 and attained normal value till day 30 in 8 Gy control. Experimental animals showed similar pattern of weight and loss, but the values were significantly higher as compared to the control ones, and attained normal value till day 30.

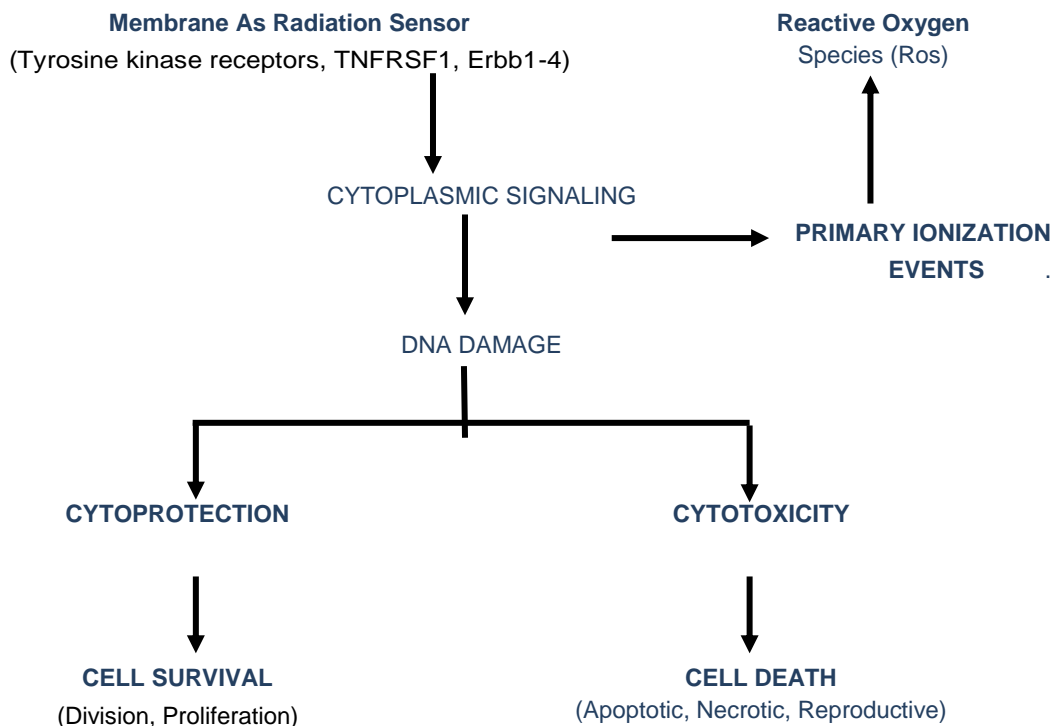
Keywords: Radio Protector, *Swiss Albino Mice*, *Brassica Compestris* Seed, Spleen.

Introduction

Life on earth has developed with an ever present background of radiation. Today we are living in the era of nuclear energy. Living organisms absorb energy from its environment in one form or another. Amongst various form of energy the radiations in one form that has always been available in nature and directly effects the living beings. Radiation is the emission, transmission and absorption of energy in space.

Interactions of Radiation with Body Cell

Recent radiobiological studies have shown that living cells activate a plethora of responses when they are challenged by ionizing radiation exposure. The ability of cellular machinery to cope with stress induced reactions and processes determine the survival or death of the cell. Lately, research has been focused to understand the initial nuclear damage and its repair. Free radicals are potentially dangerous for the cell. This damage is a major contributor to aging and degenerative diseases of aging such as cardiovascular diseases, brain dysfunction, cancer, immune system decline and cataracts.



Pathways of Cellular Response To Ionizing Radiation

As a result of the relative instability of free radicals and their potential to damage cells and tissues, there are both enzymes and small molecular weight molecules with antioxidant capabilities that can protect against the adverse effect of free radical reactions.

Cytogenetic Damage

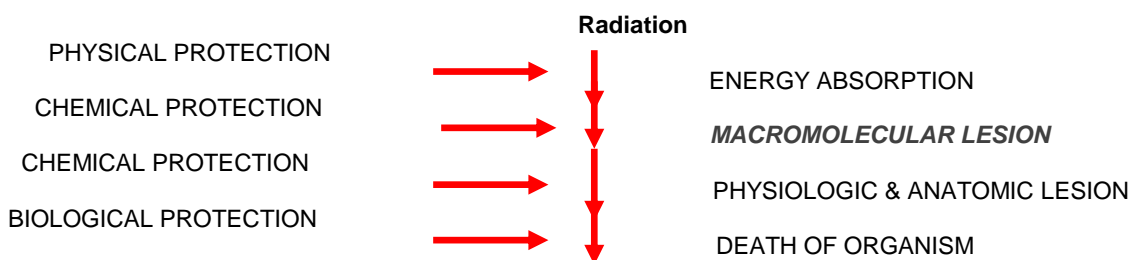
A great deal of information has been occurred on the cytogenesis response of human somatic cells to ionising radiations. One milliliter of human peripheral blood contains over 1 million small lymphocytes so that a small quantity of blood is sufficient to analyse mitotic cells for chromosome aberrations. Cell culture techniques that enable the chromosomes to be observed have been described in IAEA manual (2001). In brief phytohaemagglutinin (PHA) is added to stimulate the cells to divide and after 45 hours of culture at 37°C, colcemid is added to stop the cells at metaphase. At 48 hrs cells are harvested and cast on the slides. Chromosomes are then stained with Giemsa. Metaphase spreads are scored for the presence of dicentric under microscope and dose is estimated using dose response curve.

Need For Radiation Protection

Increasing use of ionizing radiation for diagnostic as well as therapeutic purposes has drawn the attention of many radiobiologist towards the undesired side effects of such exposures. These exposures results in the expression of severe radiation sickness. Some times the repeated therapeutic exposure might prone to be of little advantage due to the radiation sickness. Thus, there is need to screen chemicals/phytochemicals for their radioprotective potential externally in the normal tissue study will be helpful for human welfare in lince of accidental or occupational exposures or in the treatment of cancer.

There are primarily three ways to achieve radioprotection in a biological system that has been exposed to ionizing radiation (Bacq and Alexander, 1961).

- 1) Physical protection: By changing the environment by physical or chemical means.
- 2) Chemical protection: By chemical alteration of the site of the radiation injury.
- 3) Biological protection: By biological alteration to decrease the extent of the radiation injury.



Sequence of Events After Ionizing Radiation Leading To Cellular and Tissue Injury, Protection or Reversal of the Effect Can, In Part, Be Provided At Each of the Steps

Studies on *in vivo* radioprotection started within a few years of the end of World War II and the global effects of military detonation of nuclear bomb became known.

Herbal Radioprotectors

Toxicity and side effects of synthetic radioprotectors stimulated the scientists to evaluate the natural, non-toxic herbal or plant preparations for radiation protection. The Indian system of medicine offers a large number of plants in the treatment of diseases including cancer. Many phytochemicals are known to possess antioxidant properties. Indeed, human beings are consuming a variety of antioxidants in their diet and are thus protected from radiation damage. Therefore, it is necessary to assess the protective action of such commonly used phytochemicals and use their possible application in radiotherapy.

Review of Literature

Though several synthetic compounds have been used as radioprotectors, but more and more investigations are being turned to natural products, including chemicals extracted from the plants for non-toxic radioprotectors and sensitizers. Naturally occurring antioxidants provide benefits of low-toxicity, easy bioavailability and possibly increased benefits from longer use including antimutagenic effects (Weiss and Landauer, 2000). Antioxidants are known for their efficient free radical scavenging and metal chelating properties, besides enhancing the levels of intracellular antioxidant enzymes and inhibiting lipid peroxidation as well as oxidative damage to DNA and proteins (Masuda *et al*, 1988; Ramakrishna *et al*, 1998; Middleton *et al*, 2000). Flavonoids compose the largest and most studied group of polyphenols, and are an integral part of the human diet (Grace and Logan, 2000).

Radiobiologists are now evaluating the non-toxic herbal preparations for radiation protection such as Brahamarasayana (Praveen kumaret *al*, 1999; Rekhaet *al*, 2000) and chyavanaprasha (Jagetia and Baliga, 2004). Several plant extracts like *Spirulina* (Kumar *et al*, 2000), *Ocimum* flavonoids (Uma Devi *et al*, 1999; 2000), *Podophyllum hexandrum* (Kumar and Goel, 2000), *Tinosporacordifolia* (Goelet *al*, 2002), RH-3 (Agrawala and Goel, 2002), *Panax ginseng* (Kim *et al*, 2003), *Ginger* rhizome (Jagetia *et al*, 2003), *Rajgira* (Maharwalet *al*, 2003), *Ageratum conyzoides* (Jagetia *et al*, 2003), *Emblica officinalis* (Harikumaret *al*, 2004), *Brassica compestris* (var. Sarason) (Soniet

al, 2006), *Crataegus microphylla* (Hosseinimehret *al*, 2007), have been shown to have radioprotective effects.

Aim of the study

Therefore in the present investigation an attempt has been made to investigate radioprotective effects of *Brassica Compestris* Seed Extract in Swiss albino mice by calculating DRF, evaluating on spleen and lipid peroxidation (LPO) level.

Method and Material

Present Investigation Is Studied To Evaluated .The Radioprotective Effects Of Methanolic Extract Of *Brassica Compestris* Seed In Modifying The Radiation Induce Phytochemical Alterations In Spleen Of Swiss Albino Mice. And Plant identified and speci-men was placed in The Herbarium, Department of Botany, University of Rajasthan, Jaipur bearing voucher number/identification number is RUBL-19932 (Plate-I).

Endogenous Spleen Colony Assay

The endogenous spleen colony assay was done according to method of Till and McCulloch (1961). Endogenous spleen colony-forming units (CFU-S) were counted on day 10, after single total body irradiation (TBI) of 8 Gy. The surviving animals were sacrificed by cervical dislocation, whole spleens were removed out and fixed in Bouin's solution. Macro colonies, identified as distinct nodules on the surface of spleen, were scored with the help of hand lens.

Result and Discussion

In the present investigation the radioprotective effects of methanolic extract of *Brassica compestris* seed in *Swiss albino mice* has been observed.

Endogenous Spleen Colony Assay

Oral administration of MBSE to Swiss albino mice for 7 consecutive days before exposure to lethal dose (8 Gy) of gamma radiation was found to be effective in increasing the frequency of radiation induced endogenous spleen colonies (CFU-S) increase in the number of spleen colonies in MBSE pretreated irradiated animals (17.40±0.95) respectively. Furthermore, a considerable loss in spleen weight of following irradiation was noticed at day 10, however, a significant increase in such weight was evident in MBSE pretreated irradiated animals .



A: Normal

B: Control

C: Experimental

Normal : Sham irradiated

Control : 8Gy treated

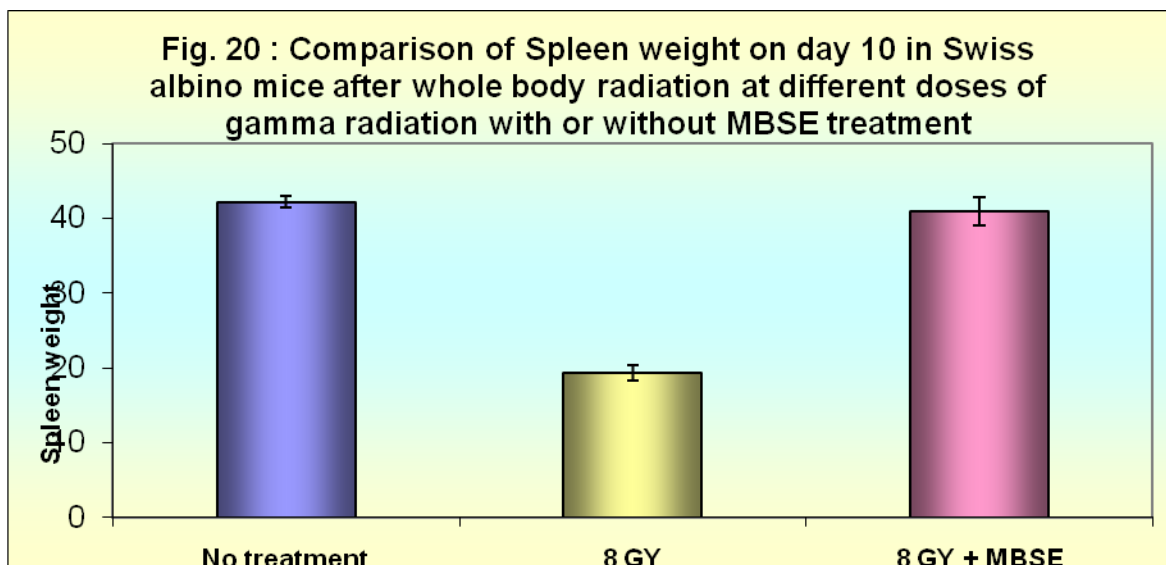
Experimental : 8 Gy radiation +MBSE treated

Plate : Spleen response on day 10 in Swiss albino mice after whole body radiated by 8 Gy gamma radiation with or without methanolic Brassica compestris seed extract treatment

Spleen response on day 10 in Swiss albino mice after whole body radiation to different doses of gamma radiation with or without MBSE treatment.

Radiation Dose	Group	Spleen weight	Number of microscopic colony
	Normal	42.24±0.81	-
8 Gy	Control	19.40±1.03 ^c	-
8 Gy + MBSE	Experiment	41.00±1.91 ^c	17.40±0.95

Each value represents Mean ± S.E.	Significance levels :
Control : Irradiation alone	a = P<0.05; b = P<0.005; c = P<0.001
Experimental : Treatment + radiation	Statistical comparison :
Drug alone : Treatment	Control Vs Normal
Normal : No treatment	Experimental Vs Control
	Drug alone Vs Normal



Bond *et al.* (1965) have reported that mortality of mice induced by mid-lethal exposure to

ionizing radiation is, primarily due to damage of haematopoietic tissues, mainly of stem cell

population. Since the CFU-S is produced by active proliferation of the surviving stem cells, it appears that the drugs afford enough protection to retain the normal reproductive capacity of a sufficient number of stem cells resulting in higher number of CFU-S. This could be brought about by two different mechanisms, (i) a direct effect on the cells during radiation to reduce the initial injury, and (ii) an action on the repair process leading to restoration of normal structure.

Conclusion

To confer an ability to protect only normal tissue cells and to differentiate tumor cells, the compound has to be modified by taking advantage of the euoxic environment of the normal cells and the hypoxic environment of the tumor cells as well as their associated biochemical characteristics. Future research for an effective radioprotectors may be directed towards compounds, which can protect normoxic cells and afford no protection under a hypoxic environment, which is a common feature in all solid tumors. Also relevant are compounds, which become enzymatically converted into toxic derivatives in hypoxic tumor cells while remaining unchanged and protecting normal cells.

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References

1. Agrawala, P.K. and Goel, H.C. (2002) : Protective effect of RH-3 with special reference to radiation induced micronuclei in mouse bone marrow. *Ind. J. Exp. Biol.*40(5), 525.
2. Bacq, Z.M. and Alexander, P. (1961) : *In Fundamentals of Radiobiology*, 2nd edn. New York: Pergamon.
3. Goel, H.C., Prasad, J., Singh, S., Sagar, R.K., Prem Kumar, I. and Sinha, A.K. (2002) : Radioprotection by a herbal preparation of *Hippophaerhamnoides*, RH-3, against whole body lethal irradiation in mice. *Phytomedicine*.9, 15.
4. Grace, S. and Logan, B.A. (2000) : Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Phil. Trans. Royal Soc. London B*.355, 1499.
5. Hosseinimehr, S.J., Azadbakht, M., Mousavi, S.M., Mahmoudzadeh, A. and Akhlaghpour, S. (2007): Radioprotective effects of hawthorn fruit extract against gamma irradiation in mouse bone marrow cells. *J. Radiat. Res.*, 48, 63-68.
6. Hari Kumar, K.B., Sabu, M.C., Lima, P.S. and Ramadasan Kuttan (2004) : Modulation of haematopoietic system and antioxidant enzymes by *Emblica officinalis* and its protective role against γ -radiation induced damages in mice. *Radiat. Res.*45, 549.
7. Jagetia, G.C. and Baliga, M.S. (2002) : Cystone, an ayurvedic herbal drug imparts protection to the mice against the lethal effects of gamma radiation: a preliminary study. *Nahrung*.46 (5), 332.
8. Jagetia, G.C. and Baliga, M.S. (2003): Evaluation of the radioprotective effect of the leaf extract of *Syzygiumcumini* (Jamun) in mice exposed to a lethal dose of gamma-irradiation. *Nahrung*.47 (3), 181.
9. Kim, J.K., Kim, J.H. and Yoon, Y.D. (2003): Evaluation of caffeine as a radioprotector in whole-body irradiated male mice. *In vivo*,17, 197-200.
10. Kumar, K.S., Srinivasan, V., Toles, R., Jobe, L. and Seed, T.M. (2002) : Nutritional approaches to radioprotection: Vitamin E. *Milit. Med.*167, 57.
11. Maharwal, J., Samarth, R.M. and Saini, M.R. (2003): Radiomodulatory influence of *Rajgira* (*Amaranthuspaniculatus*) leaf extract in Swiss albino mice. *Phytother. Res.*17 (10), 1150.
12. Masuda, A., Longo, D.L. and Kobayashi, Y. (1988) : Induction of mitochondrial manganese superoxide dismutase by interleukin 1. *FASEB. J.*2, 3087.
13. Middleton, E.J., Kandaswami, C. and Theoharides, T.C. (2000): The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacol. Rev.*52, 673.
14. Praveen kumar, V., Kuttan, R. and Kuttan, G. (1999) : Effect of 'Rasayanas' a herbal drug preparation on immune response and its significance to cancer treatment. *Ind. J. Exp. Biol.* 37, 27.
15. Ramakrishnan, N., Kalinich, J.F. and McClain, D.E. (1998) : Radioprotectors: chemical, biological and clinical perspectives, CRC Press, Boca Raton, FL.
16. Rekha, P.S., Kuttan, G. and Kuttan, R. (2000) : Effect of herbal preparation, *Brahma rasayana*, in amelioration of radiation induced damage. *Ind. J. Exp. Biol.*38, 999.
17. Soni, A.K., Samarth, R.M., Kumar, M., Shukla, S., Meena, P.D., Kumar, M. and Kumar, A. (2006): Modulation of radiation induced alterations in Swiss albino mice by *Brassica compestris* (Var. Sarason). *Pharmacologyonline*, 2, 190-199.
18. Weiss, J.F. (1997): Pharmacologic approaches to protection against radiation-induced lethality and other damage. *Environ Health Perspect*105 (Suppl 6), 1473.
19. Vano, E.; Kleiman, N.J.; Duran, A.; Romano-Miller, M.; Rehani, M.M. (2013), Radiation-associated lens opacities in catheterization personnel: Results of a survey and direct assessments. *J. Vasc. Interv. Radiol*24, 197-204.
20. Smith, J.; Willey, N.; Hancock, J. (2012) Low dose ionising radiation produces too few ROS to directly affect antioxidant concentrations in cells. *Biol. Lett.* McEvoy, C.; Young, I.S.; Woodside, J.V. Fish, n-3 polyunsaturated fatty acids, and cardiovascular disease. *Nutr. Health* 2012, 10, 221-246.
21. Chen, J.; Jiang, Y.; Liang, Y.; Tian, X.; Peng, C.; Ma, K.Y.; Liu, J.; Huang, Y.; Chen, Z.Y (2012). DPA n-3, DPA n-6 and DHA improve lipoprotein profiles and aortic function in hamsters fed a high

- cholesterol diet. Atherosclerosis*, 221, 397–404.
22. Li, X.H.; Li, C.Y.; Lu, J.M.; Tian, R.B.; Wei, J (2012). *Allicin ameliorates cognitive deficits ageing-induced learning and memory deficits through enhancing of Nrf2 antioxidant signaling pathways. Neurosci.Lett.*, 514, 46–50
23. *Preventive or Potential Therapeutic Value of*

- Nutraceuticals against Ionizing Radiation-Induced Oxidative Stress in Exposed Subjects and Frequent Fliers Maria Teresa Giardi 1,* , Eleftherios Touloupakis 2,3, Delfina Bertolotto 4 and Gabriele Mascetti 4*
24. *Int. J. Mol. Sci.* 2013, 14, 17168-17192; doi:10.3390/ijms140817168