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PROTECTION OF RADIATION INDUCED HEMATOPOIETIC INJURY BY *PODOPHYLLUM HEXANDRUM*

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INTRODUCTION

Hematopoietic system plays very important role in normal function and survival of organisms. A number of reports have validated deleterious effect of radiation on hematopoietic system. The hematopoietic system provides mature functional circulating elements since these have finite life times and cannot reproduce themselves, those lost through the bone marrow does not function if the length of time in which the hematopoietic system does not function too long, the level of vital circulating elements drops too low and the irradiated organism dies. The bone marrow tissues have very rapid turnover times that means constant source of new cells are supplied in order to balance the numbers that are lost. Exposure to a moderate or high dose of total body irradiation (TBI) causes death by Bone marrow (BM) suppression and the most common dose-limiting side effect of conventional cancer therapy using ionizing radiation. The cellular renewal depends upon presence of stem cells. Hematopoietic stem cells (HSC) provide self-renewal, differentiation capacity and a constant supply of the whole cell spectrum of all hematopoietic lineages throughout our life. HSCs are found in the bone marrow of adults, which includes femurs, hip, ribs, sternum, and other bones. Any process which inhibits or delay the division of stem cells ultimately will cause destruction of this tissue (Loss of proliferative capacity). Markers like Sca 1 have been used for identification of HSC cells and few reports indicated the radiation effect on these Sca 1 HSCs. Many plants and their isolated products have already been shown to protect whole body irradiated mice against hematopoietic injury. Earlier studies at this institute (INMAS) have shown that *Podophyllum hexandrum* afford protection against radiation induced hematopoietic injury. Hematological parameters like total leukocyte counts, Hemoglobin levels and Colony forming assay have been used to establish hematopoietic protection by *P hexandrum.* However detailed mechanisms of hematopoietic protection are still unknown.

This is an interesting approach as detailed studies on hematopoietic system will provide direct evidence of changes in bone marrow cells specifically stem cell populations against ionizing radiation. Proposed work is also an important contribution in better understanding of hematopoietic protection and will be useful in development of radioprotectors for human use.

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WHAT IS RADIATION?

Any process in which energy travels through a medium or through space, ultimately to be absorbed by another body, is called **radiation**. Non-physicists often associate the word ionizing radiation (e.g., as occurring in nuclear weapons, nuclear reactors, and radioactive substances), but it can also refer to electromagnetic radiation (i.e., radio waves, infrared light, visible light, ultraviolet light, and X-rays)

Types of radiation

1. Ionizing radiation: Has enough energy that during an interaction with an atom, it can remove tightly bound negative electrons from it, causing the atom to become charged or ionized. Examples of radiations that are ionizing are gamma rays and neutrons.

2. Non-Ionizing radiation (NIR): Refers to radiative energy that has sufficient energy only for excitation. The NIR spectrum is categorized into two regions, optical radiations and electromagnetic fields. The optical can be further sub-divided into ultraviolet, visible, and infra-red (Fig.1). The electromagnetic fields are subcategorized into radiofrequency (microwave, very high frequency and low frequency radio wave).

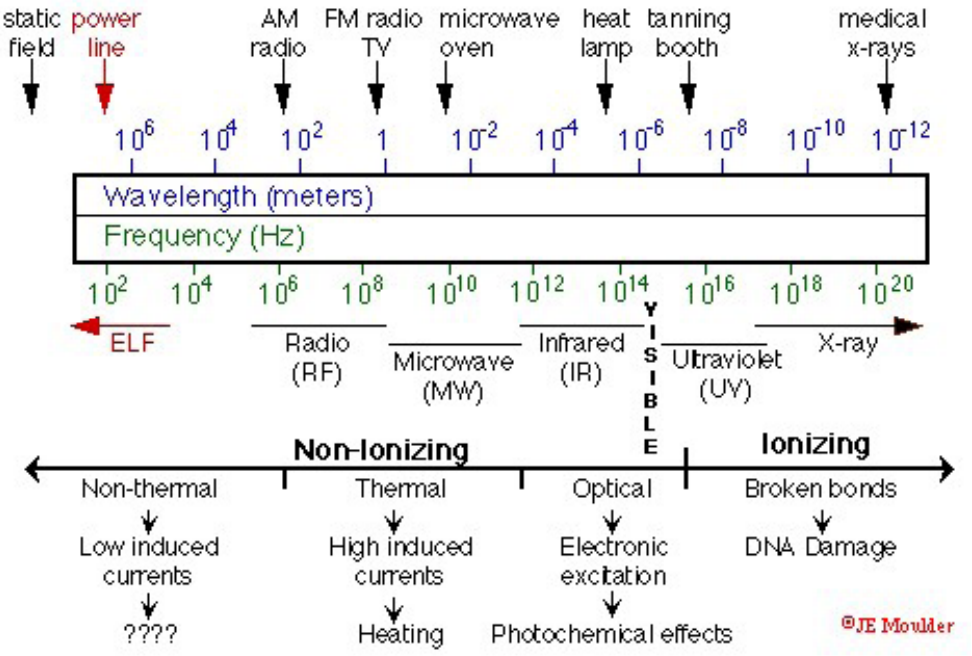

Fig.1 Electromagnetic Spectrum and its associated Biological Effects (SOURCE: www.spinspace.com).

BIOLOGICAL EFFECTS OF RADIATION

Radiation produces its effect by two ways-(a). *Direct effect*: radiation interacts directly to macromolecules of the cell (b) *Indirect effect*: radiation produces its effect through generation of free radicals by interacting

with water molecules of the cell. Free radicals are highly reactive and have a life span of 10^{-9} to 10^{-11}. Free radicals are constantly generated endogenously through several metabolic processes in cellular system and their level in the cell is maintained by homeostasis mechanisms. Ionizing radiation is major exogenous source of free radical production and their exposure to cellular system leads to imbalances in homeostatic system as a result several malfunctions and deleterious effects are observed in cellular system. Free radicals are generally categorized in two groups viz; reactive oxygen species and reactive nitrogen species. Both species are highly reactive and can damage the macromolecules of the cells leading to several types of disease or cell death. When cells are irradiated, damage is produced primarily by ionization and free radicals. It has been estimated that about two thirds of biological damage by low LET radiation is due to indirect effect. Biological damage by high LET is primarily by Ionization. The extent of radiation damage of a given cell type depends upon the total dose, dose rate, type of radiation, mode of radiation delivery, and the environmental condition of the medium. Radiation effects may be early or late and depend upon the type of damage. The types of radiation injuries in relation to approximate dose are given in following figure-2 (Prasad, 1995).

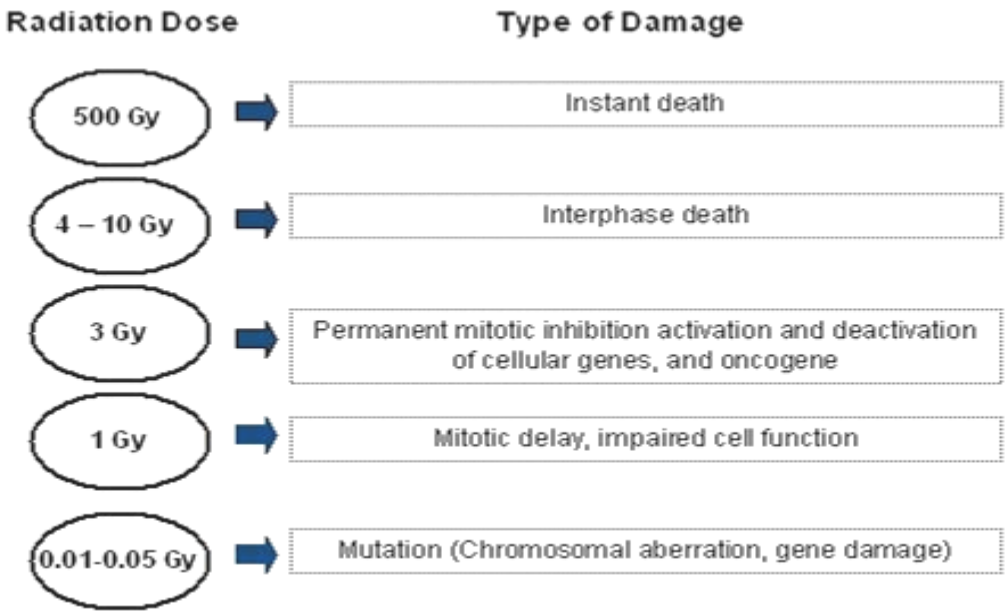

Figure-2: Biological effects of ionizing radiation.

A. *Radiation induced free radicals and their interaction with bio-molecules*

I *Effect of radiation on proteins*

It is increasingly evident that protein molecules are critical targets of free radical attack both intra and extra cellular because many of them play important role as enzymes catalyzing diverse important reactions and

transportation of different molecules present across membranes (Beal, 2002). These physical changes in the proteins after interaction with free radicals are characterized into three groups 1) fragmentation 2) aggregation and 3) susceptibility to proteolysis degradation (Pal, 1994). Oxidation of the critical -SH group (sulfhydryl) present in the calcium transport system located in the endoplasmic reticulum, mitochondria, plasma membrane may promote increase in Ca^{2+} levels in the cytosol. The elevated calcium levels have been shown to have a number of deleterious effects including induction of cell death (Franklin & Johnson, 1992).

II *Effects of radiation on membrane lipids*

Several studies have supported the idea that membrane damage induced by radiation is a critical event (Agrawal & Kale, 2001). Cregan and coworkers have suggested that damage to membrane organization is the initial step in triggering cell death (Cregan *et al*, 1999). A correlation has been observed between un-repaired membrane damage and loss of clonogenicity in cells. Lipid peroxidation is a highly destructive process and brings about change in structure, fluidity and permeability of membranes ultimately altering the structure and function of cellular membrane (Srivatsava *et al*, 1998), loss of -SH groups and inactivation of a number of membrane bound enzymes and receptors, induces swelling and alterations of respiratory functions, mediates DNA damage and alters RNA transport from the nucleus to cytoplasm (Agrawal & Kale, 2001). Spontaneous oxidation of lipid molecules in membrane by oxygen at room temperature is termed as lipid per oxidation. Among different components of membrane system, the phospholipid component of cellular membrane is highly vulnerable target of LPO due to its polyunsaturated fatty acid side chains. Lipid per oxidation is a free radical mediated chain reaction and involves three distinct steps i.e. initiation, propagation and termination.

III *Effect of radiation on genetic material*

Among different bio-molecules damage to DNA has been shown to be most important and contribute maximally to cell death (Sutherland *et al*, 2000, Olinski *et al*, 2002). Studies made on DNA irradiated *in vitro* in solution, in the dry state or *in vivo* in biological system have revealed that radiation cause a spectrum of damages to DNA.

RADIOPROTECTORS

Radioprotectors are medicines that save normal (noncancerous) cells from the damage caused by radiation therapy. These agents promote the repair of normal cells that are exposed to radiation e.g. Amifostine is the only drug approved by the FDA as a radioprotector.

Research has been carried out since 1949, on the radioprotective action of chemical substances which have shown to reduce the death rate in animals when administered prior to exposure to a lethal dose of radiation (Leathwood and Chauffard, 1982). These chemical substances reduce the radiation induced damage and provide prophylactic treatment for the damaging effects produced by radiatiotherapy. The

following radioprotection mechanisms were proposed: free radical scavenger, repair by hydrogen donation to target molecules, formation of mixed disulfides, and delay of cellular division and induction of hypoxia in the tissues (Varanda and Tavares, 2005).

Radioprotective agents have been divided into four major groups: the thiol compounds, other sulfur compounds, pharmacological agents (anesthetic drugs, analgesics, tranquilizers, etc.) and other radioprotective agents (WR-1065, WR-2721, vitamins C and E, glutathione, etc (Hosseinimehr, 2008). These chemicals are capable of scavenging free radicals, inducing oxygen depletion, antioxidants and modulators of immune response have been some of the radioprotectors extensively investigated with limited success. Mechanisms of action of some chemical radioprotectors and their combinations have been elucidated, while further understanding is required in many instances (Hoult and Paya, 1996; Upadhyay *et al.,* 2005).

Development of novel and effective approaches using non-toxic radioprotectors is of considerable interest for defence (nuclear wars), nuclear industries, radiation accidents, space flight, etc, besides playing important role in the protection of normal tissues during radiotherapy of tumors.

From the viewpoint of practical application, the radioprotector is expected to have the following capabilities:

(a) It should offer good protection against both acute and chronic radiation damage.

(b) It should be suitable for oral administration and be rapidly absorbed and distributed throughout the body.

(c) It should not show any significant toxicity, including those on behaviour.

(d) Shelf-life should be long, easy handling and storage.

(e) It should be readily available and inexpensive.

Podophyllum hexandrum

P. hexandrum is an erect, glabrous, fleshy or succulent rhizomatous herb and is found growing in Himalayan region.it belongs to the family Berberidaceae/Podophyllaceae. The roots and rhizome of the plant have been used in the Indian as well as traditional Chinese system of Medicine for the treatment of diseases. The fruit of *Podophyllum hexandrum* is ate as a mild laxative and the rhizome is consumed as a purgative, hepatic stimulant and is applied intravaginally to treat gynecological infection since ages. Podophyllotoxin, a major component of *Podophyllum* has several medicinal application e.g., as an anti-malarial, anti-fungal, and possesses immunomodulatory activity.

Botanical classification

Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Magnoliidae

Order	Ranunculales
Family	Berberidaceae (Podophyllaceae)
Genus	*Podophyllum*

Life cycle, Growth conditions, Cultivation and Geographical Distribution

The plant has a perennial life cycle and a life span of 3-10 years. The plants of *Podophyllum hexandrum* usually attains height of 1'-15". It prefers moist, peaty, sandy and loamy soil. *P.hexandrum* grow in shady alpine regions of the Himalayas (2500-4000m) and can even tolerate temperatures upto -40°F. *Podophyllum hexandrum* is found distributed in the mountainous regions of Pakistan, Northern India, Tibet, Nepal, Bhutan, China, and Afganistan. it is also found in the United States and Canada.

Chemical Constituents

Rhizomes and roots of *Podophyllum hexandrum* contain a plethora of bioactive constituents, mainly including ligans [podophyllotoxin, podophyllotoxone, demthylpodophyllotoxin, deoxypodophyllotoxin, isopicrophyllone, picropodophyllone, peltatins (α and β) etc], and flavonoids [quercetin, kaempferol and astragalin or kaempferol-3-glucoside etc]. The roots of *Podophyllum hexandrum* contain approximately 4-5% podophyllotoxin and 0.4% demethylpodophyllotoxin, while *P.peltatum* contain abouts 0.25% podophyllotoxin, 0.33% β-peltatin and 0.25% α-peltatin.

Traditional uses

P. hexandrum has been exploited in several traditional system of medicine, including Ayurveda, for the treatment of a number of ailments like constipation, cold, bacterial infections, biliary fever, septic wounds, burning sensation, erysipelas, insect bite, mental disorders, rheumatism, plaque etc. and provide symptomatic relief in some of the allergic and inflammatory conditions.

Pharmacological activity and Medicinal Uses

Podophyllum is considered a very good drug to overcome habitual constipation and maintains the functioning of normal hepatic and intestinal secretions. *P. hexandrum* is also having anticancer activity. One of the major cause of death following radiation-induced myelosuppression is infection arising from translocated endogenous gastrointestinal Gram-negative gastrointestinal bacteria. *P.hexandum* has been shown to exhibited antimicrobial activity. *P. hexandrum* also exhibited immunomodulatory and cytoprotective action, and antiviral activity. It is also used for the treatment of Rheumatism, Psoriasis, Malaria, fever and Veneral warts.

Radioprotective efficacy

Radiation-induced damage to macromolecules (DNA, protein, lipids) in the form of leaky membranes, DNA lesions, fragmented and denatured protein, is manifested as a result of increase production of reactive oxygen species (ROS/NOS) due to oxidative stress generated by low linear energy transfer (LET).

The whole extract of *Podophyllum hexandrum* rhizomes has been reported to modulate antioxidant enzymes levels, protect against ionizing radiation-induce DNA damage, protect gastrointestinal, reproductive and CNS, against radiation-induced damage.

Protection to Hepatic/Intestinal system: The radioprotective effect of an extract of *P. hexandrum* has been investigated on human hepatoma cell line (hep G2). *P. hexandrum* was found to protect the HepG2 cells against radiation damage by reducing levels of ROS/NOS, decreasing radiation-induced lipid peroxidation and augmenting glutathione levels by stimulation of neo-synthesis of glutathione (Gupta et al, 2003).

Protection to the Central Nervous System: The developing neurons within CNS are more sensitive to insult by ionizing radiation than mature neurons and their sensitivity changes with developmental stages. In this view, exposure to ionizing radiation at different periods during gestation disrupts both the morphology of specific areas e.g. hippocampus and the associated functions. Protection to neural tissue against super lethal radiation has been reported in ex vivo studies (Chawla et al, 2005).

Reduction of Radiation-induced Post-natal Physiological Alteration: Radiation is known to damage the fetal erythropoietic system leading to depleted supply to various functional regions of brain result in hypoactivity of the organism after birth. Pre-irradiation administration of *P. hexandrum* extract decreases radiation-induced post-natal physiological alterations and provides protection against the damage (in utero) caused as a result of planned radiation exposure. *P. hexamdrum* when administered prior to irradiation restores normal cellular development and differentiation that is altered by irradiation, thereby mitigates alterations in physiological markers and reflexes. Both the cerebellum and the visceral areas of cerebrum have been shown to be protected by *P. hexandrum* against radiation (Goel et al, 2002).

Gastroprotection: Administration of aqueous extract of P. hexandrum reduces the formation of apoptotic bodies in crypts and also leads to mitotic arrest. Clonogenic cells have also been shown to be protected, which is most likely due to radical scavenging activity property of extract (salin et al, 2001).

Mammalian Reproductive System Protection: Administration of *P. hexandrum* increases the weight of testis, repopulates seminal vesicle tubules, resting primary spermatocytes, stem cell survival index and decreases the abnormalities of sperm. Exposure to ionizing radiation leads to asthenospermia, hypospermia, tetratospermia, and decrease in testis weight and damages spermatogonia, which adversely influences spermatogenesis and sperm counts. Administration of aqueous extract of *P. hexandrum* rhizome has been shown to reduce sperm abnormalities in mice (Samantha and Goel, 2002).

Hemopoietic System Portection: *P. hexandrum* has been shown to protect the hemopoietic system against radiation. Hematological parameters like total leukocyte counts, Hemoglobin levels and Colony forming assay have been used to establish hematopoietic protection by *P hexandrum* (Sagar et al, 2005).

DISCUSSION

Exposure of biological matter to low LET radiation like gamma rays results in generation and enhancement of reactive oxygen species and a spectrum of damages to different biomacromolecules,

which ultimately contribute to the cell death. The hematopoietic system plays an important role in normal functioning of body system exposure of even small doses of radiation affects blood cell counts, Hb contents and other cellular biochemical and molecular alterations in hematological organs like bone marrow cells. Bone marrow transplantation is the current practice used for the treatment of radiation exposed patients. During radiotherapy one of the dose limiting adverse effects is hematopoietic toxicity. The present study was conducted to understand the changes in hematopoietic system specifically stem cell populations using mice as a model system. Exposure of 10 Gy to mice lead to lethality within 14 days. Damage in Gastrointestinal and Hematopoietic systems has been attributed to be the reasons of lethality. Exposure of lethal dose of radiation significantly decreased in the number of Sca 1 positive hematopoietic stem cells indicated the damage in hematopoietic stem cells population which could be one of the possible reason of mice lethality. However, P hexandrum treatment has significantly countered the radiation mediated hematopoietic injury by protecting and increasing the Sca1 positive cells. The result of present study clearly indicated the mechanism of hematopoietic protection of *P. hexandrum* was due to restoration of Sca 1 positive cells. Further studies are warranted to understand the mechanism of restoration of Sca 1 positive cells by *P. hexandrum* and also its effect on other hematopoietic stem cell markers.

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