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Radioprotective and immunomodulatory effects of *Mesua ferrea* (Linn.) from Western Ghats of India., in irradiated Swiss albino mice and splenic lymphocytes

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ABSTRACT

The present study reports the efficacy of *Mesua ferrea* (Linn.) extract in mitigating radiation induced toxicity along with immunomodulatory property. Swiss albino mice, splenic lymphocytes and plasmid pBR322 DNA were used to evaluate the radioprotective effect by exposing them to 6Gy EBR or 4 Gy gamma radiation. In this study, *Mesua ferrea* methanolic (MfM) or aqueous extracts (MfA) significantly protected pBR322 DNA against radiation induced strand breaks. Both the extracts significantly offered protection against radiation-induced apoptosis as indicated by propidium iodide staining and DNA ladder assay. MfM or MfA significantly scavenged radiation derived free radicals indicating their antioxidant potential. MfM or MfA were orally administered to Swiss albino mice at 250 or200 mg/kg body weight respectively for 7 days. The study showed significant increase in the levels of glutathione, and activities of endogenous antioxidant enzymes superoxide dismutase & catalase. Administration of MfA or MfM to mice significantly reduced electron beam radiation (EBR; 6Gy) induced increase in MDA levels. Immunomodulatory efficacy of MfM and MfA was evaluated using concanavalin-A (Con-A) induced proliferation of CFSE labelled splenic lymphocytes. Both the extracts significantly reduced proliferation in a dose-dependent manner. Further, MfM or MfA treatment prevented EBR induced histopathological changes in jejunum, spleen, liver and kidney. This demonstrates that the plant *Mesua ferrea* has promising antioxidant, radioprotective and immunomodulatory activity which may be attributed to the active constituents present in it.

1. Introduction

Radiotherapy is a dominant mainstay treatment modality of various solid cancers (Baskar, Dai, Wenlong, Yeo, & Yeoh, 2014), but is reported to induce secondary cancers by causing damage to rapidly multiplying normal cells (Robbins and Zhao, 2004). Though the treatment regime helps in extending the life-span of cancer patients depending on the type and stage of cancers, it has led to the development of secondary cancers, some of which are more severe than the primary cancer (Little, 2001), indicating the need for identifying drugs which can control the growth of malignant tissue leaving the normal cells with little or no side effect.

Exposure of cells to ionizing radiation generates reactive oxygen species (ROS) and reactive nitrogen species (RNS) which in turn cause cellular damage via lipid peroxidation, protein oxidation and DNA alterations. DNA damage can result in cell death or cancer. Lipid peroxidation products are reported to bind to DNA and induce mutagenesis. Free radical mediated damage to the protein can result in loss of function of DNA repair enzymes leading to mutagenesis and carcinogenesis (Devasagayam et al., 2004; Gracy, Talent, Kong, & Conard, 1999; & Cheeseman & Slater, 1993). Plant derived molecules with potent antioxidant activity could mitigate radiation induced damage to normal cells/tissues.

M. ferrea (Linn.) is a well-known evergreen tropical tree (Clusiaceae) commonly known as Naga sampige (Kannada), Naga kesara (Sanskrit), and Cobra saffron (English) widely grow in Southern Asia, used in the Indian traditional medicinal system for the treatment of fever, dyspepsia, microbial infections (Sumitra,

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Kalpana, & Jigna, 2013), renal diseases (Dennis & Akshaya, 1998), liver disorders (Garg, Sharm, Ranjan, Atrri, & Mishra, 2009). In Malaysia and India, a mixture of pounded kernels and seed oil is used for poulticing wounds. The seed-oil of *M. ferrea* is used for treating itch, other skin eruptions and rheumatism (Orwa, Mutua, Kindt, Jamnadass, & Simons, 2009). The plant is reported for its wound healing (Choudhary, 2012) anti-inflammatory, antioxidant, anticancer (Abhilash & Ramesh, 2012), analgesic (Hassan, Ali, Alimuzzaman, & Rihan, 2006), antiarthritic (Rastogi and Mehrotra, 1990-94) and immunomodulatory activity (Manoj, Sanjay-Kumar, Lokesh, & Manohara, 2012). The stem bark contains 4-alkyl and 4-phenylcoumarin (Luisella-Verotta et al., 2004), pyranoxanthones, mesuaferrin-A, mesuaferrin-B, caloxanthone-C, 1-8-dihydro-3-methoxy-6-methylanthraquinone, β-sitosterol, friedelin and betulinic acid (Soek Sin The et al., 2011) which are promising therapeutic compounds. Seed oil contains phenyl coumarin, mesuol (5,7-dihydroxy-8-isopentenyl-6-isobutyryl-4-phenylcoumarin), mesuone, mammeigin, mesaugin and coumarin. The stamen extract contains α-amyrin, β-amyrin, β-sistosterol mesuaxanthone-B and mesuanic acid (Raju, Srimannarayana, & Subbarao, 1976).

Development of radioprotective agents has been the subject of intense research in recent years, in view of their potential to improve the therapeutic index in radiotherapy. Hence the search for alternative sources, including bioactive principles of plant origin, has been an ongoing task worldwide. Since radiation-induced genotoxicity is predominantly a free radical mediated effect on DNA, plant-derived bioactive compounds with their antioxidant potential may render radioprotection to normal tissues. In this context, the present work was carried out to screen the plant *M. ferrea* for its radioprotective and anti-proliferative activity.

2. Materials and methods

2.1. Reagents and chemicals

m-phosphoric acid, sodium chloride, EDTA, disodium hydrogen phosphate, 5, 5′-dithiobis (2 nitro-benzoic acid), trichloroacetic acid, 2-thiobarbituric acid, HCl, malonaldehyde bis (dimethyl acetal),dimethyl sulfoxide (DMSO), 5-(and-6)-carboxy-2,7-dihydrodichlorofluorescein diacetate (H_2DCF-DA), RPMI 1640, fetal bovine serum (FBS), agarose, propidium iodide (PI), trizma base, triton X-100, sodium citrate, Tween 20 and ethidium bromide were procured from Sigma-Aldrich USA. Hydrogen peroxide, potassium dihydrogen phosphate, nitroblue tetrazolium chloride, riboflavin, and methionine were purchased from HiMedia, Mumbai. Concanavalin-A wasobtained from Calbiochem, USA. Carboxyfluorescein diacetate succinimidyl ester (CFSE) was procured from Molecular Probes, USA. Plasmid pBR322 DNA was obtained from Aristogene, Bangalore.

2.2. Collection and extraction

The stem bark of *Mesua ferrea* (Linn.) was collected from the Shettyhalli reserve forest, Western Ghats of Karnataka. Identified and authenticated sample was deposited for further reference in the Department of Biotechnology, TOCE Bangalore. The plant material was shade dried and powdered mechanically using a domestic electric blender, powdered samples were stored in airtight glass containers for further study. About 250 g of plant material was subjected to soxhlation using methanol for approximately 48 h. An aqueous extract was also prepared using double distilled water. Extracts were filtered, concentrated to dryness in vacuum under reduced pressure using rotary flash evaporator (IKA-German) (Shobowale, Ogbulie, Itoandon, Oresegun, & Olatope, 2013;; Akinmoladun, Ibukun, Afor, Obuotor, & Farombi, 2007).

2.3. Irradiation

The electron beam irradiation (EBR) work was carried out at Microtron center, Mangalore University, Mangalore, Karnataka, India. The un-anaesthetised animals were restrained in well-ventilated perspex boxes and exposed to whole-body EBR at a distance of 30 cm from the beam exit point of the microtron accelerator at a dose rate of ~100 cGy/min. pBR322 plasmid DNA and murine splenic lymphocytes suspended in RPMI medium were exposed to ã -radiation using a^{60}Co source at a dose rate of 0.89 Gy/min in Blood Irradiator, BRIT, Mumbai, India.

2.4. Plasmid pBR322 DNA damage studies

Plasmid pBR322 DNA was used to study the radioprotective efficacy of MfM or MfA in cell free system (Umang, Kunwar, Srinivasan, Nanjan, & Priyadarsini, 2009). Agarose gel (1%) was prepared in130 mM tris-borate/2.5 mM EDTA (TBE) buffer. Ethidium bromide (0.5 μg/ml) was added in the gel preparation to enable the visualization of the DNA bands in a UV transilluminator. The gel was submerged in an electrophoresis tank filled with TBE buffer. About 200 ng of pBR322 DNA was suspended in 20 μl of 10 mM sodium phosphate buffer, pH 7.4 and exposed to an absorbed dose of 50Gy γ-radiation dose both in the absence or presence of varying concentration of MfM and MfA. The samples were mixed with loading dye (0.25% bromophenol blue and 30% glycerol) and loaded into the wells. Electrophoresis was carried out at 60 V to separate the open circular (OC) and the super coiled (SC) form of DNA. The movement of the DNA bands was visualized on a UV transilluminator.

2.5. Isolation of lymphocytes

Lymphocytes were isolated from the spleen of Swiss albino mice following the method of Sharma, Sandur, Rashmi, Khanam & Sainis, 2007; Sharma, Sandur, & Sainis, 2007. The whole spleen was put in a 15 ml tube containing RPMI medium, squeezed gently against a sterile mesh using the piston. Then erythrocytes were lysed by brief hypotonic shock and cells were counted in a hemocytometer. Cells were cultured in a 24-well tissue culture plate containing RPMI media with 1× antibiotics (penicillin & streptomycin), 10% FBS, at cell density of 1×10^6 cells/ml. Cells were cultured for indicated time points at 37 °C in a 95% relative humidity and at 5% CO_2 atmosphere.

2.6. Estimation of apoptosis

Apoptosis of irradiated splenic lymphocytes was studied following the method of Sharma et al., 2007a,b. Lymphocytes were pre-incubated with various concentration of *Mesua ferrea* methanol extract (100, 50 25 and 10 μg/ml) for 2 h and then exposed to 4 Gy γ-radiation dose. The cultured cells were harvested by centrifugation for 2 min at 13500 rpm and the pellet was resuspended in 1 ml propidium solution and stored at 4 °C in dark. After 24 h, cells were acquired using a flow cytometer and percent apoptotic cells was determined by analyzing pre-G1 population (less than 2n DNA content) using FlowJo® software.

2.7. DNA ladder assay

DNA ladder assay was performed according to the Checker et al., 2008. The cultured lymphocytes were pre-incubated with various concentrations of *Mesua ferrea* methanol (50, 25 and 10 μg/ml) and