Radioprotective mechanism of *Podophyllum hexandrum* during spermatogenesis

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Abstract

RP-1, a herbal preparation of *Podophyllum hexandrum* has already been reported to provide protection against whole body lethal gamma irradiation (10 Gy). It has also been reported to render radioprotection to germ cells during spermatogenesis. Present study was undertaken to unravel the cellular and molecular mechanism of action of RP-1 on testicular system in strain ‘A’ mice. Various antioxidant parameters such as thiol content, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST) enzyme activity, lipid peroxidation (LPO) and total protein levels were investigated. Thiol content was seen to increase significantly \((p < 0.05)\) in both RP-1 alone and RP-1 pretreated irradiated groups over the irradiated groups at 8, 16 and 24 h. Irradiation (10 Gy) significantly decreased GPx, GST and GR activity in comparison to untreated control but RP-1 treatment before irradiation significantly \((p < 0.05)\) countered radiation-induced decrease in the activity of these enzymes. Radiation-induced LPO was also found to be reduced at all time intervals by RP-1 treatment before irradiation. As compared to irradiated group the protein content in testicular tissue was increased in RP-1 pretreated irradiated group at 4 and 16 h significantly \((p < 0.05)\). Comets revealed by single-cell gel electrophoresis were significantly longer \((p < 0.001)\) in irradiated mice than in unirradiated control. RP-1 treatment before irradiation, however, rendered significant increase \((p < 0.05)\) in comet length over the corresponding control and irradiated group initially at 4 h but at later time points, this was reduced significantly \((p < 0.01)\) as compared to the irradiated group. RP-1 treatment alone rendered shorter comets at 8, 16 and 24 h than irradiated groups \((p < 0.001)\). This study implies that RP-1 offers radioprotection at biochemical and cytogenetic level by protecting antioxidant enzymes, reducing LPO and increasing thiol content. (Mol Cell Biochem 267: 167–176, 2004)

Key words: *Podophyllum hexandrum*, glutathione peroxidase, glutathione reductase, radioprotection, spermatogenesis

Introduction

There have been many reports which implicate the role of environmental pollutants in decreasing male fertility in terms of sperm count, structure and quality [1, 2]. Reactive oxygen species (ROS) and oxidative stress play a major role in the genesis of such events. Exposure to X-rays and gamma rays have been exhaustively reported to induce DNA damage in germ cells causing mutagenic, carcinogenic and teratogenic effects [3, 4] in the progeny and produce reversible or permanent sterility in males depending on the radiation dose.

Low-linear energy transfer gamma radiation is known to cause oxidative damage by generating species like hydroxyl, superoxide, peroxyl radicals and hydrogen peroxide. The free radicals disturb the cellular homeostasis by peroxidation of membrane lipids, oxidation of proteins, base damage and adduct formation in DNA which ultimately lead to cell death if the damage is beyond repair [5]. The inherent antioxidant defence system of the cell including glutathione peroxidase, glutathione–S-transferase, catalase and superoxide dismutase competitively counteract the oxidative stress. However, the free-radical insult surpassing the inherent cellular defence mechanism warrants external antioxidant supplementation.
Herbal medicines have been in use since time immemorial for curing various diseases such as diabetes, arthritis, etc. that are alleged to be mediated by free radicals. Since radiation damage also involves oxidative damage, agents capable of antioxidant action could protect against radiation-induced lethality. Though molecular agents like cysteamine, WR-2721, MPG, etc. have also been investigated for their radioprotective properties, the side effects associated with them have restrained their use [6].

*Podophyllum hexandrum*, widely used in Ayurveda has been studied for its radioprotective manifestations at various levels of organization. The extract of *P. hexandrum*, (RP-1) has shown substantial protection to gastrointestinal system and testicular system in vivo [7, 8]. The protective effect has been attributed to free-radical scavenging ability, metal chelation and inhibition of lipid peroxidation in vitro [9].

Our earlier studies have indicated protection of germ cells during spermatogenesis at cellular and histological level by pretreatment with RP-1. Therefore, it was necessary to investigate the mode of action of RP-1 at biochemical and cytogenetic level at short time intervals in the testis.

### Material and methods

#### Chemicals

Chemicals were procured from different sources: 1-chloro 2,4-dinitrobenzene (CDNB) from Merck, Germany; reduced glutathione (GSH), oxidised glutathione (GSSG), reduced nicotinamide dinucleotide phosphate (NADPH), glutathione reductase (from baker’s yeast), agarose, sodium lauryl sarcosine, ethidium bromide (EtBr), triton-X-100, RNase, ethylenediamine-tetraacetic acid sodium salt (EDTA-Na), 2-thiobarbituric acid (TBA), and trichloroacetic acid (TCA) from Sigma Chemical Co., St. Louis, MO, USA; propidium iodide (PI) from Molecular Probes, USA. All other chemicals used were from reputed Indian firms and were of standard grade and high purity.

#### Animals

Swiss albino strain ‘A’ adult male mice (10–12 weeks) weighing 25 ± 2 g were maintained under standard laboratory conditions (25 ± 2 ºC; photoperiod 12 h light/dark cycle) and fed standard animal food pellets (Amrut Laboratory feed, India) and water ad libitum. Four to five animals were kept in polypropylene cages. All experiments involving animals were done following Animal Ethics Committee Rules and Regulations of this Institute.

### Preparation of the extract

Dried rhizome of *Podophyllum hexandrum* supplied by Field Research Laboratory, Leh (J&K, India) was powdered mechanically and known quantity of the powder was mixed in distilled water and kept at 37 ºC in an incubator for 24 h and filtered thereafter using Whatman No 1 filter paper. The filtrate was passed through Millipore filter (0.22 µ), lyophilized and stored at 4 ºC. The powder was suitably diluted in triple distilled water before use.

#### Irradiation

Whole body irradiation was given through Co$^{60}$ gamma cell (model 220 – Atomic Energy Commission, Canada Ltd.) and fresh air was continuously circulated in the irradiation chamber to avoid hypoxic conditions. Mice were kept in perforated plastic bottles for irradiation individually. The dose rate was 0.92 rad/s during the experimental period.

#### Treatment groups

Animals were divided into following four groups and sacrificed at 4, 8, 16 and 24 h after various treatments:

- **Group I**: ($n = 5 \times 2$): injected saline i.p., unirradiated control
- **Group II**: ($n = 5 \times 2$): 10 Gy whole body gamma irradiated
- **Group III**: ($n = 5 \times 2$): RP-1 (200 mg/kg b.w., i.p.) injected 2 h before 10 Gy whole body gamma irradiation
- **Group IV**: ($n = 5 \times 2$): RP-1 (200 mg/kg b.w.), injected i.p.

#### Preparation of homogenate

Testes were dissected out and kept in PBS (pH 7.4). One of the testis was used to make single-cell suspension in PBS and processed for single-cell gel electrophoresis. The testis was made free of fats and connective tissues, blotted dry, weighed and homogenized in ice-cold PBS (pH 7.4) to yield a 10% w/v homogenate which was subsequently used to measure lipid peroxidation and thiol content. The remaining homogenate was centrifuged at 10,000×g for 30 min at 4 ºC and the supernatant was used for assaying GST, GR and GPx enzyme activities. All the experiments were repeated twice.

#### Lipid peroxidation

Peroxidized membrane lipids were estimated by the method described by Beuge and Aust [10]. To the 250 µl of crude homogenate in duplicate, 0.1 M phosphate buffer (pH 7.4) was added to make the volume 1 ml. Thereafter, 2 ml of Beuge...