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Cellular radioprotecting potential of glyzyrrhizic acid, silver nanoparticle and their complex

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ABSTRACT

Silver nanoparticles (SN) of particle size of less than 50 nm were redispersed in aqueous solution of Pluronic F127 and complexed with the phytoceutical, glyzyrrhizic acid (GLY). Radioprotecting ability of the obtained nanoparticle–glyzyrrhizic acid complex (SN–GLY) was evaluated in an *in vivo* model using Swiss albino mice. Oral administration of SN–GLY, SN and GLY one hour prior to radiation exposure reduced the radiation induced damage in peripheral blood leucocytes, bone marrow cells and spleen cells of mice as revealed by comet assay. Exposure of mice to whole body gamma irradiation resulted in formation of micronuclei in blood reticulocytes and chromosomal aberrations in bone marrow cells while SN–GLY, SN or GLY administration resulted in reduction in micronucleus formation and chromosomal aberrations indicating radioprotection. In SN–GLY treated mice the cellular DNA was found protected to a greater extent compared to GLY or SN treated mice. The studies, under *in vivo* radiation exposure conditions, showed effective radiation protection.

1. Introduction

The interactions of radiation with the components of living system results in the generation of several oxidative free radicals (OFRs). OFRs so formed are responsible for many of the detrimental effects of radiation in living system as these attacks virtually all components including DNA, protein and membranes causing their dysfunction and damage [1] and impairs the indigenous cellular antioxidant defense system [2]. Ionizing radiations induce many forms of cellular damage as a consequence of irreversible changes resulting from the deposition of energy in the DNA. The major DNA damages induced by radiation are intra or inter strand cross-linking and single and double strand breaks, alteration and elimination of bases and sugar damages. The cellular responses include arrest in cell cycle, progression at cell cycle checkpoints, reproductive death, interphase death, division delay, chromosome aberrations, mutations, etc. and the induction of DNA repair. In a multicellular mammalian organism radiation induced oxidative damage to membranes as well as to cellular DNA damage ultimately result in development of radiation induced deleterious effects in various tissues and radiation induced pathological condition manifested as the radiation sickness, a condition characterized by bone marrow failure, gastro intestinal tract problems, susceptibility to bacterial infections, and other symptoms that develop days or months after exposure to ionizing radiation.

Protection of biological systems from ionizing radiation is of paramount importance during accidental and unavoidable exposures to radiation [3] and development of novel and effective approaches to combat radiation damages using non-toxic radioprotectors is of considerable interest for defence, nuclear industries, radiation accidents, space travels, etc., besides the protection of normal tissues during radiotherapy of tumors. Many synthetic as well as natural compounds have been investigated for their efficacy to protect against irradiation damage. They include sulfhydryl compounds, antioxidants, plant extracts, immunomodulators, and other agents [4].

Short-term *in vitro* tests such as lipid peroxidation, assay of free radicals and antioxidant status, cell survival and micronuclei assays, etc. can provide an idea about the radioprotective activity of an agent. However the gold standard for radioprotective activity is the evaluation of 30-day survival in rodents, because the 30-day survival after lethal whole body irradiation clearly indicates the capacity of the agent in test to modulate the recovery and regeneration of the gastrointestinal epithelium and the hemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for sustenance of the life [5]. The GI syndrome in mice can be assessed by determining survival up to ten days (measure of GI death) after exposure to comparatively high doses of whole-body radiation, whereas hemopoeitic syndrome

Abbreviations: OFR, oxygen free radicals; GLY, glyzyrrhizic acid; SN, silver nanoparticle; SN–GLY, silver nanoparticle–glyzyrrhizic acid complex.

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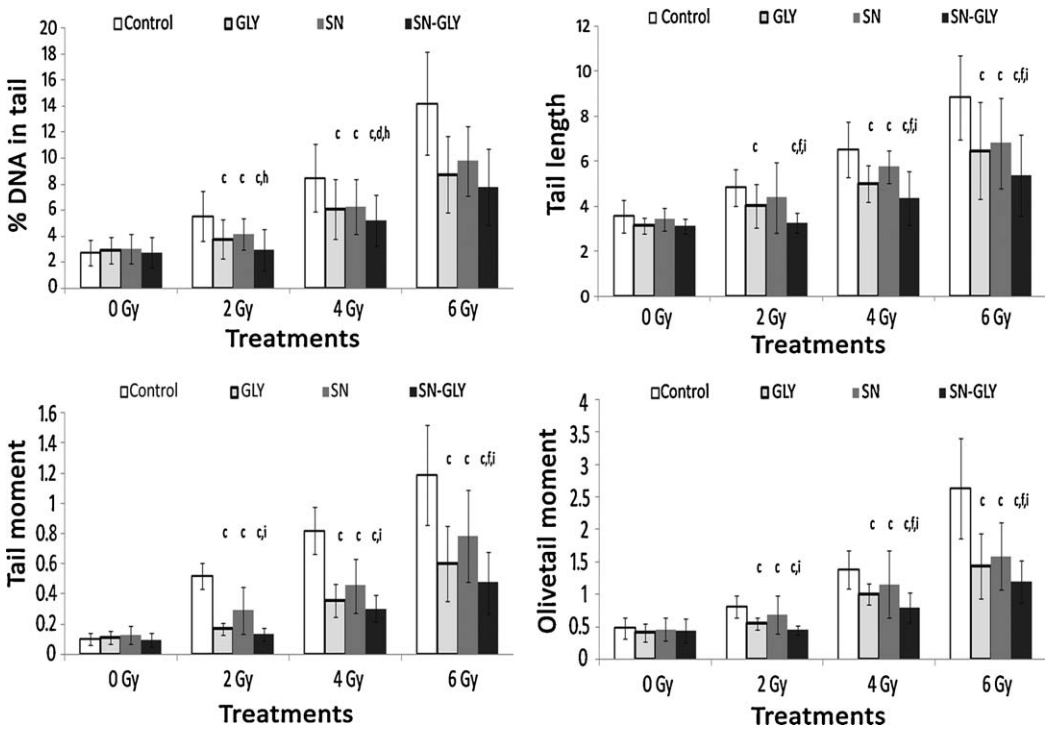
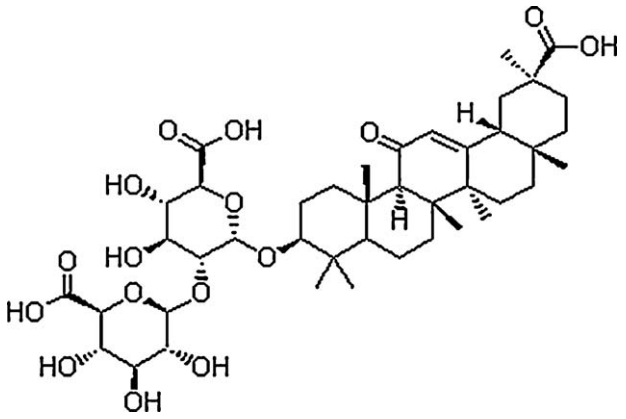


Fig. 1. Effect of oral administration of glyzyrrhizic acid (GLY), silver nanoparticles (SN) or glyzyrrhizic acid–silver nanoparticle complex (SN–GLY) on DNA damage in murine blood leucocytes induced by whole body exposure to gamma radiation (0–6 Gy) analyzed by comet assay. The percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD (c, $p < 0.001$ compared to respective radiation; d, $p < 0.05$ compared to respective GLY; f, $p < 0.001$ compared to respective GLY; h, $p < 0.01$ compared to respective SN, i, $p < 0.001$ compared to respective SN).

can be assessed by monitoring the survival of irradiated animals up to 30 days post-irradiation [6,7]. Even though there will not be any loss of survival following exposures to low doses of ionizing radiation, alterations do occur in cellular, subcellular, biochemical and molecular parameters. The deleterious consequences of exposure to low dose of ionizing radiation such as gene mutations, cancer, etc. stem from radiation induced genotoxicity. To study the ability of a compound to offer protection against the low dose whole body radiation induced genomic insults, alkaline comet assay, micronucleus assay and chromosomal aberration analysis could be the most elegant techniques [8–10].

Glyzyrrhizic acid (GLY) (Fig. 1) is a major bioactive triterpene glycoside of licorice root (*Glyzyrrhiza*) extracts possessing a wide range of pharmacological properties. The content of GLY in licorice root is 2–24% of the dry weight (Scheme 1).

Glyzyrrhizic acid (GLY) is 50 times sweeter than sugar, making it widely used as a sweetening additive in the food industry



Scheme 1. Glyzyrrhizic acid.

[11]. In many countries, GLY is used as a major therapeutic agent to treat chronic viral hepatitis and allergic dermatitis [12,13]. It is also known to have anti-inflammatory [14], anti-ulcer [15], anti-hepatotoxic [16] and antiviral activities [17,18]. Glyzyrrhizic acid is also reported to possess radioprotective properties [19]. GLY enhanced the recovery of organs and the cellular immunocompetence in mice following gamma radiation exposure [20,21].

Nanoparticles of carbon, fullerenes, have been shown to have antioxidant activity [22] and elicited *in vivo* radioprotective ability in irradiated rats [23–25]. It has been speculated that the fullerenes have the potential to scavenge reactive oxygen species (ROS) due to the electron clouds that surrounds [26,27] behaving as a "free radical sponge" [28]. Also studies have demonstrated that nanoceria could act as an antioxidant and radioprotector because of the presence of the mixed valence states of Ce^{3+} and Ce^{4+} on the surface [29–31]. Silver nanoparticle complex of palmitoyl ascorbic acid-2-glucoside (SN–PAsAG complex) is ascertained to be more radioprotective from the results on *ex vivo* and *in vivo* radiation protection studies on murine system [32]

In the present work, the radioprotecting property of silver nanoparticles of a particle size less than 50 nm [33] and glyzyrrhizic acid was explored. Efforts were made to prepare a glyzyrrhizic acid–silver nanoparticle complex which was explored for radioprotecting property. Protection of cellular DNA by silver nanoparticles, glyzyrrhizic acid and glyzyrrhizic acid–silver nanoparticle complex from radiation induced damages under *in vivo* conditions was studied in terms of comet assay, micronucleus assay and chromosomal aberrations analysis.

2. Materials and methods

2.1. Animals

Swiss albino mice of 8–10 weeks old, weighing 22–25 g were obtained from the Small Animal Breeding Section (SABS), Kerala Agricultural University, Mannuthy, Thrissur, Kerala. They were kept under standard conditions of temperature and