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Radio-protective dosimetry of *Pangasius sutchi* as a biomarker, against gamma radiation dosages perceived by genotoxic assays

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ABSTRACT

Exposure to ionizing radiation is harmful to any living organism. It may cause varying levels of genetic mutation or ultimately death. Synthetic compounds have been used to counteract the hazardous effect of radiation on the live cells, but the possibility of these synthetic compounds being harmful to the organism being treated also exists. Herbal formulations are thus being explored as a possible alternative for the synthetic radioprotectant. Induction of DNA damage in fishes caused by ionizing radiation and its protection by phytocompounds is a hardly studied topic. In this study, we analyzed the radioprotective effect of *Gymnema sylvestre* leaves extract (GS) and its active compound gymnemagenin (GG) against different doses of gamma radiation (^{60}Co) on the freshwater fish *Pangasius sutchi*. The radioprotective efficacy was assessed by micronuclei and alkaline comet assays. The freshwater fish *P. sutchi* was pre-treated with intramuscular injection (IM) of amifostine (83.3 mg/kg of B.W.), GS (25 mg/kg of B.W.) and GG (0.3 mg/kg of B.W.), 1 h prior to the gamma radiation. The fishes were exposed to LD_{30}, LD_{50} and LD_{70} of gamma radiation and the protection activities were assessed by analyzing the number of micronuclei (MN) and erythrocytic abnormalities in the blood after 2, 4, 8, 16 and 32 days after exposure. Compared to the irradiated fishes, frequency of erythrocytic abnormalities were decreased in response to the radio-protection in the amifostine treated groups for all three doses of gamma radiation (LD_{70} – 77.62%), (LD_{50} – 80.11%) and (LD_{30} – 82.30%); GS (LD_{70} – 62.66%), (LD_{50} – 69.74%) and (LD_{30} – 70.81%); and GG (LD_{70} – 49.42%), (LD_{50} – 53.43%) and (LD_{30} – 58.42%). Similarly, a significant radio-protective effect in terms of decremented DNA damage was observed using the comet assay after post exposure. The percentage of protection noted for amifostine was (LD_{70} – 58.68%), (LD_{50} – 64.52%) and (LD_{30} – 74.40%); GS (LD_{70} – 53.84%), (LD_{50} – 59.02%) and (LD_{30} – 65.97%); GG (LD_{70} – 49.85%), (LD_{50} – 52.56%) and (LD_{30} – 64.30%). From the current study, we can conclude that the radioprotective efficacy of the GS is similar to the synthetic compound (amifostine) and also greater than the bioactive compound (GG). The synergetic effect of the plant extract which leads to a better protection than the bioactive compound must be further studied. MN and Comet assays can easily identify the damage due to radiation exposure and thus can be used as predictive biomarkers for aquatic organisms exposed to radiation.

1. Introduction

An outline for protecting humans from ionizing radiation has been developed by the International Commission on Radiological Protection (ICRP) (ICRP, 2007) which has helped to protect the general public from radioactive waste from atomic weapon production and nuclear power generation, to control radiation exposure in workplace, and medical practices (Pentreath, 2002a; Delistraty, 2008). But unintended results of human focus become obliviousness to assess the impact of ionizing radiation on non-human biota. Recently, the ICRP task group published an outline for evaluating the impact of ionizing radiation on non-human species (ICRP, 2003). It is also mentioned that environmental protection should not consider the presence or absence of human as a factor (Pentreath, 2002a; Delistraty, 2008) because this depends on the significance and susceptibility of the biotic environment and promote the maintenance of biodiversity and sustainability.

Ionizing radiation generally causes the atoms and molecules to be ionized or excited to produce free radicals such as hydroxyl (OH*), superoxide anion (O_2*); disruption or formation chemical bonds and modify molecules (DNA, RNA, and proteins) that regulate cellular processes (Gulgun et al., 2016). Whenever the creation of free- radicals exceeds the antioxidant capacity of the organism, there is damage in the

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structural integrity and function of cell membranes, enzymes; and genetic material due to the impact on lipids, protein and DNA (Bhatia et al., 2008; Byers and Perry, 1992). Various living organisms can be used as biomarkers of genotoxicity (Cristaldi et al., 2004). Fish are proficient organisms for observing cytogenetic destruction and to screen the presence of the agents in surface water, not only in the laboratory, where the genotoxic agents can be identified in advance, but also in the different aquatic ecosystem. Fish species response to genotoxic agents similarly to that of higher vertebrates, which makes them as a good model organism to estimate the teratogenic, mutagenic or carcinogenic substances to humans (Udroiu, 2006; Lemos et al., 2007). So, there is a need to identify the substances that are able to protect the organisms from ionizing radiation as a countermeasure for radiation protection which is the core concern of our study.

Amifostine is an organic thiophosphate compound, clinically approved by FDA as a radio-protectant which acts as a ROS scavenging agent for hydroxyl and oxygen free radicals. It is a nucleophilic sulfur prodrug that is dephosphorylated in vivo by membrane-bound alkaline phosphatase to the active, free thiol metabolite which further oxidized to disulfide. The uptake of this active metabolites results in greater alkaline phosphatase activity, larger vascularization, and higher pH. (Grdina et al., 2002; Culy and Spencer, 2001). It is reported that amifostine ameliorates the effect of ionizing radiation on zebrafishes, when their embryos were exposed to various doses of gamma radiation (5, 10 and 20 Gy) and pre-treated with amifostine (4 mmol/L) (Geoffrey et al., 2006). It is also clinically proved that amifostine (200 mg/m^2 intravenously over 3 min) concentration deliver protect against radiation doses of 50–70 Gy and reduced the xerostomia within 90days (David et al., 2002). In relation to therapeutic use, the concentrations of amifostine used clinically are in the range 1.5–3.85 mmol/l (Shaw et al., 1999), which suggests that the degree of cytoprotection provided at antimutagenic dosages are insufficient to increase survival of either normal or neoplastic cells. But it has been rarely reported that the amifostine induced an allergic reaction and considered in the etiology of Stevens-Johnson Syndrome (SJS) and Toxic epidermal necrolysis (TEN). Patients had developed SJS and TEN during the amifostine treatment (200 and 250 mg/m^2 intravenously for 15–30 min) before each fraction of radiotherapy (Lale Atahan et al., 2000; Ayse Nur et al., 2002). So, on the other hand, usage of plants and natural products are beneficial in protecting the organisms from radiation damage since they are less lethal compared to manmade compounds at their optimum protective dose level (Bhatia et al., 2008). It is reported that the most active free radical scavenging fraction (CDF1) confers maximum in vivo radioprotection of 70% at a dose of 400 mg/kg b.wt. of *C. didymus* in mice, prior to 10 Gy radiation dose (Piya et al., 2011). Thus, the curiosity generated in developing the probable plant based-drug for the alteration of the radiation effect as mentioned by Bhatia et al. (2008). India has the rich legacy of therapeutic plants, most of which have been explored for their numerous bioactive compounds, but the plant's radioprotective potential has barely been explored. Some plants have strong antioxidant activity (Scartezzini, Speroni, 2000).

In this perspective, *Gymnema sylvestre* belongs to Asclepiadaceae which is a slow growing, perennial climber found in peninsular and central India, (Stocklin, 1969) has been evaluated for its radioprotective efficacy. The plant contains gurmarin (Arai et al., 1995), conduritol A, gymnemasaponins (Satdive et al., 2003), gymnemasins (Sahu et al., 1996), gymnemosides (Yoshikawa et al., 1997) stigmasterol, lupeol, quercitol (Gaurav et al., 2007), glycosides of kaempferol, quercetin (Liu et al., 2004) and gymnemic acid, which contains at least 17 different saponins, is the main constituent of Gymnema (Leach, 2007). Bhatia et al. (2008) has reported that *Gymnema sylvestre* plays a significant role in detoxifying drugs but its radio-protective efficacy is still not well evaluated and documented. Moreover, owing to the increased use of nuclear power for electricity generation in developing countries like India, establishing radioprotectants is inevitable for the protection of the environmental and human health during the accidental and suspected radiation exposures scenarios.

Hence the current investigation was undertaken to underpin the radioprotective efficacy of *Gymnema sylvestre* leaves extract and its active principle for the prevention of the radiation effects in the fish model and further extrapolates to the human model. Our findings on the radio protecting the efficacy of the herbal plant *Gymnema sylvestre* can be recommended as an herbal supplement for radiotherapy to the population living in high background radiation areas where the residents are constantly exposed to radiation.

2. Material and methods

2.1. Experimental fish specimens and chemicals

Pangasius sutchi is one of the fast growing catfishes, cultured in many places due to its market demand and commonly known as freshwater shark belongs to the family pangasidae (Uma et al., 2015). The single breed of *Pangasius sutchi* with an average length of 15 ± 1.00 cm and an average weight of 15 ± 1 g were obtained from the commercial fish seed hatchery and carefully transported to the laboratory and transferred to the large tank containing oxygenated water and disinfected with potassium permanganate. The fishes were acclimatized for 30 days under laboratory conditions of temperature 27 °C, pH 7.5, the hardness of water 220–240 ppm and 12 ± 12 h. Light-dark cycle. The fishes were fed with commercial feed, and the wastes were removed every day to decrease the ammonia percentage in the water. The chemicals used for the protection studies, amifostine was purchased from Sigma Company and gymnemagenin the bioactive compound from Natural remedies Pvt, Ltd. Bengaluru, Karnataka, India. The *Gymnema sylvestre* leaves were obtained from the Herbal Garden, Tamil University, Thanjavur, Tamil Nadu.

2.2. Determination of $LD_{50/30}$ dose of gamma radiation

Determination of $LD_{50/30}$ dose of gamma radiation was done by following the protocol of Anbumani and Mary Mohankumar (2012). The fishes were segregated into seven groups, 10 fishes per group were kept for 2 weeks under the laboratory conditions at 27 °C, 7.5 pH, and 220–240 ppm of hardness. One group was kept as a control without any irradiation and six groups were exposed to gamma radiation with the help of a gamma irradiation chamber at Radiological Safety Division (RSD), Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Tamil Nadu. The gamma radiation doses such as 2.5 Gy, 5 Gy, 7.5 Gy, 10 Gy, 15 Gy, and 20 Gy were given to the fishes and transported back to the laboratory. Then the fishes were maintained for 30 days to determine the $LD_{50/30}$ doses (Anbumani and Mary Mohankumar, 2012). Every 12 h., the quantity of dead fish in each group were recorded. Apart from the fishes mortality, their behavior in relation to lethality as well as the physical manifestation of radiation effects was also recorded (Praveen Kumar et al., 2015). The behavioral changes like hyperactivity, the rate of opercular activity, swimming rate and loss of balance, were also observed (Ashish Mishra and Mohanty, 2008). The data were tabulated to determine the 50% of mortality by Probit method using the stat-direct version3 software. The $LD_{50/30}$ value was 10.2 Gy (Pamela et al., 2018). Based on $LD_{50/30}$ value and its slope value, LD_{70} and LD_{30} doses were determined. The LD_{30} and LD_{70} values were 9.2 Gy and 11.4 Gy respectively. The study was approved by the Institutional Animal Ethics Committee (IAEC), Centre for Environmental and Nuclear Research (CENR) of SRM University (Ethics Clearance Number: 15/IAEC/CENR/04, Date: 28.02.2015) to perform the experiments using fish species in duplicate.

2.3. Determination of low observed effect level (LOEL) of amifostine, *Gymnema sylvestre* leaves extract and gymnemagenin

The lowest observed effects level of any chemicals or substance is