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Morphological and histological studies on freshwater prawn *Macrobrachium rosenbergii* (de man) irradiated with ^{60}Co gamma radiation

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ABSTRACT

This study was framed to investigate the ^{60}Co gamma radiation induced morphological and histological variations in freshwater prawn *Macrobrachium rosenbergii*. The LD_{50} value of ^{60}Co gamma irradiated *M. rosenbergii* observed (by probit analysis) at 30 Gy. Prawns were irradiated to four different dose levels (3 mGy, 30 mGy, 300 mGy and 3000 mGy) using Theratron Phoenix TeleCobalt Unit [P-33] and one control group (without irradiation) maintained separately. Irradiated groups exhibited several morphological variations such as discoloration; damaged rostrum; opaque coloration in cephalothorax; black bands and dot formation in abdomen; deformed uropods and telson in tail regions when compared with control group. The Hepato Somatic Index reflected the severity of radiation on hepatopancreas. Histological variations in gills, hepatopancreas and muscles of irradiated groups were observed. In gills, structural changes such as swollen and fused lamellae, abnormal gill tips, hyperplasic, necrotic and clavate-globate lamellae were observed in gamma irradiated prawns. Accumulation of hemocytes in hemocoelic space, interstitial sinuses filled with abnormal infiltrated hemocytes, the tubular epithelium with ruptured basal laminae, abnormal and coagulated lumen, necrotic tubules, thickened basal laminae, tissue debris, necrotic hepatocytes were observed in irradiated prawn hepatopancreas. In muscle, shrinkage of muscular fiber and necrotic musculature were observed in irradiated prawns. These structural alterations of the organs it is felt could affect the vital physiological functions such as respiration, osmotic and ionic regulation in gills and muscles; absorption, storage and secretion of the hepatopancreas which in turn could adversely affect the growth and survival of freshwater prawn *M. rosenbergii*.

1. Introduction

Nowadays the usage of nuclear radiation in many countries has increased (Anbumani and Mohankumar, 2012). Aquatic ecosystems were contaminated with a wide range of pollutants has become a matter of concern over the last few decades (Voegborlo et al., 1999; Dirilgen, 2001; Vutukuru, 2005). Sources of radioactivity in the aquatic environment include naturally occurring radionuclides, fallout from atmospheric runoff, watersheds that have received atmospheric deposition, and radioactive effluents from medical, industrial, and nuclear facilities released either accidentally or intentionally (Anbumani and Mohankumar, 2012).

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) assessed and reported 2.4 mSv/year as the worldwide average radiation exposure from natural sources, of which, 0.48 mSv/year was due to external terrestrial radiation (from indoors and outdoors sources), 0.39 mSv/year due to cosmic and cosmogenic radiation, 1.26 mSv/year from inhalation exposure (including radon) and, finally, 0.29 mSv/year was due to ingestion exposures (UNSCEAR, 2008).

All living organisms are exposed to a certain amount of ionizing radiation during their life span (Anbumani and Mohankumar, 2012). The aquatic organisms received external radiation exposure from radionuclides in water and sediment. They received internal radiation from radionuclides absorbed through the skin, muscle and respiratory organs (Iger et al., 1994; Kilemade and Mothersill, 2001). The International Commission on Radiological Protection

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(ICRP) emphasized the importance of protecting non-human biota from the potential effects of ionizing radiation (ICRP, 1991, 2007), and to achieve this, a regulatory framework have been developed by IAEA and ICRP (Douglas et al., 2006).

Ionizing radiation exposure creates oxidative stress and decomposes around 80% of the water in cell (Ucuncu et al., 2006) and induces cellular damage (Orhan et al., 2008). Radionuclides released either knowingly or unknowingly; during a nuclear disaster leads to contamination of inland water bodies. Biomonitoring methods are required for assessing the impacts of high and low levels of radiation that may ultimately result in ionizing radiation exposure to both humans and non-human biota (Anbumani and Mohankumar, 2012).

The necessity for investigation of sensitivity of aquatic biota in low and high dose of radiation was due to the uncertain nature of the biological response to low doses (Mothersill et al., 2002; Prise et al., 2003). Concern has also mounted on the adverse effects of low-level radionuclide contamination on aquatic ecosystems (Real et al., 2004; Stark et al., 2004; O'Dowd et al., 2006).

The giant freshwater prawn (*Macrobrachium rosenbergii*, de man, 1879) is a major commercially important crustacean species (New, 1995). It has an elongated body, usually of greenish to brownish grey in color. The head (Cephalothorax) covered with hard carapace, long rostrum (tooth) at anterior dorsal end and the abdomen consists of six cephalon and pair of eyes and five pairs of appendages (pleopods) (New, 2002; Ibrahim, 2009).

In crustaceans, gill acts as a multifunctional organ, performing vital functions such as respiration, osmoregulation, acid–base balance and nitrogenous waste excretion (Evans et al., 2005). It also acts as an important route for the entry of waterborne radioisotopes from environment (Iger et al., 1994; Kilemade and Mothersill, 2001). Hepatopancreas is the largest center of organic and inorganic reserves of decapods (Adiyodi, 1969), involved in various metabolistic activities (McLaughlin, 1983; Garcia et al., 2002). Muscles are protein-rich (Bhavan and Geraldine, 2001) and high concentration of free amino acids (Konosu and Yamaguchi, 1982; Shamasunder and Prakash, 1994). Crustacean muscle tissues contain free amino acids pool, about ten times more than that in mammalian tissues (Huggins and Munday, 1968).

Detection of responses at cellular or tissue level has of great value (Moore, 1985). Histopathological studies are very sensitive and crucial parameter, reflects the effect of toxicants on organ (Triebskorn and Kohler, 1996; Mathur and Gupta, 2008; Abdel-Warith et al., 2011).

Ionizing radiation induced morphological and histological studies in prawn is scarcely studied. Available literature mostly focusing on the effects of ionizing radiation on the biochemical, physiological, reproductive and growth aspects of aquatic organisms. In crustaceans, studies about juveniles, mortality, molting, reproductive aspects such as egg hatching, maturity are available. So, this studies being reported as a novel attempt to investigate the effects of gamma radiation on *M. rosenbergii*.

The aim of the present investigation was to evaluate the morphological and histological features in ^{60}Co gamma irradiated freshwater prawn *Macrobrachium rosenbergii*. Vital organs gills, hepatopancreas and muscle tissue were selected for examination.

2. Materials and methods

2.1. Experimental animal

The prawns (*M. rosenbergii*) were collected from the river Kaveri, Tiruchirappalli, Tamil Nadu (latitude 11°29′; longitude 79°50′ E). The collected prawns were acclimatized in Environmental Research Laboratory (Jamal Mohamed College, Tiruchirappalli) condition under 12:12 light-dark cycle. After 30 days, the prawns were divided into groups (10 each) and kept in indoor polypropylene fiber tanks (1.5 m × 1 m) with adequate aeration, and 20% of water volume changed daily. The water temperature was maintained at 27–28 °C. The water parameters maintained throughout the experiment as per Stalin et al. (2013). The prawns were fed with boiled and chopped goat liver ad libitum (twice daily).

Table 1
Instrument specifications of Theratron Phoenix [P-33] Tele Cobalt Unit.

Source strength	Source to isocentre distance	Collimator
143 RMM	80 cm	Field size min: 0 cm × 0 cm at 80 cm Field size max: 35 cm × 35 cm at 80 cm Rotation: ±90° 207 from its central axis Field size indicator: Field Camp Field size adjustment: Mechanical

Source: GVN cancer cure research center and hospital, Tiruchirappalli, Tamil Nadu, India.

2.2. Lethal Dose (LD_{50}) determination

Two hundred and fifty acclimatized prawns (25 groups) were transported to GVN hospital (at Tiruchirappalli) in aerated polypropylene fiber tanks for gamma irradiation process. One group act as control and others (three replicates/group) were exposed to 5, 10, 15, 20, 25, 30, 35 and 40 Gy gamma radiations respectively from Theratron Phoenix [P-33] TeleCobalt unit having a dose rate of 360 mGy/min. After irradiation, prawns were returned to indoor polypropylene fiber tanks in laboratory. The prawns were closely observed for a period of 96 h (every 6 h). The collected data were analyzed further by probit analysis.

2.3. Irradiation process

The acclimatized prawns (5 groups) were transported to GVN hospital (at Tiruchirappalli). One control was maintained separately. Four groups (10 each) were kept in four separate polypropylene rectangular (0.25 m × 0.05 m) tubs. For irradiation, the tubs (four no.) were placed on the TeleCobalt unit couch (table) and exposed to four different dose levels of (3, 30, 300, 3000 mGy) gamma radiation respectively (Table 1). Three different TLD (Thermoluminescence disk) were kept in each tub along with prawns for measuring the dose level emerged from the Telecobalt unit and the dosage received (entered) into the tub (Table 2). After irradiation, prawns were returned to indoor polypropylene fiber tanks in laboratory.

2.4. Morphological studies

Morphology of control and exposed groups were observed based on the modified methodology from Daisy and Ng (2007). The weight of each prawn (totally 50 prawns in 5 groups) was measured after irradiation. Body color changes, variations in cephalothorax, abdomen (uropod, telson) among control and exposed groups were observed as per standard methods.

2.5. Assessment of Hepato Somatic Index

At the end of the experiment (96 h), the weight of each prawn was measured. Control and irradiated prawns hepatopancreas were dissected and weighed. The Hepato Somatic Index (HSI) was calculated following the procedure outlined by Jiliang et al. (2007).