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Histological variations in liver of freshwater fish *Oreochromis mossambicus* exposed to ^{60}Co gamma irradiation

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ABSTRACT

The irradiation effect of ^{60}Co at the three dose level of 3 mGy, 30 mGy and 300 mGy on the histology of liver of the freshwater fish *Oreochromis mossambicus* was investigated. The liver of *O. mossambicus* was dissected out and processed for light microscopy studies. ^{60}Co exposed *O. mossambicus* were found to result in several alterations in the histoarchitecture of liver. The alterations included mild congestion of blood vessels, structural alteration, cellular swelling, vacuolation and necrotic liver cells, indicating a definite response to ^{60}Co irradiation. The results suggest that the liver of *O. mossambicus* exposed to ^{60}Co were structurally altered with increasing dose levels. It is to record that alteration in the liver does not affect the physiology, behaviour or lethality of the individuals. Self regulating mechanisms would have influenced the liver to remain sustained. To confirm the same further studies in the direction by increasing dose level is required.

1. Introduction

It has been demonstrated that Ecological Risk Assessment (ERA) approach developed for chemicals could be applied to the protection of the environment from radiation (Copplestone et al., 2004). One problem in adapting ERA to the case of radioactive substances relates to the lack of data describing effects of chronic exposure to low levels of radiation for non-human species (Garnier-Laplace et al., 2004).

Much of the current research into the radiosensitivity of non-human biota comes from the realisation that the International Commission on Radiological Protection (ICRP) conclusion is either ethical or defensible in scientific terms. Several recent reports and international bodies are grappling with the problems of regulating exposure of biota (Amiro, 1997; Hinton and Brechignac, 2004; Borretzen et al., 2005; ICRP, 2005, 2008) and the most fundamental issue is due to the lack of adequate scientific data concerning low dose exposure effects.

The risks of ionizing radiation to non-human biota (biota) are of considerable current interest, and both the International Commission on Radiological Protection (ICRP) and the International Atomic Energy Agency (IAEA), among others, have ongoing activities in this area (Chambers et al., 2006). In recent years, considerable international efforts have been undertaken to develop a regulatory framework for protection of the environment from the effects of ionizing radiation. Two innovative EC projects were started in 2000: FASSET (Framework for Assessment of Environmental Impact) and EPIC (Environmental Protection from Ionizing Contaminants in the Arctic) with the general aim to develop a methodology for protecting non-human organisms from ionizing contaminants (EPIC Project, 2001; FASSET Project, 2001).

There are few reports in the literature relating to the effects of low levels of ionizing radiation on fish. Much of the work details the effects of very high doses of radiation, which are not relevant for environmental protection applications. Much new research in the area of cellular stress biology suggests that many toxins, including radiation, produce very different cellular effects at low doses as compared to high dose effects (Calabrese and Baldwin, 2001, 2002). Effects of non-ionizing metals in combination with relatively low doses of ionizing radiation are receiving considerable attention at present (Mothersill et al., 2007; Salbu et al., 2008). Keys for understanding an environmental impact of multiple stressors are the understanding the mechanism of their interactions. However, Olsvik et al., 2010 recently showed that irradiation (3.1 mGy d^{-1})

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exposed for 5 h induced significant up-regulation of genes (caspase 6A, GR, GSH-px, Mt-A) known to respond to ROS (Reactive Oxygen Species) generating agents in the fish *Salmo salar.*

The present study is an attempt to meet the lacuna over the scientific data on the effect of ionizing radiation on non-human species. Fish is the one of the abundant population of an aquatic environment. They are easily susceptible to any alteration in the physico-chemical characteristics of the habitat. Hence, commonly available the fish *Oreochromis mossambicus* was selected and subjected to histological assessment of liver. The selection of liver cells as appropriate targets is due to their cytological sensitivity as biomarkers of organic contaminants and environmental pollution. Ultra structural alterations of fish hepatocytes have repeatedly been used as monitor systems to study sub-lethal effects of organic contaminants with great success (Braunbeck and Volkl, 1993). It is the first organ to be exposed by the portal circulation to toxicants ingested by the body. According to Hinton and Lauren (1990), the liver is a detoxification organ and is an essential for both the metabolism and the excretion of toxic substances in the body. Brusle and Gonza lez I Anadon (1996) state that fish liver histology could serve as a model for studying the interactions between environmental factors and hepatic structures and functions.

Organo-somatic indices generally express organ weight as a percentage of total body weight. These indices reflect the status of organ systems, which may change in size due to environmental factors and stressors more rapidly than the organism weights and lengths change (Goede and Barton, 1990). Organo-somatic indices serve as an initial screening biomarker to indicate exposure and effects (Mayer et al., 1992). Because of the energy storage and metabolic functions of the liver, alterations in liver size due to environmental stressors are of interest. The hepato somatic index (HSI) is the weight of the liver expressed as a percentage of body weight (Slooff et al., 1983). Evaluation of the HSI consider the role of both endogenous and exogenous factors (Schmitt and Dethloff, 2000). The HSI varies with seasonal cycles (Beamish et al., 1996; Saborowski and Buchholz, 1996; Slooff et al., 1983).

In this particular study ^{60}Co gamma irradiation were exposed to the dose level of 3 mGy, 30 mGy and 300 mGy to the fish *O. mossambicus* and to establish the impact on liver by studying its histological variations.

2. Materials and methods

2.1. Experimental animals

The freshwater fish *O. mossambicus* was collected from the Cauvery River, Tiruchirappalli District, Tamilnadu, and South India which have been relatively free from pollutants and introduced to a Natural pond (Environmental Research Lab, Jamal Mohamed College Tiruchirappalli.). The water temperature was maintained between 28 and 32 °C, Dissolved O2(DO)3.0 mg/l, Carbon dioxide 14 mg/l, Salinity 0–28ppt, Turbidity 25–100 mg/l, pH 6.0–8.5, Alkalinity 50–700 mg/l, and Total ammonia Nitrogen (TAN)-0.5–1 mg/l. Commercial fish feed was provided daily at the rate of 3% of the body weight.

2.2. Irradiation process

Sixty *O. mossambicus* fish were collected from natural earthen pond and placed in three separate polypropylene rectangular boxes with twenty fishes each. The dimensions of the box were 0.25 × 0.05 m (L × B × H) of capacity 1.5 L water. The three tubs were exposed to three different dose rates of 3 mGy, 30 mGy and 300 mGy with duration of 0.01, 0.09, 0.90 min respectively using Theratron Phoenix [P-33] tele cobalt unit having a dose rate 360 mGy/m. After 4 days, exposed fishes were subjected to histological studies. In order to take the geometrical consideration, a TL (Thermoluminescence disk) was put in a polythene bag along with fishes (Each box contains three different TLD disk) Dose received by the TLD is measured subsequently.

2.3. Hepato somatic index (HSI)

Calculation of HSI:

$$\mathrm{HSI} = \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}} \times 100$$

2.4. Tissue preparation for histological observations

For the present study liver was dissected and immediately fixed in Bouin's fixative for 48 h. The preserved tissues were processed by a routine histological method (Gurr, 1962), dehydrated in an alcohol series, cleared in xylene, infiltrated with liquid paraffin at 58 °C, and finally embedded in paraffin blocks. The blocks were trimmed and sectioned at 5–8 μm thick cut on a rotary microtome (Weswox MT Chennai, India), were stained with the Harris' Hematoxylin and counter-stained with Eosin (H&E stain). Then the slides were mounted with DPX and observed under a light microscope.

2.5. Assessment of histological characteristics

A histological grading system was used as a method to objectively estimate the histological changes or lesions identified in livers of exposed fish specimens (Table 1). Using this grading system, adapted from Pierce et al. (1978), a numerical value (grade) was assigned to each liver according to its histological characteristics. The grading system by Pierce et al. (1978) enables an objective assessment of the histological integrity of fish liver in a histological investigation such as this study, where the histology of exposed specimens is compared to that of a control group. This system clearly distinguishes between normal liver histology and liver pathology by indicating the histological conditions associated with normal and pathological conditions. Each grade represents specific histological characteristics and is assigned from 0 to 2 (0, 1, and 2), representing normal histological structure (as applied in this study), and 3–4indicating histological changes in the liver histology.

The liver histology of all 80 specimens including all exposed and control specimens were assessed individually by using the grading

Table 1
Assessment of liver histological changes observed in *Oreochromis mossambicus* specimens after exposure to gamma radiation (^{60}Co) (adapted from Pierce et al., 1978).

Grade	Histological characteristics (0–2, considered normal, 3 and 4 considered pathological)
0	Typical hepatic cord structure and hepatopancreatic structure
1	Typical hepatic cord structure and hepatopancreatic structure Mild lipid accumulation within hepatocytes
2	Typical hepatic cord structure and hepatopancreatic structure Mild lipid accumulation with in hepatocytes Mild congestion of hepatic blood vessels
3	Typical hepatic cord structure and hepatopancreatic structure +2–5 of the following : Severe lipid accumulation within hepatocytes Moderate to severe congestion of hepatic blood vessels Increase in perivascular connective tissue Hyalinization within hepatocytes Cellular swelling due to hydropic change Increase macrophage aggregates Lymphocyte infiltration
4	Loss of hepatic cord structure with two to five of the histological changes listed under 3