Low dose X–ray effects on catalase activity in animal tissue

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Abstract. This study was intended to investigate the effect of low-dose X-ray irradiation upon the activity of catalase (CAT) in freshly excised chicken tissues (liver, kidney, brain, muscle). The tissue samples were irradiated with 0.5Gy and 2Gy respectively, in a 6 MV photon beam produced by a clinical linear accelerator (VARIAN CLINAC 2100SC). The dose rate was of 260.88cGy/min. at 100 cm source to sample distance. The catalase level was assayed spectrophotometrically, based on reaction kinetics, using a catalase UV assay kit (SIGMA). Catalase increased activity in various tissue samples exposed to the studied X ray doses (for example with 24 % in the liver cells, p<0.05) suggested the stimulation of the antioxidant enzyme biosynthesis within several hours after exposure at doses of 0.5 Gy and 2 Gy; the putative enzyme inactivation could also occur (due to the injuries on the hydrogen bonds that ensure the specificity of CAT active site) but the resulted balance of the two concurrent processes indicates the cell ability of decomposing the hydrogen peroxide–with benefits for the cell physiology restoration for the chosen low dose radiation.

1. Introduction
Since the discovery of X-ray over 100 years ago, radiation has been used increasingly in medicine and industry to help for diagnosis, treatment, and technology purposes. However, radiation hazards present an enormous challenge for the biological and medical safety. The deleterious effects of ionizing radiation in biological systems are mainly mediated through the generation of reactive oxygen species (ROS) in cells as a result of water radiolysis [1]. Among them, particularly, the highly damaging hydroxyl radical (‘OH) can cause injury by reacting with biomolecules [2, 3]. ROS and oxidative stress may contribute to radiation-induced cytotoxicity and to metabolic and morphologic changes in animals and humans during radiotherapy, experimentation, or even space flight [4]. Against oxidative stress, cells are equipped with several natural enzymatic and non-enzymatic antioxidant defenses [5]. The exposure of human body to ionizing radiation could lead, however, to depletion of these endogenous antioxidants [6] and ultimately to the development of systemic diseases. Irradiation up to 14.4 Gy caused marked decrease in serum melatonin and its pineal biosynthesis three and five days after radiation exposure [7]. In addition, total antioxidant capacity of plasma was reduced in patients exposed to whole body irradiation for the purpose of reducing tumor growth. Consequently, the cellular antioxidant capacity was decreased and the organs become more susceptible to the deleterious effects of ROS [8]. On the other hand, more than 35 countries worldwide allow the irradiation of food. In the USA, in addition to raw beef, pork and lamb, spices, wheat, flower, potatoes, pork, fruits and vegetables, have been irradiated. Irradiation was approved to control Trichinea in pork in 1990, Salmonella in chickens in 1994, and E. coli in row meat in 1999. In Canada, irradiation is used to prepare meals for hospital patients. French food processors also use irradiation. The World Health Organization designated food irradiation as a perfectly sound food preservation technology that is needed in the world where food borne diseases are on the increase, and where in between 1/4 to 1/3 of the global food supply is lost post – harvest. The approved doses for food
preservation are: 4 kGy for chilled beef meat, 7 kGy for frozen beef meat, maximum 30 kGy for spices, and less than 1 kGy for fresh vegetables. Exposure of living organisms to low-dose ionizing radiation – still considered a new radiobiological concept - could trigger various metabolic reactions that help cells to defend against oxidative stress such as that related to hydrogen peroxide - enhanced following water radiolysis, often generating the indirect effect of radiations. Hydrogen peroxide is highly deleterious to the cell and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death [9-11]. In the context of food preservation, this study was intended to investigate the effect of low-dose X-ray-irradiation upon the activity of catalase (CAT) in freshly excised chicken tissues (liver, kidney, brain, muscle).

2. Materials and methods

2.1. X-ray exposure
The biological material was represented by freshly excised chicken tissues (kidney, muscle, liver, brain).

The tissue samples were irradiated with 0.5 Gy and 2 Gy respectively, in a 6 MV photon beam produced by a clinical linear accelerator (VARIAN CLINAC 2100SC – Regional Institute of Oncology, Iasi, Romania). The dose rate was of 260.88 Gy/min. at 100 cm source to sample distance. The exposure time for the studied doses delivery was calculated using the following formula [12]:

$$ t = \frac{D}{\dot{D}(z_{max}, 10, hv)} \times RDF(A, hv) \times PDD(1.5\text{cm}, A, hv) \times 0.005029 $$

(1)

were $t$ is the irradiation time, $D$ is the absorbed dose in the sample; $\dot{D}(z_{max})$ is the dose rate at the point $z$ where it reaches its maximum value on the central axis of a 10 x10 cm$^2$ photon beam; RDF ($A$) is the relative dose factor (depending on the radiation type); PDD ($1.5\text{cm}, A, hv$) is the percentage depth dose at 1.5 cm depth, for a $A$ field size. The parameters for the given irradiation geometry (6 MV photon beam) were obtained after beam calibration procedures (in accordance with dosimetric standard IAEA TRS-398) using a 3D Blue Water Phantom and a PTW Unidose Electrometer.

2.2. Catalase activity
The catalase activity was determined using UV analysis method described by Hugo Aebi [13], using a catalase UV assay kit (SIGMA). The assay involves spectrophotometrically survey of the decrease in absorbance of hydrogen peroxide at 240 nm with kinetic recording software. The assay was performed within a short time period (60 seconds), because catalase reaction is very fast.

Unit definition: One unit of catalase will decompose 1.0 micromole of hydrogen peroxide to oxygen and water per minute at pH 7.0 at 25 °C at a substrate concentration of 10 mM hydrogen peroxide.

2.3. Statistical analysis
To ensure statistical significance, three replays of every sample and control were analyzed in identical conditions. Average values and standard deviations were considered for graphic representations. Student $t$-test was applied to assess the statistical significance of differences between controls and irradiated samples, with the threshold of 0.05 in all experiments.

3. Results and discussions
The study on the action of low-dose X-rays on the antioxidant defense system in the kidney tissue is represented by monitoring the absorbance decrease of hydrogen peroxide and calculating the enzymatic activity of catalase. The absorbance dynamics of hydrogen peroxide for 60 s for the kidney samples is shown in figure 1, where the downward trend of the absorbance of hydrogen peroxide as a result of its decomposition into $O_2$ and water by the action of catalase can be seen.
Figure 1. Hydrogen peroxide absorbance dynamics at 240 nm for control and irradiated chicken kidney tissue

The catalase activity (figure 2) for the kidney tissue revealed a significant increase in the enzymatic activity, with about 40% and 100% (p<0.05) for 0.5 Gy and respectively 2 Gy, compared to control.

Figure 2. Catalase activity in chicken kidney tissue vs. X-ray dose

The exposure of brain tissue to low doses of X-ray led to an increase in catalase activity in this tissue, as shown in figure 3. Thus, in brain tissue samples irradiated with 0.5 Gy the levels of catalase activity were increased by 25%, while for samples irradiated with 2 Gy the catalase activity was increased almost 2.4 times, compared the non-irradiated samples (figure 4).

Next sets of samples were consistent with muscle tissue. Their exposure to the chosen radiation doses also led to a decrease in absorbance of H$_2$O$_2$ as a result of increased catalase activity.

Figure 3. Hydrogen peroxide absorbance dynamics at 240 nm for the control and irradiated chicken brain
Figure 4. Catalase activity in chicken brain tissue vs. X-ray dose

Figure 5 illustrates the dynamics of H$_2$O$_2$ absorbance spectra in the chicken muscle tissue.

Figure 5. Hydrogen peroxide absorbance dynamics at 240 nm for the control and irradiated muscle tissue

The experimental data illustrated in figure 6 show a stimulation of antioxidant defense system activity in muscle samples after exposure to low doses of radiation - after 0.5 Gy X-ray exposure, the catalase activity being intensified by up to 20% compared with non-irradiated tissue (p < 0.05).

Figure 6. Catalase activity in chicken muscle tissue vs. X-ray dose

A major defense mechanism involves antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), converts active oxygen molecules into non-toxic compounds. The liver has the highest content of antioxidants and antioxidant enzymes, indicating the important role that it plays in antioxidant detoxification. Figure 7 presents the dynamics of hydrogen peroxide absorbance at 240 nm recorded for the extracts obtained from irradiated and non-irradiated liver tissue processing.
From figure 8 a significant increase in catalase activity in liver tissue irradiated compared to the non-irradiated can be seen. An exposure to a low dose of 0.5 Gy X radiation caused an increase in hepatic antioxidant defense process of about 2.3 times compared with non-irradiated samples (p < 0.05) while the exposure to 2 Gy resulted in tripling the intensity of catalase activity compared to non-irradiated samples.

4. Conclusions

In biological systems, in addition to normal metabolism, ionizing radiation is a source of active oxygen radicals. Cells can be damaged and even lead to cell death under considerable exposure to ionizing radiation when reactive oxygen species content cannot be controlled by cellular antioxidants. In this study we observed the stimulation of antioxidant defense system status in different types of chicken tissues after low-dose (0.5Gy) and medium dose (2Gy) of X radiation. It can be concluded that under moderate oxidative stress (exposure to low and medium doses of ionizing radiation) cells are able to regulate a variety of physiological mechanisms in an attempt to cope with oxidative stress. On the other hand, exposure to higher doses caused a decrease in catalase activity, possibly due to inhibition or oxidative inactivation of the enzyme protein caused by reactive oxygen species generation. However, catalase is one of the three main families of enzymes in mammalian cells critical for removing peroxide. Reduction of catalase activity may be due to increased consumption of this enzyme to prevent lipid peroxidation.

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