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RESEARCH ARTICLE

Impact of 60CO-γ radiation on testicular lactate dehydrogenase of Swiss albino mice

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ABSTRACT

Radiation induced decrease in lactate dehydrogenase (LDH) activity as compare to control. In control mice and ones radiated by 0.05 Gy 0.10 Gy, 0.15 Gy, 0.20 Gy and 0.25 Gy, LDH values were 860.00 IU, 822.50 IU, 819.80 IU, 827.30 IU, 681.35 IU and 730.50 IU, respectively. Statistically the significant value at a dose of 0.10 Gy was 189.80 IU. This decrement may be contributed to 'switch off' of glycolytic pathway to some extent or use of some other Kreb cycle intermediates other than lactates. These decrement(s) possibly may be due to injured metabolism and may thus effects male fertility.

Key words: *fertility, LDH, radiation, Swiss albino mice*

INTRODUCTION

Natural background radiation of various forms exists in the biosphere and comes from three well known and studied sources *i.e.*, cosmic rays, living cells and earth crust. Living cells, which have the inherent capability to bio-accumulate and bio-amplify radioactive isotopes from the environment. Mammalian testes are an ideal organ to study a variety of cellular processes example cell division, growth, differentiation and maturation. Radiation induced damage to testis have been subject of absorbing interest in addition of emanation of natural radiations from earth crust, the increased use of radionucleotides in medicine, veterinary research, and therapies has increased the vulnerability and sensitivity of human, animals and plant population to radiation hazards [1]. Radiation damages occur through collision of photon particles with atoms and molecules in cells which ionize to give rise to ions and free reactive radicals. Free radicals are believed to play a major role in more than sixty different health conditions including the ageing process, cancer and arthrosclerosis [2 and 3].

Lactate dehydrogenase [LDH] is one of the key enzymes of the glycolytic cycle which catalyses the reversible reaction lactate ↔ pyruvate in the presence of NAD which act as electron acceptor. It is very active in the interstitium and absent in leydig cells, and exhibited relatively attenuated activity in the seminiferous tubules [4 & 5]. Experimental studies have shown that testicular cells are responsive to such physical factors as temperature and radiation. Radiosensetivity of pre-meiotic spermatocytes and differentiating spermatids has been documented by several investigators [6, 7, 8 and 9].

Alteration in LDH of mammalian testes in response of various forms and doses of radiation has not been adequately studied; no tangible and comparative information is available on gamma radiation induced alterations, therefore in present study, impact of 60CO-γ radiation has been studied on testicular LDH of Swiss albino mice.

MATERIALS AND METHODS

Procedure of Radiation: Swiss albino mice were restrained in position by tying rubber bands around the fore, and hind limbs. They were exposed to single pulse of various doses of γ-radiation

for different times by Cobalt-60 camera. Radiations were applied to the abdominal region at a point where the paired abdominal testes were located. Control groups were sham irradiated and maintained for comparison with γ-irradiated males under similar conditions.

Surgical Processes and Preparation of Testicular Homogenates: Mice of control and experimental groups were weighed before and after radiation. They were sacrificed by cervical dislocation after 24 h of radiation. Testes were surgically excised under aseptic conditions. They were freed off of excess fascia and blood clots; rinsed several times in chilled physiological saline (4°C). After blotting the tissue the wet weight of each testis was separately recorded on monopan electric balance. Homogenate of testes (100 mg/ml) were prepared in normal saline (0.9% w/v) in ice bath in Potter (Elvehjem homogenizer for 5 min). The homogenate were centrifuge at 3000 rpm for 20 min to obtain the subcellular fractions. The supernatant was decanted and utilized for biochemical assay of lactate dehydrogenase (LDH), as per procedures detailed below.

Lactate dehydrogenase (LDH): LDH was biochemically quantitated in the testes by using 2-4-DNPH method of King [10].

Principle: LDH catalyses the following reaction

$$\text{Lactate} + \text{NAD} \overset{LDH}{\Longleftrightarrow} \text{Pyruvate} + \text{NADH}$$

Products so formed are coupled with 2,4-dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and is measured colorimetrically.

Procedure: Two tubes labelled as 'Control' (C) and 'Test' (T) were set up in pairs. Buffer lactate substrate pH 10 (0.5 ml) was added to both tubes. Distilled water (0.1 ml) and tissue homogenate (0.05 ml) were added to 'C' and 'T', respectively. They were vortexed and incubated at 37°C for 5 min. Then NAD solution (0.1 ml) was added to test 'T' and again incubated at 37°C for 15 min. To this colour reagent DNPH (0.5 ml) was added while in the test tube 'C' tissue homogenate (0.05 ml) was added and vortexed. Incubation was carried on for 15 min at 37°C. Finally 4M NaOH (0.5 ml) was added to both tubes and absorbency was measured at 440 nm. The standard curve was prepared by changing the concentration of 1.0 M working pyruvate standard, NADH, buffer, and lactate substrate (pH 10.0) in the total assay system containing NAD, DNPH colour reagent and 4 M NaOH incubated for 15 min at 37°C.

Calculation:

Net O.D. of the Test = O.D. Test – O.D. Control

The enzyme activity was computed and represent in the IU (international unit)

RESULTS AND DISCUSSION

Concentration of LDH in the testicular homogenates of control and experimental groups manifested their minimal and maximum values vis-a-vis control (860 units) in the following manner. The activity of LDH testes irradiated by 0.05 Gy was 822.5 units; by 0.1 Gy, 819.8 units; by 0.15 Gy, 827.3 units by 0.2 Gy and by 0.25 Gy these values were 681.35 units and 730.5 units, respectively. These values are less than the control. Testicular LDH activity was maximally decreased on exposure to 0.20 Gy and minimal decrease was observed in case of 0.15 Gy of irradiation γ-radiation induced decrease in testicular LDH of mice vis-a-vis control. Maximum decremental change was observed in the testes of mice exposed to 0.2 Gy and it was 20.77%. Exposure to 0.05 Gy, 0.1 Gy, 0.15 Gy and 0.25 Gy caused the percentile decrement which was computed to be 4.36%, 4.67%, 3.80% and 15.06% respectively. Decrease in LDH may be attributed to 'Switching off of glycolytic pathway for meeting the minimal energy demand of cell types for survival. It seems that testes may use other preferred Krebs cycle intermediate substrate other than lactates. In contrast

to the present findings, Liubimova et al. [11], observed elevation in LDH activity after radiation in children treated for nephroblastoma.

Table 1: Quantitative perturbations in the amount of lactate dehydrogenase (LDH) in the testis of Swiss albino mice challenged by various doses of ^{60}CO-γ radiation

S.No.	Dose (Gy)/min	LDH (in IU)
1.	Control	860.00±10.00
2.	0.05	822.50±02.50
3.	0.10	819.50±01.50
4.	0.15	827.30±28.30
5.	0.20	681.35±11.05
6.	0.25	730.50±05.50

Values are mean ± SE, IU = International Unit

Pant et al., [12] reported increase in testicular LDH level by oral administration of carbofuran. However, reduction in the kinetics of spermatogenesis, and enzyme activity after ionizing irradiation was observed by Ivanitskaia et al, [13].

A variety of dehydrogenesis are involved in metabolic pathways that are critical in the bioenergetics of cells. LDH is an important enzyme of the glycolytic cycle which catalyses the reverse transformation of pyruvate to lactate in the presence of NAD which acts as a co-factor [14].

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