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RESEARCH ARTICLE

BIOCHEMICAL ALTERATIONS IN MOUSE TESTIS AFTER THE COMBINED TREATMENT OF RADIATION AND CADMIUM CHLORIDE

C. L. Verma[1], Keshar Dev[2], M. L. Gupta[3] and R. K. Purohit[4]

1 Department of Zoology, Radiation Biology Laboratory, Govt. Lohia College Churu, India.
2 Department of Zoology, Radiation Biology Laboratory, Govt. Lohia College Churu, India.
3 Former Joint Director, College Education, Rajasthan, India.
4 Radiation Biology Laboratory, Department of Zoology, Govt. Dungar College, Bikaner, India.

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Corresponding Author

Dr. C. L. Verma

Department of Zoology,
Radiation Biology Laboratory
Govt. Lohia College Churu, India

Email address: drvermacl@gmail.com

Abstract

In today's world a combined exposure to physical, chemical and biological agents is unavoidable. Such situation can occur in the environment as well as in the work place. For risk estimates, it is desirable and often necessary to take such combined exposure into account. Combined action of ionizing radiation and other agents are of potentially great importance, because there are many occasions where interactions might occur in our environment.

In the light of above, the present study was aimed to evaluate the combined effect of radiation and cadmium on the testis of Swiss albino mice. The animals were sacrificed at 1, 2, 4, 7, 10, 14 and 28 days post- treatment. To investigate the existence of the synergistic or additive effects of radiation and cadmium chloride, biochemical changes in the testis were performed. It was found that sialic acid decreased while the activity of acid phosphatase and alkaline phosphatase increased in all the three exposures. Such decline was observed with higher dose (5.00Gy) at early intervals. Recovery started at day 10 or 14 but standard value could not be attained upto the last intervals. A similar decreasing pattern was observed with 2.5 and 1.25 Gy, but it was found comparatively very less with 5.0 Gy group. The recovery was also faster in these groups. Results indicated that the infliction of radiation insults and subsequent repair in the testes to be dose dependent.

In combined treatment the pattern of biochemical changes was similar to individual treatment but the magnitude of their occurrences was statistically higher and the normal value could not be attained even at the last autopsy (28 days). Thus the damage and recovery pattern in combined treatment (radiation and cadmium chloride) indicates the synergistic effect of these two agents when given simultaneously.

Introduction

Risk management of substances is often based on health risk assessments which generally take into account data from studies on individual substances. However, humans are simultaneously exposed to a large number of chemicals in their living environment. It is necessary to consider whether combined exposures to some substances could induce toxic effects when they appear in the same time. Therefore, the research hotspots of environmental pollutants are gradually turning from individual to joint action. Living organisms are exposed to a mixture of environmental stressors, and the resultant effects are referred to as multiple stressor effects (Ng *et al*., 2013). Cadmium is a highly toxic and carcinogenic environmental contaminant, which has no special biological role in animal organism (Smith *et al*., 1994; Korenekova *et al*., 2002). It is accumulated in the human body with a half-life exceeding 10 years and

has been linked with a number of health problems including marked damage to testes and fertility reduction (Kelley, 1999; Bench *et al*., 1999).

Many cadmium-induced changes are similar to those caused by other harmful factors, especially by ionizing radiation. The most striking effects of ionizing radiation include inhibition of DNA and protein synthesis (Verma *et al*., 2009).Therefore, increasing level of cadmium and radiation in the environment, the biological effects of these agents should be considered in the context of combined exposure of an organism to multiply agents. In the present study, therefore, the biochemical changes in acid and alkaline phosphate activity and sialic acid were investigated in mice testis after administration of cadmium chloride and gamma irradiation alone or after combined treatment.

Material and Methods

Animals:

Healthy male Swiss albino mice (6-8 weeks old) were procured from CCS Agricultural University, Hissar and maintained at 20-25^{o}C. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*.

Source of radiation:

Cobalt gamma radiotherapy source (Theratron, AECL, Canada) was used to irradiate the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan), India. The animals were irradiated at the dose rate of 0.97 Gy/min.

Cadmium chloride treatment:

The aqueous solution of the cadmium chloride (SDS chemicals, India) was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, thus giving a concentration of 20 ppm and then administered orally in drinking water.

Plan of experiment: The animals were divided into different groups according to the treatment given to them:-

Group- I:-Sham-irradiated animals serve as standard.
Group- II:-Only cadmium chloride treated animals.
Group- IIIa: - 1.25 Gy of gamma irradiated animals.
Group- IIIb: - 2.50 Gy of gamma irradiated animals.
Group- IIIc: - 5.00 Gy of gamma irradiated animals.
Group- IVa: - $CdCl_2$ + 1.25 Gy of gamma irradiated animals.
Group- IVb: - $CdCl_2$ + 2.50 Gy of gamma irradiated animals.
Group- IVc: - $CdCl_2$ + 5.00 Gy of gamma irradiated animals.

Autopsy of animals

Five animals were autopsied by cervical dislocation from every set of experiment at each post-treatment interval of 1, 2, 4, 7, 14 and 28 days. The weight of the animals was recorded and their testis was removed. The biochemical parameters studied were acid phosphatase, alkaline phosphatase and sialic acid.

Result and Discussion

The present study indicated alterations in the activities of some key enzymes reflecting the oxidative stress. The role of phosphatases in the testis has been shown to be associated with the transport of substances across the cell membrane and with the growth process and differentiation (Tice and Barrnett, 1963). The present study revealed an increase in acid phosphatase activity after radiation exposure (Fig- 1). The increase was found to be dose dependent, maximum in the higher dose group (IIIc). A similar increase in activity of acid phosphatase after irradiation has also been reported by many workers (Gupta and Bawa, 1975a; Shah and Gadhia, 1977; Gehlot *et al*., 2007). The increase in the activity of acid phosphatase during the first week of irradiation may, therefore, be due to lysosomal breakdown of testicular cells or to increased phagocytic activity of the sertoli cells (Nebel, 1959; Hugon and Borgers, 1966).

Acid phosphatase is localized in cellular lysosomes and change in activity of lysosomal enzymes take place following whole-body irradiation. An enhanced golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of the enzymes are attributed to an increase acid phosphatase level (Will and Wilkinson, 1966) The discharge of enzymes from lysosomes may be due to activation of pre-existing latent enzymes or due to synthesis of new lysosomes as a consequence of irradiation (Rene *et al*., 1971). It is suggested that there might be destruction of lysosomes, which release the acid phosphatase and thus resulting in the increased activity of the enzyme. The animals of group IV (combined group) also exhibited more increase in the activity of acid phosphatase in testis as compared to individual effect of radiation and cadmium chloride. This may be due to combined action of radiation and cadmium (Fig- 1).

An increase in the activity of alkaline phosphatase in testes was observed in the present study (Fig- 2). The activity increased upto day 7 in all irradiated sub-groups and thereafter, the value decreased. The increase was found to be dose dependent, maximum in the IIIc sub-group, comparatively lower in IIIb and minimum in the IIIa sub-group. A Similar pattern of increase in rats, Indian desert gerbil and mice at various sublethal and lethal doses also reported (Noamen *et al*., 1968a; Dixit *et al*., 1976; Hishida *et al*., 1979) The increase in alkaline phosphatase activity may be in response to increased requirement of both protein and nucleic acids to meet radiation damages (Kaur *et al*., 1975). An increased activity of alkaline phosphatase was observed in mouse testis after administration of cadmium chloride. This increased activity might be due to increased phosphorylation or tissue damage caused by excess amount of heavy metal (cadmium). The animals of group IV also exhibited the same pattern of increase in the activity of alkaline phosphatase in testis, but there was more increase as compared to individual effect of radiation and cadmium chloride. This may be due to combined action of radiation and cadmium. Ionizing radiation disturbs the sialic acid metabolism in several tissues of experimental rats (Jeanlog and Codington 1976; Sarkar *et al*., 1982). Sialic acid content of the testes per gram tissue weight decreases after exposure to 1.25, 2.50 and 5.0 Gy of gamma rays and this decrease is higher in the testis irradiated with higher dose used in the present study (Fig- 3). After decrease at day 1 interval, the sialic acid content increased upto day 4 in all the experimental groups. Then it decreased again at day 7 and continued upto day 14. Our results are similar with those of (Jaimala and Bhartiya, 1987; Sharma *et al*., 2010).

The decrease observed in the sialic acid content might be due to abrupt slow down in the rate of spermatogenesis after irradiation and cellular degeneration. After day 1, the sialic acid concentration of the mouse testes tended to increase towards the normal value but remained below the mark. This tendency continued upto day 4, after which the downward trend started again. In the irradiated testes when degenerative changes begin, the degenerated cells are removed from the tissue continuously. This leads to reduction in the testicular weight. This reduced weight of the testes seems to be responsible for the increased sialic acid concentration per gram tissue weight observed between days 1 and 4. It was observed that decrease in the testicular weight was quite high during this period.

The animals of group II (Cadmium chloride treated) also exhibited decreased concentration of sialic acid in the testes after cadmium chloride treatment. A significant decrease in the concentration of sialic acid of testes was associated with the degenerative changes of spermatogenic elements. Reduced sialic acid concentration in the seminal vesicle of cadmium chloride treated animals indicate an inhibition of androgen production (Lohia *et al*., 1976).

The animals of group IV also exhibited more decrease in the sialic acid concentration in testes as compared to individual effect of radiation and cadmium chloride. This may be due to combined action of radiation and cadmium. In general, alterations induced by administration of cadmium chloride alone were milder than those induced by gamma irradiation alone; however, the character of alterations was similar. In majority of cases, after combined treatment the alterations of biochemical parameters were more profound than after single treatment.

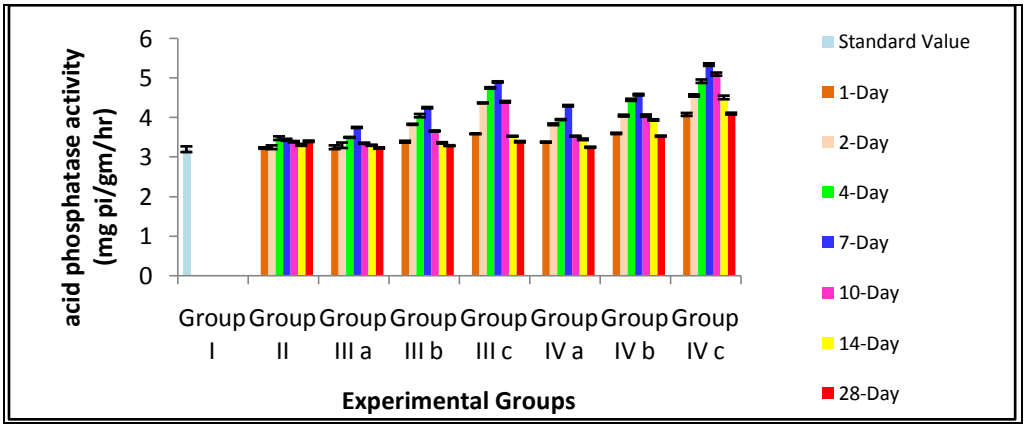

Figure- 1: Variations in the acid phosphatase activity in the testes of mice in various groups [Mean ± S.E.]

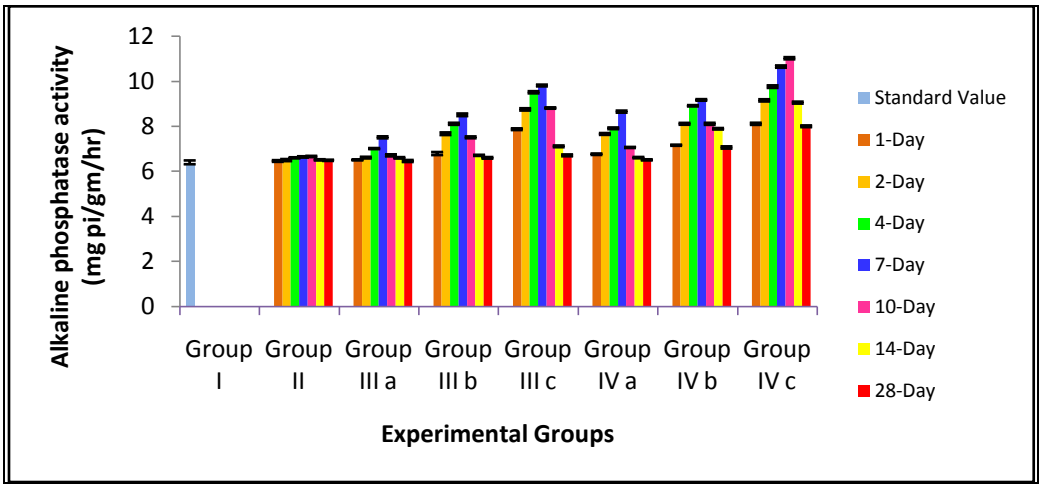

Figure- 2: Variations in the alkaline phosphatase activity in the testes of mice in various groups [Mean ± S.E.]

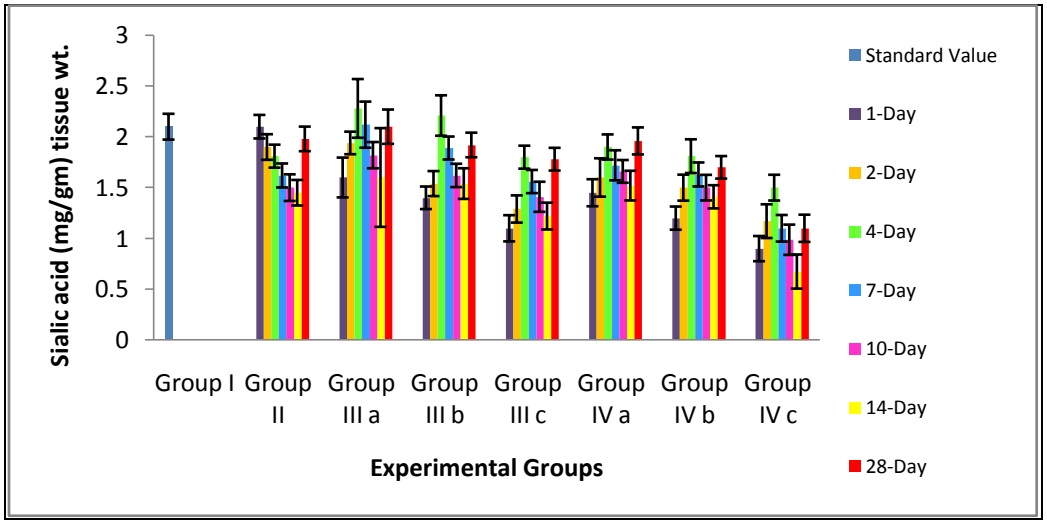

Figure- 3: Variations in the Sialic acid content in the testes of mice in various groups [Mean ± S.E.]

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