

## X-RAY INDUCED CHANGES IN BIOCHEMICAL AND HISTOCHEMICAL PARAMETERS IN THE TESTIS OF MALE WILD INDIAN HOUSE RAT, *RATTUS RATTUS*

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### ABSTRACT

To observe the complementary effects, whole body of the male wild Indian house rat, *Rattus rattus* was irradiated with X-rays at 100 r, 200 r, 300 r and 500 r (single dose) doses. The changes in the histophysiological parameters of the testis were observed after 2, 7, 15 and 30 days of post treatment. Determination of biochemical parameters: ascorbic acid, cholesterol, acid and alkaline phosphatases in the testicular tissues indicated that concentrations of ascorbic acid and cholesterol (2 to 15 days post treatment) had increased while acid and alkaline phosphatases (2-30 days) had decreased. Changes in these parameters were observed in all four X-ray treated groups (100 r, 200 r, 300 r and 500 r). The histochemical study of the different X-ray treated groups clearly showed the intense accumulation of lipid substances and depletion of acid, alkaline phosphatases,  $\Delta^5$ -3 $\beta$ -HSDH and 17 $\beta$ -HSDH in the testicular tissues of 7 to 30 day - post treated groups exposed to 300 r and 500 r X-irradiation. In view of the changes observed in the testicular tissues, it is likely that a single dose of X-ray may cause certain histophysiological changes in the testis of wild rodents at least for certain periods. As a single dose of X-rays of 200r or 300r was sufficient to cause sterility in male rats, this may be included as a controlling agent that adversely effect the fertility of the rodent pests. Possible mode of action is discussed.

**Key Words:** rodent pest, acid and alkaline phosphatases, lipid, fertility, spermatogenesis

### INTRODUCTION

Alberts-Schonberg's observation in 1903 showed that radiation causes a rapid regression of the mammalian testis. Numerous studies have shown that the testis is one of the most important radiosensitive organs in the body (Ellis and Berliner, 1969). The testis decreases in size and a gradual cessation of spermatogenesis occur due to radiation (Santra and Manna, 2002; Heller, 1948). Ellinger (1957) reported the arrest of cell division, followed by the disappearance of various tubular elements in the following order : spermatogonia, spermatocytes, spermatid and spermatozoa.

Craig *et al.* (1961) reported that, of the 3 different doses of X-ray (200 r, 300 r and 500 r) to which mice and rats were exposed, the dose 300 r produced a period of complete sterility in both mice and rats. Hopkinson *et al.* (1978) reported the progressive reduction of total tubular area due to shrinkage of cytoplasm,

spermatocyte and spermatid nuclear area due to X-irradiation at 300 r.

Abbott (1959) concluded that the endocrine functions of the testis remain unchanged after radiation. Fogg and Cowing (1951) cited evidence proposed by other workers for an altered pituitary-testis axis. Some workers have shown that endogenic substances or pituitary gonadotrophins have a radioprotective action on the testis (Johnson and Witschi, 1963; Binhammer, 1967). Gunn *et al.* (1960; 1961), have since shown that androgen secretion by the testis is altered after radiation. The steroid biotransformations can be altered by irradiation and this was first shown by Rosenfeld *et al.* (1955) and Berliner *et al.* (1962), for the adrenal glands. Testicular steroid biotransformations were shown to be altered by various modes of X-irradiation by Shikita and Tamaoki (1965) and Simpson and Ellis (1967).

The adverse effect of X-rays on the testicular tissues has been well studied in many

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mammalian species. But there is no substantial information on the effects of X-ray on the testicular histochemistry and biochemistry of the wild rats to our knowledge. In order to fill this gap in information, the effect of X-rays on the testicular tissues of the wild Indian house rat (*Rattus rattus*) was studied.

## MATERIALS AND METHODS

In the present study 120 adult male wild Indian house rats (*Rattus rattus*) were used. Prior to the experiment, rats were collected from the surrounding fields of the Kalyani University and were maintained under normal laboratory conditions (L 12h: D 12h). To determine the effect of radiation on the testis, rats were exposed to four different doses of X-ray 100 r, 200 r, 300 r and 500 r. After exposure of X-ray, animals were maintained under laboratory conditions. Observations were made in four successive phases. Six rats from each group (control, 100 r, 200 r, 300 r, 500 r) were autopsied after 2 days (1<sup>st</sup> phase), 7 days (2<sup>nd</sup> phase), 15 days (3<sup>rd</sup> phase) and 30 days (4<sup>th</sup> phase).

For the quantitative estimation of various biochemical components eg. total cholesterol (Zarrow *et al.*, 1964), ascorbic acid (Nino and Prasad, 1980), acid and alkaline phosphatases (Walter and Schutt, 1974) within the testis of control and the four experimental groups, at least six treated rats were used. At autopsy, the testis was removed, weighed in a torsion balance, homogenised in different required media for the extraction of cholesterol, ascorbic acid, acid and alkaline phosphatase. After homogenisation they were centrifuged and the supernatants were used to estimate the biochemical components. The measurements were made at relevant wavelengths using a spectrophotometer (Spectronic 20 Genesys). After assessing for each biochemical component, the data were analysed using Student's 't' test.

The reference to the procedure used for cytochemical analysis is given against each of the constituents. For lipids (Kay and Whitehead, 1941),  $\Delta^5$ -3 $\beta$ -hydroxysteroid dehydrogenase (Wattenberg, 1958 modified by Bilaspuri and Guraya, 1984), 17 $\beta$ -hydroxysteroid dehydrogenase (Pearson and Grose, 1959 modified by Bilaspuri and Guraya, 1984), alkaline phosphatase Butcher and Chayan, 1966 and acid phosphatase (Bitensky, 1963). At the

end of cytochemical procedures, tissue sections were mounted in glycerine jelly and observations were made under light microscopy. Histochemical changes within the testis of control and experimental rats were carefully examined and their changes were recorded. Photomicrographs were taken from stained sections.

## RESULTS

### Biochemical components

Testicular extracts of control rats contained very low concentrations of ascorbic acid and cholesterol (Tables 1 and 2). The concentration of acid and alkaline phosphatases were very high (Tables 1 and 2). The different biochemical components in the testicular extract of 2 day post-irradiated rats are given in Table 1. The concentration of cholesterol had increased with an increasing in dosage from 100 r to 500 r. The concentration of ascorbic acid in the 100 r and 200 r treated rats has also increased compared to the control. The concentration of acid phosphatase in the 300 r and 500 r treated and the concentration of alkaline phosphatase in the 100 r, 200 r, 300 r and 500 r treated rats are quite low in the 2 day- post treated rats compared to the control (Table 1). In the 7 day- post treated rats the concentration of ascorbic acid in the 200 r, 300 r and 500 r X-ray irradiated groups and cholesterol in the 200 r, 300 r, 500 r groups had increased than the control rats. The concentration of ascorbic acid higher than in the 2 day-treated rats (Table 1). The concentration of acid phosphatase in the 300 r and 500 r groups and alkaline phosphatase in all four treated groups had decreased. The concentration of cholesterol in the 200 r, 300 r and 500 r treated groups in 15 day- post treated rats was very high compared to the control. The maximum concentration was in the 300 r group. In the 100 r, 300 r and 500 r groups the concentration of ascorbic acid in the testicular extract was higher (Table 2) but the concentration of alkaline phosphatase in the 100 r, 200 r, 300 r and 500 r groups and alkaline phosphatase in the 100 r, 200 r, 300 r and 500 r groups had decreased in comparison to the control. The concentration of alkaline phosphatase in the 500 r group was very low than in the control group. In 30 day- post treated rats, the concentration of ascorbic acid was closer to the control group. But the concentration of cholesterol in the 300 r and 500 r groups was higher than in the control group. The activity of

alkaline phosphatase in the 100 r, 200 r, 300 r and 500 r groups and acid phosphatase in the 500 r group had decreased in the 30 day - post

treated rats in comparison to the control (Table 2).

**Table 1. Effect of different dosages of radiation (X-ray) on biochemical components of testes 2 and 7 days after exposure of adult male *Rattus rattus*.**

Control and treatment Groups (No. exposed)	(mmol/100mg fresh testicular tissue)		(mg/100mg fresh testicular tissue)	
	Acid phosphatase	Alkaline phosphatase	Ascorbic acid	Cholesterol
Control (6)***	0.694 ± 0.054*	0.312 ± 0.013	0.018 ± 0.0009	0.529 ± 0.02
100r- 2 days (6)	0.770 ± 0.086	0.192 ± 0.014	0.019 ± 0.0004	0.720 ± 0.09
200r- 2 days (6)	0.669 ± 0.014	0.189 ± 0.024	0.022 ± 0.001	0.774 ± 0.02
300r- 2 days (6)	0.547 ± 0.033	0.169 ± 0.007	0.017 ± 0.0009	0.793 ± 0.07
500r- 2 days (6)	0.452 ± 0.036	0.236 ± 0.04	0.018 ± 0.0008	0.808 ± 0.03
F-Table 5%	2.76	2.76	2.76	2.76
F- Calculated	6.14	6.27	4.84	4.32
CD- value at 5%	0.12	0.06	0.002	0.17
	<u>T<sub>1</sub> &gt; C &gt; T<sub>2</sub> &gt; T<sub>3</sub> &gt; T<sub>5</sub>**</u>	<u>C &gt; T<sub>5</sub> &gt; T<sub>1</sub> &gt; T<sub>2</sub> &gt; T<sub>3</sub></u>	<u>T<sub>2</sub> &gt; T<sub>1</sub> &gt; C = T<sub>5</sub> &gt; T<sub>3</sub></u>	<u>T<sub>5</sub> &gt; T<sub>3</sub> &gt; T<sub>2</sub> &gt; T<sub>1</sub> &gt; C</u>
Control (6)	0.568 ± 0.016	0.246 ± 0.013	0.019 ± 0.001	0.670 ± 0.05
100r- 7 days (6)	0.591 ± 0.054	0.167 ± 0.01	0.018 ± 0.001	0.499 ± 0.03
200r- 7 days (6)	0.595 ± 0.047	0.165 ± 0.026	0.025 ± 0.002	0.749 ± 0.04
300r- 7 days (6)	0.490 ± 0.026	0.147 ± 0.013	0.028 ± 0.002	0.783 ± 0.04
500r- 7 days (6)	0.417 ± 0.013	0.123 ± 0.015	0.038 ± 0.009	0.783 ± 0.03
F-Table 5%	2.76	2.76	2.76	2.76
F- Calculated	4.73	7.85	3.60	10.29
CD- value at 5%	0.10	0.02	0.01	0.11
	<u>T<sub>2</sub> &gt; T<sub>1</sub> &gt; C &gt; T<sub>3</sub> &gt; T<sub>5</sub></u>	<u>C &gt; T<sub>1</sub> &gt; T<sub>2</sub> &gt; T<sub>3</sub> &gt; T<sub>5</sub></u>	<u>T<sub>5</sub> &gt; T<sub>3</sub> &gt; T<sub>2</sub> &gt; C &gt; T<sub>1</sub></u>	<u>T<sub>5</sub> = T<sub>3</sub> &gt; T<sub>2</sub> &gt; C &gt; T<sub>1</sub></u>

\*\* Underline indicates insignificant difference among the groups at 5% level

C, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>5</sub> are the mean value of control, 100r, 200r, 300r and 500r of X-ray irradiated groups respectively.

**Table 2. Effect of different dosages of radiation (X-ray) on biochemical components of testes 15 and 30 days after exposure of adult male *Rattus rattus*.**

Control and treatment Groups (No. exposed)	(mmol/100mg fresh testicular tissue)		(mg/100mg fresh testicular tissue)	
	Acid phosphatase	Alkaline phosphatase	Ascorbic acid	Cholesterol
Control (6)***	0.630 ± 0.036*	0.242 ± 0.002	0.019 ± 0.009	0.627 ± 0.05
100r- 15 days (6)	0.550 ± 0.077	0.122 ± 0.019	0.020 ± 0.001	0.575 ± 0.03
200r- 15 days (6)	0.493 ± 0.029	0.124 ± 0.005	0.019 ± 0.001	0.698 ± 0.04
300r- 15 days (6)	0.504 ± 0.027	0.119 ± 0.006	0.021 ± 0.0004	1.010 ± 0.41
500r- 15 days (6)	0.461 ± 0.02	0.111 ± 0.002	0.023 ± 0.002	0.701 ± 0.02
F-Table 5%	2.76	2.76	2.76	2.76
F- Calculated	2.31	36.67	1.65	18.13
CD- value at 5%	0.12	0.03	0.004	0.11
	<u>C&gt;T<sub>1</sub>&gt;T<sub>3</sub>&gt;T<sub>2</sub>&gt;T<sub>5</sub>**</u>	<u>C&gt;T<sub>2</sub>&gt;T<sub>1</sub>&gt;T<sub>3</sub>&gt;T<sub>5</sub></u>	<u>T<sub>5</sub>&gt;T<sub>3</sub>&gt;T<sub>1</sub>&gt;T<sub>2</sub>=C</u>	<u>T<sub>3</sub>&gt;T<sub>5</sub>&gt;T<sub>2</sub>&gt;C&gt;T<sub>1</sub></u>
Control (6)	0.599 ± 0.029	0.274 ± 0.019	0.018 ± 0.001	0.537 ± 0.04
100r- 30 days (6)	0.643 ± 0.052	0.177 ± 0.005	0.017 ± 0.0007	0.453 ± 0.03
200r- 30 days (6)	0.608 ± 0.034	0.152 ± 0.026	0.018 ± 0.001	0.595 ± 0.05
300r- 30 days (6)	0.538 ± 0.021	0.143 ± 0.006	0.021 ± 0.002	0.846 ± 0.06
500r- 30 days (6)	0.366 ± 0.044	0.130 ± 0.016	0.021 ± 0.0008	0.910 ± 0.15
F-Table 5%	2.76	2.76	2.76	2.76
F- Calculated	8.60	12.39	1.90	6.41
CD- value at 5%	0.11	0.05	0.004	0.24
	<u>T<sub>1</sub>&gt;T<sub>2</sub>&gt;C&gt;T<sub>3</sub>&gt;T<sub>5</sub></u>	<u>C&gt;T<sub>1</sub>&gt;T<sub>2</sub>&gt;T<sub>3</sub>&gt;T<sub>5</sub></u>	<u>T<sub>5</sub>=T<sub>3</sub>&gt;T<sub>2</sub>=C&gt;T<sub>1</sub></u>	<u>T<sub>5</sub>&gt;T<sub>3</sub>&gt;T<sub>2</sub>&gt;C&gt;T<sub>1</sub></u>

\*\* Underline indicates insignificant difference among the groups at 5% level

C, T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>5</sub> are the mean value of control, 100r, 200r, 300r and 500r of X-ray irradiated groups respectively.

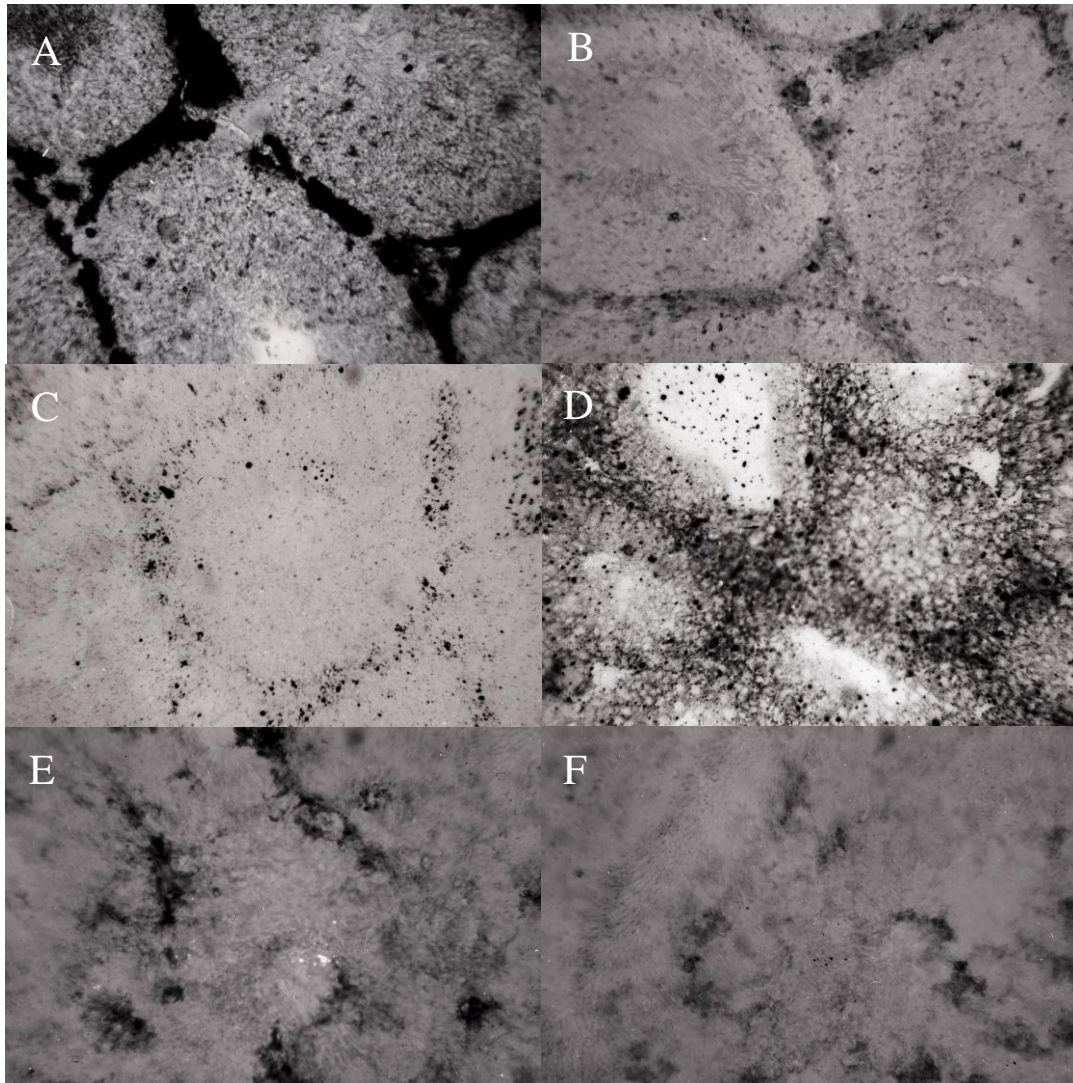
### Histochemical components

The control group contained lesser amount of sudanophilic lipids (Fig. 1C). The intensity of  $\Delta^5$ -3 $\beta$ -hydroxysteroid dehydrogenase, 17 $\beta$ -hydroxysteroid dehydrogenase, alkaline phosphatase (Fig. 1A) and acid (Fig. 1E) phosphatase were quite high (Table 3a, b, c, d). The histochemical distribution of different components in the testicular tissue of 2 day - post treated rats are presented in Table 3 a. Sudanophilic lipid granules were found with

higher intensities especially in the 300 r and 500 r irradiated rats than in control rats. The intensity of acid and alkaline phosphatases and 17 $\beta$ -HSDH of 300 r and 500 r groups had also decreased, but no remarkable change in  $\Delta^5$ -3 $\beta$  HSDH within the seminiferous tubules were found in the 2 day- post treated rats compared to the control. In comparison to the control rats, a significant depletion in the activity of acid and alkaline phosphatases,  $\Delta^5$ -3 $\beta$ -HSDH and 17 $\beta$ -HSDH were found in the 7 days post treated

groups (Table 3b). Sudanophilic lipid granules are found with higher intensities in the 300 r (Fig. 1D) and 500 r post irradiated groups of 7 day - post treated rats than in the control. Reduction in the intensity of  $\Delta^5$ -3 $\beta$ -HSDH, 17 $\beta$ -HSDH, acid (Fig. 1F) and alkaline (Fig. 1B) phosphatases in the 300 r and 500 r groups were found following 15 days of post irradiation in

comparison to the control, but the sudanophilic lipid granules regained towards normal levels (Table 3c). In 30 day post treated rats a decrease in the intensity of acid and alkaline phosphatases were observed in the 300 r and 500 r treated groups. But the activity of sudanophilic lipids,  $\Delta^5$ -3 $\beta$ -HSDH and 17 $\beta$  HSDH were closer towards the control level (Table 3d).



**Figure 1.** (A) Cryocut section of testis in control rats showing strong alkaline phosphatase activity within interstitial cells and basement membrane ( $\times 200$ ). (B) Very low alkaline phosphatase activity within interstitial cells and basement membrane of the seminiferous tubules in 300r X-irradiated rats after 15 days ( $\times 200$ ). (C) Section of testis of control rats showing the distribution of Sudanophilic lipids in interstitial cells, basement membrane and spermatogonial cells. ( $\times 200$ ). (D) Section of the testis of 300r X-irradiated rats 7 days after treatment showing increased lipid granules within seminiferous tubular region. Interstitial cells show similar lipid content as in control rats. ( $\times 200$ ). (E) Strong acid phosphatase activity within interstitial cells of testis in control rats ( $\times 200$ ). (F) Low acid phosphatase activity within interstitial cells of testicular sections in rats 15 days after 300r X-irradiation ( $\times 200$ ).

**Table 3 a. Histochemical changes in the testis of male *Rattus rattus* 2 days after treatment with different doses of X-rays.**

Tested for	Regions	Control	2 days -100r	2 days -200r	2 days -300r	2 days -500r
Sudan III & IV	i) Basement membrane	++	++	++	++	+++
	ii) Seminiferous tubule	+	+	+	++	++
	iii) Interstitium	++	++	++	++	++
Acid phosphatase	i) Basement membrane	++	++	++	++	++
	ii) Seminiferous tubule	±	±	±	±	±
	iii) Interstitium	++	++	++	++	++
Alkaline phosphatase	i) Basement membrane	+	+	±	±	+
	ii) Seminiferous tubule	+	+	±	±	+
	iii) Interstitium	+	+	+	+	+
17β-hydroxy steroid dehydrogenase	i) Basement membrane	++	++	+	+	+
	ii) Seminiferous tubule	++	++	+	+	+
	iii) Interstitium	++	++	++	++	++
Δ <sup>5</sup> -3β- hydroxy steroid dehydrogenase	i) Basement membrane	+++	++	++	+	+
	ii) Seminiferous tubule	+	+	+	±	±
	iii) Interstitium	+++	++	++	+	±

**Intensity of reaction:** + : Positive ; - : Negative ; ++ : Moderate ; +++ : Highly positive or intensified;  
± : Not significant

**Table 3 b. Histochemical changes in the testis of male *Rattus rattus* 7 days after treatment with different doses of X-rays.**

Methods	Regions	Control	7 days -100r	7 days -200r	7 days -300r	7 days -500r
Sudan III & IV	i) Basement membrane	++	++	++	++	+++
	ii) Seminiferous tubule	+	+	++	+++	++
	iii) Interstitium	++	++	++	++	+++
AAcid p phosphatases	i) Basement membrane	++	++	++	+	+
	ii) Seminiferous tubule	±	±	±	-	-
	iii) Interstitium	++	+	±	-	-
Alkaline phosphatase	i) Basement membrane	+	+	±	±	±
	ii) Seminiferous tubule	+	+	-	-	-
	iii) Interstitium	+	±	±	±	-
17β-hydroxy steroid dehydrogenase	i) Basement membrane	++	++	+	±	±
	ii) Seminiferous tubule	++	+	+	+	+
	iii) Interstitium	++	++	+	+	±
Δ <sup>5</sup> -3β- hydroxy steroid dehydrogenase	i) Basement membrane	+++	++	++	++	++
	ii) Seminiferous tubule	+	+	+	+	+
	iii) Interstitium	+++	++	++	++	+

**Intensity of reaction:** + : Positive ; - : Negative ; ++ : Moderate ; +++ : Highly positive or intensified;  
± : Not significant

**Table 3 c. Histochemical changes in the testis of male *Rattus rattus* 15 days after treatment with different doses of X-rays**

Methods	Regions	Control	15 days -100r	15 days -200r	15 days -300r	15 days -500r
Sudan III & IV	i) Basement membrane	++	++	++	+++	+++
	ii) Seminiferous tubule	+	+	+	+	++
	iii) Interstitium	++	++	++	+++	+++
Acid phosphatase	i) Basement membrane	++	++	++	±	±
	ii) Seminiferous tubule	±	±	+	-	-
	iii) Interstitium	++	++	+	±	-
Alkaline phosphatase	i) Basement membrane	+	+	+	+	+
	ii) Seminiferous tubule	+	+	+	±	±
	iii) Interstitium	+	+	+	-	-
17β-hydroxy steroid dehydrogenase	i) Basement membrane	++	++	+	±	±
	ii) Seminiferous tubule	++	+	+	±	±
	iii) Interstitium	++	++	+	+	+
Δ <sup>5</sup> -3β- hydroxy steroid dehydrogenase	i) Basement membrane	+++	++	++	+	+
	ii) Seminiferous tubule	+	+	+	+	+
	iii) Interstitium	+++	++	+	+	±

**Intensity of reaction:** + : Positive ; - : Negative ; ++ : Moderate ; +++ : Highly positive or intensified; ± : Not significant

**Table 3 d. Histochemical changes in the testis of male *Rattus rattus* 30 days after treatment with different doses of X-rays.**

Methods	Regions	Control	30 days -100r	30 days -200r	30 days -300r	30 days -500r
Sudan III & IV	i) Basement membrane	++	++	++	++	++
	ii) Seminiferous tubule	+	+	+	+	++
	iii) Interstitium	++	++	++	++	+
Acid phosphatase	i) Basement membrane	++	++	++	+	±
	ii) Seminiferous tubule	±	±	+	+	±
	iii) Interstitium	++	++	+	+	+
Alkaline phosphatase	i) Basement membrane	+	+	+	+	+
	ii) Seminiferous tubule	+	+	+	+	±
	iii) Interstitium	+	+	+	+	+
17β-hydroxy steroid dehydrogenase	i) Basement membrane	++	++	++	+	+
	ii) Seminiferous tubule	++	++	++	+	+
	iii) Interstitium	++	++	++	++	+
Δ <sup>5</sup> -3β- hydroxy steroid dehydrogenase	i) Basement membrane	+++	+++	+++	++	+
	ii) Seminiferous tubule	+	+	+	+	±
	iii) Interstitium	+++	+++	++	++	±

**Intensity of reaction:** + : Positive ; - : Negative ; ++ : Moderate ; +++ : Highly positive or intensified; ± : Not significant

## DISCUSSION

The present investigation demonstrates structural damages to the testis, of the adult wild male Indian house rat (*Rattus rattus*) after X-ray irradiation at doses of 100 r, 200 r, 300 r and 500 r. From the histochemical study, high amount of lipid materials were observed in X-ray treated groups. In the 300 r and 500 r groups 7 and 15 days of post treatment, the accumulation of lipid granules were maximum. Lipid accumulation in mammals within the testicular tissues, especially in the Sertoli cells due to local irradiation or after high dose of ionizing radiation has been described by Lacy *et al.* (1965); Collins and Lacy (1969). High amount of lipid granules in the Sertoli cells of mammals seem to be due to the phagocytosed lipid materials of the degenerating germ cells (Lacy, 1960). It is also known that the lipid inclusion in Sertoli cell is under the control of FSH secretion from the pituitary (Kerr and de Kretser, 1975). Serum FSH has been shown to be related to the germinal cell component, particularly spermatogonial numbers (de Kretser *et al.*, 1974). Irradiation and local heat application to the testis are known to disrupt spermatogenesis and cause an increase in the lipid inclusion in Sertoli cells of rat (Collins and Lacy, 1969) and to be associated with elevated levels of FSH, presumably resulting from a decrease in the feedback signal from the testis (Kerr and de Kretser, 1975).

Some alteration in the steroid dehydrogenase enzymes ( $\Delta^5$ -3 $\beta$ -HSDH and 17 $\beta$ -HSDH) has been observed in rats exposed to various doses of X-rays. But when the lipids and steroid enzymes are taken together, some inverse relationship has been observed in the testicular tissues due to X-ray treatment. The lower activity levels of the dehydrogenase enzymes within the interstitial cells of various X-ray treated rats seem to be due to the spermatogenetically inactive gonads. Although there are some controversy, most are of the opinion that in mammals, the Leydig cells are not affected by radiation, i.e., they are radioresistant (Ellis, 1970; Setchell, 1978). Both lipids and steroid dehydrogenases are usually known to show an inverse relationship. Higher content of lipids and lower activity levels of dehydrogenases are characteristic of steroidogenically inactive gonads.

Not only steroid enzymes but the activities of phosphatase enzymes were also found to

decrease in various X-ray treated groups of rats (both biochemically and histochemically). The alkaline phosphatase is said to be a histochemical marker for primordial germ cells of various species, including rat (Mc Alpine, 1955) and mouse (Mintz, 1957). It is known that alkaline phosphatase enzyme is required for the synthesis of glycogen, which in turn apparently participates in the metabolic process of spermatogenesis (Sohval, 1958). Mann (1964) reported an intense activity of acid phosphatase in the seminal plasma of several mammalian species including humans. Seminal and prostatic acid phosphatase have been associated with the nutrition of spermatozoa (Mann, 1964; Serrano *et al.*, 1976) with their fertilizing ability (Singer *et al.*, 1980). Szego (1972, 1974) reported that lysosomal acid phosphatase of the rat preputial gland is implicated with the mediation of steroids and hypophysial gonadotrophins.

It is also known in the present investigation that ascorbic acid in the testicular tissues increased due to X-ray irradiation. In mammals, ascorbic acid has been found to exert an inhibitory role on steroidogenesis (Kitabchi, 1967). Also ascorbic acid is a known catalyst for both lipid peroxidation and alteration of unsaturated fatty acid composition (Shimizu, 1970). Hence, the involvement of ascorbic acid in the process of steroidogenesis in the testis of control and treated groups may be taken into consideration. It is well established that cholesterol plays an important role in the inhibition or stimulation of sperm formation in the testis and is the primary substrate for androgen biosynthesis. Increased testicular cholesterol content after the X-ray irradiation is also suggested for reduction in steroid production (Mohanty and Chainy, 1988). Elevated testicular cholesterol level may be implicated with the altered lipid metabolism, which is frequently associated with testicular atrophy (Santra and Manna, 2002).

It has been suggested (Ellis and Berliner, 1969) that 17  $\beta$ -ol-dehydrogenase (oxidase) is located in both tubules and interstitial elements and diminishes progressively during the interval after irradiation. This phenomenon is directly related to the destructive changes associated with the depopulation of the seminiferous tubules. They also suggested the destruction of the germinal epithelium with direct or whole body irradiation, that affect both the reduction of androstenedione to testosterone and the oxidation of testosterone to androstenedione. Thus, a very dynamic relationship seems to exist

between androgen synthesis and spermatogenesis. Closely associated with the changes in androgen synthesis is the effect of radiation on DNA synthesis. Thus, it can be said that although Sertoli and Leydig cells are histologically insensitive they are "biochemically radiosensitive" (Ellis and Berliner, 1969).

The findings infer that X-ray irradiation causes significant damages to the testis. Considering the changes observed it can be stated that the single dose of X-ray i.e. 300r is sufficient for the suppression of spermatogenic activity at least for few days in the wild Indian house rat, *Rattus rattus*. It also suggests that exposure to a single dose of X-ray of 200r or 300r may affect the fertility of the rodent pests and thereby bring about their control.

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