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RESEARCH ARTICLE

Effect of gamma radiation on total testicular triglyceride of Swiss Albino Mice

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ABSTRACT

The observations on testicular triglyceride (TG) revealed that the value in control mice was 47.96 mg/dl. A decremental trend in the values after 0.05 Gy 0.15 Gy, 0.25 Gy of irradiation was seen and the corresponding values for these doses were 42.9 mg/dl, 31.8 mg/dl; 47.16 mg/dl, respectively. However, the activity of TG showed elevation in the testes which were irradiated by 0.1 Gy and 0.2 Gy i.e., they were 53.33 mg/dl and 51.4 mg/dl, respectively. The disturbance in TG activity may be due to inhibition of lipase enzyme and radio-sensitivity of sertoli cells in which lipase is known to concentrate and accumulate. The fertility of male mice is reported to be influence by the lipid concentration, the higher concentration of lipids reveal the lower fertility. This may also true for the present study since higher concentration of lipid was estimated in response of γ-radiation.

Key words: *ionized radiation, fertility, lipids.*

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-amplifies radioactive isotopes from the environmental, ionized radiation were the first to be recognized as environmental tetrogen to effect human. Therapeutic doses of gamma radiations administered to testes have significant effect upon the quality of life manifested by sexual and psychological problems .In extreme cases, although unusual, variety of morphological aberration may occur e.g., reduction in growth of facial hair, total loss of body hair, alteration in muscle mass and redistribution of body fat to a more feminine pattern and atrophy of testes. Radiation induced pathologies have been studied in several placental mammals [1, 2 and 3]. A significant decrease in body weight also occurs in rats exposed to infrared radiation [4]. However, it has been reported that γ-radiation does not affect body weight although a significant reduction was observed in the weight of the testes and other genital organs [5, 6 and 7].

Mammalian testes represent an intricate association of heterogenous cell population whose primary exocrine function is to produce spermatozoa; and endocrine function is to synthesize and release a variety of androgens. These functions are distinctly compartmentalized *i.e.*, spermatogenesis in the semineferous tubules and androgenesis in the leydig cells. A sustained generation of precursors via enzymatic intervention occurs in both processes. In leydig cells cholesterol is used for biosynthesis of wide array of androgens which initiate, sustain, and maintain sexual libido and behavior. While in the later cholesterol is used for biosynthesis of wide array of androgens which initiate sustain and maintain sexual "libido", and behavior. The metabolic status of germ cells, endocrine cells (Leydig), and somatic cells (Sertoli cells) of the testes is known to undergo cyclic changes that coincide with the cycle of the semineferous epithelium and hormone production.

MATERIALS AND METHODS

Test animal: Sexually mature Swiss albino mice weighted 18±2 gm was used as a "model" for the present study to investigate the effect of various doses of gamma radiations on the testes. The mice are maintained on standard rodent chow *adlibitum* access to clean sterilized water. They were kept in mice cages at 26±2°C in 12 h dark 12 h day light.
Group 1: served as control, and were sham irradiated.
Group 2: were irradiated by 0.1Gy of gamma radiations
Group 3: were irradiated by 0.20Gy of gamma radiations
Group 4: were irradiated by 0.30Gy of gamma radiations All experimental groups and control group sacrificed after 24 h after giving single dose of irradiation.
These experiments were repeated twice.

Procedure of radiation: The animals were restrained in position by tying rubber bands around the forelimb and hind limbs. They were exposed to single pulse of various doses of gamma radiation by Cobalt-60 camera. Radiation was applied to the abdominal region where the paired testes were located.

Surgical process and preparation of Testicular homogenate:
Mice of control and experimental groups 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 of fascia and blood clots; rinsed several times in chilled physiologic saline (4 deg). After blotting the tissue the wet weight of each testes were 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 centrifuged at 3000 rpm for 20 min to obtain the sub cellular fraction. The supernatant was decanted and utilized for biochemical assay of triglyceride as per procedure detailed below.
Triglyceride was quantitated biochemically in the testes of control and radiated mice by ENZOKIT GPO-PAP of Bucolo and David [8].

Triglycerides (TG): TG was quantitated biochemically in the testes of control and radiated mice by ENZOKIT GPO-PAP method of Bucolo and David [8].

Principle: Triglycerides (TG) are hydrolysed to glycerol and free fatty acids by lipase enzyme. In the presence of ATP and glycerokinase the glycerol is converted to Glycerol-3-phosphate. The glycerol-3-phosphate is then oxidized by glycerol-3-phosphate oxidase to yield hydrogen peroxide. Hydrogen peroxide reacts in the presence of peroxidase with ESPAS (N-ethyl-N-suphopropyl-m-anisidine) and 4-aminoantipyrine to form a coloured complex. The intensity of the colour developed is proportional to triglycerides concentration and is measured spectrophotometrically at 546 nm (range 530 to 570 nm).

(a) Triglycerides + H_2O $\xrightarrow{\textit{Lipoprotein lipase}}$ Glycerol fatty acids

(b) Glycerol + ATP $\xrightarrow{\textit{Glycerokinase}}$ Glyerol-3-phosphate + ADP

(c) Glycerol-3-Phosphate + O_2 $\xrightarrow{\textit{Glycerol–Phosphate oxide}}$ H2O + Dihydroxy acetone phosphate

(d) H2O + Aminoantipyrine + ESPAS Pyrine $\xrightarrow{\textit{Peroxidase}}$ Quinoneimine + H2O (pink colour complex)

Procedure:
(i) Working solution: The contents of one bottle of enzyme (Lipoprotein lipase, glycerokinase, glycerol 3-phosphate oxidase, per oxidase, 4-amino-antipyrine and ATP) with the contents of

one bottle of buffer. (Pipes buffer and ESPAS) were dissolved and mixed thoroughly. This served as the chromogen reagent.

(ii) Sets of three test tubes were prepared and designated as 'Test' (T), 'Standard' (S), 'Blank' (B),

(iii) 0.01 ml testes homogenate was added to 'T'

(iv) Standard solution 0.01 ml was added into 'S'

(v) Chromogen reagent (1.0 ml) was added in all the three test tubes 'T', 'S' and 'B'

(vi) The test tubes were vortexed and incubated for 5 min, at 37°C. The absorbency of test (AT), standard (AS), blank (AB), or against distilled water was read at 546 nm.

$$TG = \frac{AT - AB}{AS - AB} X\ 200\ mg/dl.$$

RESULTS AND OBSERVATIONS

Triglycerides (TG): In the homogenate of the testes of Swiss albino mice challenged by various doses of γ-radiation, the values of TG showed fluctuations. The TG value in control mice was 47.96 mg/dl. A decremental trend in the values after 0.05 Gy 0.15 Gy, 0.25 Gy of irradiation was seen and the corresponding values for these doses were 42.9 mg/dl, 31.8 mg/dl; 47.16 mg/dl, respectively, same decreamental trend observe in weight of the testes (Table 1 & 2). However, the activity of TG showed elevation in the testes which were irradiated by 0.1 Gy and 0.2 Gy *i.e.*, they were 53.33 mg/dl and 51.4 mg/dl, respectively but decline observed in weight of the testes on same doses (Table 1 & 2).

Table 1: Changes in genadosomatic index of sexually mature adults of Swiss albino mice challenged by various doses of γ-radiation vis-a-vis control

S. No.	Doses (Gy)	Weight of mice(gm)	Weight of testes(gm)
1.	Control	17.240 ± 0.431	0.123 ± 0.002
2.	0.05	17.160 ± 0.739	0.120 ± 0.002
3.	0.10	18.140 ± 0.624	0.065 ± 0.002
4.	0.15	17.780 ± 0.574	0.098 ± 0.001
5.	0.20	17.722 ± 0.691	0.050 ± 0.001
6.	0.25	18.800 ± 0.533	0.065 ± 0.002

Values are mean ± SE, $p \leq 0.001$

Table 2: Quantitative profile of triglyceride (mg/dl) in testis of Swiss albino mice challenged by various doses of γ-radiation

S. No.	Doses (Gy)	Total T.G.(mg/dl)
1.	Control	47.967±0.549
2.	0.05	42.900±0.520
3.	0.10	53.333±0.296
4.	0.15	31.800±0.351
5.	0.20	51.400±0.702
6.	0.25	47.167±0.296

Values are mean ± SE, $p \leq 0.001$

DISCUSSION

Triglyceride are a major component of depot of 'storage' lipids in animal cells. The lipids may be altered by abnormal conditions such as γ-radiation.

Testicular TG in control and various experimental groups showed differential values. In the groups of mice exposed to 0.05 Gy, 0.15 Gy and 0.25 Gy of γ-radiation showed 10.55%, 7.17% and 1.66%

decrement as compared to control. The decremental effect of 0.25 Gy was considered to be negligible. The groups of mice challenged by 0.1 Gy, 0.20 Gy showed percentile increment which were 11.19%, 7.17% vis-a-vis control (100%). A comparison of the result of the present studies with others shows several interesting parallels but also some sharp differences.

Thus increased activity of triglyceride as observed in the present studies agrees with the findings of Savina et al. [5], Nehru et al. [9], El-Ghazaly and Ramadan [10] and Abdel-Magied and Ahmed [11] observed this increment after exposure of testes and reported increment in body weight and lipids.

Free radical impairs liver functions and can be a major reason of hormonal imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of cholesterol and triglycerides [12] radiation causes depletion of spermatogonia cells and their subsequent generations, thereby negatively impact on weight of testes [13, 14 and 15]. Moreover, in the body, the activity of triglyceride and V_E are inversely proportional to each other. On the other hand, Umegaki et al. [16] suggested that neither increase of V_E nor its decrease was observed in the testes after irradiation by a dose of 6.0 Gy. In addition, Johnson [17] reported that in rats with lowered fertility the level of total lipid tended to be higher than in once with higher fertility. Judy [18] studied the sperm from the testes containing higher lipid levels. There fusion with ovum appeared to bring about higher rate of embryo death.

Lipids are known to be concentrated in the Sertoli cells which are highly radiosensitive [19]. The enzyme lipase is also affected by radiation which altered its activity. Inhibition of lipase increases the level of lipids and vice-versa.

Total testicular triglyceride in the irradiated mice showed the following pattern of abundance

Dose (Gy)	→ Control	: 0.05	: 0.10	: 0.15	:0.20	: 0.25
TG (mg/dl)	→ 47.96	: 42.9	: 53.33	: 31.8	:51.4	: 47.16
% change	→ 10.55(d)	: 11.19(I)	: 33.69(d)	: 7.17 (I)	: 1.66 (d)	

This change may be due to radiosensitivity of Sertoli cell which may accumulate triglyceride or disturbances in the level of lipase may result in this. Further higher level of lipid may lower fertility.

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