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**Original Research Article**

# Prophylactic role of soya oil against radiations damage on reproductive performance of male Albino rat

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## Abstract

Soyabeen (*Glycine max*) oil is widely consumed as edible food in many countries of the world. Its versatility allows them to be incorporated into dietary substances. The radioprotective efficacy of soya oil against gamma irradiation was studied in Swiss albino rats. Rats were administered soya oil orally once a day for 15 consecutive days then exposed to the dose of 5 Gy of gamma radiations. The gamma radiations induced DNA strands break in spermatogonia, depleted in seminiferous tubules as well as other abnormalities in sperm motility, sperm count, sperm viability, and longevity, total antioxidant capacity (TAC), LH, FSH and testosterone hormones in testis. These abnormalities were markedly prevented by soya oil pretreatment. Soya oil pretreatment inhibited apoptotic signaling proteins, improving germinal epithelium deterioration believed to be injured by gamma irradiations. This produced a dose reduction factor for soya oil. The protective role of soya oil against gamma radiations may be attributed due to its constituent, the isoflavone. Isoflavone plays a significant role in scavenging of free radicals. The study highlights the gonado-protective role of soya oil against radiation induced degenerative changes in testes of swiss albino rat.

**Keywords:** Soya oil, Reproductive performance, Prophylaxis, Radiation damage, Albino rat, testicular histology.

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## 1. Introduction

Recently, researchers have received an increasing focus on food containing bioactive components due to their functionality in disease prevention and treatment. Soya oil is derived from the dried ripen seeds of soyabeen (*Glycine max*). Isoflavone is the principle constituent substance of Soya oil having antioxidant properties. Isoflavone occur in soybean almost as glycosides. Researchers took it as a challenging task of the results of in-vitro animal studies for the sake of well being of human. However, the relevance of these types of research is of doubtful to understanding the effects of soya foods in human health due to many physiological and anatomical differences between humans and rodents. In the case of soya, there is an additional advantage to the non-human primates and rodent animals that they can metabolize isoflavone more efficiently than human. Evidences claimed that isoflavone content has a number of health benefits in human being. It alleviates the level of important omega-3 fatty acid. With improving skin health in menopausal women and reduces the risk of prostate and breast cancer. It is well established fact that soya food is consumed by most of the people in non-Asian countries except those who are allergic to soya protein. Furthermore, the biological impact of one food constituent can be affected by the presence of others [1].

Gamma rays are the electromagnetic radiations occasionally accompanying the emission of alpha and beta particles. Exposure of such radiations can cause cellular damage, chromosomal aberration, mutation and other abnormalities in the different organs of living organisms depending upon the total amount of energy, duration of exposure and intensity of radiations. Ionizing radiations can impair spermatogenesis and can cause mutations in germinal epithelium in male gonads. Generally dividing spermatogonia are more sensitive to such type of radiations. The main objective of this study was to discuss the prophylactic role of soya oil against radiations effects on reproductive performance of male Swiss Albino Rat.

## 2. Materials and Methods

### 2.1 Animal selection:

A colony of 50 Adult (6-8 week old) Swiss albino rat (*Rattus norvegicus*) of body weight 150–200 gm have been selected from Animal Care Centre, Department of Zoology, University of Rajasthan, Jaipur. The colony took care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals, prepared by the Academy of Sciences, 1996. Rats were kept in a cycle of 12-hour light and 12 hours darkness, at a temperature of 20°C ± 2°C with relative humidity 50% ± 20%, and ventilated with filtered non-recycled air. They were fed on standard chow pellets which were purchased from Jatia bajar, Sikar and tap water for the entire test period. The experimental procedure was approved by the local ethics committee, of Pandit Deendayal Upadhyay Shekhawati University, Sikar (Rajasthan). Most of the experiments were designed and performed in the Department of Zoology of S. K. Govt. Girls College, Sikar and some of them like irradiation facilities were carried out in radiotherapy department, S. K. Medical College and Hospital, Sikar, India.

### 2.2 Gamma-irradiation:

Animals were exposed to 5 Gy whole body γ-irradiations (dose rate 1 Gy/minute) for 8 hours using a $^{60}$Co teletherapy unit. The dose rate was calibrated by radiation safety team of the Institute as a part of routine calibration requirement in accordance to the Atomic Energy Regulatory Agency, India (Figure-2). This irradiation work was carried out in Radiotherapy Department, S. K. Medical College and Hospital, Sikar, India.

### 2.3 Experimental Design

To evaluate the adverse effects of gamma rays and the possible radioprotective efficacy of soya oil, 50 male Swiss albino rats were selected from an inbreed colony and randomly divided into following three groups.

**Group I (Control group):**

Rat ($n$ = 16) of this group were neither received soya oil nor be irradiated for 30 consecutive days.

**Group II (γ-irradiated group):**

Rats ($n$ = 17) of this group of testes region exposed to 5 Gy intermittent dose for 8 hours of gamma radiations. This group served as irradiated control group.

**Group III (Soya oil treated+ γ-irradiated):**

Rats ($n$ = 17) of this group were given soya oil, and after 30 min of the last dose administration such animals were exposed to 5 Gy gamma radiations for 8 hours duration. This served as experimental group.

Rats of all three were under regular and thorough observation for 30 days for visible abnormalities and mortality. After animal experiments, the tissue sections were prepared taking under consideration of different histological and histomorphological parameters of seminiferous tubules and the biological characteristics of Leydig`s cells. They were evaluated applying quantitative assessment of Johnson scoring method (Table-1).

### 2.4 Histo-morphological examination of mouse sperm

Animals were scarified on 3$^{rd}$, 10$^{th}$, 15$^{th}$, 25$^{th}$ and 30$^{th}$ days of post-irradiation. Testes were immersed in Bouin's fixative, harvested cauda epididymis, embedded in parafin blocks, sectioned at 5 mm and stained with hematoxylin/eosin. Johnson's score was used to evaluate spermatic activity. A drop of aliquot was transferred to heamocytometer which was prepared with nylon mess strainer and observed under bifocal compound microscope to ensure deformities and architecture in the size and shape of testicular cells. Histological examinations of both peripheral and central seminiferous tubules were done in the testes of each group at every interval with the help of a bifocal microscope.

### 2.5 Evaluation of sperm count

The epididymal sperm count was evaluated using the method of Yarube *et al* [2]. Briefly, the right caudal epididymis of each rat was carefully removed, washed with phosphate buffer, dried with blotting paper and immediately homogenized in 1 ml of 0.5% formal saline. 1 ml of the aliquot was 200 times diluted using RBC diluting pipette. Counting was done using new improved Neubaur`s counting chamber (made in Germany) and the cells were counted by using a compound microscope at the magnification of x400.

### 2.6 Sperm motility

To assess the effect of irradiation on sperm motility after 2h, 4h and 8h post-irradiation, 10-15 µL single cell suspensions (1 × 10$^6$ cells/ml) were transferred to haemocytometer and observed under compound microscope. All sperms were observed individually under microscope with 400X magnification and considered as motile if they had shown any movement. Each sample was counted at least two times. Results presented in percentage sperm motility index was determined by dividing number of motile sperms with sum of total number of sperms.

$$\text{Sperm mortality} = \frac{\text{Motile sperms}}{\text{Sum total number of spems}} \times 100$$

### 2.7 Sperm viability test

To evaluate sperm viability, 0.1 ml of sperm suspension (1×0$^6$ cells/ml) was mixed with 2% eosin stain. After 3 or 4 minutes both stained and unstained were counted using Neubour`s heamocytometer with inverted microscope. Slide was examined at least 2 times or 3 times for stained and unstained cells and presented the proportion eosin positive and eosin negative sperms. Unstained sperms were eosin negative and were found viable.

### 2.8 Total antioxidant capacity (TAC) in testes

Rats of experimental group III were killed; testes were carefully removed, washed dried with blotting paper. Testes homogenate was prepared in pre chilled PBS by

tissue homogenizer and centrifuge with 10,000 g at 4°C for 25 min. supernatant was stored immediately and further analyzed. By using spectrophotometer, total antioxidant capacity of testes was determined.

**2.9 Evaluation of hormones**

The FSH and LH and testosterone concentrations were evaluated using commercial kits (Microwell Enzyme linked immunosorbent assay kit)

**2.10 Statistical analysis:**

ANOVA (Analysis of Variance) was applied to compare the average of the values within each group of irradiations. The p-values are two-sided at a significance level of $\leq 0.05$.

## 3. Results and discussion

The radioprotective ability of photochemical may offer an insight into the alteration of germ cells of testis radio-sensitivity. The optical photomicrographs of the cross-sections for the control group indicated the integrity of the tubules and interstitial tissue. The seminiferous tubules were completely intact and clearly observed in control or normal group (Figure-1). While in experimental group, all sperm cell lines were seen and the basal membrane of the seminiferous tubules as well as the spermatogonia lines on it could be clearly observed. The Leydig`s cells in the interstitial tissue were observed with acidophilic cytoplasm. The result of II irradiated group showed that the testes significantly reduced in all histological parameters of cauda epididymis e.g. frequency of spermatozoa, spermatids, spermatogonia, spermatocytes etc. This may be due to damage in the testicular tissue by irradiations.

Irradiated animals exhibited sickness within 2-3 days with significant mortality rate during 7 days of exposure and remaining animals died within next 10 days after exposure which might be occur due to haematopoitic syndrome [4,5]. According to Cordelli *et al* [6] radiations cause morphological changes in the testis histological architecture mainly due to the killing of spermatogenetic cells. Whole body or partial body exposure has potential threat for germ cells population, but literature on radioprotection by soya oil in reproductive organ is scanty and, therefore, required more attention for investigation its role in reproductive system. Meistrich (1993) Studied that whole-body radiation exposure can cause reversible or permanent damages in male reproductive system [7]. Testis is one of most radiosensitive reproductive organs, sensitive to radiation dose as low as 0.1 Gy, because of highly proliferating spermatogonial cells [8, 9]. Significant decrease in sperm count and morphological abnormalities has been reported at radiation doses as low as 1-2 Gy in rats. Testes possesses germ cells at different stages of development, a process known as spermatogenesis, and developing sperms are very sensitive to ionizing radiation, known to affect morphology, function and ultimately the spermatogenesis [10, 11]. This may have apparently leads to acceleration of germ cell apoptosis, resulting in a decline in sperm count, and altered gonadal integrity and function [12]. Thus, the sertoli cells were considered as reference standard cells, only if they had nucleolus in them. The total number of spermatogonia was divided by total number of sertoli cells and expressed as ratio of spermatogonia/sertoli cells. The results showed that more number of spermatogonia per sertoli cell were present in pre-radiated rats ($p < 0.001$) on 7$^{th}$ day following irradiation.

On the other hand, soya treated and irradiated rats (Group-III) results in a significant decrease in the number of spermatogonia per sertoli cell as observed on 3rd day (Figure 3). The present results suggest that a single prophylactic dose of soya oil recover spermatogenic cells in irradiated testes of rats as a function of post-irradiation days. Soya oil treatment alone did not show any change in the histological architecture and spermatogenic population of testes till 30$^{th}$ day of observation. Spermatogenesis is affected after radiation as both Leydig and Sertoli cells die during cell division. Radiation doses required to kill spermatocyte is higher than spermatogonia, eventually lead to disappearance of spermatids, spermatogonia and spermatocyte (Figure-2). Scientists reported that mice exposed to high doses of gamma radiation, the presence of abnormal sperm in the epididymis of mice increased. In table-2, the average value of different histological parameters of testes show significant decrease in second group of gamma radiations exposure alone in comparison to control group ($p = 0.0095$). While a remarkable increase for Johnson score were found in group-III. Similar results were found for the other parameters too i.e. permatozoa, spermatids, spermatocytes and spermatogonia.

These findings were supported by Lynn *et al* and Agarwal *et al*[13,14] who resulted the active components of soya oil are well known to suppress radiation induced lipid peroxidation and reduce the radiation induced cellular damage. Gamma radiations affect both Ledig`s and Sertoli`s cells during spermatogenetic cell divisions in the testis. Radiation doses required to kill spermatocyte is higher than spermatogonia, eventually lead to disappearance of spermatids, spermatogonia and sperm deformities like hook less, folded, amorphous type, banana like, short tailed, two tailed, and two headed sperms (Figure-4). Radiation exposure carried out radiolysis of water which in turn generate free radicals in the cells. These free radicals are reactive oxygen species, highly damaging for all biomolecules, DNA, proteins and lipids in cells. The biological manifestations are in the form of radiation-induced injuries in various organs with increasing radiation doses [15]. Antioxidant properties of soya oil are well documented and understood at both molecular and cellular levels [16–18]. Irradiations (2Gy) to the testes significantly

affected the frequency of spermatogonia, primary spermatocytes, spermatids, spermatozoa, seminiferous tubules, lumen diameter, thickness of epithelium, Ledig`s cell nucleus diameter, volume of epithelium height and apoptotic cells, while administration of 200 mg/kg silimarin dose improved all the above parameters [19]. FSH and LH concentrations in the radiation exposed group were relatively lower compared to that recorded in Soya oil (6%). There was a significant decrease in the serum testosterone concentration in the radiation exposed group ($P<0.01$). Significant decreases in the levels of serum sex hormones are known to be associated with suppressed reproductive functions. Exposure to several radiation agents has been reported to cause reproductive dysfunctions in different subjects [20]. Recovery and re-population will depend on proliferation of the surviving stem cell that is spermatogonia and ultimately the germinal epithelium with germ cells [21, 220].

**Table-1: Johnson`s scoring method**

| Seminiferous tubules description | Levels |
|---|---|
| Full spermatogenesis, a large number of sperm heads are located on the sidelines of a round and regular lumen and perfect tubules | 10 |
| Although there are a large number of sperms, no round and regular lumen can be seen. Many spermatozoa present but disorganized spermatogenesis | 9 |
| Sperm count is very low | 8 |
| No spermatozoa but a large number of round spermatids are visible | 7 |
| A few round spermatids can be seen | 6 |
| No spermatozoa and spermatids present but there is a large number of primary spermatocytes. | 5 |
| Only a few spermatocytes present | 4 |
| There are no primary spermatocytes. Only spermatogonia are observed | 3 |
| There are no germ cells, only Sertoli cells can be seen. | 2 |
| Neither germ cells nor Sertoli cells are observed and the tubules are atrophic. | 1 |

**Table 2: Histological parameters of seminiferous tubules**

| Groups→ | Control group | γ-irradiation group | S/O fed + γ-irradiate group |
|---|---|---|---|
| **Johnson`s classification** | 9.76 ± 0.25 | 3.36 ± 0.32 | 6.24 ± 0.12 |
| **Number of spermatogonia** | 3.55 ± 0.22 | 1.25 ± 0.23 | 0.02 ± 0.25 |
| **Number of primary spermatocyte** | 65.71 ± 0.12 | 34.83 ± 0.25 | 42.21 ± 0.23 |
| **Number of round spermatid** | 122.23 ± 0.21 | 70.41 ± 0.53 | 84.32 ± 0.24 |
| **Number of spermatozoa** | 128.12 ± 0.45 | 87.66 ± 0.34 | 92.12 ± 1.22 |

Significant difference compared to the control group the group 1 ($p < 0.05$)

The sperm morphology is important for direct assessment of sperm quality. Therefore, we have performed morphological evaluation of spermatozoa and analyzed.

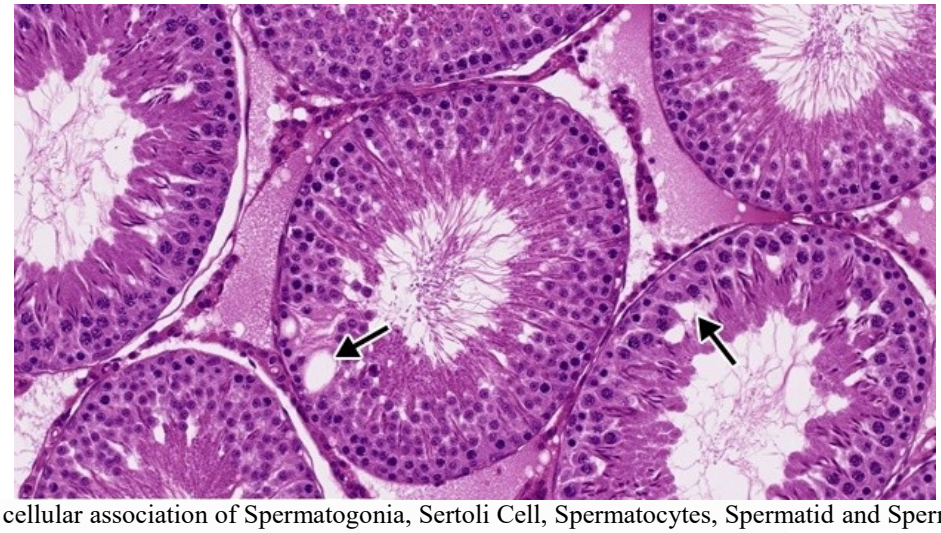

Figure 1: Normal cellular association of Spermatogonia, Sertoli Cell, Spermatocytes, Spermatid and Spermatozoa) in stages of development were observed in intact seminiferous tubules of the testes of control group. (Source: confocal microscopy)

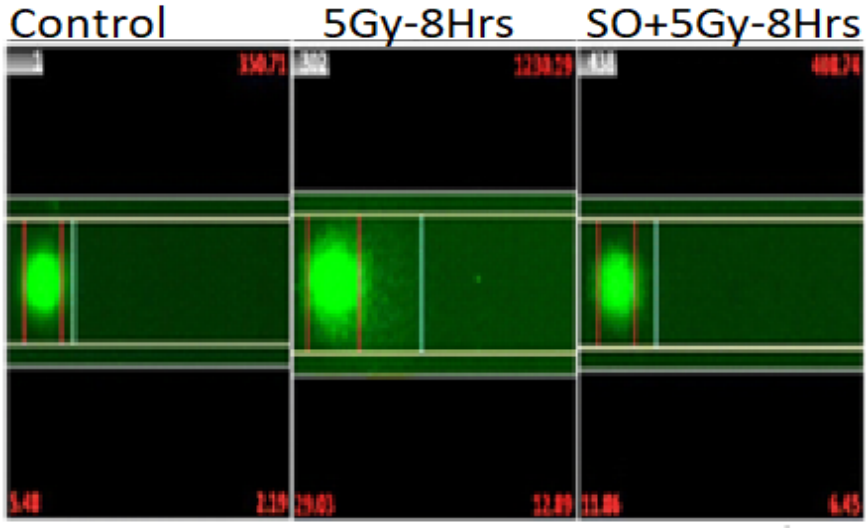


**Figure-2: Gamma irradiations (Source:High power digital camera)**

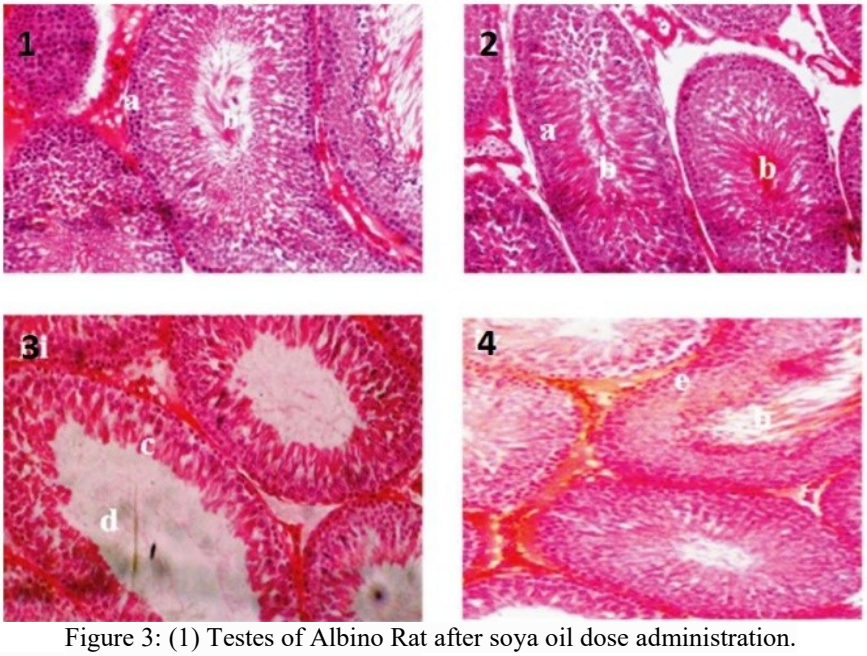


Figure 3: (1) Testes of Albino Rat after soya oil dose administration.
(2) Short term exposure of gamma rays on testis (spermatozoa in the lumen of seminiferous tubules).
(3) Long term exposure of gamma irradiated testes showing degenerative seminiferous tubules
(4) Gamma irradiated with soya oil treated recovering damages of spermatogonia.
**Source:** (confocal microscopy)

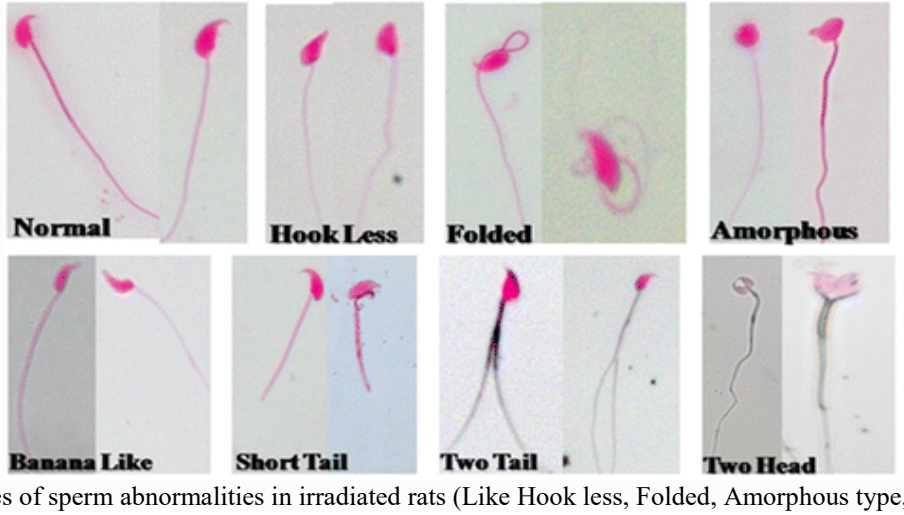


Figure-4 Seven types of sperm abnormalities in irradiated rats (Like Hook less, Folded, Amorphous type, Banana like, short tailed, Two tailed, and two headed sperms). (Source: Confocal microscopy)

## 4. Conclusion

This study highlights the gonadoprotective role of soya oil improving germinal epithelium deterioration believed to be caused by gamma irradiations. Radiation-induced spermatogenic cells depletion in seminiferous tubules as well as sperm abnormalities, motility and viability in testes were markedly prevented by soya oil pre-treatment. These results will be useful for understanding the radioprotective potential of soya oil in male reproductive system and, can be exploited for its use in cancer radiotherapy patients undergoing hemibody and abdominopelvic region radiation exposure.

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