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HISTOLOGICAL ALTERATIONS IN THE TESTES OF GAMMA-IRRADIATED MICE INDUCED BY *ADHATODA VASICA* LEAF EXTRACT

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Keywords:

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ABSTRACT: Extract of *Adhatoda vasica* leaves has been used to treat various diseases and disorders in Ayurvedic and Unani medicine. Effect of ethanolic extract of *Adhatoda vasica* against gamma radiation-induced changes in terms of histological alterations in testes of Swiss albino mice were studied at post-irradiation intervals between 1-30 days. Mice exposed to 6 Gy gamma radiation showed radiation-induced sickness, including changes in the histology of testes. When ethanolic leaf extract of *Adhatoda vasica* was given orally at a dose of 900 mg/kg, body weight prior to irradiation showed significant protection. Leaf extract of *Adhatoda vasica* was also reported for the protective role in the spleen of Swiss albino mice exposed to 6 Gy γ-radiation. Mice were divided into four groups *i.e.*, group (i) containing normal mice served as a control for each experimental stage; group (ii) mice given 900 mg/kg body wt. of *Adhatoda vasica* extract orally for 15 days; group (iii) mice exposed to gamma radiation (6 Gy) and group (iv) mice given *Adhatoda vasica* extract orally for 15 days and then exposed to gamma radiation (6 Gy). There was a significantly lesser degree of alterations to extract treated plus irradiated mice testes. However, there was a higher degree of alterations in irradiated testes architecture.

INTRODUCTION: It is well-known that the demand for herbal drug treatment of various ailments is increasing and plant drugs from the ayurvedic system are being explored more in India and globally. The leaves of the plant are the main source of drug preparation. Leaf extract of *Adhatoda vasica* was also reported for the protective role in the spleen of Swiss albino mice exposed to 6 Gy γ-radiation [1]. The radioprotective efficacy of a hydroalcoholic extract of *Pterocarpus santalinus* was reported in Balb-C mice exposed to γ-radiation [2]. Extracts of various plants have been reported beneficial for free radical-mediated conditions in humans, as they contain compounds with antioxidant activity that can prevent damage induced by reactive oxygen species.

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Many more medicinally useful plants are present in the Himalayas. Among them is *Adhatoda vasica* used in the present study; it belongs to the family Acanthaceae and is found throughout India up to 1300 m. The plant has been used in the indigenous system of medicine worldwide as an herbal remedy for treating cold, cough, chronic bronchitis, asthma, sedative expectorant, rheumatism, and rheumatic painful inflammatory swellings. Plant extracts contain phytochemical constituents for miscellaneous medicinal activities, which are bioactive in nature [3].

Polyphenols from medicinal plants, such as were found to decrease irradiation-induced oxidative stress in normal lymphocytes using ROS mechanisms, acting as a radiation modifier for normal cells [4]. Medicinal plants have played an important role in pharmacology and medicine for many years.

Today, it is estimated that about 80% of the world relies on botanical preparations as medicine to meet their health needs [5]. Plants provide complicated, mixed, and distinct non-nutrient elements which act as the main basis of drug discovery [6].

Radio protectors and radiomitigators are important modulators, and their delivery occurs simultaneously with the radiation administration to reduce normal tissue toxicity [7]. Exposure to whole-body irradiation may induce cancer by a number of different mechanisms.

Free radicals have been directly associated with biological effects of ionizing radiation such as lethality, physiological disorders, mutation, and carcinogenesis. Life on Earth has evolved through continuous exposure to ionizing radiation, whose mode of action at the bio-molecular level is unique among all known mutagens and carcinogenic agents [8].

Ionizing radiation affects somatic and germ cells, leading to mutation, cell death, malformation and cancer. Interaction of ionizing radiation with living cells causes a variety of changes, whose damage intensity depends fundamentally on the absorbed dose, type of radiation, conditions of irradiation, and intrinsic radiosensitivity of cell [9].

The testes are situated outside the abdominal cavity within a pouch called the scrotum. The scrotum helps maintain the testes' low temperature necessary for spermatogenesis. Testes are the main reproductive organ in males and are responsible for sperm production. Ionizing radiation is found to produce marked effects on testes in terms of lethality and impaired spermatogenesis. Testes are known to be reduced in size and weight after radiation exposure [10, 11, 12, 13].

MATERIALS AND METHODS: Swiss albino mice of Balb-C strain weighing 22-25g were procured from Central Research Institute (CRI) Kasauli, Himachal Pradesh, India. These were maintained in the animal house of the Department of Biosciences of Himachal Pradesh University, Shimla, under proper hygienic conditions (24+2 C temp. and light).

Mice were provided Hindustan lever feed and water *ad libitum*. The Institutional animal ethics committee approved the entire animal care and experimental procedures of Himachal Pradesh University, Shimla (IAEC/Bio/12-2009).

Plant Material: Leaves of *Adhatoda vasica* were collected from herbal garden Joginder Nagar, Himachal Pradesh, India.

Plant Materials: Dried leaves powder was extracted five times with 80% ethanolic solution. Extraction was done after every twenty-four hours. The collected suspension was concentrated under reduced pressure.

Source of Irradiation: About 6-8 weeks old male mice were irradiated in "Gamma chamber-900" (BARC) with an automatic timer having cobalt- 60 as the source of gamma rays.

Experimental design: Normal healthy animals showing no sign of morbidity were divided into the following groups:

(i) Mice in the first group serve as control (ii) Mice of the second group were administered *Adhatoda vasica* extract (900mg/kg body wt.) for 15 days.

(iii) Mice of the third group were exposed to gamma radiation (6Gy).

(iv) Mice of the fourth group were administered *Adhatoda vasica* extract (900mg/kg body wt.) for 15 days and then exposed to gamma radiation (6Gy).

RESULT AND DISCUSSION: The results obtained for histopathological studies on testes of mice were presented in **Fig. 1-10** and discussed as follows.

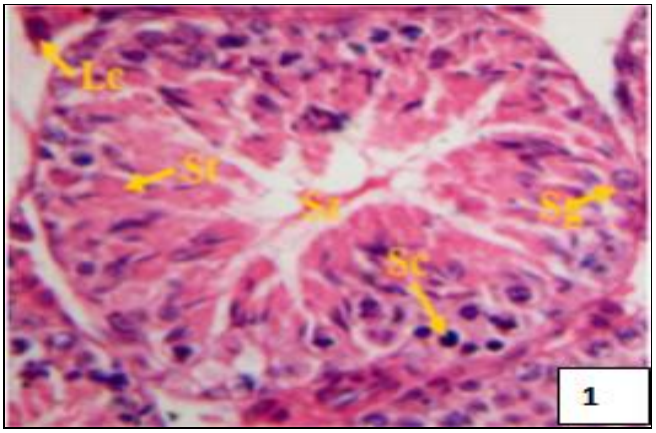

FIG. 1: T. S. OF CONTROL MICE TESTES DEMON-STRATING DARK STAINED SPERMATOGONIA (SG), SPERMATOCYTES (SC), LEYDIG CELLS (LC) AND SPERMATOZOA (SZ). LIGHTLY STAINED SERTOLI CELLS (ST) ARE ALSO VISIBLE X400

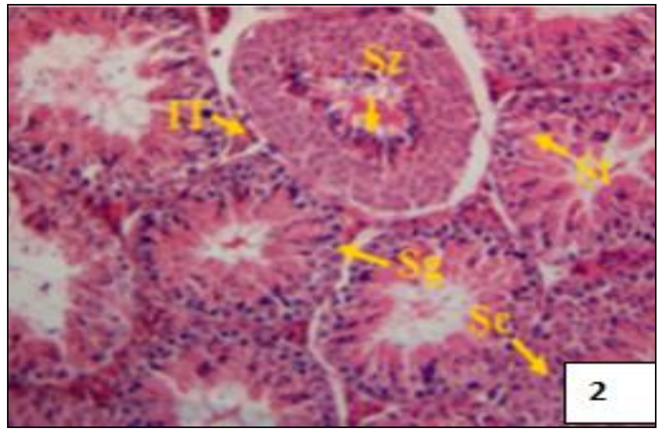

FIG. 2: T. S. OF *ADHATODA VASICA* EXTRACT TREATED MICE TESTES AT 5 DAY STAGE EXHIBITING SERTOLI CELLS (ST), SPERMATO-CYTES (SC), INTERSTITIAL TISSUES (IT) AND SPERMATOZOA (SZ) IN THE LUMEN OF SEMINIFEROUS TUBULES X200

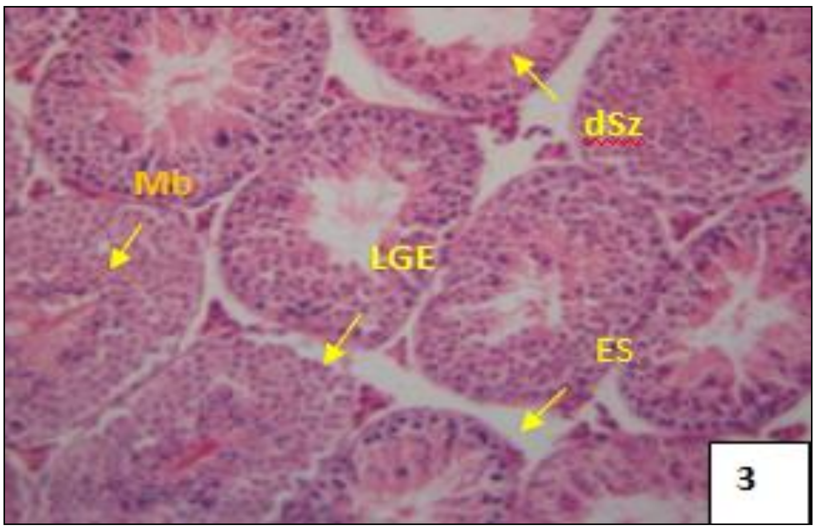

FIG. 3: T. S. OF *ADHATODA VASICA* EXTRACT TREATED MICE TESTES AT 15 DAYS STAGE SHOWING PRESENCE OF SOME MICRO BALLOONS (MB) AND SOME SEMINIFEROUS TUBULES DEVOID OF SPERMATOZOA (DSZ) X200

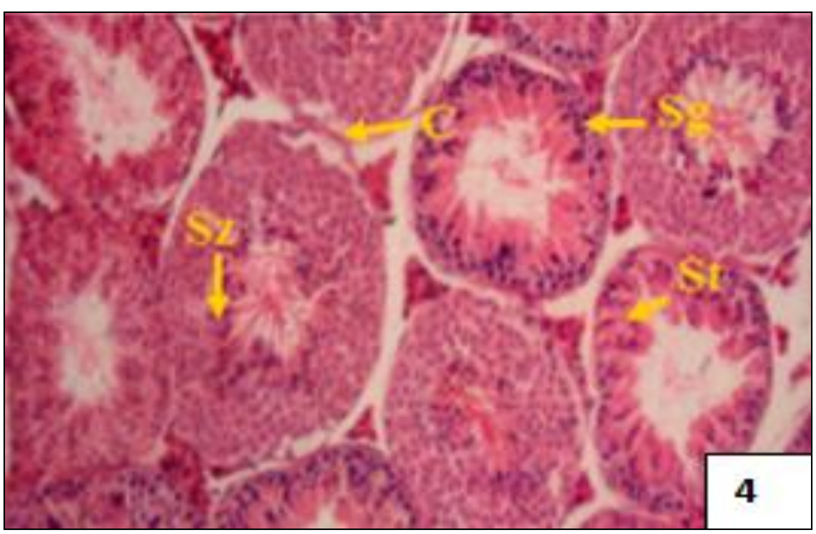

FIG. 4: T. S. OF *ADHATODA VASICA* EXTRACT TREATED MICE TESTES AT 30 DAYS STAGE REVEALING CRACKS (C) OR RUPTURING BETWEEN SEMINIFEROUS TUBULES AND NORMAL SPERMATOGONIA (SG). NORMAL SPERMATOZOA (SZ) AND NORMAL SERTOLI CELLS (ST) ARE CLEARLY SEEN X200

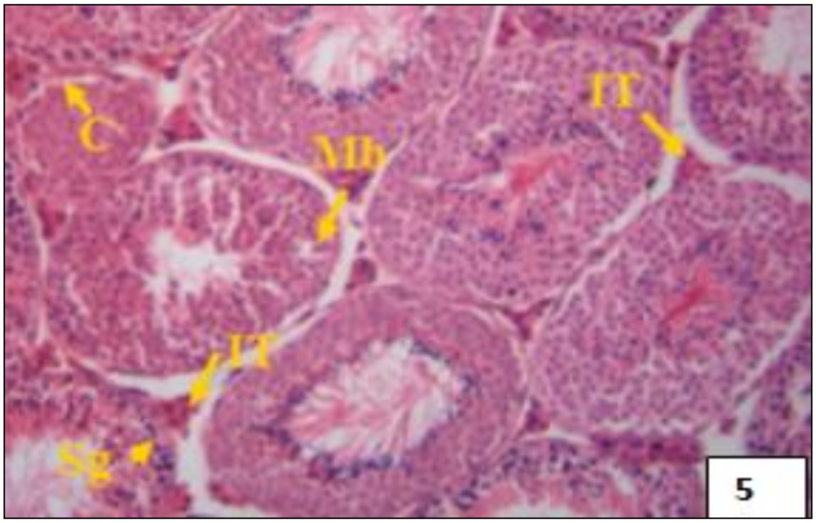

FIG. 5: T. S. OF TESTES OF IRRADIATED MICE AT 1 DAY STAGE DEPICTING NUMEROUS MICRO BALLOONS (MB) LIKE STRUCTURE AND CRACKS (C) BETWEEN SEMINIFEROUS TUBULES AND SPERMATOGONIA (SG). INTERSTITIAL TISSUES (IT) AND SPERMATOGONIA (SG) ARE ALSO SEEN MERGED X200

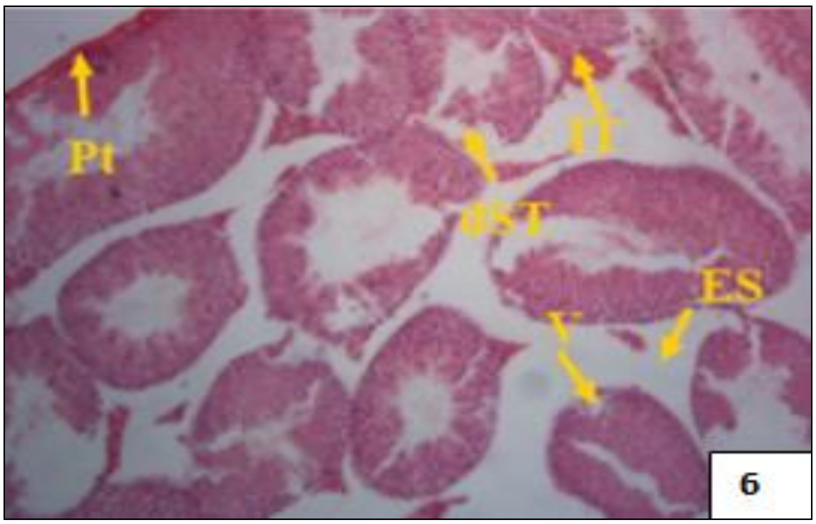

FIG. 6: T. S. OF TESTES OF IRRADIATED MICE AT 5 DAYS STAGE SHOWING DEGENERATION OF SEMINIFEROUS TUBULES (DST) ALONG WITH VACUOLE (V) FORMATION. PERITONEAL MEMBRANE (PT) AND EMPTY SPACES ARE ALSO SEEN X100

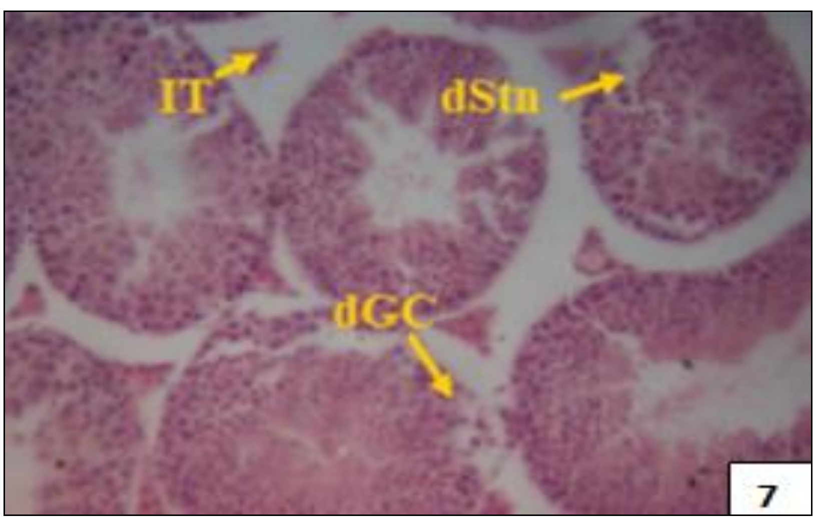

FIG. 7: T. S. OF TESTES OF IRRADIATED MICE AT 15 DAYS STAGE REVEALING DEGENE-RATION OF GERM CELLS (DGC) AND DEGENERATED SERTOLI CELL NUCLEI (DSTN). ENLARGED INTERSTITIAL TISSUE (IT) SPACES ARE ALSO VISIBLE X200

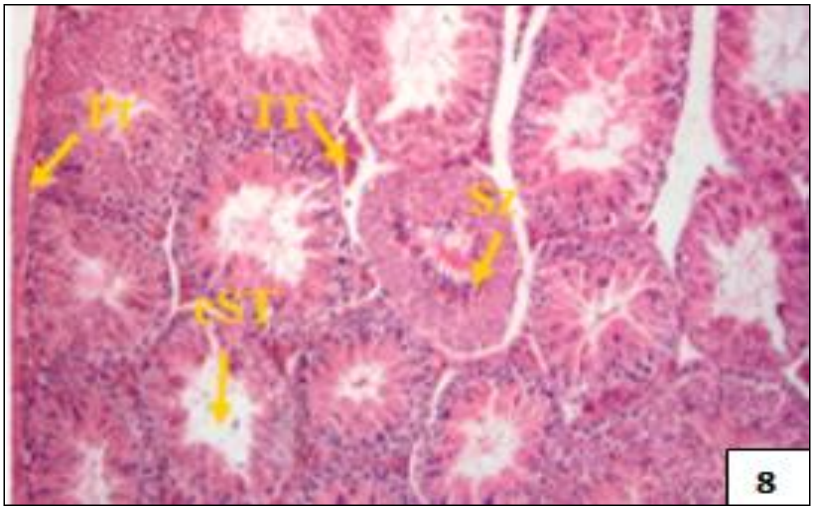

FIG. 8: T. S. OF *ADHATODA VASICA* EXTRACT TREATED MICE TESTES AT 5 DAYS STAGE DEMONSTRATING OUTER MEMBRANE OF SEMINIFEROUS TUBULES PERITONEUM (PT) AND NORMAL SPERMATOZOA (SZ). THE SOME EMPTY SEMINIFEROUS TUBULES (EST) ARE ALSO OBSERVED X100

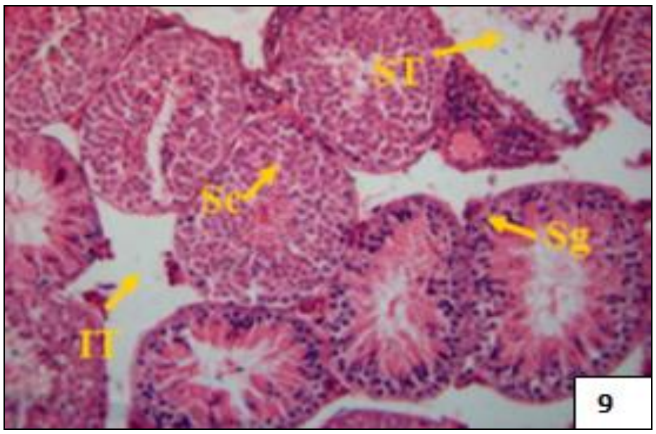

FIG. 9: T. S. OF *ADHATODA VASICA* EXTRACT TREATED PLUS IRRADIATED MICE TESTES AT 15 DAYS STAGE SHOWING LIFTING OF MASS OF SEMINIFEROUS TUBULES (ST) AND ENLARGED INTERSTITIAL TISSUES (IT) SPACES. THE SPERMATOGONIA (SG) AND SPERMATOCYTES (SC) ARE LARGE IN NUMBER X200

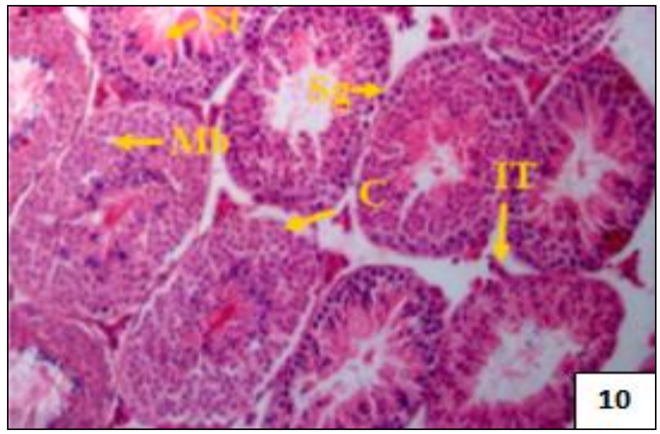

FIG. 10: T. S. OF *ADHATODA VASICA* EXTRACT TREATED PLUS IRRADIATED MICE TESTES AT 30 DAYS STAGE DEPICTING MICRO BALLOONS (MB) FORMATION AND INTERSTITIAL TISSUES (IT). THE NORMAL SPERMATOGONIA (SG) AND SERTOLI CELLS (ST) ARE SEEN IN THE SECTIONX200

Control Mice Testes: Microscopic examination of the control testes of mice had normal structure and was completely enveloped by a thick capsule, tunica albuginea composed mainly of dense collagenous fibrous connective tissue. The structural components of the testes are the seminiferous tubules and interstitial tissues. The seminiferous tubules have two types of cells, the Sertoli cells, resting on thin basement membrane and the spermatogenic cells. These cells are mainly layered, namely the spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa **Fig. 1.** Sperms may appear in some tubules with condensed nuclei with tails directed towards lumen. There were very few gaps between the seminiferous tubules and the successive stages of transformations of spermatogonia into spermatozoa and were easily visible in control mice testes. The interstitial tissue appeared as a granular acidophilic ground substance containing Leydig cells and blood vessels.

***Adhatoda vasica* Extract Treated Mice Testes:** The mice treated with *Adhatoda vasica* extract showed no significant change in the architecture of testes. The seminiferous tubules showed all stages of normal germ cells with active spermatogenesis histological features of testes. The majority of tubules were active. The normal interstitial tissues spaces were observed.

At 5 days stage, some empty seminiferous tubules were seen, and some elongated tubules were also noticed **Fig. 2.** The seminiferous epithelium exhibited the presence of vacuoles. Compact arrangement of cells was also visible. Round spermatid nuclei had condensed chromatin along with the nuclear envelope. The usual feature of successive stages of transformations of spermatogonia into spermatozoa was seen. At 15 days stage, some seminiferous tubules appeared devoid of spermatozoa and loosening of germinal epithelium **Fig. 3**. The seminiferous tubules exhibited peritoneum membrane at 30 days stage of extract treatment **Fig. 4**. The different germ cells, especially mature sperms, were rarely seen, and sertoli cells disappeared in most tubules.

Irradiated Mice Testes: Irradiated mice testes resulted in severe effects on the histoarchitecture of testes. The majority of the tubules were inactive and demonstrated an overall absence of spermatogenesis and such seminiferous tubules were lined by only a single or double layer of cells. At one day stage, irradiated mice testes exhibited micro balloons-like formation. Seminiferous tubules appeared damaged due to the loosening of germinal epithelium and were no more rounded **Fig. 5**. There was a drastic depletion of the spermatogonial population with necrotic and pyknotic nuclei compared with control mice.

At 5 days stage, seminiferous tubules showed vacuole formation, and some tubules degenerated in the sections. The spermatogonia were also seen enlarged in size. The interstitial tissue spaces were loosening their boundaries. Tubular epithelium appeared extremely pycnotic, and no signs of spermatogenic activity were seen, which showed a complete spermatogenic arrest **Fig. 6**. The germinal epithelium was highly disorganized with shrinkage of tubules and cytoplasmic vacuolization. Sertoli cells and leydig cells showed shrinkage in their size. In irradiated mice testes, there was a significant decrease in the number of spermatogonia. A similar decrease was also found in the number of primary spermatocytes, secondary spermatocyte and spermatid. At 15 days stage, testes demonstrated degenerated sertoli cell nuclei and degenerated germ cells. There was hardly any distinction between spermatogonia, spermatocytes, or spermatid. The enlarged interstitial tissue was also noticed. Tubular epithelium became loose and began to detach from the basement membrane **Fig. 7**. Many workers have reported a reduction in testicular weight due to spermatogenic arrest on the administration of drugs, radiations, chemicals, and pesticides [14]. The results of the present study conform with these findings, showing a significant reduction in the weight of testes and a significant increase in the number of sperm abnormalities after radiation exposure.

***Adhatoda vasica* extract plus Irradiated Testes:** The mice treated with *Adhatoda vasica* extract and then exposed to 6 Gy gamma radiation dose showed lesser degree of disorganization in testes architecture. The number of germ cells increased by day 30, histology of testes revealed near-normal histoarchitecture except for some cytoplasmic vacuolization and lumen with full of sperms. At 5 day stage, *Adhatoda vasica* extract-treated plus irradiated mice testes exhibited some micro balloons like structure and some debris in seminiferous tubules. However, the cellular content of the tubules was compact, multilayered, and arranged in a more or less radial configuration in most tubules showing signs of normal spermatogenesis **Fig. 8**. The interstitial tissue spaces were seen to increase. At 15 day stage, lifting of mass of seminiferous tubules and enlarged interstitial tissues spaces were noticed **Fig. 9**. The spermatozoa was observed aggregated in the lumen of seminiferous tubules. The chromatin material of spermatogonial nuclei appeared condensed and continuous sloughing of germ cells. The spermatogonia had dense globules of chromatin with a nuclear membrane. The germinal epithelium was broken down, and tubular diameters were abnormal. The basement membrane was not always visible, and the lumen of the seminiferous tubules was devoid of spermatozoa and filled with fibrous material.

At 30 days stage, normal spermatogonia and sertoli cells were observed in the sections. The cellular contents of most of the tubules were compact and a sign of normal spermatogenic activity. There was a significant increase in spermatozoa in the lumen of seminiferous tubule **Fig. 10**. Infertility has been a major medical and social problem. *Adhatoda vasica* extract pre-treatment rendered a high degree of recovery in spermatogenic cells, and almost a normal testicular architecture was re-established by the end of the experiment, but some pathological lesions like adhesion of tubules, mild cytoplasmic vacuolation, and less number of mature spermatozoa still persisted in the lumen of some tubules. A similar observation has also been reported while using *Panax ginseng* [15], *Podophyllum hexandrum* [16] and *Mentha piperita* [17] as radio-protector for the modulation of testicular injuries after irradiation. The protective ability of the phytochemicals against radiation-induced male reproductive abnormalities may offer new insight into the modification of testicular germ cell radiosensitivity, which may have implications in amelioration of testicular injuries.

CONCLUSION: The oral administration of *Adhatoda vasica* in the present work has shown that it provides protection against irradiation. Thus *Adhatoda vasica* may safely be used as a cancer chemopreventive agent, which may help enhance the cell's detoxification reaction and thus may be effective as a blocking agent against chemically induced carcinogenesis.

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CONFLICTS OF INTEREST: Nil

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