Role of Septilin in Treatment of Radiation Induced Testicular Injury

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Abstract

The protective effects of Septilin (herbal extract) against radiation-induced damage in testis of male rats have been studied. Animals were divided into four main groups:

1- Control group, 2- Septilin group (100mg/kg body weight/day), 3- Irradiated group (4Gy gamma-radiation), 4- Protected group in which animals (Male rats) were given Septilin orally (100mg/kg body weight/day) for two weeks before radiation exposure (4Gy gamma-radiation), then rats were re-given Septilin treatment again following irradiation and were autopsied at the third and seventh days after treatment to evaluate the effectiveness of the treatment before and following radiation in terms of histological alterations in rats testis. The irradiated group showed marked testicular degeneration, interstitial edema and degeneration of spermatogonial cells in seminiferous tubules. The tubules were damaged and greatly depleted of germ cells. However, animals treated with Septilin pre and post-irradiation showed almost normal testicular morphology with regular arrangement of germ cells. Slight testicular degeneration was still observed. Also, presence of some spermatozoa with in the lumen of some tubules was noticed.

Conclusion: Thus, this study concluded that gamma-irradiation exerts damaging effects on rat testes. Pre (protective) and post-treatment with Septilin inhibited most of these toxic effects. The protection afforded with Septilin in the present study proved to be beneficial for the clinical use of such herbal extract as a radio protector.

Key Words: Gamma radiation — Septilin — Rat testis.

Introduction

RADIATION is an important modality in cancer treatment and estimates are that between one third and one half of all patients will require ionizing irradiation therapy during some point in their clinical management. However, the radiation-induced damage to the normal tissues restricts the therapeutic doses of radiation that can be delivered to tumors and thereby limits the effectiveness of the treatment ill. The use of chemical compounds (radioprotectors) represents an obvious strategy to improve the therapeutic index in radiotherapy. However, most of the synthetic radioprotective compounds studied have shown inadequate clinical application owing to their inherent toxicity and high cost [2]. These observations necessitated a search for alternative agents that are less toxic and highly effective. Studies in the recent past have shown that some medicinal plants possess radioprotective effects 131.

The radioprotective agents are compounds that are administered before or/after exposure to ionizing radiation to reduce its damaging effects, including radiation-induced lethality [4]. Although synthetic radioprotectors such as the aminothiols have yielded the highest protective factors; yet they are proven to be more toxic [5] than naturally occurring protectors [6]. For example Spinach was proven to be very efficient against radiation induced oxidative stress as their leaves are rich in antioxidants and was reported as pre-treatment radioprotector [7]. Also different plant extracts were tested against radiation effects and showed potential radioprotective activities in mammals [8-12]. The effect of various doses of 50% ethanolic extract of Septilin (a herbal preparation) on the radiation-induced mortality was studied in mice exposed to 10Gy of gamma-irradiation daily. Treatment of mice with Septilin, for 5 days before irradiation, delayed the onset of mortality and reduced the symptoms of radiation sickness when compared with untreated irradiated controls [13]. In vivo studies have suggested the immunomodulatory properties of Septilin, an herbal preparation. It is being used against various types of inflammatory disorders. However, the mechanism of action of Septilin in the modulation of inflammation is not explored using suitable in vitro models [14].

Spermatogenesis is a long, complex and finely tuned process. During this process, the developing
sperm cell is sensitive to endogenous or exogenous stresses. Exposure to reproductive cytotoxic agents may damage somatic testicular cells or germ cells at different stages of differentiation, leading to a temporary or permanent impairment of fertility [15].

Ionized radiation, being one of the environmental cytotoxic factors and reproductive toxic agent, affects testicular function, morphology and causes death of the germinal cells and therefore, reversible or permanent sterility [16].

The testis is a main target organ of radiation damage; it has been reported that irradiation induces several histopathological consequences. Also, clear testicular degeneration and death of spermatogenic cells especially primary spermatocytes and apoptosis [17]. The differentiating spermatogonia are very sensitive to radiation and are killed under the effect of doses less than 3 Gy in the Sprague-Dawley rat [18], in humans [19] and in mice [20].

The present work aimed to discuss the damaging effects of gamma radiation on rat testis and investigate whether Septilin administration pre and post irradiation could prevent gamma radiation-induced histological damage in rat testes.

Material and Methods

The experimental animals in the present investigation were 56 male albino rats with average body weights 130-150 gm. The animals were housed in metal cages, under normal temperature, pressure, humidity, good ventilation and illumination conditions during the whole period of the experiment. All animals were fed with semi purified diet and water ad libitum for 10 days before the start of the experiment. They were maintained at standard condition 24±10 relative humidity.

Exclusion criteria:

Rats which were inactive or showed signs of infection were discarded before treatment.

Animals were divided into four main groups:

- The first group served as control group (7 rats).
- The second group (positive control): Healthy rats were given Septilin in a daily oral dose (100 mg/kg b.wt.) for two weeks (7 rats).
- The third group served as irradiated rats that received (4 Gy gamma-radiation) as single dose. Some rats were sacrificed on the third day (7 rats) and the rest were sacrificed on the seventh days (7 rats).

- The fourth group (14 rats) was divided into two equal subgroups; both were given Septilin for two equal weeks and both were subjected to whole body y-irradiation at a sub-lethal single dose of 4 Gy. Then both groups were re-given Septilin (the same oral dose). The first sub-group (7 rats) was sacrificed three days following Septilin administration. The second sub-group (7 rats) was given Septilin for seven days following irradiation then was sacrificed.

Whole body irradiation technique:

Whole body y-irradiation of animals was performed at irradiation facility of National Centre for Radiation Research and Technology (NCRRT), Cairo, Egypt using Cesium 137 Gamma Cell 40 giving a dose rate of 0.89 Gray/min at the time of experiment with a total of 4 grays.

The herb used in the present investigation was Septilin Himalaya (a product of herbal healthcare), Drug Co. Private Ltd; it is an Ayurvedic herbal preparation containing various herbs and minerals. Its main ingredients are (in mg): Balsamodendron mukul 162; Sank Bhassma 32; Maharasni qoath 65; Tinospora cordifolia 49; Rubia cordifolia 32; Emblica officinalis 16; Moringa pterigos-perma 16; Glycyrrhiza glabra 6. It has been reported to have anti-bacterial, anti-inflammatory, anti-exudative and immuno-stimulatory effect [21]. The dose used in this study was 100 mg/kg b.wt./day as oral administration using a stomach tube for 14 days before radiation and same dose following radiation [13].

For histological examination small pieces of rat testis tissue samples were fixed in 10% formalin in phosphate buffer for 3 days. Afterwards, testis tissues were processed by routine histological methods and embedded in paraffin blocks. Paraffin blocks were placed in rotary microtome, and sections of 5 gm thickness were obtained with disposable metal microtome blades. After deparaffinization and rehydration, all sections were stained with hematoxylin-eosin (H-E). Sections were examined under light microscope.

Results

The control group:

The light microscopy examination of the testes of the control rats had normal structure and completely enveloped by a thick capsule, tunica albuginea, which is composed mainly of dense collagenous fibrous connective tissue. The structural components of the testis are the seminiferous tubules and interstitial tissues. In the seminiferous
tubules there are two types of cells: The Sertoli cells, resting on the thin basal lamina (basement membrane) and the spermatogenic cells. These cells are many layers, namely, the spermatogonia, primary and secondary spermatocytes; spermatids and finally mature spermatozoa (Figs. 1,2).

**Septilin group:**

Testicular section obtained from rats treated with Septilin at a daily oral dose of 100mg/kg b. Wt. For two weeks exhibited closely similar structure to those of the control animals and normal spermatogenesis denoting that Septilin has no cytotoxic effects on the testes of rats (Fig. 3).

**Effect of γ-irradiation:**

In this group rats were exposed to single dose (4Gy) of gamma irradiation. There was marked testicular degeneration detected in the majority of the seminiferous tubules. The organized structure of cells forming the seminiferous tubules was completely disrupted and diffuse intercellular edema was observed. Spermatogenesis was absent in most seminiferous tubules.

Three days post exposure to a single dose (4Gy) of gamma irradiation, the seminiferous tubules were lined with few spermatogenic cells, Sertoli cells, and few primary spermatocytes. The seminiferous tubules were mostly atrophied and the interstitial connective tissue was thickened with less number of interstitial cells (Fig. 4). The few primary spermatocytes remained contained dark condensed nuclei. Most of the degenerated spermatozoa were seen in the lumen of the tubule. The spermatogenic cells were irregularly distributed within the seminiferous tubules with formation of giant multinucleated cells and the basement membrane of the tubules was abnormal. The interstitial connective tissue showed blood vessels congestion (Fig. 5).

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**Fig. (1):** Photomicrograph of T.S testis showing: The seminiferous tubules contain sertoli cells and germ cells of various stages (primary spermatocytes (PS); secondary spermatocytes (SS); spermatids (ST) and spermatozoa. The interstitial tissue (ICT) contains normal interstitial cells (IC). (Control group). H&E x200.

**Fig. (2):** Photomicrograph of T.S of rat testis showing normal structure of seminiferous tubule lined by several layers of spermatogonia and sertoli cells are resting on basement membrane. The lumen contain mature spermatozoa (SP). (Control group) H & E x200.

**Fig. (3):** Photomicrograph of T.S of testis showing: Normal structure of seminiferous tubule lined by several layers of spermatogenic cells (SG) and sertoli cells are resting on the basement membrane. (Septilin group) H & E x200.

**Fig. (4):** Photomicrograph of T.S of testis showing marked testicular degeneration in the majority of the seminiferous tubules with few lining spermatogenic cells (SG) and widening of spaces between the seminiferous tubules (ICT). (Radiated group after 3 days) H & E x100.
After seven days of radiation: The results showed marked testicular atrophy. There were marked shrunken seminiferous tubules with absence of spermatozoa in the lumen. Also degenerated, scattered spermatogenic cells, and irregular basement membrane that was detached from the overlying cells. Depletion of the spermatogonia was prevailing in all testicular tissue. There was apparent widening of the interstitial connective tissue and depletion of the interstitial cells (Fig. 6).

**Septilin treated before and after γ irradiation:**

Moderate ameliorative effect was detected in the testes of 4 Gy gamma-irradiated rats treated with Septilin pre- and three days following gamma radiation exposure. Results showed improvement in the seminiferous tubules architecture with some tubules remained degenerated. There was degeneration of spermatogonial cells lining seminiferous tubules associated with formation of spermatid giant cells. The number of the cells in the spermatogenic series exhibited large increase when compared with those of irradiated rats but still much less as compared with the control group. In addition, the interstitial spaces was still abnormally increased and contained less number of the interstitial cells. Tubular basement membrane was irregular. Interstitial widening with faintly stained acidophilic material (edema) was detected (Fig. 7).

Marked ameliorations were noticed in the testes after seven days treatment with Septilin following gamma-irradiation exposure. The ameliorative effects were represented by the normal appearance of the seminiferous tubules, they appeared arranged in lobules separated by interstitial tissue. The spermatogenic cells appeared regularly arranged within the tubules with normal tubular lumen containing spermatozoa. The basement membrane of the seminiferous tubules was mostly normal. The primary spermatocytes are closer to the lumen and spermatogonia are rested on the basement membrane of the seminiferous tubules. The interstitial tissue appeared almost normal containing the interstitial cells of Leydig. Interstitial widening was (edema) barely detected (Fig. 8).

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**Fig. (5):** Photomicrograph of T.S of testis showing marked testicular degeneration. Seminiferous tubules are lined by few spermatogonia (SG). Thickened interstitial tissue (ICT) with congested blood vessels (BV). (Radiated group after 3 days) (H&E x200).

**Fig. (6):** Photomicrograph of T.S of testis showing marked shrinkage of seminiferous tubules and testicular atrophy associated with interstitial widening (IE). (Radiated group after 7 days) (H & E x100).

**Fig. (7):** Photomicrograph of T.S of testis: Showing degeneration of spermatogonial cells lining seminiferous tubules associated with formation of spermatid giant cells (SGC) and interstitial widening with faintly stained acidophilic material (IE) (Radiated and Septilin treated group after 3 days) (H & E x200).

**Fig. (8):** Photomicrograph of T.S of testis irradiation showing slight testicular degeneration. The seminiferous tubules (ST) are arranged in lobules. Sperms are present in the lumen and spermatogonia (SG) are rested on the basement membrane. (Radiated and Septilin treated group after 7 days) (H & E x200).
Discussion

Ionizing radiation (IR)-induced cellular damage is implicated in carcinogenesis as well as therapy of cancer. Advances in radiation therapy have led to the decrease in dosage and localizing the effects to the tumor; however, the development of radio resistance in cancer cells and radiation toxicity to normal tissues are still the major concerns. Thus agents are needed that could prevent the damage to normal cells and tissues caused by the direct and bystander effects of radiation, without have its own systemic toxicity [24].

The use of radioprotective compounds, which can selectively protect normal tissues against radiation injury, is of immense use. It will also permit use of higher doses of radiation to obtain better cancer control and possible cure. However, till date no ideal radioprotectors are available as most synthetic compounds are toxic at their optimal concentrations. Plants commonly used as dietary and or therapeutic agents have recently been the focus of attention since in most cases they are nontoxic and are easily accepted for human use [25].

Germ cells are responsible for transmitting genetic information from generation to generation. If mutations occur in male germ cells, they are transmitted to offspring and may induce malformations or diseases [26].

In the present study, histopathological changes of the testes were assessed after three, and seven days post gamma-irradiation. Severe atrophy of the seminiferous tubules with loss of germ cells were observed in most seminiferous tubules in the rats irradiated with 4Gy of gamma-radiation. There was interstitial tissue widening indicating edema, also blood vessels congestion were detected. However, the damage was more apparent seven days post irradiation. The response of rat testis to gamma-irradiation was studied with use of single dose but on two different days (the third and the seventh) to show the variable damaging effects resulting compared to time.

The results of the current study agreed with Samarth and Samarth [27], who reported that radiation (8Gy) induced moderate to severe testicular atrophy with degeneration of germ cells in seminiferous tubules. The tubules were shrunken and greatly depleted of germ cells. Sertoli cells with few germ cells were observed in the lumen.

Also, histological examination of testes of the irradiated rats (5Gy) showed extensive degenerative changes in the seminiferous tubules and defoliation of spermatocytes. Irradiation resulted in 59% and 40% decreases in spermatozoa motility and live/dead sperm count, respectively, and a 161% increase in total sperm abnormalities [28].

The histological lesions observed in the rat testes in Eissa and Moustafa study [29], varied between vacuolation, swelling, pyknosis and even necrosis in some spermatogenic cells as well as significant depletion in the number of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. The histochemical observations revealed diminution in the polysaccharides content and increase in the collagen fibres in the testis of irradiated animals. These effects were mostly perceptive with the high dose of the radiation (6Gy) than with the lower one (3Gy).

Zhang et al., [30] results suggested that radiation and chemotherapeutic drugs cause permanent sterility in male rats, not by killing most of the spermatogonial stem cells, but by blocking their differentiation in a testosterone-dependent manner. However, they found that transplantation of spermatogonia, harvested from prepubertal testes to adult testes that have been exposed to cytotoxic therapy might be limited by the somatic damage and may require hormonal treatments or transplantation of somatic elements to restore the ability of the tissue to support spermatogenesis.

Germ cells have variable sensitivity to radiation; type B spermatogonia are most sensitive, spermatids are the least sensitive. Therapeutic radiation may cause only temporary infertility with recovery in 30-80 weeks, delayed if combined with chemotherapy. Irradiation of mice testis created a gap in spermatogenesis, which was initiated by loss of A1 to B-spermatogonia and lasted for approximately 10 days. Irradiation with 2 times (1Gy) showed a more pronounced effect on germ cell elimination than with (1Gy), but spermatogenesis was in both cases completely reconstituted 42 days after irradiation [31].

Germ cells were initially depleted as a result of killing the radiosensitive differentiating spermatogonia [32]. Spermatogonial depletion due to testicular toxicants and seminiferous tubule atrophy by impairment of spermatogenesis was documented [33]. In irradiated mice, (8Gy) the germinal epithelium was highly disorganized with shrinkage of tubules, absence of sperm and spermatids shrinkage in their size of Sertoli cells and Leydig cells [34].

Morphologically, examination of irradiated testis revealed presence of disorganization and
Role of Septilin in Treatment of Radiation Induced desquamation of germinal cells and the reduction in sperm count in seminiferous tubule lumen [35].

Another study that agrees with our results, examination of gamma-irradiated testis revealed presence of marked disorganization and depletion of germ cells, arrest of spermatogenesis, formation of multinucleated giant cells, and vacuolization in the germinal epithelium [36].

Porter et al., study [37] found a relationship between increased interstitial fluid and the block in spermatogonial arrest when male rats were exposed to 3.5, (6Gy) gamma radiation. They postulated that the mechanism by which interstitial edema inhibits spermatogonial differentiation might involve direct or indirect effects. Edematous interstitial fluid may contain proteins or other factors or generate reactive oxygen species that directly suppress spermatogonial differentiation or induce apoptosis of differentiating spermatogonia.

Throughout germ cell differentiation, somatic Sertoli cells nurture the developing sperm cells, regulate proper germ cell development, and maintain the integrity of the seminiferous tubules [38]. This study showed marked decrease in Sertoli cells number as a direct damaging effect of radiation induced damage.

Regarding giant cells observed in the testis of gamma-irradiated animals, Labib [39] suggested that the multinucleated giant cells were derived from the fusion of two or three primary spermatocytes.

The present data showed that the interstitial spaces in the testis of irradiated animals appeared larger than those in the control animals and most Leydig cells became degenerated. Comparable observations were reported by Labib [39]. Interstitial edema was still detected after seven days post Septilin treatment.

Radioprotective agents are defined as compounds that help to diminish the biological effects of ionizing radiation when administered before exposure to radiation [4]. The herbal drugs offer an alternative to the synthetic compounds and are considered either non-toxic or less toxic than their synthetic counterparts. Plants and their phytochemicals, especially with free radical scavenging, antioxidant properties, and immunostimulatory effects have been evaluated for their radioprotective effects. Preclinical studies in the past two decades have shown that some commonly used medicinal plants and their phytochemicals possess radioprotective effects [40]. Various other experimental approaches, aimed at preventing radiation-induced quantitative damage to the gonads, have been explored [1,7,11,12]. The present study showed that Septilin has strong protective effects against gamma-radiation induced testicular histological alterations in rats. Septilin in oral daily dose of 100 mg/kg b. Wt. For two weeks prior to gamma-irradiation and following radiation could attenuate the histological lesions observed under the effect of gamma-radiation especially with the low dose of this radiation. The current study has proven the efficacy of Septilin as the treated irradiated rats testes regained their normal tubular arrangement, spermatogenic cells, and the tubules contained spermatozoa suggesting regaining normal spermatogenesis.

Conclusion:

The present study proved that Septilin exerts a strong protective effect against whole body gamma irradiation-induced testicular damage. Septilin was found to be more effective in low dose irradiated group. It has the ability to minimize, and in some cases to prevent the deleterious influences of ionizing radiation when administered pre, and post-exposure. Thus in clinical therapy the amount of ionized radiation dose applied is thought to be very important. These results have implication for the potential use of Septilin as a radioprotector. The possible clinical and therapeutic ramifications of Septilin require further investigations.

References


