

Effects of *Chlorophytum borivillianum* Sant. F against gamma radiation-induced testicular injuries in Swiss albino mice

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Abstract

Background: Radiation therapy is considered as an important tool in cancer treatment. Despite its impressive role in treating cancer, severe side effects in organs have been reported. To address these therapeutic side effects, several combination methods have been identified to minimize adverse effects caused by radiation therapy. **Aims and Objectives:** Based on higher radioactive sensitivity of testicular tissues, administration of *Chlorophytum borivillianum* (CB) Sant. F extracts was evaluated for its protective effects against radiation in testis. **Materials and methods:** Two forms of CB extracts (CB alone and CB-silver nanoparticles [AgNPs]) were administered at a dose of 50 mg/kg body weight in Swiss albino male mice for 7 consecutive days. Following 6 Gy gamma radiation, animals were observed for 30 days in four phases. Sperm counts, body weight, testicular weight and stereological and histological evaluation of testis were evaluated. **Results:** Following irradiation, a significant decline in body weight ($P = 0.008$) and testicular weight ($P = 0.001$) was noted when compared with control. Ununiformed type A and B spermatogonia, partially filled tubules, inter-tubular vacuoles, and disrupted epithelium were the main types of damages caused by irradiation. Reorganization and resumption of histological features emerged from the 15th day postirradiation in CB extract (CBE)-treated animals. **Conclusion:** Testicular response was observed against radiation in animals treated with CB extracts, while CB-AgNPs indicated better toleration when compared to CB extract alone.

Keywords: *Chlorophytum borivillianum*, gamma radiation, spermatogenesis, testicular damages

Introduction

The use of radiation in therapeutics has increased in recent times with an increasing number of cancer patients. Recent data indicate that nearly 60% of all cancer patients receive radiation therapy during the course of cancer treatment.^[1-3] Principally, high-energy radiation disintegrates DNA of targeted cells and encourages apoptosis causing arrest in proliferation.^[4] Although radiation treatment is highly efficient and cost-effective, it causes mild-to-severe side effects.^[5] Some of the highly documented side effects of radiation therapy are cardiovascular disease, cystitis, erectile dysfunction, vaginal dryness and stenosis, and infertility.^[6-8]

There is ample information available, claiming various degrees of damages to the testicular cells by both low- and high-dose radiations.^[9-12] Application of external beam radiotherapy (20–600 cGy) in cancer patients has resulted in a significant decline in the number of germ cells and development of azoospermia.^[12] Testicular cells are predominantly sensitive to radiation exposures. An earlier study reported that doses as

low as 3.5–6 Gy can severely impair testicular functions such as long-term reduced spermatogenesis.^[13] Previous studies have reported that there are two types of tissue damages that occur following radiation exposure in testicular cells, seminiferous tubules, and Leydig cells, affecting both spermatogenesis and secretion of testosterone, respectively.^[14]

A number of combinational chemotherapies are identified to minimize the tissue damage caused by radiation during therapy. In principle, radioprotectants are compounds that have antioxidant, anti-inflammatory, anti-proliferative and peroxidation inhibitory properties.^[15-19] Some herbal medicines have been found to have similar properties with significantly

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less side effects. *Chlorophytum borivilianum* (CB) Santapau and Fernandes (Family: Liliaceae) or *Shweta Musli* has been recognized for its medicinal property against various medical conditions in the Indian subcontinent.^[20] It is known in Ayurveda for its antimicrobial, anti-inflammatory, hepatoprotective, and anti-impotency properties.^[21] In this study, CB root extract (CBE) has been used to evaluate its potential protective effects against testicular injury by gamma radiation. The defensive efficacy of the plant extract following tagging with silver nanoparticle (AgNP) has also been examined.

The aim of the study was to evaluate and compare the protective effect of CB extract (CBE) and CB-AgNPs against testicular damages due to gamma radiation.

Materials and methods

Collection and preparation of plant extract

The pure dried root powder of CB was purchased commercially from Naturemed, UPC-797079478464 (Hyderabad, Telangana, India). The plant root extract was prepared by mixing 1%–5% of plant root powder with deionized water in a 250 ml conical flask (Borosil, India). The solution was boiled in water and then incubated at 50°C–70°C for an hour. The extract was then filtered twice through Whatman No. 1 filter paper to remove particulate matter and to get clear solution that was then refrigerated (4°C) in 250 ml Erlenmeyer flask until further use. In each and every step of the experiment, sterile conditions were maintained for the effectiveness and accuracy in results without contamination.

Biosynthesis of silver nanoparticles

The plant extract tagged AgNPs were synthesized using a constant volume of the plant extract under various experimental conditions. Five milliliters of CBE (aqueous extract) was mixed with 95 ml of AgNO₃⁽⁺⁾ for the synthesis of CB-AgNPs. The formation of CB-AgNPs is confirmed by color change from whitish to reddish brown. The appearance of reddish-brown color indicates the formation of AgNPs.^[22-24] Desired nano-range was selected for further experiment. Morphological and structural evaluations of synthesized particles were confirmed by transmission electron microscope (TEM).

Source of irradiation

The Cobalt teletherapy unit (ACT-C9) (Bhabhatron-II TAW telecobalt machine) at Cancer Treatment Center, Radiotherapy

Department, SMS Medical College and Hospital, Jaipur, was used for irradiation. Unanesthetized animals were restrained in a well-ventilated perspex box and whole body was exposed to gamma radiation. Dosimetry was then calculated as 1.07 Gy/min from the source to surface distance that is 80 cm.

Animals

Random-bred male Swiss albino mice (*Mus musculus*) (6–8 weeks), weighing between 20 and 30 g, were used for the present experiment. These animals were maintained in the departmental animal house facility at temperature of 24°C ± 3°C and 12-h light and 12-h dark periods. The animals were fed mice pellet diet (Ashirwad Pvt. Ltd., India) and provided with open access to safe drinking water (IAEC Approval Number: 1678/90/re/S/12/CPCSEA, Date June 16, 2017).

Experimental design

Based on an earlier study, an optimum dose of 50 mg/kg body weight of CB root extracts (CBEs) was applied.^[25] To evaluate the adverse effects of gamma radiation in testes and the possible radioprotective efficacy of CBE, male mice of proven fertility were randomly selected from an inbred colony and divided into the following groups (control group – group I [*n* = 20]), (test groups – group II–IV [group II gamma-irradiated group, group III –CBE+ irradiated, group IV – CB and CB-AgNPs+ irradiated] contained 20 animals each) [Table 1].

Pretreatment with CBE (CB and CB-AgNPs) was continued for 7 days. Later, on the 7th day, mice were irradiated with 6 Gy gamma radiation. Following irradiation, the efficacy of CBE was evaluated for 30 days. Five animals from control group (I) and each test group (II–IV) were sacrificed by cervical dislocation on an observational day (*viz.* 1, 7, 15 and 30 days).

Body and organ weight

The body weight was observed at the 1st, 7th, 15th and 30th days of experimental schedule. Subsequently, weights of testes were recorded for each group following sacrifice at the observational day.

Sperm count

The left cauda epididymis of the mice was minced in 0.5 ml phosphate buffer and then, the supernatant was diluted with sperm counting solution. Sperm numbers per milliliter were determined using a hemocytometer.^[26]

Table 1: Animal groups assigned for the current study with their respective specifications

Groups	Specification
Group I (control)	Sham-irradiated animals were given double distilled water through oral gavages once a day for 7 consecutive days (dose equivalent to CBE)
Group II	Animals of this group were given double distilled water through oral gavages once a day for 7 consecutive days. On the 7 th day, mice were irradiated with 6 Gy gamma radiation
Group III	Animals of this group were treated daily once with optimum dose of CBE dissolved in distilled water through oral gavage for 7 consecutive days. On the 7 th day, mice were irradiated with 6 Gy gamma radiation
Group IV	Animals of this group were treated daily once with optimum dose of CB-AgNPs dissolved in distilled water through oral gavage for 7 consecutive days. On the 7 th day, mice were irradiated with 6 Gy gamma radiation

CBE: *Chlorophytum borivilianum* extract, CB-AgNPs: *Chlorophytum borivilianum*-silver nanoparticles

Quantification of spermatogenesis and Sertoli cells

The number of germ cells per testis was determined using the optical dissector method.^[27,28] Nuclear number was assumed as equal to one cell in number. Sections were analyzed using a 100x oil immersion lens on an axioscopic microscope equipped with a high definition camera. Cells were counted manually with the help of Image J software (National Institute of Health, USA) for enhanced imaging and clear nuclear counting. Microscopic fields for counting were selected using a systematic uniform random sampling scheme.^[29] Fifty frames of 100 μm² corresponding to 5000 μm² were evaluated per animal.

The numerical density (N_v) of each cell type was calculated by dividing the number of cells counted by the volume of all dissectors:

$$N_v = \text{number of cells counted/area of frame} \times \text{number of frames} \times \text{depth}$$

The number of cells (Nc) per testis was calculated as:

$$Nc = N_v \times \text{testis weight}$$

The germ cells were grouped into spermatogonia (SG), spermatocytes (S) and round and elongated spermatids (S). A number of Sertoli cells (SCs) were determined as described for the germ cells.

Histopathological studies

Testicular tissues were collected posts carification and fixed in 4% paraformaldehyde for 24 h, later dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax. Further 5 μm thin sections were cut and fixed on glass slides followed by staining with Harris's hematoxylin and eosin for light microscopic observations.

Statistical analysis

All the above parameters were statistically analyzed using various biometric tests. Values were expressed in mean ± standard error. Multiple parametric analyses were conducted by one-way ANOVA with addition of Tukey's multiple comparison test (MINITAB, Pennsylvania, USA). For paired analysis, Student's *t*-test was applied when and where required (EXCEL, Microsoft, USA). A radar plot was applied for comparative analysis of variation within each group for estimation of spermatogenesis.

Results

Biosynthesis of *Chlorophytum borivilianum*-silver nanoparticles

TEM micrographs indicated that CB-AgNPs were distinct, uniformly spherical in shape, and were well separated from each other. The average particle size was estimated by measuring more than 100 particles from TEM images. The sizes ranged between 20 and 30 nm with an average particle size of 25.01 ± 3.76 nm [Figure 1a and b].

Body and organ weight

Body weight of animals in Group I ranged between 21 and 28 g on days 1, 7, 15 and 30, respectively. Body weight of Group II

animals declined significantly (*P* = 0.008) when compared with control. Group II showed a continuous progressive decline in the body weight which was minimum on day 1 and maximum on day 30. Variation in body weight of group III animals was also found significant when compared with group I (*P* = 0.013). However, in this case, an arbitrary increase in body weight was observed. In group IV, animals had a consolidated increase in body weight following minor initial decrease. By the 15th–30th day of the experiment, body weight returned close to normal range. Nevertheless, the overall variation in body weight of group IV remained significant when compared with group I (*P* = 0.027) [Table 2].

Both left and right testes of group I animals were recorded within the range of 111–113 mg on days 1, 7, 15 and 30, respectively. For group II, the weight of both left and right testes declined sharply (*P* = 0.001) and continued to decline further as the day of investigation progressed (i.e., 1st–30th day). Group III animals showed an uninterrupted elevation in testis weight following initial decline. However, despite progressive improvement, the weight of testes was significantly lower in group III animals when compared with group I (*P* = 0.004). Although the weight of testes in group IV animals was close to control range by the 30th day of observation, it remained overall significantly less in comparison to control (*P* = 0.01) [Table 3].

Sperm count

Sperm count remained unaltered during all observational phases (i.e., 1st, 7th, 15th and 30th day) in control animals. Sperm

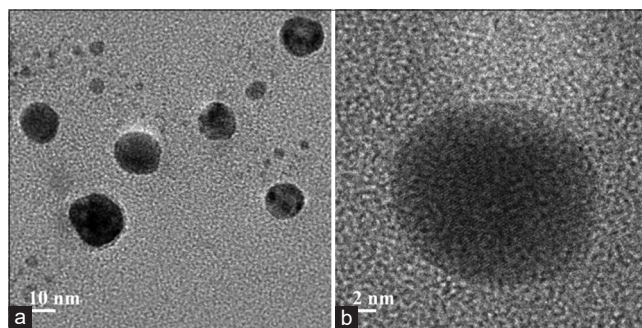


Figure 1: Transmission electron microscope image of *Chlorophytum borivilianum*-silver nanoparticles: (a) high-resolution image of nanocrystals at 10 nm (b) high-resolution image of a single nanocrystal at 2 nm

Table 2: Respective body weight (g) of mice in experimental groups (values are presented in mean ± standard error)

Day	Group I	Group II	Group III	Group IV
1 st	24.11±1.01	18.58±0.64**	19.1±0.64*	21.76±0.74*
7 th	26.31±1.08	15.21±0.75**	14.36±0.59**	18.21±0.65*
15 th	21.39±1.11	16.39±0.69**	18.2±0.78*	22.36±0.85
30 th	27.55±1.02	11.47±0.54**	21.55±0.59*	20.9±0.61*

P*<0.05, *P*<0.01, indicate significant, highly significant, and extremely significant differences, respectively, against Group I (control)

count in the control group ranged between 43 and 44 mil/ml, whereas in Group II, sperm count declined evidently and recorded as 32.16 ± 0.61 , 29.2 ± 1.42 , 27.37 ± 1.23 , and 23.6 ± 1.36 mil/ml on days 1, 7, 15, and 30, respectively. Consolidated improvement in sperm count was noted following initial depletion in both Groups III and IV. In contrast to Group II, where sperm count declined continuously up to the 30th day of observation, in Groups III and IV, sperm count gradually elevated as the day progressed. Sperm count in Group III was measured as 33.56 ± 1.56 , 32.94 ± 1.32 , 34.56 ± 0.80 , and 35.6 ± 1.03 mil/ml on days 1, 7, 15, and 30, respectively. Similarly, in Group IV, it was recorded as 35.6 ± 1.63 , 37.6 ± 1.63 , 37.1 ± 0.92 , and 39.9 ± 0.64 mil/ml on days 1, 7, 15, and 30, respectively. Nevertheless, despite improvement in Groups III and IV, the count remained significantly less in comparison to control ($P = 0.001$ and $P = 0.002$) [Figure 2].

Quantification of spermatogenesis and Sertoli cells

Following irradiation, patterns of effect were observed in number of germ cells and SC counts. Initially, sharp depletion in sperm count was evident in all the test groups with or without CBE treatment. Group II animals showed a gradual decline in sperm count as the day of observation progressed through the 1st day to the 30th day. However, Groups III and IV showed protection against irradiation from the 1st day of observation. The reversal sustained as the day progressed to the 30th day. Regardless of improved number of SG, spermatocytes, and spermatids in animals treated

with CBE (CB and CB-AgNPs), the number was found to be significantly less in comparison to control (Group I) [Table 4]. SCs showed rapid restoration in Groups III and IV; nonetheless, restoration in Group IV was distinctively immediate and highly responsive to CB-AgNP treatment. The changes in SC count was nonsignificant for Group IV animals when compared with control ($P = 0.053$) [Table 4].

Histopathological study of testis in comparison with stereological analysis

The histology of testis of Group I showed curved or oval seminiferous tubules with the epithelium-containing SCs and germ cells of various stages covering the complete spermatogenesis. The basal lamina was thick showing a closer association with SG and SCs. Structures of SG were oval in shape, resting on the basement membrane. Germ cell differentiation appeared normal, and the spermatocytes and spermatids were prominent with well-defined nuclei and granular cytoplasm. The type A and type B SG can be distinguished in pattern. SC cytoplasm showed closer association with germ cells and the elongated spermatids; lumen contained mature spermatozoa. The interstitium had distinct Leydig cells and intertubular elements, observed with round, granular, and prominent nucleus [Figure 3]. Observations in group II and group III were comparable to control and remained similar throughout the investigation period, i.e., 1–30 days [Figure 3].

Following irradiation, group II animals commonly showed disrupted epithelium causing disoriented germ cells, mostly and largely empty tubules, pyknotic nuclei, and intertubular edema in all 20 animals at each time interval. On the 15th day postexposure, SG and spermatocytes appeared to have fallen disoriented into the intratubular vacuoles (witnessed in four animals). On the 30th day postexposure, epithelium-containing SCs were still disoriented, however, little organization seemed to have taken place with the undifferentiated SG and spermatocytes (observed in two animals). In comparison to stereological analysis, the histological architecture of group II showed mostly anticipated observation. The count of all germ cells falls into inner zones and distinctly deviated polygonal structure was evident [Figure 3]. Partially filled lumen was also visible, indicative of insufficient spermatogenesis.

Similar, results were observed in group III and group IV animals with slightly improved cellular architecture. Disruption in the epithelium was observed on the 1st day

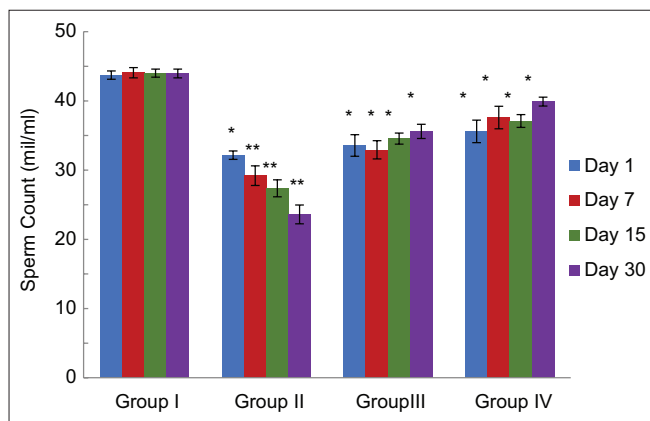


Figure 2: Epididymal sperm count of mice in experimental groups (values are presented in mean \pm standard error). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant, highly significant and extremely significant differences, respectively, against group I (control)

Table 3: Respective testis weight (g) of mice in experimental groups (values are presented in mean \pm standard error)

Group	Days 1		Days 7		Days 15		Days 30	
	Left	Right	Left	Right	Left	Right	Left	Right
Group I	111.57 \pm 1.32	112.21 \pm 1.34	111.91 \pm 1.58	110.31 \pm 1.62	112.16 \pm 1.66	112.45 \pm 1.69	111.83 \pm 1.59	112.99 \pm 1.57
Group II	75.39 \pm 1.50**	74.54 \pm 1.52**	56.72 \pm 1.42**	55.47 \pm 1.45***	55.58 \pm 1.25***	57.33 \pm 1.21***	50.84 \pm 1.77***	49.65 \pm 1.74***
Group III	81.44 \pm 0.72*	80.19 \pm 0.73*	63.20 \pm 0.86**	62.56 \pm 0.82**	71.80 \pm 1.62**	73.69 \pm 1.61**	88.88 \pm 0.62*	88.91 \pm 0.66*
Group IV	89.84 \pm 1.82*	90.48 \pm 1.85*	68.34 \pm 1.83**	67.48 \pm 1.84**	78.60 \pm 1.40**	77.59 \pm 1.42**	96.72 \pm 1.21*	94.15 \pm 1.20*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant, highly significant, and extremely significant differences, respectively, against Group I (control)

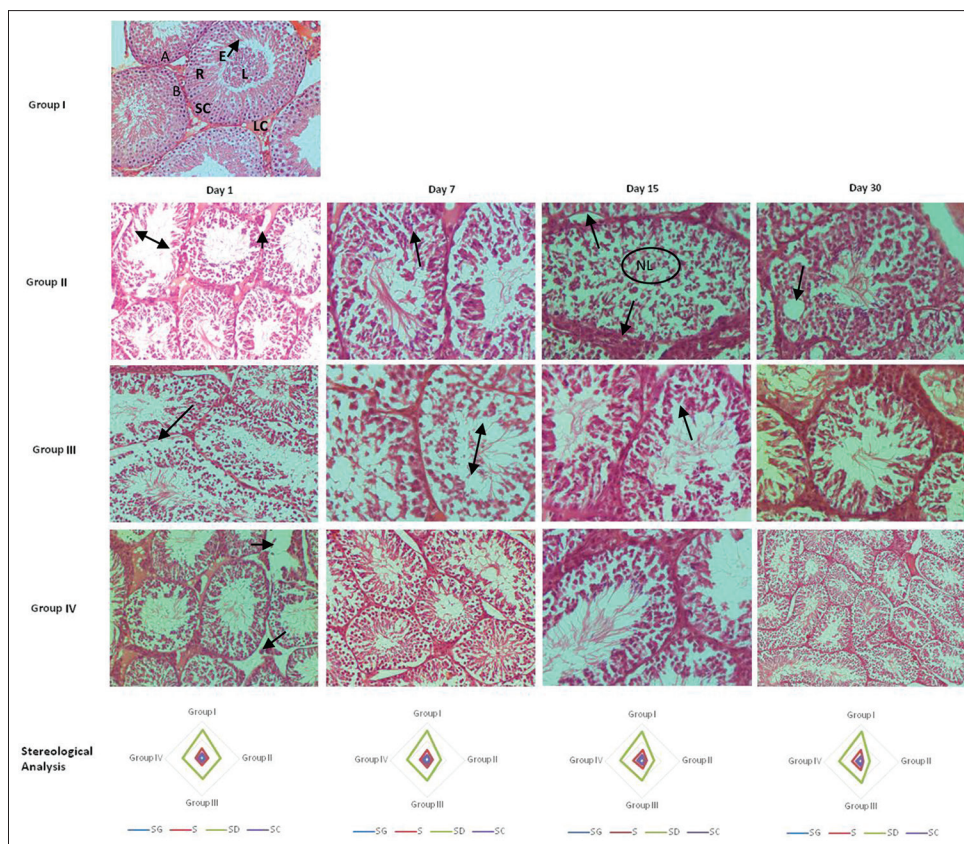


Figure 3: Histological architecture of testis of group I (control) and test groups (II–IV) at various observational days (1, 7, 15 and 30). When compared to control group II animals showed emptied lumen, disintegrated epithelium, vacuole formation, and spermatogonia falling away from Sertoli cells. Leydig cells were found dilated and vacuolation was evident. On the 15th day of observation, most seminiferous tubules lost luminal area (no lumen) and spermatids and spermatocytes were completely disoriented (magnification $\times 400$; H and E). Types A and B spermatogonia (A and B); Spermatocytes (S); Round (R) and Elongated (E) spermatid; Lumen (L); Sertoli cells (SC); Leydig cells (LC)

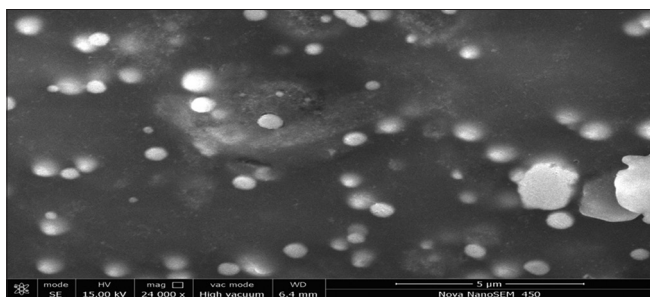


Figure 4: Standard error of the mean image of *Chlorophytum borivilianum* root extract conjugated silver nanoparticle

and 7th day in all animals of each group, however, from the 15th day onward, partial integration was observed in epithelium-containing SCs (observed in 3 animals of group III and 4 animals of group IV). Waves of SG, spermatocytes, and spermatids were observed in both groups (in all 20 animals of each group), respectively. Insufficient-to-partial spermatogenesis was observed in all animals during entire period of investigation in group III and group IV animals [Figure 3]. Stereological evaluation of group III and group IV animals indicated better counts for germ cells and SCs comparing to group II. It was also evident that corners representing group III and group IV were not in the

control zone, however, better spermatogenesis was evident comparing to group II. Histologically, there was not much difference visible between groups V and VI; it was also evident in stereological radar plot.

Discussion

There are many studies available which report on errors in radiation oncology.^[30-32] Besides, normal therapeutic intervention with ionizing radiation also has mild-to-moderate side effects, causing significant damage to various tissues and organs.

Effects of radiation on male reproductive organs are well documented.^[13] Testis is reported as most radiosensitive tissue which showed significant alteration at dose levels as low as 0.15–0.3 Gy.^[33] It was observed that there was a significant loss of testicular weight following gamma radiation and the study clearly indicated loss of testicular weight without restoration over observational days. A study was done in accordance with Gong *et al.*,^[9] who reported that irradiation with 2 Gy gamma-rays led to reduction of testicular weight significantly ($P < 0.05$). Resumption in body weight or testicular weight of irradiated animals until the 30th day was not observed, however, it is not confirmed that the declination

Table 4: Stereological evaluation of germ cells and Sertoli cells in control and test groups (values are presented in mean±standard error; counts of germ cells are in numbers)

Day	Group I	Group II	Group III	Group IV
Spermatogonia				
1	21.36±1.50	12.71±1.14	17.47±1.28	16.52±1.31
7	23.17±1.50	10.49±1.18	15.75±1.34	18.41±1.36
15	22.46±1.46	9.96±1.09	18.37±1.41	19.35±1.48
30	25.84±1.62	7.85±1.04	16.80±1.42	20.56±1.45
P	-	0.003**	0.008**	0.001**
Spermatocytes				
1	51.28±4.28	34.36±4.12	38.86±4.20	36.29±4.34
7	53.73±4.30	28.37±3.98	40.91±4.33	39.33±4.48
15	58.15±4.42	22.54±3.73	42.70±4.49	47.56±4.54
30	59.24±4.44	11.76±3.65	44.82±4.54	49.37±4.59
P	-	0.009**	0.000***	0.001**
Spermatids				
1	151.66±3.64	96.56±3.41	111.96±3.10	106.88±3.48
7	154.26±3.67	72.19±3.28	109.71±3.12	109.67±3.57
15	156.44±3.74	61.24±3.15	105.64±3.08	115.54±3.61
30	158.33±3.71	44.51±3.23	114.18±3.19	119.56±3.72
P	-	0.003**	0.000***	0.000***
Sertoli cell				
1	26.43±2.59	19.98±2.17	22.45±2.43	23.65±2.44
7	27.65±2.68	14.30±2.26	23.63±2.46	26.14±2.49
15	29.90±2.72	11.52±2.14	21.27±2.62	27.96±2.52
30	28.25±2.75	8.77±2.26	24.81±2.76	28.55±2.60
P	-	0.008**	0.012*	0.053

*P<0.05, **P<0.01, ***P<0.001 indicate significant, highly significant, and extremely significant differences, respectively, against Group I (control), SE: Standard error

of both body weight and testicular weight was permanent as possibly long-term observation may have slower resumption in irradiated animals. A study by Newman *et al.*^[34] claimed that the amount of loss of weight following irradiation and following eating and dietary habit leads to restoration of weight.

A study also revealed severe damages in histological architecture of testis at 6 Gy radiation dose level. Damages by irradiation specifically reduced the size of lumen of seminiferous tubules. SG appeared falling into lumen, and in some cases, no lumen was present at the center of seminiferous tubules. This distortion in spermatogenesis was also confirmed by cauda epididymal sperm count which indicated a significant reduction in sperm production. A study by Gong *et al.*^[9] revealed that following irradiation at dose 2 Gy, the epithelial height of seminiferous tubules was decreased significantly when compared to control. Furthermore, the report mentions a decrease in the diameter of seminiferous tubules, which is in accordance with the results of this study. Likewise, the overall spermatogenesis was also affected; stereological analysis confirmed a reduced number of germ cells and SCs following gamma irradiation.

Nearly 60% of cancer patients receive radiation therapy; thus, associated toxicity and adverse effects pose a significant

concern.^[35] Only a limited number of compounds and agents have been identified to protect against radiation-induced injuries. The use of CB against various medical conditions in South East Asia is highly acclaimed. The study of CBE’s protective effect against gamma radiation revealed an interesting outcome. It is observed that an apparent difference in body weight and testicular weight between irradiated animals treated with CBE (CB and CB-AgNPs) and animals irradiated without any treatment. Better initial toleration against the gamma radiation followed by quick recovery during 30-day observational period was noticed in the study. Many compounds have shown a protective effect against chemotherapy to reduce adverse side effects such as amifostine, glutamine, pentoxifylline, benzydamine and sulfasalazine.^[9] It was predicted that 50 mg/kg body weight of CB root extract has a protective effect against radioactivity.

The histological architecture of testis following initial irradiation insult indicated similar damages in animals administered with 50 mg/kg body weight of CB root extract to that of animals irradiated without any treatment. Nevertheless, a rapid organization was later assumed in the testicular tissues. Partially filled lumen was also evident through histological evaluation. A study also revealed that regardless of high-dose (6 Gy) radiation animals those treated with CB and CB-AgNPs indicated continued spermatogenesis. This assumption was also reconfirmed through stereological evaluation of germ cells. Complete resumption of SCs following initial depletion by the 30th day of observation was found in animals those were treated with CB-AgNPs. The reason behind complete resumption of numbers of SCs is due to elevated efficacy of CB root extract through nanonization and biotagging with AgNP [Figure 4]. Principally, nanostructures have the ability to preserve, target and bypass first-pass metabolism; thus, it increases the bioavailability.^[36,37] The improvement in histological architecture was also evident through cauda epididymal sperm count, which reflected better count every passing observation day. It was assumed by stereological analysis that except for group II, both CBE-treated groups (III and IV) maintained lower but steady spermatogenesis.

Although the protective activity of CBE and CB-AgNPs against radiation-induced testicular injury was observed, both treatment groups failed to restore complete resumption of spermatogenesis. Disorientation of germ cells was still evident in testicular histology of animals treated with CBE. The number of germ cells also could not resume back to normal range by the 30th day of observation and remained significantly lower comparing to control. It was, however, expected that longer duration of observational period may have resumed complete histological architecture. It was also hypothesized that continuous treatment pre- and postradiation may have also elevated the efficacy of CBE.

Conclusion

The present study confirmed that 50 mg/kg body weight of *Chlorophytum borivilianum* root extract has a protective

effect against gamma radiation. The study also confirmed that nanonization of extract and its tagging with AgNP increases extract's bioavailability. Thus, radiation-induced testicular injury in CB-AgNP-administered mice was comparatively lower and subsequent recovery was faster.

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Conflicts of interest

There are no conflicts of interest.

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