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Ameliorating Effects of Silver Nanoparticles Synthesized Using *Chlorophytum borivillianum* against Gamma Radiation Induced Oxidative Stress in Testis of Swiss Albino Mice

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Abstract*—Chlorophytum borivillianum* root extract (CBE) was chosen as a reducing agent to fabricate silver nanoparticles with the aim of studying its radioprotective efficacy. The formation of synthesized nanoparticles was characterized by UV–visible analysis (UV–vis), Fourier transform infra-red (FT-IR), Transmission electron microscopy (TEM), Scanning electron microscope (SEM). TEM analysis showed particles size in the range of 20-30 nm. For this study, Swiss albino mice were selected from inbred colony and were divided into 4 groups: group I- control (irradiated-6 Gy), group II- normal (vehicle treated), group III- plant extract alone and group IV- CB-AgNPs (dose of 50 mg/kg body wt./day) administered orally for 7 consecutive days before irradiation to serve as experimental. CB-AgNPs pretreatment rendered significant increase in body weight and testes weight at various post irradiation intervals in comparison to irradiated group. Supplementation of CB-AgNPs reversed the adverse effects of gamma radiation on biochemical parameters as it notably ameliorated the elevation in lipid peroxidation and decline in glutathione concentration in testes. These observations indicate the radio-protective potential of CB-AgNPs in testicular constituents against gamma irradiation in mice.

Keywords*—Chlorophytum borivillianum,* gamma radiation, radioprotective, silver nanoparticles.

I. Introduction

THE use of nanoparticles in synthesizing medical products is attracting a significant attention in recent time because of their potential application as therapeutics, diagnostics, surgical devices and nano-medicine based antimicrobial agents [1]-[3]. Nowadays, cerium oxide nanoparticles, yttrium oxide nanoparticles and few more are gaining popularity in the field of radioprotection, as these were found to possess antioxidant properties [4], [5].

Ionizing radiation generates oxidative stress which causes large-scale destruction and damage to various essential biomolecules. Disorders related to reproductive health became prominent issues in recent times after numerous reports of adverse effects on ionizing radiation on reproductive functions [6]. Oxidative stress is a major factor in male infertility. Since the cells of spermatogenic lineage are vulnerable to radiation-induced reactive oxygen species (ROS). This makes testes a highly radiosensitive organ with wide range of radiosensitive germ cells [7]. Under normal conditions, the testis is protected by an elaborate array of antioxidants enzymes and free radical scavengers, in order to support the dual action of this organ as germ cells spermatogenic [8] as well as Leydig cells steroidogenic function [9]. However, a wide variety of endogenous and exogenous factors exist that are capable to disturb these defenses and compromise the male fertility by generating free radicals in testes. It is within this context that we studied convergence of radiation with nanotechnology. Most applications in nanomedicine harness the unique chemical and physical properties. These particles can be employed to sense, image, measure, and manipulate biologic processes and functions.

Nanoparticles (NPs) have been shown to easily trespass biological membranes such as epithelia and the walls of very small capillaries. Thus, by following systemic administration, NPs can easily move throughout the body, potentially affecting number of different tissues [10]. For example, in addition to passing through the blood-brain barrier [11], NPs have been shown to penetrate the blood-testis barrier and reach the seminiferous epithelium[12]. Thus, due to this advantage, *Chlorophytum borivillianum* (family: Liliaceae) was selected which constitutes an important class of Ayurvedic herb known as Rasayana, its antioxidant approach to disease management has been reported [13] along with its immunostimulatory and adaptogenic properties [14] and it is used to cure impotency and sterility. It contains protein, rootfibre, saponin, sapogenins, vitamins, fructans, fructo-oligosaccharides, acetylated mannans and phenolic compounds [15]. Its root shows anti-aging [16] anti-diabetic [13], anti-inflammatory [17], antipyretic [18], aphrodisiac [19] and immunomoduladory activity [14]. The objective of the conducted study was to investigate the protective role of biosynthesized AgNP using *Chlorophytum borivilianum* against oxidative stress in testis of swiss albino mice.

II. Materials and Methods

A. Materials

The *C. borivillianum* tuber roots were collected directly

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from local market. Sigma Aldreich (USA) provided $AgNO_3$ (99.98%) as a silver precursor. Solutions were freshly prepared and kept in dark to avoid any reaction.

B. Root Extract Preparation

The dried *C. borivillianum* root tubers powder 5 g was boiled in 100 ml of distilled water for 1 hour at 70 °C. The extract was cooled to room temperature filtered through Whatman No. 1 filter paper (pore size 25 µm). Further final aqueous extract (CBE) was stored at 4 °C in refrigerator.

C. Synthesis of AgNPs

The AgNPs were synthesized using a constant volume of the plant extract under various experimental conditions. Aqueous solution of 1 mM $AgNO_3$ was prepared and used for the synthesis of AgNPs. 5 ml of *Chlorophytum borivillianum* aqueous extract is mixed with 95 ml of $AgNO_3$. The appearance of reddish brown color indicates the formation of AgNPs.

D. Physicochemical Characterization of AgNPs

UV-vis spectroscopy: After formation of biosynthesized silver nanoparticles (CB-AgNPs), the absorbance of the CBE and CB-AgNPs was measured over the wavelength range of 300 to 800 nm. The preparation of Ag-NPs was studied by UV-Vis spectroscopy (Systronics Double beam spectrophotometer 2203).

FT-IR spectroscopy: With the help of FT-IR, possible biomolecules can be identified, which are responsible for the reduction of Ag+ ions and capping of the bioreduced Ag-NPs synthesized using root extract. The FT-IR spectra were recorded over the range of 400-4000 cm^{-1} using Shimadzu IR affinity. After complete bioreduction of Ag+, the *C. borivillianum* root-powder extract was centrifuged at 15,000 rpm for 20 min. to isolate the Ag-NPs from other compounds.

TEM and SEM measurements: SEM analysis of AgNPs was done using Nova nano FESEM 450 (FEI). TEM analysis of CB-AgNPs was done using the Tecnai G^2 20 (FEI) S-Twin operating at 200 kV. Placing a drop of CB-AgNPs water dispersion on a carbon coated copper grid and drying at room temperature prepared samples.

E. Animal Care and Handling

The animal care and handling were according to the guidelines of CPCEA, New Delhi, India. 6-8 weeks old Swiss albino mice weighing 23 ± 2 gm from an inbred colony were used in the study. They were kept under standard laboratory conditions viz. proper room temperature and light conditions (14 and 10 hrs of light and dark time period, resp.). The animals were given standard mice feed (from Ashirwad Industries, Chandigarh, India) and water *ad libitum.* Antibiotics such as tetracycline were also given once in two days, to prevent unnecessary infections.

F. Source of Irradiation

For irradiation, Cobalt therapy unit of Cancer treatment Centre, Department of Radiotherapy, SMS Medical College and Hospital, Jaipur, India was selected where unanaesthestized mice were restrained in well-ventilated boxes and exposed whole body to gamma radiation (6 Gy) with the distance (SSD) of 77.5cm from the source to deliver the dose rate of 1.07 Gy/ min.

G. Dose Selection of CB-AgNPs

The optimum dose selection of *C. borivilianum* root extract was decided on the basis of previously performed experiments in our own laboratory (20). Among the doses 50 mg/(kg bw·day) was selected for the study.

H. Experimental Design

Adverse effects of gamma rays were evaluated by selecting Swiss albino mice from an inbred colony and were divided into following groups

- *Group I (normal):* Mice of this group were given distilled water (as vehicle) through oral gavages once in a day for 7 consecutive days.
- *Group II (irradiated control):* Mice of this group were given distilled water for 7 days (as in Group-I) and then exposed to 6 Gy dose of gamma radiation. This group served as irradiated positive control.
- *Group III (negative control):* (CB+IR) Mice of this group were treated with 50 mg/kg b. w.t/day of CB (root extract) dissolved in double distilled water through oral gavage for 7 consecutive days and then were exposed to 6 Gy dose of gamma radiation.
- *Group IV (experimental):* (CB-AgNPs+IR) Mice of this group were given 50-mg/kg b. w.t/day of CB-AgNPs dissolved in double distilled water and were then exposed to 6 Gy gamma radiations.

I. Autopsy Schedule

Animals from all the above groups (I, II, III & IV) were continuously observed till 30 days for their weight change, any sign of sickness, any visible abnormalities, and mortality. All animals were autopsied at 1, 7, 15, and 30 days post treatment and irradiation for the evaluation of biochemical variations in testes.

J. Biochemical Analysis

Testes were used for biochemical analysis to measure lipid peroxidation [21] and glutathione levels [22] at each autopsy interval in all above groups.

K. Statistical Analysis

The results obtained in the present study were expressed as the mean ± SEM. Statistical analysis (ANOVA) followed by Tukey's multicomparison test was applied to find significant difference between irradiated control and experimental group.

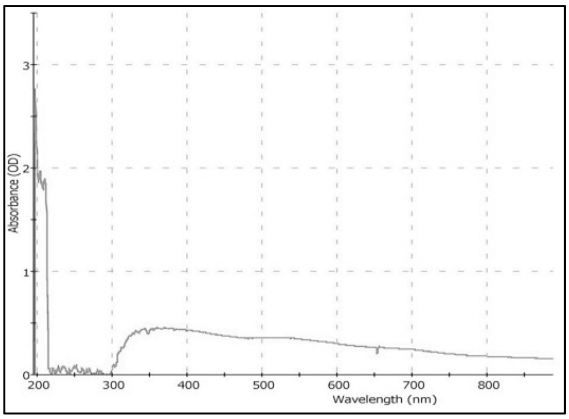
III. Results and Discussion

Photosynthesized AgNps are extensively used in various drug deliveries and drug carrier systems, as they are efficient for active and passive targeting of drugs. These AgNPs can carry small drug molecules or large biomolecules such as proteins, DNA, or RNA and can release these therapeutic agents to a target site for effective therapy. In the present

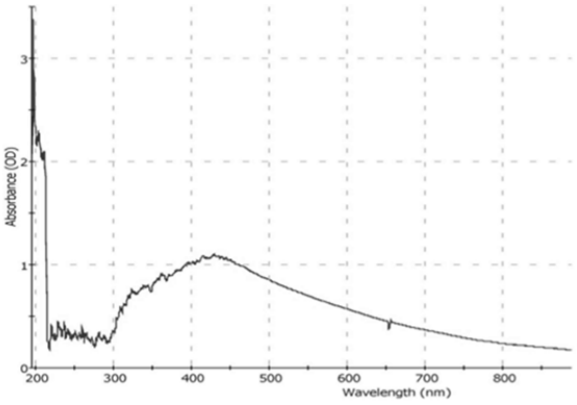
study, silver nitrate and plant extract were used for the biosynthesis of CB-AgNPs. During synthesis, color of reaction mixture changes from white to brown, which is the primary indicator of formation of AgNPs. This occurs due to the surface plasmon resonance or in other words, collective oscillations of the conduction electrons confined to AgNPs. Lastly, the approach employed in the production of these materials (AgNPs) is low cost and ecofriendly [23].

A. UV-Vis. Spectroscopy Analysis

Primary characterization of CB-AgNPs was done by UV-Spectroscopy. The absorption spectrum of the AgNPs depicts in Figs. 1 (a) & (b). As shown in figure, a characteristic and well-defined SPR band for AgNPs was obtained at around 433 nm. Mulvaney et al. reported similar results where AgNPs exhibit well defined SPR bands at around 433 nm [24].



(a)



(b)

Fig. 1 (a) UV-vis spectrum analysis of CBE; 1(b) UV-vis spectrum analysis of *Chlorophytum borivillianum* conjugated AgNPs

B. FTIR Analysis

In our study, FTIR bands of plant extract were inferred at 3300, 2100 and 1650, 1140 cm^{-1} (in black color), Fig. 2 and FTIR spectrum of the AgNPs shows peaks at 3500, 2200, 1660, 1550, 1500, 1480, 1146 cm^{-1} (in red color). The strong band at 3400 cm^{-1} in CBE was attributed to the O-H stretching band of alcohols and phenols which shifted to 3500 cm^{-1} in AgNPs apparently because of protein binding [25].

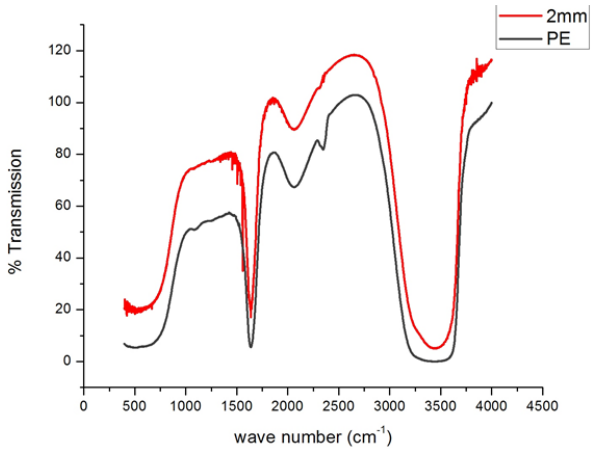


Fig. 2 FT-IR spectra for the *Chlorophytum borivillianum* root-powder extract (black) and CB-AgNPs (Red) after biosynthesis reaction

C. SEM Analysis

SEM analysis gave a detailed insight of morphology of CB-AgNPs. It shows that the synthesized AgNPs were well dispersed without aggregation and posses' spherical shapes.

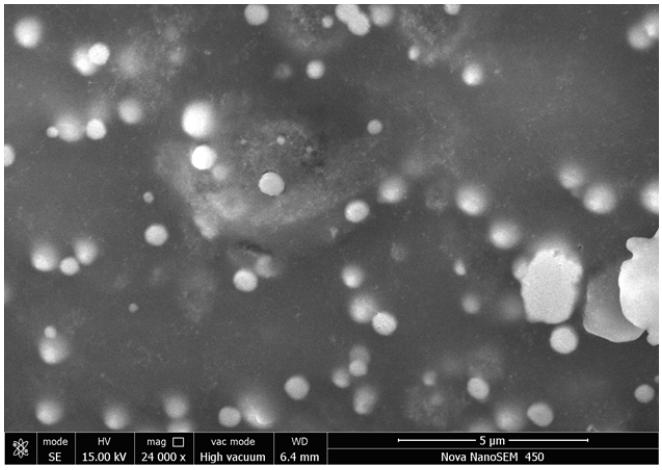
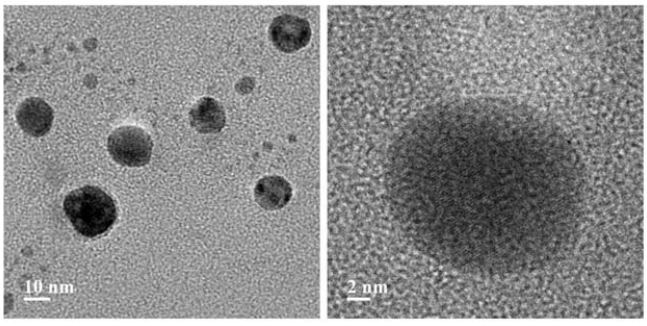


Fig. 3 SEM images of *Chlorophytum borivillianum* conjugated AgNP

D. TEM Analysis



(a) (b)

Fig. 4 TEM image of CB-AgNPs: (a) high resolution image of nanocrystals at 10 nm (b) high resolution image of a single nanocrystal at 2 nm

TEM was used to obtain essential information on primary

NP size and morphology. TEM results were in sync with SEM micrographs revealing distinct, uniform spherical shapes of CB-AgNPs that 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 to 30 nm with an average particle size of 25 nm (Fig. 3).

E. Body Weight and Testes Weight

The mice exposed to 6 Gy gamma rays exhibited some signs of radiation sickness within 2-3 days after radiation exposure. Irradiated ones showed reduction in food and water intake and irritability within 15 days of exposure. No symptoms of radiation sickness and mortality were observed in CB-AgNPs pretreated group. Animals given DW (Group I), CB alone (Group III) and CB-AgNPs (Group IV) showed a significant increase in body and tissue weight.

In group III, CB alone supplemented group animals were healthier in comparison to the control as indicated by statistically significant ($p < 0.005$) higher body weights. In group III, initially, gradual decline in body weights by 14.6% was recorded up to day 7-post irradiation; thereafter, significant increase in the body weights was noticed indicating recovery. In group IV (CB-AgNPs treated + irradiated group) similar pattern of initial weight loss was observed but much lesser in comparison to other groups, and soon after recovery was noticed, with a difference that the body weight was significantly ($p < 0.001$) higher at all the *post irradiation* intervals studied. This indicates that supplementation of CB-AgNPs before irradiation protected the body weights to an extent.

CB-AgNPs pretreated animals outstand the hazardous effects of irradiation according to results observed. Decrease in body weight was significantly recovered in 30 days post irradiation. These results suggest the possible protection of gastro-intestinal tract by CB-AgNPs, as radiation induces gastrointestinal damage due to which decrease in food and water intake is observed, as also described by Griffiths et al. [26]. Similarly, CB-AgNPs have also shown positive effect on testicular weight. All irradiated animal experience testicular weight loss may be due to the damages in germinal epithelial cells or sertoli cells. Similar results with decrease in testicular weights were also observed by Lin et al. [27] and Jagetia et al. [28] in irradiated mice.

After day 7, Group II and III animals experienced significant testicular weight loss, whereas group IV animals within 30 days achieved near to healthy testicular weights. This can be attributed to possible aphrodisiac and antioxidants properties present in CB-AgNPs.

F. Lipid Peroxidation

LPO levels in testes were found to be significantly ($P < .001$) higher in irradiated control animals at all autopsy intervals as compared to Group III and IV. LPO levels were increased in CB-AgNPs but the values were significantly ($P < .001$) lower than their respective irradiated controls. Thereafter, a significant ($P < .001$) and continuous fall in LPO was observed on remaining intervals and only CB-AgNPs gained normal level.

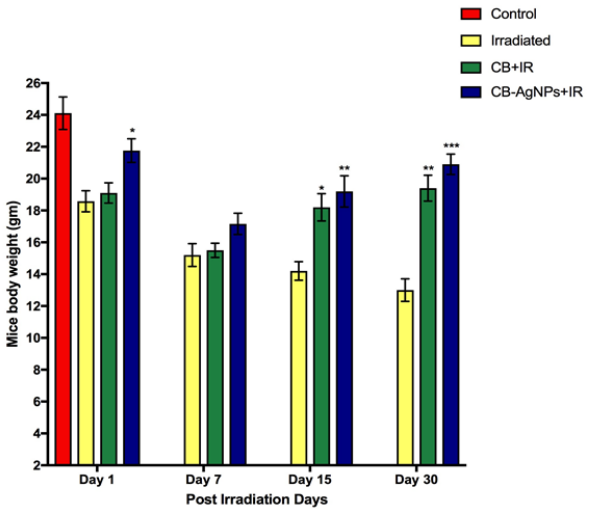


Fig. 5 Ameliorating role of CB-AgNPs on body weight in Swiss albino mice exposed to 6 Gy gamma radiations. The values are means ± SEM

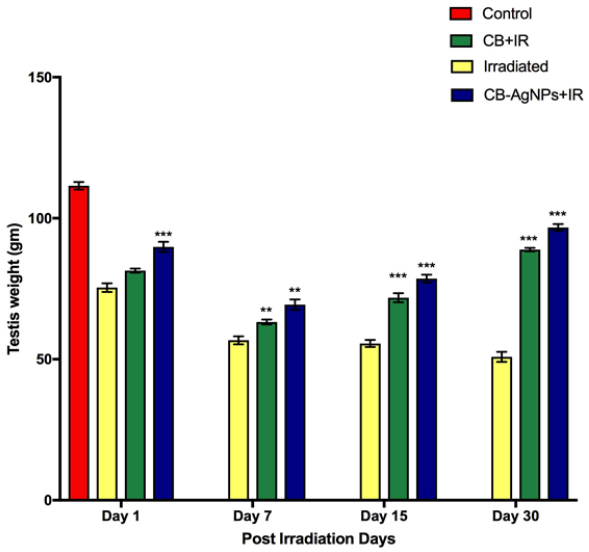


Fig. 6 Ameliorating role of CB-AgNPs on testicular weight in Swiss albino mice exposed to 6 Gy gamma radiations. The values are means ± SEM

In the present study, both irradiated and experimental groups showed a gradual and continuous augmentation in the level of LPO till day 15 postirradiation, which may be due to increased oxidative stress and decrease in body weight, testis weight, and protein value after radiation exposure as also suggested by Yadav et al. [29]. Preirradiation treatment of CB-AgNPs significantly decreased LPO at all the autopsy intervals in comparison to irradiated control, which is in accordance to our belief that, one of the possible mechanisms of radioprotection by CB-AgNPS may be owing to the scavenging of free radicals generated by radiation exposure.

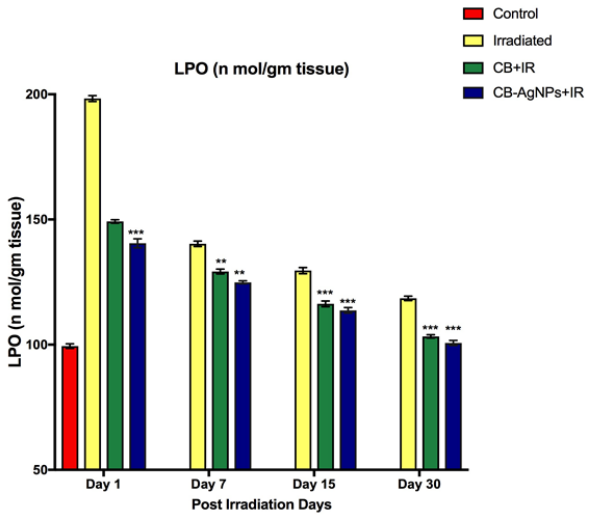


Fig. 7 Protective role of CB-AgNPs on LPO levels in testis of Swiss albino mice exposed to 6 Gy gamma radiations. The values are means ± SEM

Glutathione: Reduced glutathione (GSH) measured as acid soluble sulfhydryl group (-SH) in tissue homogenate showed a significant decline after exposure to 6 Gy gamma radiations. However, CB-AgNPs pre-treated groups caused a significant elevation.

GSH can provide protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation [30]. GSH works as protector by scavenging free radicals and restoring various damaged molecules by hydrogen donation as well as reduction of peroxides [31].

Depletion of GSH was lower in CB-AgNPs pretreated animals as the animals of this group had a high level of phytoantioxidants after CB-AgNPs administration. Afterwards, GSH tended to be utilized less due to the declining impact of radiation and endogenous reparative homeostatic activity.

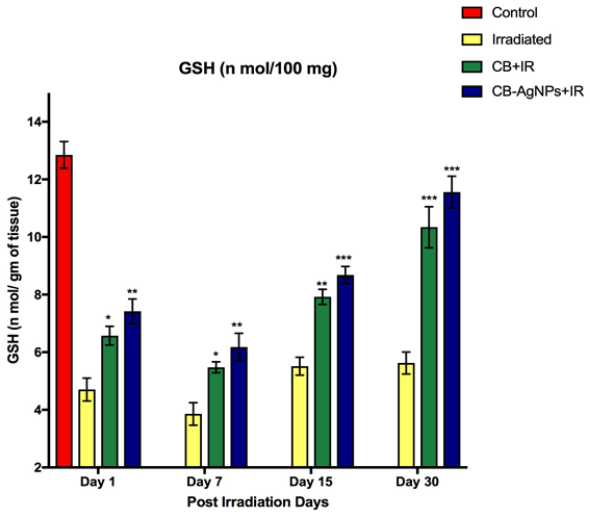


Fig. 8 Protective role of CB-AgNPs on GSH levels in testis of Swiss albino mice exposed to 6 Gy gamma radiations. The values are mean ± SEM

Previous findings [32] suggested that radiation-induced depletion of glutathione resulted in an enhanced LPO, and this was also observed in testicular tissue by Faidan et al. [33]. Our results suggest that CB-AgNPs contains a very efficient and natural antioxidant system (NAO) that is enough in preventing oxidative damage, which is mediated through the generation of ROS. The exact mechanism of action is not known yet. However, scavenging of free radicals and increased concentration of endogenous antioxidant system may be considered as important mechanisms of protection provided by *Chlorophytum borivillianum* against radiation-induced damage to the testicular tissue. Thakur et al. [14] impart the support to this contention by the experiments on free radical scavenging, where CB has been found to scavenge OH and O^{2-} radicals and also exhibit immunomodulatory properties.

Inbuilt properties of *Chlorophytum borivillianum* present in CB-AgNPs might have increased the intracellular level of reduced glutathione, and stimulated the immune systems that could have provided protection against the radiation-induced mortality. Since significant radioprotection is obtained at a nontoxic dose of CB-AgNPs, it may have an advantage over the contemporary radioprotectors available at experimental level. Further, intensive studies are required to unravel the underlying mechanism of such plant against ROS-mediated damage for improving its efficiency better.

IV. Conclusion

Based on the above promising results, it can be concluded that AgNPs synthesized from root extract of *Chlorophytum borivillianum* (CB-AgNPs) have the potential to mitigate the oxidative stress produced due to 6 Gy dose of gamma radiation. This was reflected in the form of body weight, tissue weight, significant decline in LPO levels, and an enhancement in GSH content in CB-AgNPs pretreated mice as compared to the irradiated control group.

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