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Research Article

ANTI-RADIATION EFFICACY OF SILVER NANO-PARTICLES PREPARED FROM *CHLOROPHYTUM BORIVILLIANUM* ROOT EXTRACT

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A B S T R A C T

In present study, we evaluated radio-protective potential of green synthesized silver nanoparticles (CBE-AgNPs) against sublethal γ-radiations. The 50 mg/kg b.wt. dose of CBE-AgNPs was found most effective on the basis of survivability assay performed in male Swiss albino mice. This dose was used as treatment for further experimentation. Animals were then divided in three major experimental groups; a) radiation control, b) radiation+ plant extract and c) radiation+ CBE-AgNPs. Mice were autopsied at regular intervals to study the alterations in various biochemical parameters in liver homogenate. The results of this study showed that pretreatment of irradiated mice with CBE-AgNPs and plant extract produced a significant increase in liver glutathione, superoxide dismutase, catalase and total protein level compared to corresponding values of irradiated control. In contrast, liver lipid peroxidation level was significantly declined in irradiated mice groups pretreated with CBE-AgNPs and plant extract in comparison to gamma irradiated untreated mice. Results also demonstrated that AgNPs synthesized from *C. borivillianum* possess radioprotective efficacy significantly higher than the plant extract. In conclusion, CBE-AgNPs offer a physiological approach to ameliorate the radiation induced biochemical alterations. In addition, they provide sufficient protection against radiation injuries in liver of Swiss albino mice.

INTRODUCTION

Nanotechnology is one of the most dynamic fields of research in modern material science. This is the promising field that deals with specific substances having the size ranging in nanoscale. Nanomaterials often demonstrate distinctive and considerably changed aspects in contrast to their large sized counterparts.

Nanoparticles can be broadly synthesized by three major routes i.e. physical, chemical and biological approaches. Physical approaches comprise evaporation-condensation and laser ablation technique; chemical approaches take account of chemical reduction by organic and inorganic reagents; and biological approaches include reduction by microorganisms and plants products (Singha *et al.,* 2014). Nowadays, physical and chemical methods are not in trend due to their high cost and limited accessibility. Chemicals can lead to the formation of certain toxic compounds that are absorbed on the surface of nanoparticles and these nanoparticles generate negative impacts on medical applications (Prabhu and Poulose, 2012). Since, toxic chemicals are not used during the fabrication of nanoparticles in green chemistry therefore it is environmentally benign and more compatible than other methods in pharmaceutical and biomedical applications (Geetha *et al.,* 2012). Currently, nanoparticles can be biologically synthesized by using plant extract, bacteria, fungi and enzymes. Moreover, using a plant extract can decrease the cost of preparation and eliminate the need for any special culture preparation and isolation technique compared with using bacteria and fungi for fabrication of nanoparticles (Saxena *et al.,* 2010).

Silver nano-particles have a high specific surface area, high thermal stability, huge mechanical strength as well as antibacterial activity (Murphy, 2008; Jain *et al.,* 2009; Udapudi *et al.,* 2012). Many reports are available on plant mediated synthesis of silver nanoparticles, particularly leaf extracts of *Azadiracta indica* (Sarkar *et al.,* 2004), *Oscimum sanctum* (Ramteke *et al.,* 2013), *Magnolia kobus* (Song *et al.,* 2012), *Mangifera indica* (Sarsar *et al.,* 2013), *Calotropis gigantean* (Sivakumar *et al.,* 2011), *Annona squamosa* (Singaravelu *et al.,* 2013) and seed extracts of *Coriandrum sativum* (Manisha *et al.,* 2014) pomegranate (Chauhan *et al.,* 2011).

Nowadays, researchers are focusing on herbal products as possible anti-radiation drugs because of their nontoxic effects in comparison to synthetic drugs. In the past years different plant extracts and herbal preparations have been reported to

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have radio protective action such as garlic (Gupta, 1988), ginseng (Pandey *et al.,* 1984), Podophyllum (Goel *et al.,* 1999), Ocimum (Uma Devi *et al.,* 2000) and Mentha (Samarth and Kumar, 2003) have been found to have an advantage over the synthetic compounds in terms of low/no toxicity at the effective dose.

It is obvious that several plants comprise the potential to demonstrate a broad range of physiological activities that may be essential to the alleviation of ionizing radiation-induced damage in biological systems. In Ayurveda, Safed musli (*Chlorophytum borivilianum*) is considered as white gold and typically used as herbal medicine by native communities of India. It is an eminent medicinal plant of India. Various pharmacological studies on *Chlorophytum borivilianum* have revealed a wide range of pharmacological activities, most importantly aphrodisiac, immunomodulatory, antimicrobial and anticancer activities (Khanam *et al.,* 2012).

Based on the properties of silver nanoparticles and significance of *Chlorophytum borivilianum*, the present study has been undertaken to investigate the radio protective efficacy of green synthesized Ag-NPs from root extract of *Chlorophytum borivilianum* against radiation induced oxidative stress.

MATERIAL AND METHODS

Animal Care and Handling

Six weeks adult male Swiss albino mice, weighing 25±2 gm, from an inbred colony were used for the present study. These mice were maintained under controlled conditions of temperature and light (light: dark, 10h: 14h). The animal care and handling was done according to the guide-lines set by INSA (Indian National Science Academy, New Delhi, India). The Departmental Animal Ethical Committee (DAEC) approved this study. Mice were housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed (procured from Hindustan Levers Ltd, India) and water *ad libitum*.

Source of Irradiation

Animals were irradiated by a Co^{60} source (Bhabatron-II TAW telecobalt machine) in the cobalt therapy unit at Cancer Treatment Center, Department of Radiotherapy, SMS Medical College and Hospital, Jaipur, India. Unanaesthestized mice were restrained in well-ventilated boxes and exposed whole body to gamma radiation at the dose rate of 1.07 Gy/min from the source to surface distance (SSD), that is, 80 cm.

Preparation of plant extract

Tubers of *C. borivilianum* were collected commercially from local market. Roots were air-dried, powdered and extracted in double distilled water by continuous stirring at 50°C. The extract was filtered with whatman paper and the filtrate dried for 48 hrs in oven at 50°C and then a powdered form was obtained. This powder form was dissolved in double-distilled water just before oral administration.

Preparation of nanoparticles

CBE-AgNPs were prepared by mixing and stirring 2mM $AgNO_3$ solution with Whatmann paper filtered aqueous root extract of *chlorophytum borivillianum* in 1:10 ratio. After 4 hrs of reaction the transparent solution of $AgNO_3$ changed into orange colored solution. Change in color of solution clearly indicated reduction of Ag^+ to Ag^0 and formation of silver nanoparticles. To get the nanoparticles free from residual organic contents of CBE, the solution was centrifuged at 1000 rpm and the supernatant was taken out by aspiration. This supernatant was then ultra centrifuged at 10000 rpm for 30 minutes. Thereafter the supernatant was discarded from the centrifuge tube and the loose precipitate formed at the bottom was dispersed in a small volume of de-ionized water. It was then dried at room temperature and used as CBE-AgNPs for anti-radiation study.

Determination of optimum dose

The mice were divided into five groups of 10 animals each and given NP's (25, 50, 100, 150, 200 mg/kg b.wt.) orally for 7 consecutive days. Thirty minutes after the final administration the animals were exposed whole body to 10 Gy gamma radiations. All animals were observed for 30 days for any sign of sickness, morbidity and mortality. The optimum dose thus obtained was used for further experiments.

Experimental design

To evaluate the radio protective potential of CBE, mice were randomly selected from an inbred colony and divided into following groups.

- ***Group I:*** Radiation alone treated (Irradiated control): Animals of this group were given double distilled water (DDW) through oral gavages once in a day for 7 consecutive days. On 7th day, mice were irradiated with 6 Gy gamma radiations.
- ***Group II:*** CBE + Radiation (Experimental 1): Mice of this group were treated with CBE (dose equivalent to CBE-AgNPs) dissolved in distilled water through oral gavage for 7 consecutive days once daily before 6 Gy gamma irradiation.
- ***Group III:*** CBE-AgNPs + Radiation (Experimental 2): Mice of this group were treated with optimum dose of CBE-AgNPs (50 mg/kg b.wt.) dissolved in distilled water through oral gavage for 7 consecutive days once daily. After an hour on 7th day, mice were irradiated with 6 Gy gamma radiation.

Animals were necropsied at different time intervals viz. 1st day, 7th day, 15th day and 30th day post irradiation to determine the changes in various parameters of liver.

Biochemical analysis

Liver was perfused with saline and removed from the sacrificed animal. The tissue was then homogenized in ice cold buffer to yield 10% (w/v) homogenate. This homogenate was then centrifuged to remove debris, and the supernatant was immediately used for further experimentation. The following biochemical parameters were measured in the liver of Swiss albino mice:-

Glutathione (GSH): Reduced glutathione was estimated in liver as total non-protein sulphydryl group by the method as described by Moron *et al.* (1979).

Lipid peroxidation (LPO): Lipid peroxidation estimated in liver spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method given by Okhawa *et al* (1979).

Superoxide dismutase (SOD): Superoxide dismutase was assayed utilizing the technique of Marklund and Marklund (1974).

Total plasma proteins: Estimation of protein was based on the method proposed by Lowery *et al.* (1951).

Catalase: It was estimated in liver homogenate according to the protocol given by Aebi (1984).

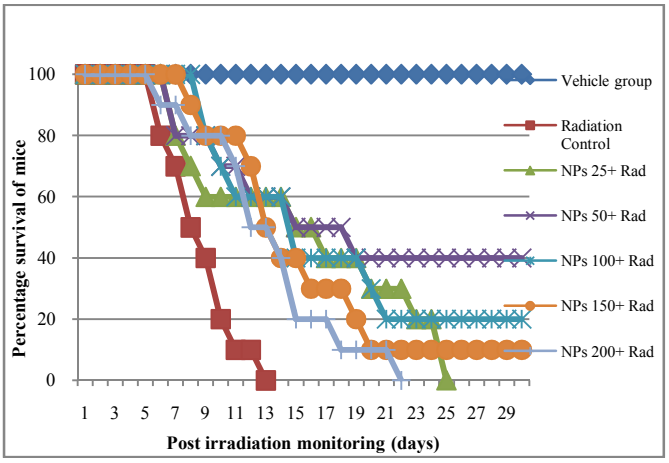
Statistical analysis

The results obtained in the present study were expressed as mean ± SEM. The statistical differences between various groups were analyzed by ANOVA and the significance was observed at the $p<0.001$, $p<0.01$ and $p<0.05$ level. All groups were compared by Bonferroni's multiple comparison tests.

RESULTS

Determination of optimum dose of CBE-AgNPs

Different doses of Ag-NPs (25-200 mg/kg b.wt.) were found to be effective against sub-lethal 10 Gy gamma irradiations, but maximum survivability (40%) was observed at 50 mg/kg b.wt. dose group (Graph 1). This dose was selected as optimum dose for further experimentation.

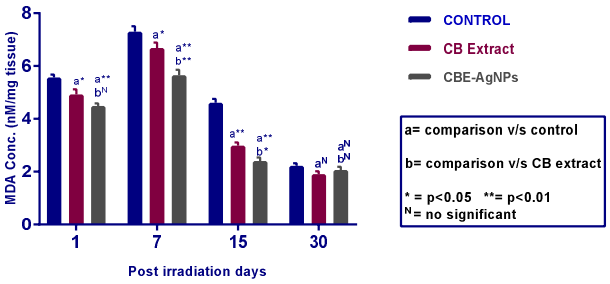


Graph 1 Thirty (30) days survival of mice with or without *C. borivillianum* AgNPs after exposure to sub-lethal 10 Gy gamma radiations.

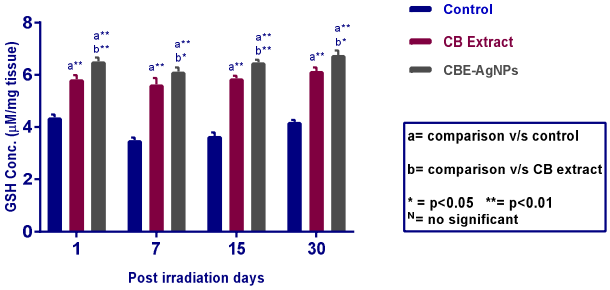
Biochemical assay

In lipid peroxidation assay, TBARS level of mice pretreated with CBE (group II) and CBE-AgNPs (group III) was found significantly lower in compare to irradiated-control (group I) at all the follow up intervals and nearly achieved their normal values on day 30. The comparative study between CBE and CBE-AgNPs groups showed that the extent of lipid peroxidation in CBE-AgNPs pretreated animals was found significantly lower ($p<0.001$) than CBE pretreated group on 15th and 30th day post irradiation (Graph II).

In reduced glutathione assay, both experimental groups (CBE and CBE-AgNPs groups) showed significantly higher values of GSH at all autopsy intervals when it was compared to irradiated-control (group I). Further, a significantly higher values ($p<0.05$) of GSH in CBE-AgNPs animals was also observed in compare to CBE group at all autopsy intervals (Graph III).

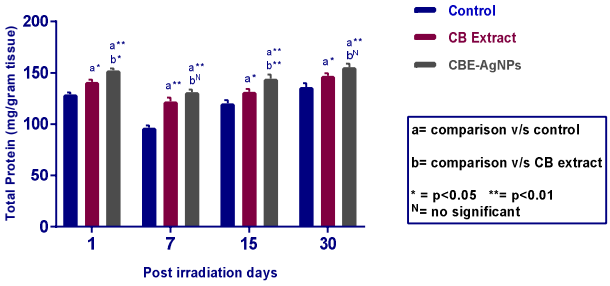


Graph II Variations in LPO (TBARS level) in liver of Swiss albino mice.



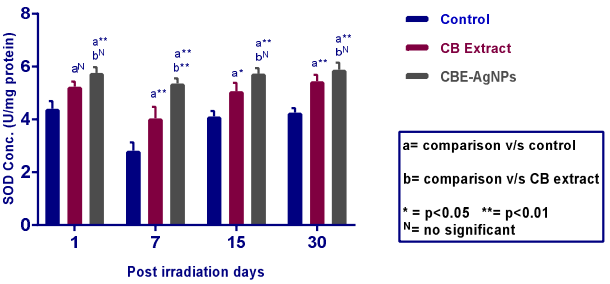
Graph III Variations in GSH concentration in liver of Swiss albino mice

In total liver protein measurement assay, total protein content in tissue of CBE pretreated group and CBE-AgNPs pretreated group was observed significantly higher at all follow up days compare to the irradiated control (group I) animals. The difference between total protein content of CBE and CBE-AgNPs animals was also found significant ($p<0.05$) (Graph IV).



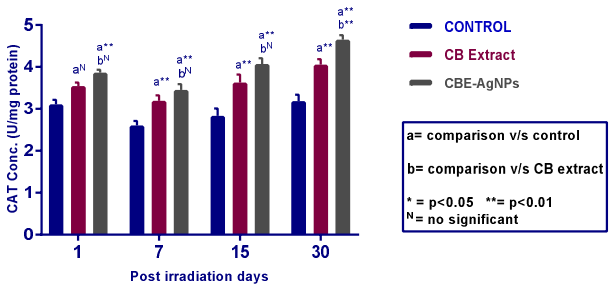
Graph IV Variations in Total protein content in liver of Swiss albino mice

In Superoxide dismutase assay, animals supplemented with CBE (group II) as well as CBE-AgNPs (group III) before irradiation exhibited a higher ($p<0.05$) SOD concentration than irradiated control animals (group I). The values of SOD content in the CBE-AgNPs pretreated were significantly higher ($p<0.05$) on 7th day than corresponding CBE pretreated mice (Graph V).



Graph V Variations in SOD level in liver of Swiss albino mice.

In catalase assay, Catalase level of both experimental groups (group II and III) animals was found significantly increased throughout the experiment in compare to irradiated control animals (group I). Besides, a significant increase ($p > 0.05$) was also observed in CBE-AgNPs pretreated irradiated animals (group III) than CBE pretreated irradiated animals (group II) at all autopsy interval (Graph VI).



Graph VI Variations in catalase level in liver of Swiss albino mice

DISCUSSION

Radiation protection is an important aspect in radiotherapy research. Ionizing radiations such as X-rays, γ- rays etc. interact with biological systems and result in generation of reactive oxygen species (ROS). These ROS molecules then attack on various essential cellular macromolecules such as nucleic acids and membrane proteins etc. and cause damages which result in abnormal functionality of system (Anandjiwala *et. al.,* 2008).Various anti-oxidative materials confer radioprotection against free radical mediated oxidative stress (Devasagayam *et. al.,* 2004). Present study indicates that green synthesized silver nanoparticles obtained from *C. borivillianum* root extract may act as radioprotective agent and render protection against radiation induced oxidative damages. Similar results of nanoparticles mediated radioprotection were also observed by earlier researchers (Chandrasekharan and Nair, 2013; Ramachandran and Nair, 2011).

In survivability experiment, all animals of irradiated control group were found unwell and survived merely up to day 13th post irradiation. The principal cause behind this radiation induced quick mortality were post irradiation damages in gastrointestinal epithelium cells leading to improper absorption of nutrients and water (Jagetia *et al.,* 2003). The pattern of survival in different CBE-AgNPs groups was same as irradiated control group except the slow mortality rate. This obviously signifies the efficiency of CBE-AgNPs. This diminution in GI death may be due to the shielding of intestinal epithelium, which would have permitted appropriate absorption of the nutrients. Thus, pretreatment of mice with AgNPs provided protection against radiation sickness and mortality. Furthermore, we observed optimum radiation protection (40% survival) at 50 mg/kg b wt dose of CBE-AgNPs, although higher doses showed a decline in shielding action of AgNPs. These results were in favor of earlier study that believed radioprotective agent works precisely at a particular dose level, beyond this it may not be protective as much as it was (Thomson, 1962).

It is well known that ionizing radiations affect biological system by producing free radicals and reactive oxygen species which results in oxidative cellular damages viz. lipid peroxidation of cellular membrane. Various biological molecules which provide protection against these free radicals such as glutathione, superoxide dismutase (SOD), and catalase increase in quantity and strengthen DNA repair mechanisms to nullify these adverse effects (Prdhan *et al.,* 1973). In case of severe damages, these protective molecules are not capable of blocking all of the damage of intense radiation which results in reduction of these molecules along with increased lipid peroxidation (Shaban *et al.,* 2003; Van Der Vliet *et al.,* 1997; Zhang *et al.,* 2011). Thus, maintaining a balance between the rate of generation of radicals and scavenging of free radicals is an essential part of biological homeostasis.

Literature study has demonstrated that the antioxidant properties of plant compounds could be correlated with oxidative stress defense (Amarowicz *et al.,* 2004). In present study, we observed that the level of various biochemical parameters had a significant tendency to normal values for CBE pretreated as well as CBE-AgNPs pretreated group as compare to the irradiated control group. It may be due to intrinsic anti-oxidative makeup of CBE or green synthesized AgNPs. These compounds shield the cellular GSH from radiation induced diminution. This GSH executes its function against free radical induced oxidative damages by sustaining the reduce state of cellular protein thiol group. It results in reduction of lipid peroxidation level along with augmentation in total protein, SOD, CAT level by protection of these molecules from radiation induced denaturation (Bump and Brown, 1990). Our findings also detailed that anti-oxidative potential of CBE-AgNPs pretreated group was found significantly higher over CBE pretreated group. This may be due to the fact that green synthesized nano-particles are working here as anti-oxidative materials (Rana and Asia, 2016) and their greater surface area to volume ratio increase the pharmacological activity of drug over plant extract by attaching various anti-oxidative phytochemical constituents on small sized metal ions. (Shankar and Mohan, 2017).

CONCLUSION

This study discussed a novel approach for the protection of Swiss albino mice against radiation-induced oxidative damage in the liver by using green synthesized CBE-AgNPs. However, further studies are required on the mechanistic aspects of CBE-AgNPs which could be potent anti-radiation drug of the future.

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