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Research Article

RADIATION INDUCED OXIDATIVE STRESS IN LIVER AND ITS MITIGATION BY *CHLOROPHYTUMBORIVILIANUM* ROOT EXTRACT: *IN VIVO* STUDY

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ABSTRACT

In this study, protective effects of *C. borivilianum* root extract (CBE) against radiation-induced oxidative stress have been investigated in liver of Swiss albino mice. Adult mice selected from an inbred colony were administered with CBE (50 mg/kg body wt) for 7 days prior to treatment with 6 $GyCo^{60}$ γ-radiations. After irradiation, mice were sacrificed at different time intervals (i.e. day 1, 7, 15 and 30) to determine the alterations due to irradiation in hepatic lipid peroxidation (LPO), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and total protein contents and its probable amelioration by CBE. We found that, pretreatment of CBE significantly restored GSH (p<0.01), CAT (p<0.01), SOD (p<0.05) and total protein content (p<0.05) in liver and precluded from radiation induced augmentation in lipid peroxidation (p<0.01). In conclusion, it can be said that *C. borivilianum* root extract plays a significant role in radioprotection.

Keywords: CBE, γ-radiations, Radioprotection, CAT, SOD, GSH, LPO, Protein.

INTRODUCTION

Radiation induced injuriesare chiefly mediatedby various free radicals that affectsvarious molecular targets such as DNA, membrane lipids and proteins.The deleterious effects of radiation can now be strategically counterpoised by the use of many drugs and chemicals. However,chemicalradioprotectorsproducedhitherto are found noxious at their optimumdoses (Sweeney, 1979).Therefore, development ofreliable and non- toxic radioprotectors forhuman safety has been an area of immense interestfor radiation biologists. It is obvious that several plants have potential to mitigate radiation induced damages in biological systems(Samarth *et al.*, 2017; Jagetia, 2007). The use of plants and natural products as radio-protective agentsis advantageousoverother radioprotectors, as they are less toxic or practically non-toxic compared to the synthetic compounds.

Chlorophytumborivilianum(Liliaceae) is a conventional rare herb which has many therapeutic applications. Different pharmacological studies on tubers of *C. borivilianum*has indicated its antioxidative(Govindarajan*et al.*, 2005), immunomodulatory (Thakur *et al.*, 2006), antidiabetic (Panda *et al.*, 2007), antistress (Kenjale*et al.*, 2007), aphrodisiac (Thakur and Dixit, 2007), antimicrobial (Deore and Khadabadi, 2007), hypolipidemic(Visavadiya and Narsimhacharya, 2007), anti-inflammatory (Deore and Khadabadi, 2008), hypocholesteremic(Deore and Khadabadi, 2009) activities. Major therapeutic components of *C. borivilianum* are saponins,stigmasterol, Beta sitosterol, hecogenin,polysaccharides and mucilage.

In present investigation, an attempt has been made to study the protective effect of *C. borivilianum* tuber extract against gamma radiation induced oxidative stress in liver of Swiss albino mice.

MATERIAL AND METHODS

Animal Care and Handling

The animal care and handling was done according to the guide-lines set by INSA (Indian National Science

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Academy, New Delhi, India). The Institutional Animal Ethical Committee (IAEC) approved this study. Six weeks adult Swiss albino mice (*Mus musculus*), weighing 25±2 gm, from an inbred colony were used for the present investigation. These mice were maintained under controlled conditions of temperature and light (light: dark, 10h: 14h). They were provided standard mouse feed (procured from AshriwadPvt.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°Cand then a powdered form was obtained. This powder form was dissolved in double-distilled water (dose-50mg/kg b.wt.) just before oral administration.

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. Normal (Vehicle treatment): Animals of this group were given double distilled water (DDW) through oral gavage once in a day for 7 consecutive days.

Group II. CBE treated: Mice of this group were treated with CBE (50 mg/Kg b.wt.) dissolved in distilled water through oral gavage for 7 consecutive days once daily.

Group III. Radiation control: Animals of this group were given double distilled water (DDW) through oral gavages once in a day for 7 successive days. On the day 7^{th}, mice were irradiated with 6 Gyγ-radiations.

Group IV. CBE + Radiation treated: Mice of this group were treated with CBE (50 mg/Kg b.wt.) dissolvedin distilled water for 7 consecutive days (one time daily) before 6 Gyγ-irradiation.

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

Quantitative analysis

The body weight of each experimental animal was recorded just prior to autopsy to know the effects of different treatments. Animals were autopsied by cervical dislocation at different autopsy intervals. Liver was then perfused with saline and removed from the sacrificed animal and placed on a piece of filter paper to remove excess of moisture. The complete liver was then weighed and a part of liver tissue was homogenized in ice cold buffer to yield 10% (w/v) homogenate. This homogenate was then centrifuged and the supernatant was immediately used for the estimation of various biochemical parameters. Activity of SOD and CAT were estimated by the methods suggested by Marklund and Marklund (1974) and Aebi (1974) respectively whereas Lipid peroxidation estimation was done to assess MDA level in liver tissue by the method of Ohkhawa*et. al.,* 1979.Reduced glutathione was estimated as total non-protein sulphydryl group by the method as described by Moron *et al.* (1979). The total liver protein content was also determined through the protocol mentioned by Lowry *et al.* (1951).

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

Body weight

Body weight of irradiated control (group III) animals was significantly reduced ($p<0.001$) at all autopsy intervals as compared to the normal (group I).However, bodyweights of group IV animalsi.e. mice supplemented with CBE before irradiation were also reduced after irradiation but the reduction level was not as much as group IIIanimals. Body weight of group IV was found significantly higher ($p<0.01$) after 7^{th} day post irradiation compared to group III(Table 1).

Liver weight

In group I and group II, no significant changes in liver weight were observed.After irradiation in group III, Liver weight of micewas significantly increasedat all autopsy intervals with maximum increased on 7^{th} day post irradiation as compared to the normal (group I). However, statistically non-significant changes in liver weight was

evident in group IV animals when compare to group III up to day 15 post irradiation but at day 30 post irradiation a significantreduction ($p<0.05$) in liver weight was observed (Table 1).

Lipid Peroxidation assay

Lipid peroxidation product as reflected by thiobarbituric acid substances (TBARS) level in liver was augmented significantly (maximum at day 7) in group III animals at all the follow up intervals except day 30, as compared to group I ($p<0.001$). LPO level also increased in group IV up to day 7 but the values were significantly lower than group III ($p<0.01$). Both groupsnearly achieved the normal range of TBARS level on day 30 post irradiation (Figure 1).

Reduced glutathione assay

A significant difference ($p<0.001$) in the GSH content of liver was observed throughout the experiment between group III and group I animals. Group IV also showed a similar mode of variation in GSH throughout the experiment, but the observed values were significantly higher ($p<0.01$) compared to group III at all autopsy intervals (Figure 2).

Total protein assay

A significant reduction ($p<0.001$) in total liver protein was found in group III at all autopsy intervals in comparison to normal (group I). Similarly, total protein content in group IV was significantly higher at all autopsy intervals compare to the group III animals. The significant level was highest on 7^{th} day post irradiation ($p<0.01$) afterwards it was declined up to 30^{th} day ($p<0.05$) (Figure 3).

Superoxide dismutase assay

SOD concentration in irradiated control (group III) was found significantly lower compared to the group I ($p<0.001$). Animals supplemented with CBE extract (group IV) before irradiation exhibited a higher ($p<0.05$) SOD concentrationthan control (group III). In both irradiated group, a maximum decline in SOD concentration was observed on 7^{th} day(Figure 4).

Catalase assay

No significant difference in the catalase content of liver was observed among non-irradiated groups (group I and II animals) throughout the experiment. However, in group III animals, a statistically significant decrease ($p<0.001$) in catalase level was evident at all follow up intervals as compared to group I. CBE-treated irradiated animals (group IV) also showed a similar mode of variation in catalase throughout the experiment, but the observed values were significantly higher after day 7 as compared to group III (Figure 5).

Table 1. Variations in body weight and liver weight of Swiss albino mice after 6 Gy gamma irradiation in the presence or absence of *Chlorophytumborivilianum* root extract.

Parameters	Irradiated Groups	Post irradiation observations (Mean ± SEM)				Non-Irradiated Groups	Observations
		1^{st} day	7^{th} day	15^{th} day	30^{th} day		
Body weight (gm)	Group III (Control)	25.98 ± 0.432 (91.41%)***	22.84 ± 0.318 (80.37%)***	24.54 ± 0.353 (86.35%)***	25.24 ± 0.169 (88.81%)***	Group I (Normal)	28.42 ± 0.427 (100%)
	Group IV (CBE + IR)	26.79 ± 0.431 (94.26%)^{N}	24.37 ± 0.368 (85.75%)*	26.18 ± 0.335 (92.12%)*	27.03 ± 0.454 (95.11%)**	Group II (CBE only)	28.81 ± 0.402 (101.37%)^{N}
Liver weight (gm)	Group III (Control)	1.571 ± 0.0088 (106.20%)***	1.742 ± 0.0144 (117.75%)***	1.661 ± 0.013 (112.27%)***	1.625 ± 0.0157 (109.86%)***	Group I (Normal)	1.479 ± 0.0067 (100%)
	Group IV (CBE + IR)	1.550 ± 0.0073 (104.80%)^{N}	1.720 ± 0.0116 (116.32%)^{N}	1.630 ± 0.013 (110.18%)^{N}	1.572 ± 0.0100 (106.28%)*	Group II (CBE only)	1.500 ± 0.0076 (101.80%)^{N}

Statistical Comparison ($P< 0.05$ - *, $P< 0.01$ - **, $P<0.001$ - ***, N- no significant).

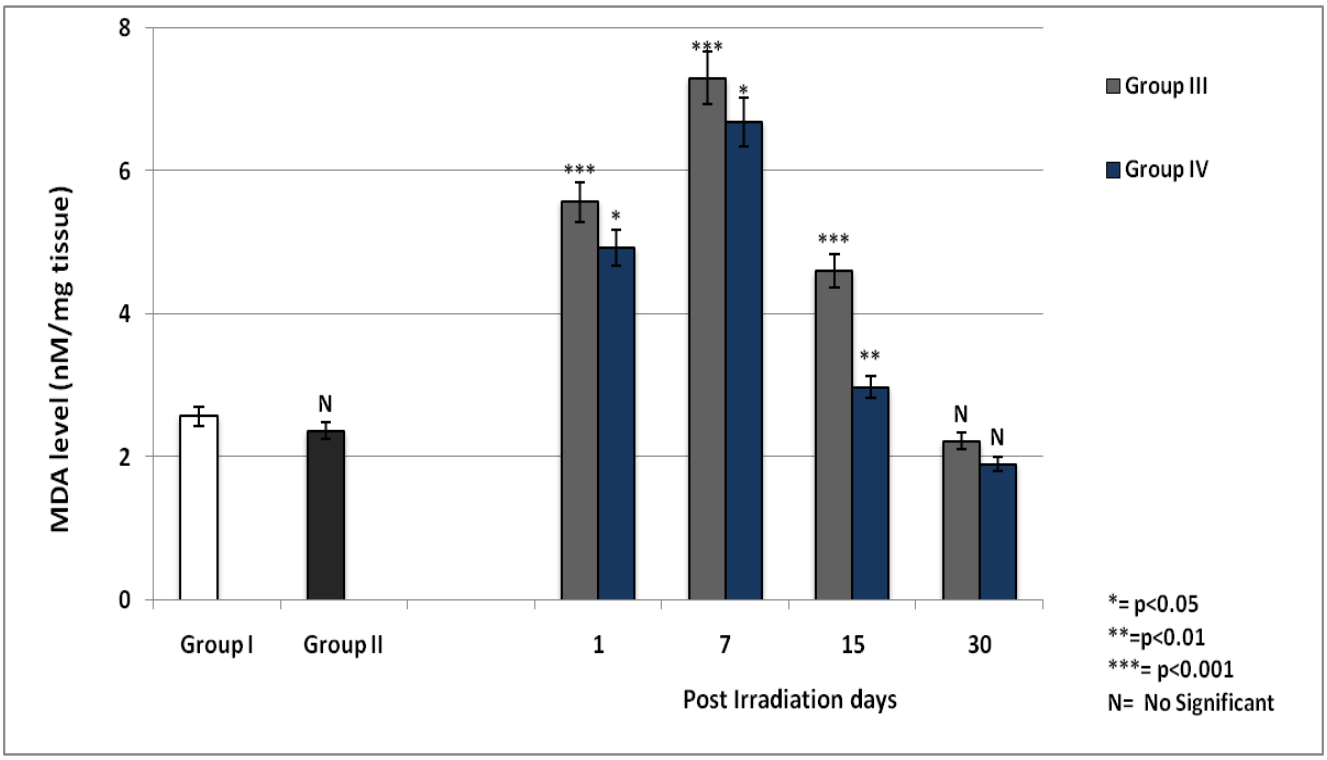

Figure 1. Variations in LPO (MDA level) in liver of Swiss albino mice after 6 Gy gamma irradiation in the presence or absence of*C. borivilianum* root extract.

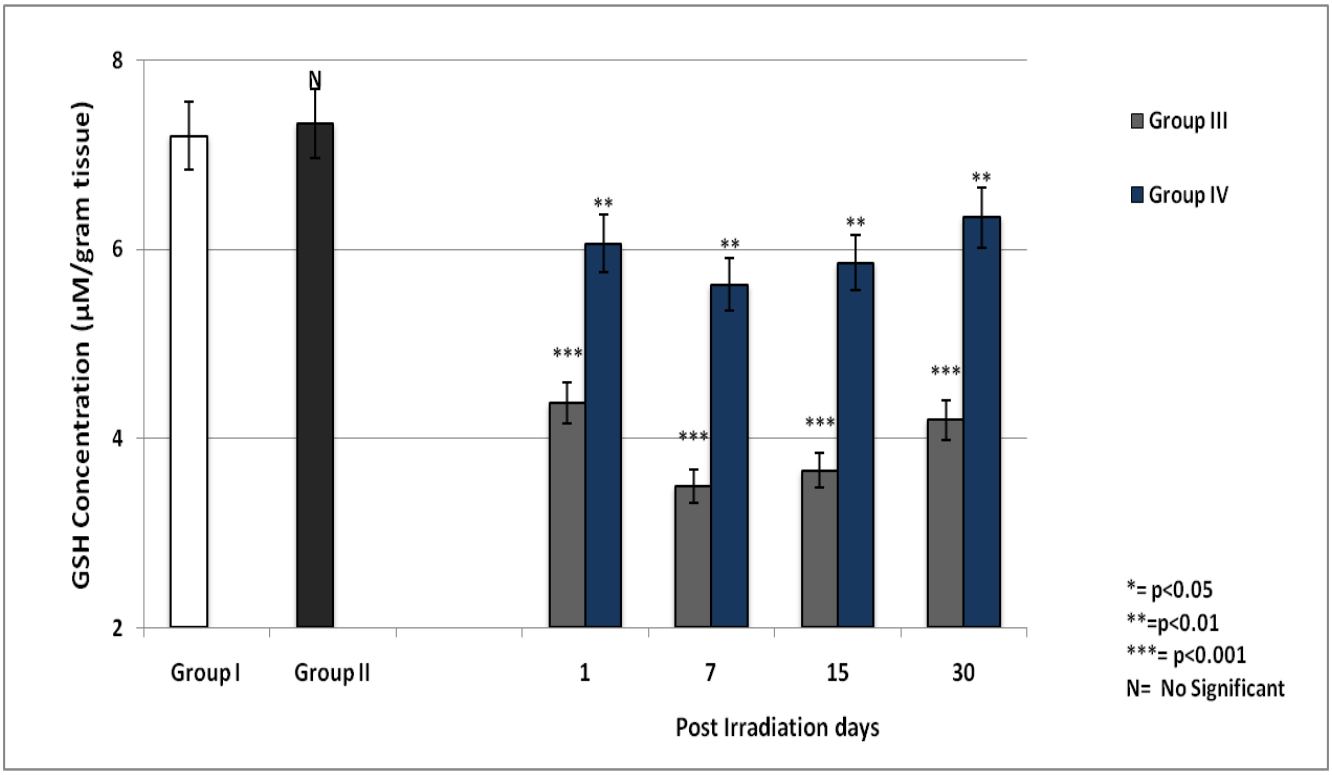

Figure 2. Variations in GSH concentration in liver of Swiss albino mice after 6 Gy gamma irradiation in the presence or absence of *C. borivilianum* root extract.

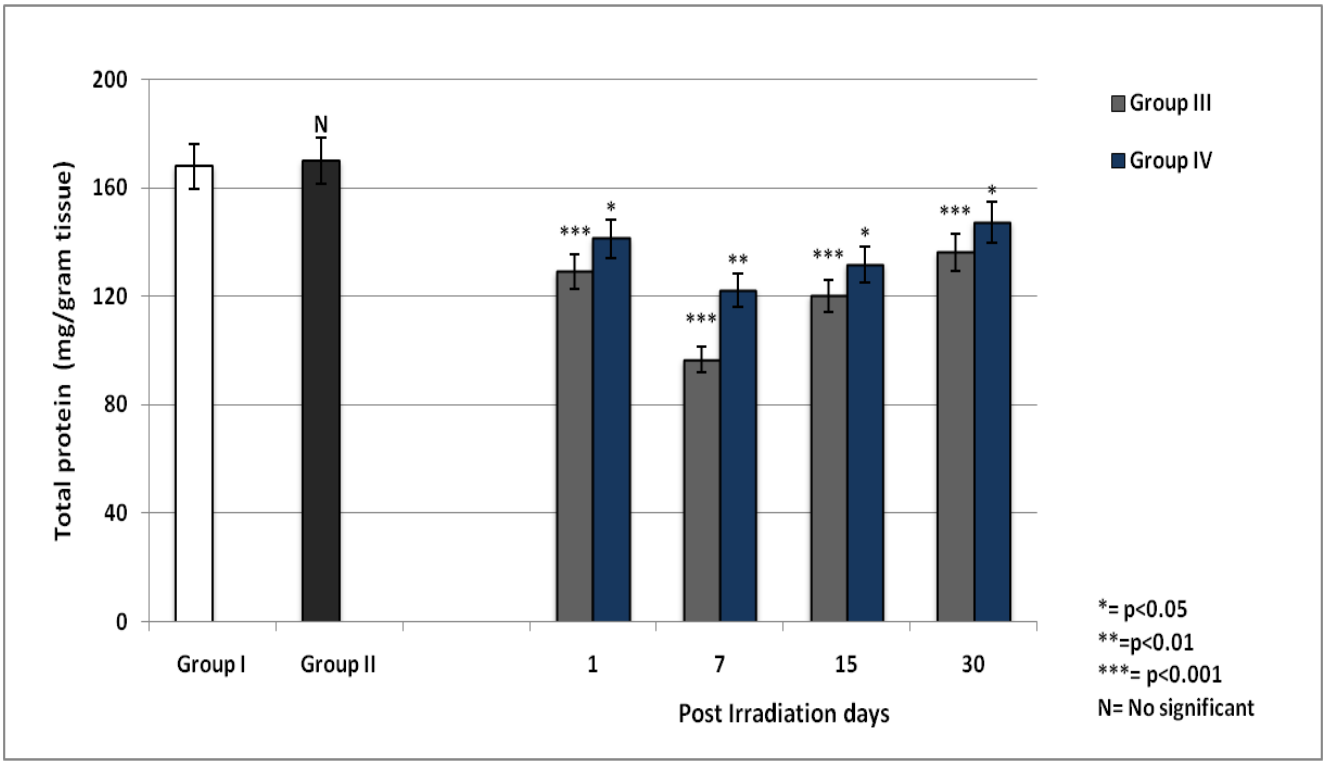

Figure 3: Variations in Total protein content in liver of Swiss albino mice after 6 Gy gamma irradiation in the presence or absence of *C. borivilianum* root extract.

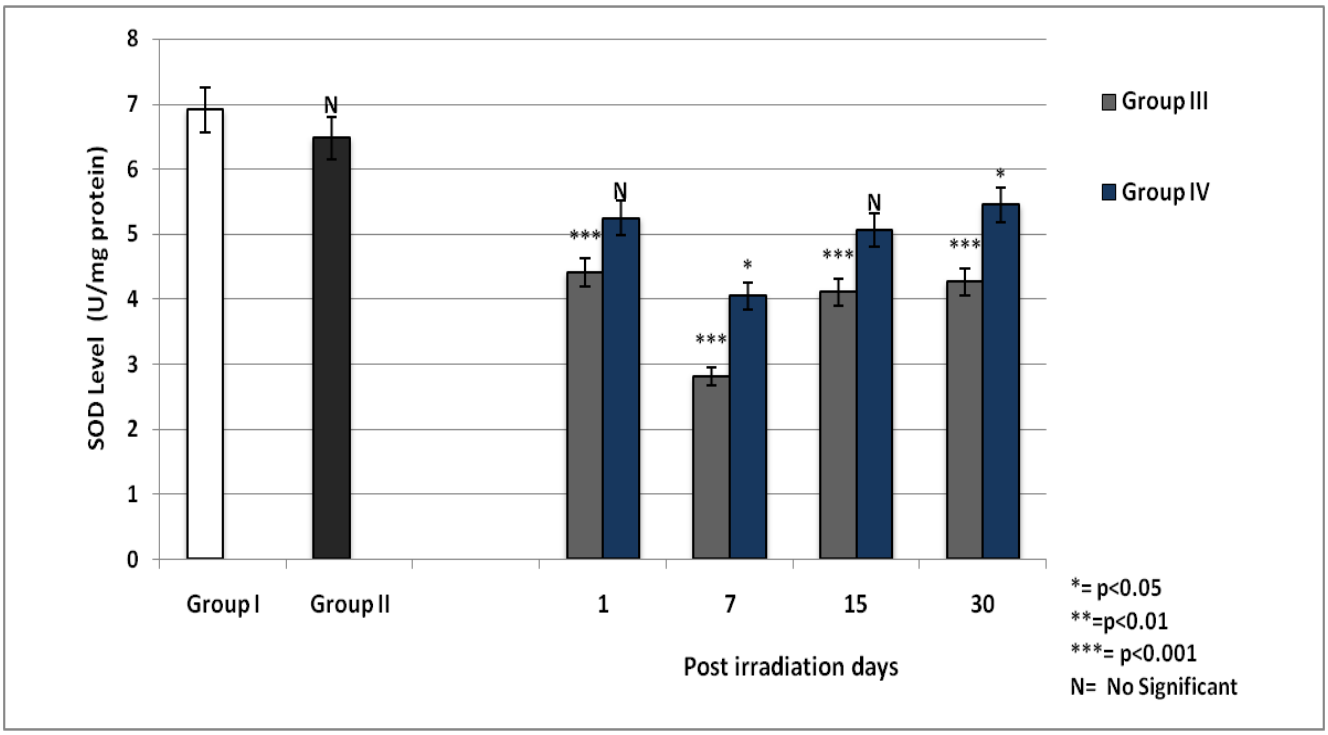

Figure 4. Variations in SOD level in liver of Swiss albino mice after 6 Gy gamma irradiation in the presence or absence of *C. borivilianum* root extract.

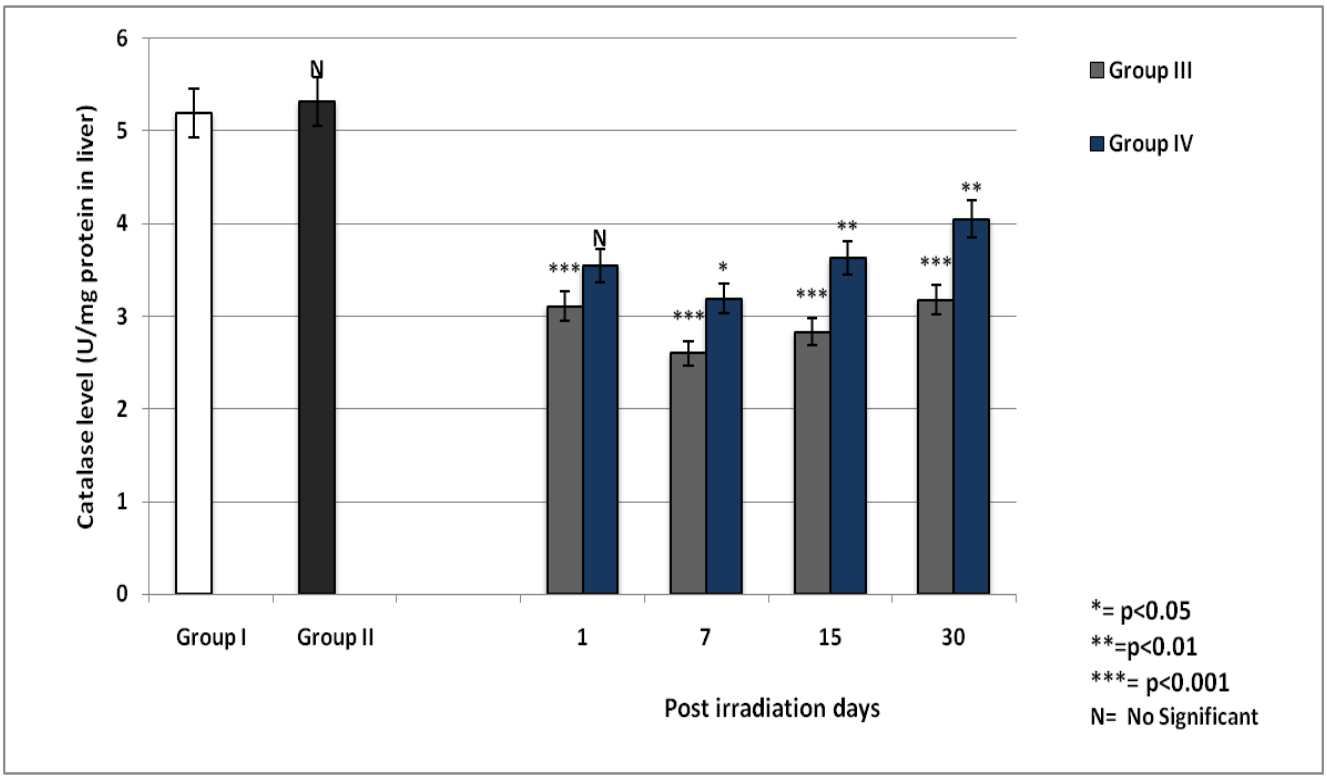

Figure 5. Variations in catalase level in liver of Swiss albino mice after 6 Gy gamma irradiation in the presence or absence of *C.borivilianum* root extract.

DISCUSSION

The major radiation-induced cellular oxidative damage is initiated by reactive oxygen species (ROS) such as superoxide, peroxide and hydroxyl radicals, generated by radiation reaction with tissue water (Zhao and Robbins, 2009). These free radicals targetcellular nucleic acid, causing their molecular modifications which results in improper segregation of chromosomes during cell division and ultimately radiation-induced mitotic death (Jonathan *et al.*, 1999; Nair*et al.*,2001). Variousbioactive compounds were studied against γ-radiations mediated toxicity i.e. ascorbate, melatonin, Vitamin E, carotenes and lipoic acid derivatives (Beckman and Ames, 1998).The extracts from some plants including *Panax ginseng* (Mansour, 2013), *Chemlliasinensis*(Pal *et al.*, 2013), *Zingiber officinalis* (Baliga, *et al.*, 2012) *Curcuma longa* (Nada *et al.*, 2012)*Zataria multiflora* (Hosseinmehr *et al.*, 2011)*Menthapiperta*(Baliga and Rao, 2010)have shown radio-protectiveeffects due to their anti-oxidative potential.

In this context, we observed a significant reduction in body weight in irradiation-controlanimals (group III) during entire experiment as reported byUrsoand Congdon(1957). Lamerton*et al.*(1953)explained that the general biphasic pattern of weight loss in the early phase may be due to the gastrointestinal damage by whole-body irradiation and in later phase due to decrease in water intake (Nakamura *et al.*, 1968). However, CBE pretreated irradiated animals (group IV) also showed radiation induced loss in body weight but values were significantly higher and reached normal by day 30 of post-irradiation. Samarth and Kumar(2003) also noted similar recovery patterninplant extract based radioprotection study.

Present study also demonstrated a significant reduction in GSH, CAT, SOD and total protein levelswhile a significant increase was found in LPO level after irradiation. These results are found in accordance with other relative studies on gamma radiations (Jindal *et al.*, 2006;Jagetia*et al.*, 2004; Sharma *et al.*, 2007).This may be due to the fact that when healthy (normal) cells are exposed to ionizing radiation, they cause the oxidative cellular damages in form of free radicals and ROS, which results in lipid peroxidation of cellular membrane. Free radicals protective system molecules such as glutathione,superoxide dismutase (SOD) and catalase increase and intensify DNA repair mechanisms (Pradhan *et al.*, 1973).In spite of these efficient repair mechanisms, they are unableto obstruct all of the damage of intense radiation which ultimately leads in reduction of these protective molecules i.e. GSH, CAT, SOD, and increased LPO level due to normal tissue death (Shaban*et al.*, 2003; Van der Vliet *et al.*, 1997; Zhang *et al.*, 2011). On the other hand, reduction in total protein level was found due to the adverse changes in structure of various enzymes and proteins by introducing carbonyl groups which leads to the oxidative modification of protein (Levin *et al.*, 1994).

Restoration ofall parameters studied (i.e. LPO, GSH, CAT, SODand total protein) to their respective normal level was observed inCBE pretreated group (group IV).

These findings arein consistent with previous plant mediated radioprotection reports (Krishna and Kumar, 2005; Sharma and Kumar, 2007; Sharma and Goyal, 2014).GSH, LPO and SOD levels act as an indicator of tissue oxidative stress hence restoration of these biochemical parameters to normal level indicates the defense potential of plant extract against radiation induced oxidativedamages.Amarowicz*et al.* (2004)also confirmed that various antioxidant properties of bioactivecompounds found in plants could be associated with oxidative stress defense. As we know that GSH is a versatile protector and executes its function through free radical scavangering, restoration of injuries by supplying singlet hydrogen ion, diminution in peroxidation and sustaining the reduce state of protein thiol group. Intrinsic anti-oxidative makeup of CBE in body shields the cellular GSH from radiation induced diminution,which results in reduction of lipid peroxidation level, augmentation in total protein, SOD, CAT level by protection of these molecules from radiation induced denaturation (Bump and Brown, 1990).

CONCLUSION

The results from the present investigation indicate that pretreatment with *C. borivillianum*root extract protects mice from γ-radiationsinduced alterations in oxidative stress parameters of liver which may be due to synergistic effects of the phytochemicals present in *C. borivillianum*root extract.

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