

## RADIOPROTECTIVE EFFECT OF *OPUNTIA ELATIOR* STEM EXTRACT AGAINST GAMMA-RADIATION INJURY IN SWISS ALBINO MICE

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### ABSTRACT

*Opuntia elatior* Mill. is a folklore medicinal plant and contains malate of manganese, resin, and many other compounds. The present study was planned to explore the preventive potential of a moderate dose of *Opuntia elatior* (OE) against toxicity induced by gamma radiation (6 Gy) in mouse spleen. A moderate dose of OE (10 mg/kg body weight) was given orally to adult mice, before whole body irradiation. The mice were irradiated whole body with 6 Gy gamma radiation with and without *Opuntia elatior* pretreatment. Chloroform and Ethanol extracts were given to two separate groups of mice. Mice were sacrificed at 1,3,5,7,10,14 and 30<sup>th</sup> days post-irradiation and the spleen was removed. Superoxide dismutase activity (SOD) and catalase activity were measured. As compared to chloroform extracts, ethanol extracts of *O. elatior* stem significantly increased the activities of CAT and SOD. Ethanolic extract of *O. elatior* was more effective than its chloroform extract. It may be due to the extraction of more powerful components of *O. elatior* in ethanol. In conclusion, the results suggested that ethanol stem extracts of *O. elatior* can be used as less expensive, non-toxic eco-friendly radioprotectors.

**Keywords:** Gamma Radiation, Spleen, Catalase Activity, Superoxide Dismutase Activity.

### Introduction

A gamma ray is a kind of high-frequency electromagnetic radiation consisting of high-energy photons that free electrons from molecules and ionizes them. It can penetrate the body and cause damage directly at a cellular level, or indirectly to water molecules, which then generate free radicals (Lee *et al.*, 2012; Wang *et al.*, 2015). Free radicals further result in oxidation, DNA damage, and inflammation in the body (Fernandez-Viadero *et al.*, 2016; Haase, G.M. and Prasad, 2016; Wang *et al.*, 2015). As a result of modern society's stronger emphasis on radiation to carry out various tasks, such as nuclear energy, and its effects on biological systems, exposure to radiation exposure is now inescapable. As a result, it is essential to develop effective radioprotective agents (Azab *et al.*, 2022). Many synthetic radio-protective drugs and compounds have been investigated in the recent past for their effectiveness against the damaging effect of radiation therapy but have some side-effects and toxic nature. Therefore, in search for less toxic and more reliable radio-protectors, interest in natural and herbal plant-based radio-protective compounds are of great interest and researches have been carried out for the high effectiveness and low-toxicity based plant extract as radio-protectors (Nadi *et al.*, 2019).

Plants are the richest source of many compounds like flavonoids, polyphenols, polysaccharides, alkaloids, vitamins, tannins, etc., as important secondary metabolites, and these natural products have a key role in scavenging the free radicals and are defensive against disorders caused by the ionizing radiations. Free radical scavenging properties, antioxidant properties, protecting DNA from strand breaks, and lipid membrane from peroxidation are generally due to the presence of different phytochemicals present in plants (Seigler, 1998; Havsteen, 2002). *Opuntia elatior* Mill. is a folklore medicinal plant, and its ripened fruits are used by local people of Gujarat in treating anemia and general debility. *Opuntia elatior* possesses varied ethnobotanical claims in different diseases due to having several nutraceuticals and pharmacologically significant secondary metabolites (Bourhia *et al.*, 2020). Fruit is a rich source of nutrients and vitamins (Sawaya *et al.*, 1983; Teles *et al.*, 1984) and it has analgesic, hematinic, and anti-

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asthmatic activity (Chauhan, 2010). Of the polyphenols, phenolic acids, anthocyanin, and flavonoid content is notable (Bayar *et al.*, 2016; Farag *et al.*, 2017; Berrabah *et al.*, 2019). The stem of *O. elatior* contains malate of manganese, fatty acid, citric acid, wax, resin, and sugar (Prajapati *et al.*, 2015). Due to the presence of cytoprotective active material flavonoid, stem extract has been reported (Sivasubramanian *et al.*, 2013). The stem of *O. elatior* has various phytochemical components, but *in vivo* studies have not been extensively reported on stem extracts. The evaluation of pharmaceutical activities of this stem part may provide natural herbal medicine. Therefore, the present study was carried out to evaluate the radioprotective activity of stem extracts of *Opuntia elatior* in the spleen, which is an important hematopoietic organ.

## Materials and Methods

### • Plant Material

The stem of *Opuntia elatior* Mill. was procured from the botanical garden (Smriti Van) of Jaipur, Rajasthan. Identification of the plant material was done with the help of a taxonomist in the Department of Botany, University of Rajasthan, Jaipur (RUBL no. 211574)

### • Preparation of Plant Extracts

The Cladodes of *O. elatior* were cut into pieces and dried in shade. The extracts of the stem of *Opuntia elatior* (OEE) were prepared by extracting powder in ethanol and chloroform separately at 68 °C and 40°C in a Soxhlet apparatus for 36 hours each. The extract was lyophilized dissolved in double distilled water (DDW). The animals were given one extract at a time by gastric intubation with different dose rates according to the designed investigations. Dose rates of plant extracts were selected on the basis of experiments it was 10 mg/kg body wt. for both the extracts.

### • Experimental Animal

Colony bred healthy 6-8 weeks old Swiss albino mice with an average initial body weight of 22±2 g was selected. The mice were maintained under controlled conditions of temperature and light (14 and 10 h of light and dark). The animals' food was standard mice feed (procured from Ashirwad Industries, Chandigarh, India) and water *ad libitum*. Animal care was carried out according to Organization for Economic Cooperation and Development (OECD) guidelines 453. The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA, Government of India (Approval No: KU/IAEC/Ph.D/145). The institutional animal ethics committee (IAEC) of the Department of Zoology, University of Rajasthan, Jaipur approved the study (CPCSEA registration no. 1678/Go/Re/S/12/CPCSEA dated 16.06.2017).

### • Source of Irradiation

Animals were treated with Cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajasthan, India. Unanaesthetized animals in well-ventilated perspex boxes (30cm×30cm× 5cm) exposed the whole body to gamma radiation with external Co<sup>60</sup> teletherapy, SMS radiation Unit with source surface distance (SSD) of 80cms to deliver the dose rate of 1.47Gy/min. The dose rate was calibrated throughout the experimental period according to the decay table of Co<sup>60</sup>.

### • Experiment Design

They were divided into four groups.

- Group I: Control mice without any treatment.
- Group II: 6 Gy Co<sup>60</sup> gamma radiation only.
- Group III: *Opuntia elatior* extract (10mg/kg body weight) only.
- Group IV: *Opuntia elatior* extract (10mg/kg body weight) + 6 Gy Co<sup>60</sup> gamma radiation

Then mice were sacrificed on 1, 3, 5, 7, 10, 14 and 30 days post irradiation. Spleen was removed for analysis of Catalase and Superoxide dismutase activity by the method of Beers and Sizer, (1952) and Marklund and Marklund, (1974).

## Statistical Analysis

The result for all the groups at various autopsy intervals were expressed as mean ± Standard error (S.E.). To find out whether mean of sample drawn from experimental (group IV deviates significantly from respective control (group III), Student's 't' test was used by the method of Bourke *et al.*, (1985). The significance level was set at different levels as P < 0.05, P<0.01 and P<0.0

## Results and Discussion

### • Effect of *O. Elatior* Stem on Biochemical Analysis

Prolonged exposure to ionizing radiation (IR) induce cellular injury through generation of reactive oxygen species (ROS) and attenuating the endogenous antioxidant enzymes, leading to severe health impairments, especially the surrounding normal tissues (Yi *et al.*, 2020; Pooja *et al.*, 2021). Gamma radiations induce reactive oxygen/ nitrogen species (ROS/RNS) like nitric oxide and superoxide radicals which react to produce reactive peroxynitrite, which is known to induce cytotoxicity by interacting with biomolecules like protein, lipids and nucleic acids. ROS also affects the antioxidant defense mechanisms, by reducing the intracellular concentration of CAT as well as SOD activity (Nair *et al.*, 2007). Mice irradiated with a sub-lethal dose of 6 Gy depleted antioxidant enzyme levels in the organ homogenate observed decreasing CAT and SOD activity was observed. These intracellular antioxidants play a critical role in scavenging free radicals (Nair and Nair, 2003).

However, numerous antioxidants are known to protect the cells from reactive oxygen and nitrogen species by free radical scavenging activity in the cellular milieu (Verma *et al.*, 2011). Endogenous antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) provide the first line of defense and the homeostasis of these enzymes in the cells is crucial for maximum radioprotection (Samarth and Kumar, 2003). Therefore, the role of SOD is to catalyze the breakdown of  $O_2^{\cdot -}$  to  $O_2$  and  $H_2O_2$ , prevents formation of  $OH^{\cdot}$ . The ROS scavenging activity of SOD is effective only when it is followed by the actions of other enzymes, because the dismutase activity of SOD generates  $H_2O_2$ , which needs to be further scavenged by CAT. Various antioxidant compounds have been reported in plants by several researchers. For example, *Phyllanthus amarus* has been reported to restore the depleted levels of enzymes to normal (Kumar and Kuttan, 2004). The presence of polyphenols and flavonoids in the methanol extracts of *T. involucrata* can be responsible for maintaining balanced antioxidant enzyme levels (Thimmaiah *et al.*, 2019).

Administration of *O. elatior* extract prior to irradiation inhibits the decline in the intracellular antioxidant enzyme levels viz., SOD and CAT. Various phenolic and bioactive compounds are found in the leaf extract of *O. elatior* such as Citric acid, 2-Hydroxyisocaproic acid and 1-Octen-4-ol. The phenolic compounds are very important plant constituents because of their scavenging ability by virtue of hydroxyl groups and are, therefore, known as powerful chain-breaking antioxidants. Till date very few researches have been conducted on the medicinal properties of *O. elatior*, and essentially nothing is known regarding their potential immunomodulatory properties (Rajeshwar *et al.*, 2005). In the present study, *O. elatior* showed potent scavenging activity of superoxide radicals and can scavenge  $H_2O_2$  formed by SOD as the ethanol and chloroform stem extract of *O. elatior* at 10 mg/kg b.wt. ameliorated the change of antioxidant enzymatic activity in gamma radiation-induced mice. The results suggests that the antioxidant activity of *O. elatior* may be due to degradation of  $H_2O_2$  and other peroxides. Therefore, it can be possible that the mechanism of action by *O. elatior* is due to the modulation of superoxide and other peroxide effects on tissues, which could provide a reasonable explanation for the enhanced radioprotection in *O. elatior* pre-treated mice.

### • Effect of Ethanol and Chloroform Extract on SOD Activity in Spleen

Exposure to gamma radiation on spleen provokes the manufacture of ROS, such as superoxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen which can lead to cellular injury (Deng *et al.*, 2019). Endogenous enzymes such as superoxide dismutase (SOD) and catalase (CAT) are principal anti-oxidant enzymes and are responsible for the ROS deactivation system in cells (Jameel and Mohammed, 2021). As shown in Table 1, in ethanol extract, the SOD level was significantly decreased (19.23 U/mg) when exposed to radiation as compared to control, which further improved when treated with plant dose (23.71 U/mg) on the same day of exposure. When further studies were done on different time intervals it was observed that the SOD level increased from 1 to 30 days in all groups. When studies were done with chloroform extract, it was observed that SOD levels decreased significantly in groups treated with gamma radiation (19.33 U). It was observed that SOD levels were increased (20.05 U/mg) after treatment with chloroform extract (Table 2). In 2016, Jothy *et al.*, observed a decrease in the SOD activity for four days but then a significant increase was observed in a dose-dependent manner when they studied the radioprotective effect of *P. longifolia* leaf extract on spleen by X-rays. In another study carried out by Rao *et al.*, in 2009, an increase in SOD activity was observed when the mouse to study liver was treated with *Zingerone* (ZO) extract 1 h prior to the 4.5 Gy gamma radiation.

**Table 1: Variation in SOD Activity Level in Spleen of an Irradiated Mouse with and Without *Opuntia Elatior* Extract (Ethanol) Treatment**

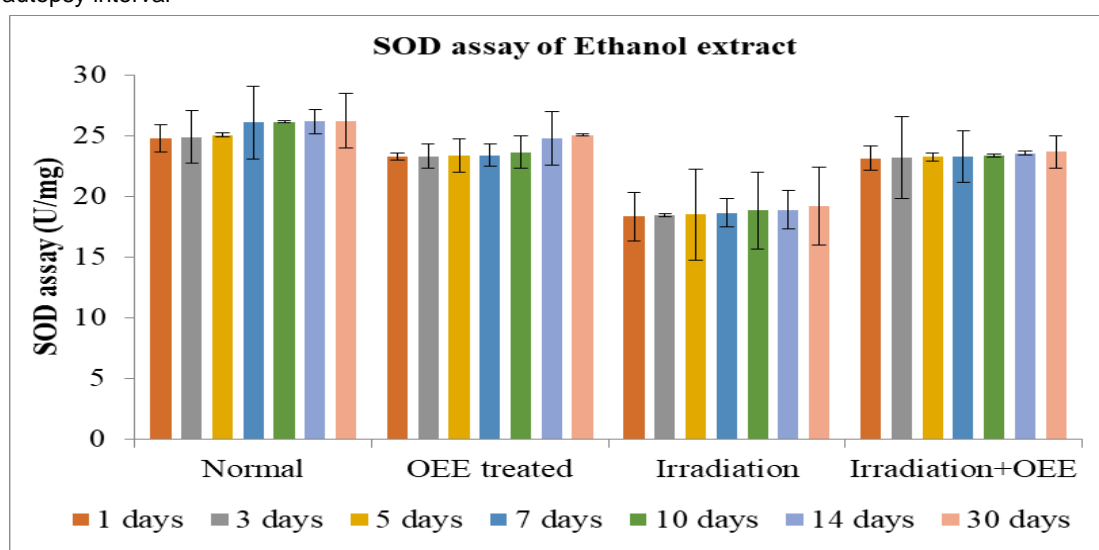
Autopsy interval	1Day	3Days	5Days	7Days	10Days	14 days	30 days
Experimental Groups							
Group 1 Normal (Without any treatment)	24.81±1.135	24.94±2.143	25.1±0.180	26.12±3.017	26.18±0.105	26.21±1.024	26.25±2.245
Group 2 (OEE treated)	23.3±0.280	23.36±1.009	23.41±1.349	23.44±0.090	23.68±1.356	24.81±2.193	25.1±0.086
Group 3 Irradiation (6 Gy)	18.37±2.001	18.48±0.141	18.55±3.751	18.68±1.179	18.87±3.178	18.94±1.593	19.23±3.200
Group 4 (OEE 10mg/kg+6Gy irradiation)	23.19±1.036	23.24±3.372	23.29±0.363	23.35±2.117	23.41±0.126	23.58±0.165	23.71±1.350

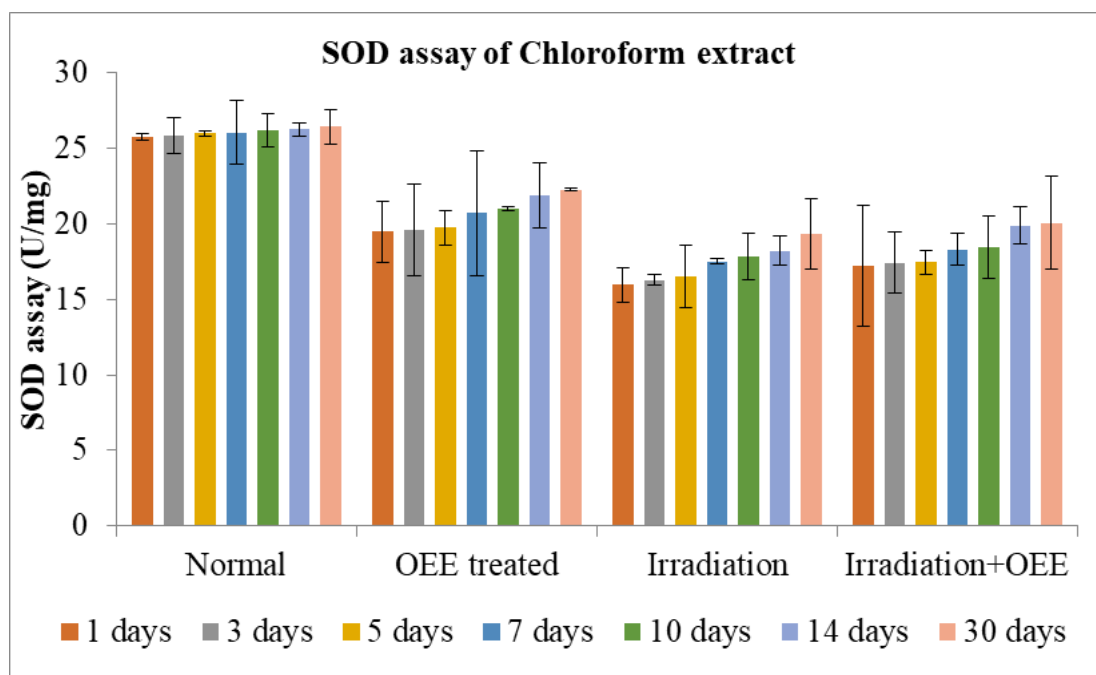
Data represented by Mean±SE (Six mice per group). n=6, P <0.05 (Data with superscript\* were significant and data without superscript\* were on significant). Data represent the mean value at that autopsy interval

**Table 2: Variation in SOD Activity Level in Spleen of Irradiated Mouse with and without *Opuntia Elatior* Extract (Chloroform) Treatment**

Autopsy interval	1Day	3Days	5Days	7Days	10Days	14 days	30 days
Experimental Groups							
Group 1 Normal (Without any treatment)	25.74±0.211	25.81±1.218	25.97±0.160	26.02±2.083	26.17±1.133	26.21±0.408	26.4±1.140
Group 2 (OEE treated)	19.45±2.035	19.56±3.006	19.71±1.160	20.69±4.114	20.99±0.116	21.87±2.147	22.25±0.083
Group 3 Irradiation (6 Gy)	15.93±1.117	16.26±0.334	16.48±2.047	17.51±0.150	17.84±1.515	18.21±0.932	19.33±2.313
Group 4 (OEE 10mg/kg+6Gy irradiation)	17.21±4.011	17.39±2.022	17.44±0.771	18.29±1.075	18.41±2.055	19.88±1.245	20.05±3.050

Data represented by Mean±SE (Six mice per group). n=6, P <0.05 (Data with superscript\* were significant, and data without superscript\* were on significant). Data represent the mean value at that autopsy interval

**Fig 1: Effect of Ethanol Extract of *O. Elatior* (OEE) Stem on SOD Activity of Swiss Albino Mice in the Spleen**



**Fig. 2: Effect of Chloroform Extract of *O. Elatior* (OEE) Stem on SOD Activity in the Spleen of Swiss Albino**

• **Effect of Ethanol and Chloroform Extract of *O. Elatior* on Catalase Activity in Spleen**

Catalase activity is one of the antioxidant activities to alleviate stress by regulating intracellular hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels (Kaur and Bhatla, 2022). When studies on catalase activity was done on ethanol extract it was observed that level was decreased in group exposed to gamma radiation (96.2 H<sub>2</sub>O<sub>2</sub> decayed/min/mg protein) which improved after treatment with plant extract (105.1 H<sub>2</sub>O<sub>2</sub> decayed/min/mg protein). It was observed that in all the groups Catalase level increased along with time and was maximum at 30<sup>th</sup> days (Table 3). In chloroform extract treated mouse, it was observed that the catalase level decreased in the group treated with gamma radiation (96.73 H<sub>2</sub>O<sub>2</sub> decayed/min/mg protein) which thereafter increased when treated with plant extract (99.83 H<sub>2</sub>O<sub>2</sub> decayed/min/mg protein). With the increase in time intervals the level was also increased (Table 4). In 2016, Jothy *et al.*, observed a decrease in the CAT activity for four days but then a significant increase was observed in a dose-dependent manner when they studied the radioprotective effect of *P. longifolia* leaf extract on spleen by X-rays. In another study carried out by Rao *et al.*, in 2009, an increase in CAT activity was observed in liver when the Swiss albino mice was treated with *Zingerone* (ZO) extract 1 h prior to the 4.5 Gy gamma radiation.

**Table 3: Variation in Catalase Activity Level in Spleen of Irradiated Mouse with and without *Opuntia Elatior* Extract (Ethanol) Treatment**

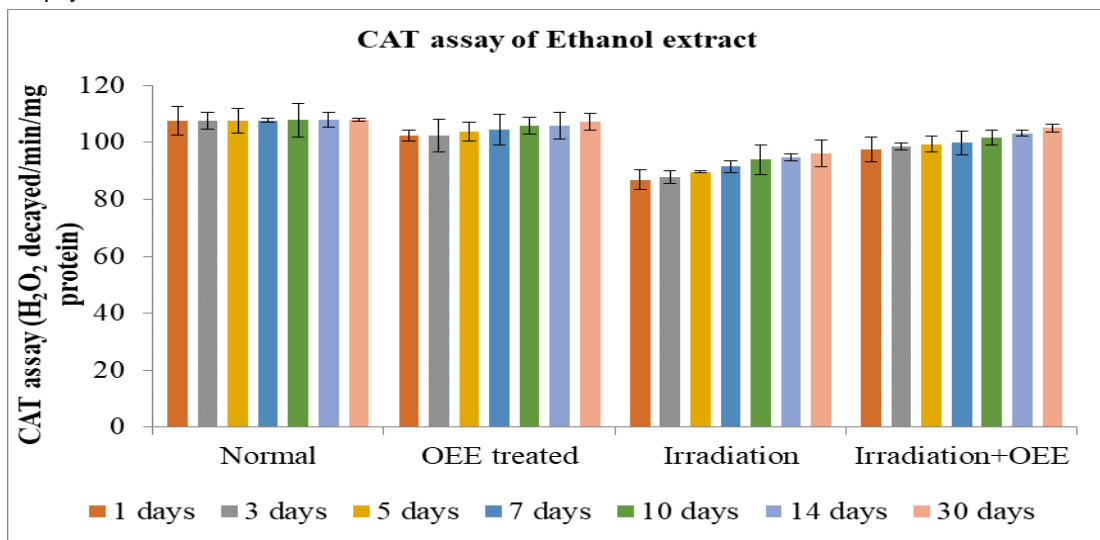
Autopsy interval	1 Day	3 Days	5 Days	7 Days	10 Days	14 days	30 days
<b>Experimental Groups</b>							
Group 1 Normal (Without any treatment)	107.63±5.103	107.68±3.003	107.72±4.327	107.77±0.542	107.81±5.828	107.89±2.672	108.08±0.522
Group 2 (OEE treated)	102.36±3.589	102.5±5.737	103.82±3.218	104.47±5.535	105.83±3.033	105.92±4.644	107.21±2.864
Group 3 Irradiation (6 Gy)	86.9±	87.71±2.278	89.63±0.271	91.57±2.045	93.91±5.179	94.66±1.255	96.2±4.652
Group 4 (OEE 10mg/kg+6Gy irradiation)	97.5±4.336	98.69±1.233	99.31±2.767	99.86±4.170	101.71±2.552	103.24±0.913	105.1±1.395

Data represented by Mean±SE (Six mice per group).n=6,P <0.05 (Data with superscript\* were significant and data without superscript\* were on significant). Data represent the mean value at that autopsy interval

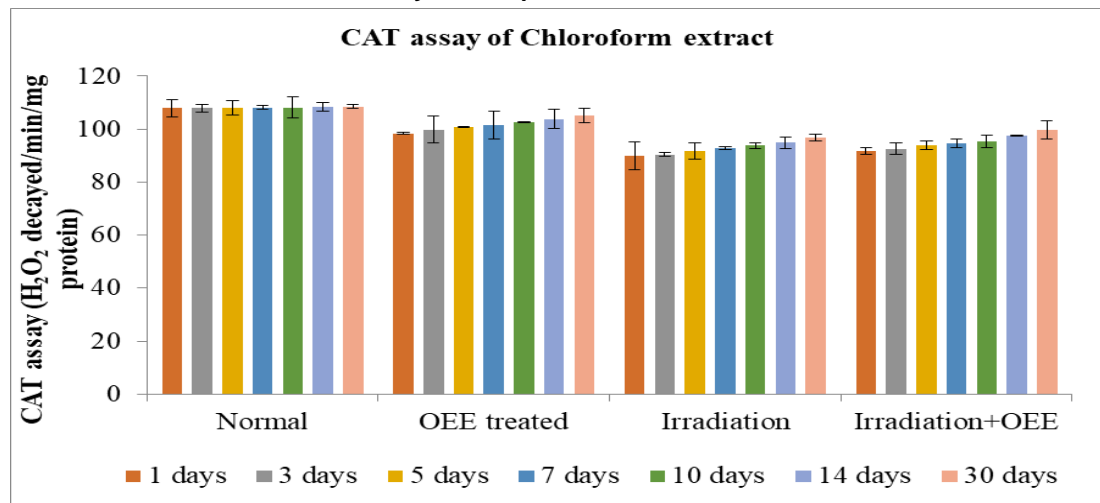
**Table 4: Variation in Catalase Activity Level in Spleen of Irradiated Mouse with and without *Opuntia Elatior* Extract (Chloroform) Treatment**

Autopsy interval	1 Day	3 Days	5 Days	7 Days	10 Days	14 days	30 days
<b>Experimental Groups</b>							
Group 1 Normal (Without any treatment)	107.89±3.234	107.92±1.536	108.04±2.597	108.17±0.630	108.23±4.050	108.49±1.729	108.51±0.744
Group 2 (OEE treated)	98.39±0.365	99.73±5.065	100.82±0.207	101.58±5.124	102.71±0.222	103.82±3.705	105.11±2.809
Group 3 Irradiation (6 Gy)	89.91±5.125	90.49±0.669	91.88±3.132	92.91±0.553	93.76±1.19	94.86±2.126	96.73±1.242
Group 4 (OEE 10mg/kg+6Gy irradiation)	91.83±1.172	92.61±2.144	93.81±1.675	94.67±1.777	95.44±2.301	97.64±0.254	99.83±3.452

Data represented by Mean±SE (Six mice per group).n=6, P <0.05 (Data with superscript\* were significant and data without superscript\* were on significant). Data represent the mean value at that autopsy interval



**Fig. 3: Effect of Ethanol Extract of *O. Elatior* (OEE) Stem on CAT Activity in the Spleen of Swiss Albino Mice**



**Fig. 4: Effect of Chloroform Extract of *O. Elatior* (OEE) Stem on CAT Activity in the Spleen of Swiss Albino Mice**

## Conclusion

It can be concluded that both the extracts of *Opuntia elatior* stem, Ethanol and Chloroform, protect Ethanol extract has given the most significant result in the spleen against 6 Gy whole body irradiation. The oral administration of ethanol extract of *O. elatior* stem protected the mice against change in antioxidant enzyme levels induced by radiation. The ethanol stem extract administration thereby increased radiation tolerance. Thus, the present study clearly suggests that *Opuntia elatior* stem extract could be a promising protective agent against radiation exposure, of course more studies required.

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