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RADIOPROTECTIVE EFFICACY OF *OPUNTIA ELATIOR* ON BIOCHEMICAL PARAMETERS IN *SWISS ALBINO MICE*

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ABSTRACT

The radioprotective efficacy of Opuntia elatior different extracts viz. Acetone and Petroleum Ether was investigated in Swiss albino mice at 6 Gy of gamma radiation.4 groups were divided in the presence (experimental) or absence (control) of Opuntia extract 10 mg/kg body wt.) to know the concentration of Reduced glutathione (GSH) and Lipid peroxidation (LPO) activity. Group I was kept without any treatment as control, Group II treated with 10 mg/kg body weight dose of plant extract, Group IV treated with Opuntia elatior extract (10 mg/kg body weight) and 6 Gy gamma irradiation dose, Group III treated with 6 Gy gamma irradiation only. These animals were scarified and their spleen was dissected out and further biochemical parameters were studied. In the present investigation effect of acetone and petroleum ether extract of stem was observed on levels of LPO and GSH. When studies on GSH was done it was observed that level was again decreased in group exposed to gamma radiation which improvement after treatment with plant dose (10.88 to 12.49). On increasing time interval it was observed that in all the groups GSH content increased along with time intervals i.e. maximum at 30 days. Further when LPO was done it was observed that it was maximum in group treated with gamma radiation only (71.44) which decreased thereafter when treated along with plant dose (66.17). It was observed that level was increased from 1 day to 30 days except on day 30, when it was decreased. In case of GSH the value decreased in all the groups. When time interval was counted we observed that GSH level increased in all the groups along with increase in time duration. Here also we observed that LPO increased in group treated with gamma radiation which thereafter reduced when treated with plant extract. With the increase in time interval its level was increased except in the group treated with plant dose along with radiation on 30th day where level decreased. Artificial chemicals generally recommended as radioprotectants but since they high toxicity and side effects, their consumption is limited, plant based radioprotectant's may act as efficient resource due to non toxic nature without side effects.

Keywords: *Opuntia Elatior, Gamma Radiation, GSH, LPO, Mouse.*

Introduction

Ionizing radiations have severe biochemical effects and are responsible for distortions due to a flow of events thus generating free radicals. Therefore, to combat side effects of these free radicals, the most frequent approach is to satiate the generated free radicals with prominent and non-toxic plant based products (Nagpal and Abraham, 2017). The requirement of potent radioprotective molecules is done both by revealing the chemical behaviour of natural products and synthesizing their derivatives, as potent radioprotectors based on scientifically validated proofs and their bioefficacy. Isolation of compounds as potential radioprotectors is a crucial process in the accretion of such efficacies, but due to little knowledge about global standardized systems for pre-selection and complete characterization of contender protectors results in grim problems.

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Besides medical and remedial applications, the application of different radiation has enhanced exponentially in various stages of human life in current scenario, like agriculture, food processing and preservation, industry, nuclear power generation, aviation, space, electronics, communications, and warfare. Therefore, most renowed application in medicine, science, and technology improved living conditions for mankind to lead a better life (Hanumakumar et al. 2018), humans including other living species are exponentially showing to various types of radiation. Various natural and chemical products like antioxidants, cytoprotective, immunomodulators, anti-inflammatory molecules, hematopoietic agents, vitamins, and DNA binding molecules have been studied broadly for their radio-protective efficacy in both *in vitro* and *in vivo* (Liu et al. 2015; Maria et al. 2017; Molkentine et al. 2019).

Opuntia elatior Mill. belongs to the family Cactaceae. Its fruit is also an adequate resource of nutrients and vitamins and consumed freshly. *O. elatior* has potent possess free radical scavenging (Itankar et al., 2014, anti-asthmatic (Chauhan et al., 2015), anti-ulcer (Subramanian et al., 2013 and anti-inflammatory efficacies (Sativa et al., 2014).

Thus in the present investigation radioprotective efficacy of Acetone and Petroleum ether extracts of *Opuntia elatior* on GSH and LPO in spleen of swiss albino mice was studied.

Materials and Methods

Male *Swiss albino mice* (*mus musculus norvegicus*) 6-8 weeks old, weighing 25±2 g each from an inbred colony at the Department of Zoology, University of Rajasthan, Jaipur, were selected for the experiments. They were maintained under the maintained environment of temperature 37±5° C and kept in the alternative day and night cycles. The study was conducted on random bred 6-8 weeks old Swiss albino mice with an average initial body weight of 22±2 g. They were maintained under controlled conditions of temperature and light (14 and 10 hr of light and dark, respectively). The animals are given with standard mice feed (procured from Ashirwad Industries, Chandigarh, India) and water *ad libitum*. 4 to 6 animals were kept in a polypropylene cage containing saw dust as bedding during the investigation.

The institutional animal ethics committee (IAEC) approval number of the Department of Zoology, University of Rajasthan, Jaipur, is 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. Unanaesthastized animals were undemonstrative in well-ventilated perspex boxes (30cm×30cm× 5cm) and exposed whole body to gamma radiation with external Co^{60}teletherapy unit at SMS Hospital 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^{60}.

Source of Plant Material

The plants were collected from the Botanical garden (Smriti van) Jaipur, Rajasthan and voucher specimen was deposited in Department of Botany, University of Rajasthan for authentication (RUBL no. 211574).

Preparation of Plant Extract

Opuntia elatior stem was peeled off, cut into pieces and shade dried, and then powdered. Plant material was taken out and shade dried and then powdered. The extract of the stem of *Opuntia elatior* was prepared by extracting powder in petroleum ether and acetone separately at 68 ºC and 40ºC in a Soxhlet apparatus for 36 hours each. The extract was lyophilized in double distilled water (DDW). The animals were given 1 extract at a time by gastric intubation with different dose rates according to the designed investigations. Both petroleum ether and acetone extracts were given at 10mg/kg body weight.

Dose Selection of Plant

Healthy adult (6-8 week old) *Swiss albino* mice were taken from inbred colony maintained in the laboratory, Specific dose of *Opuntia elatior (*cladode) extract was selected on the basis of survival assay.

Experimental Design

The animals were observed carefully everyday for symptoms of radiation sickness, behavioral toxicity and mortality. The second group which was irradiated without plant extract exhibited signs of radiation sickness within 2-4 days after exposure to 6Gy gamma radiation.

Design of Experiment

Adult, healthy, Swiss albino mice were used for the study. They were divided into four groups.

- Group I: Control mice without any treatment.
- Group II: 6 Gy Co 60gamma radiations only.
- Group III: *Opuntia elatior* extract (10mg/kg body weight only).
- Group IV: *Opuntia elatior* extract (10mg/kg body weight) + 6 Gy Co60 gamma radiation

The animals were observed for changes in their behavior, body weight, mortality (if, any). The animals were sacrificed at suitable post treatment intervals.

Autopsy

The animals from all the groups were sacrificed by cervical dislocation at 1,3,5,7 ,14 and 30 days. Six animals were sacrificed at each interval from every group and spleen was removed for analysis of colony forming units (CFU), reduced glutathione level and lipid peroxidation activity.

Biochemical Studies

Biochemical parameters like GSH and LPO activity were studied using Moron et al., 1979 and Utley et al., 1967 methods.

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

Effect of Acetone extract on GSH and LPO activity in spleen

When GSH level were studied it was found that level was decreased in group exposed to gamma radiation which improved after treatment with plant extract (10.88 to 12.49). With increasing time interval it was observed that in all the groups GSH content increased along with time intervals i.e. it was maximum at 30 days **(Table 1)**

Table 1: Variation in GSH Level in Spleen of Irradiated Mouse with and without *Opuntia Elatior* Extract (Acetone) Treatment

Autopsy Interval / Experimental Groups	1 Day	3 Day	5 Day	7 Day	10 Day	14 Day	30 Day
Group 1 Control (Without any Treatment)	18.29± 2.32158	18.31± 1.202345	18.38± 0.326548	18.41± 0.757452	18.56± 1.594125	18.72± 0.155671	18.79± 2.351574
Group 2 (Plant Extract only)	13.97± 0.320208	14.64± 3.093644	14.88± 1.815746	15.38± 2.150628	15.73± 0.493862	16.71± 2.010298	17.46± 1.493229
Group 3 Irradiation only (6 Gy)	10.88± 1.19098	11.47± 1.46238	11.76± 2.99945	12.1± 1.96267	12.39± 2.22806	13.64± 3.20677	14.97± 0.54749
Group 4 (Plant Extract-10mg/kg+6Gy irradiation)	12.49± 3.154145	13.67± 0.119304	13.81± 1.590514	14.34± 0.699738	14.61± 3.130341	15.88± 1.642285	16.46± 2.123919

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

When LPO studies was done it was observed that it was maximum in group treated with gamma radiation (71.44) which decreased thereafter when treated along with plant extract (66.17). when studies were done on the basis of time intervals it was observed that level was increased (from 1 day to 30 days) except on day 30 on which LPO was decreased **(Table 3)**

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

Autopsy interval / Experimental Groups	1 Day	3 Day	5 Day	7 Day	10 Day	14 Day	30 Day
Group 1 Control (Without any Treatment)	64.28± 1.610062	64.32± 1.847728	64.38± 2.191286	64.41± 3.978006	64.45± 1.930604	64.49± 1.523822	64.51± 4.358605
Group 2 (Plant Extract only)	64.8± 3.055591	64.83± 3.172638	64.87± 1.405216	64.91± 2.294457	64.97± 2.363564	65.51± 2.895071	66.89± 2.350858
Group 3 Irradiation only (6 Gy)	71.44± 4.269254	74.73± 2.391743	77.91± 3.061966	79.88± 3.001066	81.23± 1.432492	86.05± 3.498757	88.57± 3.486436
Group 4 (Plant Extract-10mg/kg+6y Irradiation)	66.17± 2.056113	67.52± 1.345697	68.93± 4.910014	73.53± 4.737383	74.8± 5.023186	79.33± 4.57664	78.91± 2.932053

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

Effect of Petroleum ether Extract on GSH and LPO Activity in Spleen

In the case of GSH, it was observed that value decreased in all the groups. When time interval was counted it was observed that GSH level was increased in all the groups along with increase in time duration **(Table 3)**

Table 3: Variation in GSH Level in Spleen of Irradiated Mouse with and without *Opuntia Elatior* Extract (Petroleum Ether) Treatment

Autopsy Interval / Experimental Groups	1 Day	3 Day	5 Day	7 Day	10 Day	14 Day	30 Day
Group 1 Control (Without any Treatment)	18.11± 2.175921	18.14± 1.560043	18.2± 1.008084	18.24± 0.666058	18.29± 2.326163	18.36± 1.445141	18.43± 0.364737
Group 2 (Plant Extract only)	14.57± 0.185562	14.83± 4.308809	15.34± 0.241316	15.78± 2.739148	16.43± 1.205003	16.81± 0.170098	17.93± 2.12154
Group 3 Iradiation only (6 Gy)	11.92± 3.096842	12.13± 0.310054	12.41± 2.195017	12.79± 1.263685	13.03± 0.349619	13.42± 2.280577	14.76± 1.278137
Group 4 (Plant Extract-10mg/kg+6y irradiation)	11.49± 1.484464	11.64± 2.302412	12.92± 3.033766	13.73± 0.283608	14.06± 3.06226	14.73± 0.173877	15.01± 3.124804

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

Here also it was observed that LPO increased in group treated with gamma radiation which thereafter reduced when treated with plant extract. With the increase in time interval the level was increased except in the group treated with plant extract along with radiation on 30th day where level decreased **(Table 4)**

Table 4: Variation in LPO Level in Spleen of Irradiated Mouse with and without *Opuntia Elatior* Extract (Petroleum ether) Treatment

Autopsy interval / Experimental Groups	1 Day	3 Day	5 Day	7 Day	10 Day	14 Day	30 Day
Group 1 Control (Without any Treatment)	64.31± 2.299268	64.34± 0.363731	64.39± 3.799083	64.42± 1.485407	64.46± 4.384317	64.51± 0.405832	64.6± 3.409418
Group 2 (Plant Extract only)	65.88± 1.689349	65.41± 3.812117	65.79± 0.566304	65.99± 2.151612	66.06± 0.639713	67.73± 2.115443	69.08± 1.087949
Group 3 Irradiation only (6 Gy)	73.67± 0.529937	74.9± 2.760151	77.23± 1.056709	79.46± 0.303535	82.41± 5.551949	86.55± 1.469229	88.39± 4.289876
Group 4 (Plant Extract-10mg/kg+6y irradiation)	66.09± 3.342474	66.53± 0.794313	67.2± 2.188523	72.89± 5.762849	73.42± 1.342125	78.36± 3.028663	75.7± 0.220303

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

Discussion

In current scenario there has been keen attention devoted to novel molecules bearing non toxic radioprotective efficacy which can neutralize the damaging effect of radiation. These molecules could provide defense against genetic damage, mutation, distortion in the immune system and harmful effects of radiation, which initiates free radicals. The reactive oxygen species (ROS) produced by ionization radiation are strongly regulated by various antioxidants, like non -enzymatic free radical scavengers and enzymes which can savenge these free radicals. Antioxidant mechanisms including LPO, GSH and GPx are crucial as they have important role in providing defence from radiation exposure (El –Nahas et al., 1993). The death of animals post radiation exposure to include many factors like distortion of the hematopoietic and gastrointestinal system, thus causing lead to damage in immune system (Krishna and Kumar, 2005).

In the present investigation effect of acetone and pet ether extract was observed on GSH and LPO levels. All the levels showed some variations according to group and time interval from 1 to 30th day. However, level increased in group treated with both plant extract and radiation at 30th day of interval in both the extracts when given to albino mice.

Certain doses of ionizing radiation enhance the oxidative load on the body and the endogenous free radical scavenging activity is not able to combat such stress load (Lata et al., 2009). There are reports which prove that ionizing radiation at the cellular level can induce damage in genetic material like DNA, proteins, lipids, and carbohydrates in the different parts of the body. The execution of symptoms like change in food habits, irritability, epilation, weight loss, lazyness within 3 –5 days by 10 Gy of gamma irradiation has been reported. The mortaility due to irradiation from 8 to 13 days is correlated with hemopoietic distortions. The endurance after exposure to high doses of irradiation, i.e., 10 Gy depends on amount of hemopoietic stem cells (HSC) and the potentiality of these cells to initiate prominent level of mature cells of replications to rejuvenate the damaged hematopoietic cells (Pooja et al., 2021). Electron beam irradiation also caused tremendous reduction in GSH content in the liver. The efflux of GSH from the liver is the pathway for supply of GSH to other organs (Yadav and Bhatnagar, 2007). Certain edibles and beverages like dark tea have prominent free radical scavenging capacity, could greatly alleviate the hematopoietic damage caused by ionizing radiation, by reducing the amount of ROS and elevating the levels of antioxidant enzymes like SOD, CAT, and GSH -Px (Long et al., 2018; Begum et al., 2012).

Conclusion

The search for radioprotective agents to counteract radiation damage is of immense use in radiotherapy. Radioprotective agents minimize or prevent damage from radiation exposure. Thus, it can be concluded that acetone and Petroleum ether extract of *Opuntia elatior* stem extract scavenge free radicals initiated by radiation exposure and thus controls radiation-induced oxidative stress, and are probable radioprotector having potent biomedical applications.

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