



Received on 23 August 2018; received in revised form, 03 November 2018; accepted, 09 November 2018; published 01 May 2019

RADIOPROTECTIVE EFFECT OF *OPUNTIA ELATIOR* ON BLOOD OF SWISS ALBINO MICE

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Keywords:

Opuntia elatior,
Gamma radiation, Hematological
alteration, Mouse, Blood cells, Herbal

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ABSTRACT: The modulatory effect of *Opuntia elatior* stem extract was studied in Swiss albino mice at dose 6 Gy of gamma radiation. Four groups were divided in the presence (experimental) or absence (control) of *Opuntia* extract 10 mg/kg body wt.) to observe hematological alterations. Four groups are Group I without any treatment as control, Group II treated with 10 mg/kg body weight dose of plant *Opuntia elatior* extract, Group III treated with *Opuntia elatior* extract (10 mg/kg body weight) and 6 Gy gamma irradiation dose, Group IV treated with 6 Gy gamma irradiation. These animals were scarified and their blood was collected by heart puncture from the syringe in the EDTA filled vials at days 1, 15 and 30 post-irradiations. There was no variation in RBC and WBC counts including hemoglobin content in the control group (without any treatment); but significantly decreased in 6 Gy gamma irradiation treated group IV, whereas a recovery pattern was recorded in experimental animals (Group II and Group III) and a normal value of hematological parameters were regained by day 30 post-treatment after treatment with plant extracts as compared to radiation group with some variations. Synthetic chemicals were used to as radioprotective agents but because of their high toxicity and side effects, they were not as useful as we thought, so natural products (plant extract) may offer as potential radioprotector due to their low toxicity and side effects.

INTRODUCTION: Radiations are divided into two classes on the basis of their energy potential, ionizing and non-ionizing radiation. Alpha, beta, gamma and X rays have enough energy to cause ionization when interact with matter thus named as ionizing radiation. Occupational or accidental exposure to radiation *via* nuclear weapons in war and terrorist activities and also the use of radioactive materials in electricity generation are the major concern to the increasing risk of radiation exposure^{1,2}.

The scientific outcomes of contact with ionizing radiation are enhanced by a series of physical, chemical, biochemical and cellular retorts initiated due to accumulation of radiation³. The energy associated with ionizing radiation is generally more than the bond energies of many molecules and can cause hemolytic bond scission and the creation of secondary electrons.

In current era most of the scientists has been interest in non-toxic radioprotectors that can be suggested as defence against disastrous effects of radiation in occupation as well as in therapeutic medicines for human beings. Though there are several reports and till date no molecule has been discovered as an effective non-toxic radioprotector for their use^{4, 5, 6, 7, 8}. Therefore, screening of bioactive compounds represents a major source for

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.10(5).2306-14
The article can be accessed online on www.ijpsr.com	
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(5).2306-14	

the discovery of new radioprotective drugs. The consumption of medicinal plants for combating harmful diseases is increasing worldwide, as it has been proved that the use of plants-derived compounds are used as effective remedies for the treatment and management of diseases through self-medication⁹. It is however, recommended that safety should be the main concern in the screening of these plants for health care needs¹⁰.

Plants are the richest source of many compounds like flavonoids, polyphenols, polysaccharides, alkaloids, vitamins and tannins *etc.*, as important secondary metabolites and these natural products have key role in scavenging the free radicals and defensive against disorders caused by the ionizing radiations. Free radical scavenging property, antioxidant property, protecting DNA from strand breaks and lipid membrane from peroxidation are they are generally due to presence of different phytochemicals present in plants^{11,12}.

Opuntia is a great genus of succulent shrubs, a native of the new world, now broadly cultivated in the parts of the world which has higher temperatures, on account of their exceptional morphology and attractive flowers. They are commonly called as Prickly pears, because of their edible fruits. These are initially introduced into India and other eastern countries by early European communities. The fruit of the plant is considered a refrigerant and is said to be useful in gonorrhoea. In addition to food, Indian fig is used to treat whooping cough, diabetes, prostate problems, rheumatism, nosebleed, and in dentistry in central Mexico¹³.

In Australia, it is used to relieve the symptoms and to lower the blood sugar level in diabetes. The baked fruit is used in whooping cough and syrup of the fruit enhances the secretion of bile and control spasmodic cough and expectoration¹⁴. The flowers cure bronchitis and asthma. A tea is made from the flowers used in combating kidney problems. Dried flowers are also ground into a paste and applied to the skin for measles^{15,16}.

MATERIALS AND METHODS: Male Swiss albino mice (*Mus musculus norvegicus*) which is about one and half months old, weighing 25 ± 2 g each from an inbred colony at Department of

Zoology, University of Rajasthan, Jaipur, were screened for the experiments. They were preserved under controlled conditions of temperature 37 ± 5 °C and kept in normal day light and dark night (14 and 10 h of light and dark, respectively). The animals were fed with standard mice feed (procured from Ashirwad Industries, Chandigarh, India) and water *ad libitum*. Four to six animals were housed in a polypropylene cage containing saw dust (procured locally) as a bedding throughout the experiment.

The experiment is approved by 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. Unanaesthetized animals were controlled in well ventilated perspex boxes (30 cm × 30 cm × 5cm) and whole body was exposed to gamma radiation with external Co⁶⁰ teletherapy, SMS radiation Unit with source surface distance (SSD) of 80 cm to deliver the dose rate of 1.47Gy/min. The dose rate was adjusted throughout the experimental period as per the decay table of Co⁶⁰.

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 stem of *Opuntia elatior* was prepared by extracting powder in ethanol and acetone separately at 68 °C and 40 °C in a Soxhlet apparatus for 36 h each. The extract was *in vacuo* dried in double distilled water (DDW). The animals were fed one extract at a time by gastric intubation with different dose rates according to the plan of experiments. Both ethanolic and acetone extracts were prepared at 10 mg/kg body weight.

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

Dose Selection of Plant: Healthy adult (6-8 week old) Swiss albino mice will be taken from an inbred colony maintained in the laboratory, Specific dose of *Opuntia elatior* (cladode) extract was selected based on survival assay.

Experiment Design: 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 60 gamma radiations only.
- **Group III:** *Opuntia elatior* extract (10mg/kg body weight only).
- **Group IV:** *Opuntia elatior* extract (10 mg/kg body weight) + 6 Gy Co60 gamma radiation.

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

Autopsy: The animals from all the groups were sacrificed by cervical dislocation at 1, 15 and 30 days after irradiation. Six animals were sacrificed at each interval from every group, and hematological parameters were studied.

Hematological Parameters: For this study, blood of mice were collected from heart puncture using a sterile syringe in an EDTA containing vial and following parameters were studied by Accurex cbc 360 automatic hematology analyzer.

Statistical Analysis: The result for all the groups at various necropsy 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: In the current investigation, variations in the RBC count, hemoglobin and WBC were found to possess a parallel pattern in all the groups. These were significantly reduced in the 6 Gy irradiated animals and a normal value was not reached till the last autopsy interval (*i.e.*, day 30). Prior administration of OE enhanced the recovery in these parameters, and a normal value was registered by day 30th post treatment. Initially RBC counts in acetone fraction were normal in control (day 1 to 30) and did not possess any variation in control. When mice were treated with plant extracts almost same observations was observed as compared to control. When these mice were given radiation a significant reduction in RBC level was observed from day 1 to 30 which show increase when mice were again treated with plant extracts.

In acetone fraction RBC showed increase in day 1 to 15 but decreased on day 30th which was at par with control **Table 1**. When anova test was applied it was observed that in acetone extract treatment along with days was found to be moderate significant while individually day was not significant but treatment alone was highly significant **Table 2**.

TABLE 1: VARIATION IN RBC CONTENT OF IRRADIATED MOUSE WITH AND WITHOUT OEE (ACETONE) TREATMENT (IN millions/cumm)

Autopsy interval / Experimental Groups	Day 1	Day 15	Day 30
Group 1 Control (without any treatment)	4.36 ± 0.11	4.61 ± 0.08	4.56 ± 0.09
Group 2 Plant Extract only (10 mg/kg body weight)	4.45 ± 0.07	4.53 ± 0.11	4.70 ± 0.10
Group 3 Plant +Radiation (Extract-10mg/kg+6Gy Radiation)	4.55 ± 0.10	5 ± 0.06	4.85 ± 0.07
Group 4 Radiation (6 Gy Radiation)	3.81 ± 0.12	2.23 ± 0.13	1.56 ± 0.11

TABLE 2: ANALYSIS OF VARIANCE

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	
Treatment	3	59.638	19.8794	51.7955	<2.2e16	***
Day	2	1.69	0.845	2.2016	0.1195	
Treatment: Day	6	15.372	2.562	6.6754	1.91E-05	**

In ethanolic extracts, there was no alternation in control in day 1 to 30. When mice were treated with plant extracts, there was a reduction in content at the 1st day to 15th day and increased at the 30th day which was almost equal to control. Further, when mice were treated with radiation, there was a significant reduction in RBC level from day 1 to

day 30. Finally, when mice were treated with radiation along with plant extract, there were no significant changes, and the value was almost equal to control **Table 3**. In ethanol extract treatments along with day and treatments without day and only days were found to be highly significant **Table 4**.

TABLE 3: VARIATION IN RBC CONTENT OF IRRADIATED MOUSE WITH AND WITHOUT OEE (ETHANOL)

Autopsy interval / Experimental groups	Day 1	Day 15	Day 30
Group 1 Control (without any treatment)	4.4 ± 0.05	4.4 ± 0.08	4.43 ± 0.06
Group 2 Plant Extract only (10 mg/kg body weight)	3.98 ± 0.05	1.85 ± 0.10	4.81 ± 0.07
Group 3 Plant +Radiation (Extract-10mg/kg+6Gy Radiation)	4.38 ± 0.09	4.4 ± 0.08	4.55 ± 0.05
Group 4 Radiation (6 Gy Radiation)	3.88 ± 0.10	2.56 ± 0.12	1.58 ± 0.05

TABLE 4: ANALYSIS OF VARIANCE

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	
Treatment	3	38.476	12.8253	52.665	< 2.2e-16	***
Day	2	8.674	4.3372	17.81	8.47E-07	***
Treatment: Day	6	3.55E+01	5.9146	24.287	2.26E-14	***

However, WBC also showed some variations as there were no alternations. In acetone fractions, the range was observed between 1120-7899/cumm. In mice treated with plant extracts, only the value decreased from day 1 to day 15th but increased after that almost equal to control group. In mice treated with radiations, the value continuously decreased. In the group treated with plant extracts along with

radiation, the value was at par with control, which reduced on day 15th but increased on the 30th day almost equal to control and showed recovery as compared to radiation group **Table 5**. When ANOVA test was applied, it was observed that in acetone extract treatment along with the day and without day were highly significant while individually day was less significant **Table 6**.

TABLE 5: VARIATION IN WBC CONTENT OF IRRADIATED MOUSE WITH AND WITHOUT OEE (ACETONE) TREATMENT (IN/cumm)

Autopsy interval / Experimental groups	Day 1	Day 15	Day 30
Group 1 Control (without any treatment)	6605.66 ± 95.03	6950 ± 97.00	6554.50 ± 117.03
Group 2 Plant Extract only (10 mg/kg body weight)	6221.66 ± 91.05	5213 ± 60.12	6550.33 ± 159.85
Group 3 Plant +Radiation (Extract-10mg/kg+6Gy Radiation)	6628.83 ± 94.21	6700.33 ± 94.03	6674.50 ± 112.12
Group 4 Radiation (6 Gy Radiation)	3879.50 ± 165.64	2910.50 ± 165.64	1558.83 ± 165.64

TABLE 6: ANALYSIS OF VARIANCE

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	
Treatment	3	187811250	62603750	152.56	< 2.2e-16	***
Day	2	3309568	1654784	4.0326	0.02274	*
Treatment: Day	6	19392066	3232011	7.8761	2.82E-06	***

In ethanolic extracts, it ranged from 819-10618/cumm though the value did not show significant variations from day 1 to day 30th but increased continuously in mice treated with plant extracts only. When mice were irradiated with radiations, there was significant reduction in WBC count as compared to control which show an increase when

again mice were fed with plant extracts along with radiation continuously from day 1 to 30th, but the value is less as compared to radiation group **Table 7**. In ethanol extract treatments with days and without days were found to be highly significant but day alone was not significant **Table 8**.

TABLE 7: VARIATION IN WBC CONTENT OF IRRADIATED MOUSE WITH AND WITHOUT OEE (ETHANOL) TREATMENT (IN/cumm)

Autopsy interval / Experimental groups	Day 1	Day 15	Day 30
Group 1 Control (without any treatment)	7758 ± 131.03	7052.16 ± 167.30	7658.66 ± 256.90
Group 2 Plant Extract only (10 mg/kg body weight)	6376 ± 101.68	7915.66 ± 285.18	8299.50 ± 125.00
Group 3 Plant +Radiation (Extract-10mg/kg+6Gy Radiation)	6660.33 ± 101.34	8686.16 ± 353.29	8969.5 ± 182.11
Group 4 Radiation (6 Gy Radiation)	4668 ± 228.82	1655 ± 83.02	1364 ± 105.78

TABLE 8: ANALYSIS OF VARIANCE

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	
Treatment	3	361781231	120593744	87.9304	< 2.2e-16	***
Day	2	838469	419235	0.3057	0.7378	
Treatment: Day	6	72540449	12090075	8.8154	6.81E-07	***

Individually in acetone extract in control group hemoglobin value did not change. In the group treated with plant extract only, there were no variations in all 30 days. In mice treated with radiation, there was a continuous reduction in level in all 1, 15 and 30th day which decreased after supplementation of plant extract at a dose level of

10 mg/kg till 15th day and increased thereafter almost equal to control **Table 9**. When ANOVA test was applied, it was observed that in acetone treatment along with a day and without day were found to be highly significant and individually day was less significant **Table 10**.

TABLE 9: VARIATION IN HEMOGLOBIN CONTENT OF IRRADIATED MOUSE WITH AND WITHOUT OEE (ACETONE) TREATMENT (IN gm/dl)

Autopsy interval / Experimental groups	Day 1	Day 15	Day 30
Group 1 Control (without any treatment)	13.45 ± 0.15	15.11 ± 0.15	13.73 ± 0.11
Group 2 Plant Extract only (10 mg/kg body weight)	13.68 ± 0.23	15.13 ± 0.13	14.21 ± 0.18
Group 3 Plant +Radiation (Extract-10mg/kg+6Gy Radiation)	13.08 ± 0.09	10.6 ± 0.19	13.83 ± 0.20
Group 4 Radiation (6 Gy Radiation)	11.56 ± 0.21	10.15 ± 0.24	6.73 ± 0.22

TABLE 10: ANALYSIS OF VARIANCE

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	
Treatment	3	270.276	90.092	72.645	< 2.2e-16	***
Day	2	8.726	4.363	3.518	0.03592	*
Treatment: Day	6	115.723	19.287	15.552	1.15E-10	***

In the ethanolic extract, no changes were observed till 30th day in control, but it increased in mice continuously till day 30th treated with plant extract only. When mice were irradiated with radiations, there was a significant reduction in hemoglobin level till 30th day. However, when radiated mice

were treated with plant extract the level of hemoglobin level slightly increased as compared to control **Table 11**. In ethanol extract treatment alone and with the day were highly significant while individually day was moderate significant **Table 12**.

TABLE 11: VARIATION IN HEMOGLOBIN CONTENT OF IRRADIATED MOUSE WITH AND WITHOUT OEE (ETHANOL) TREATMENT (IN gm/dl)

Autopsy interval / Experimental groups	Day 1	Day 15	Day 30
Group 1 Control (without any treatment)	13.66 ± 0.13	13.81 ± 0.19	14.30 ± 0.10
Group 2 Plant Extract only (10 mg/kg body weight)	13.83 ± 0.16	16.13 ± 0.19	17.21 ± 0.15
Group 3 Plant +Radiation (Extract-10mg/kg+6Gy Radiation)	12.21 ± 0.14	15.75 ± 0.17	16.63 ± 0.15
Group 4 Radiation (6 Gy Radiation)	10.80 ± 0.29	8.71 ± 0.23	5.25 ± 0.16

TABLE 12: ANALYSIS OF VARIANCE

	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	
Treatment	3	614.6	204.867	168.8078	< 2.2e-16	***
Day	2	12.28	6.139	5.0587	0.009327	**
Treatment: Day	6	184.72	30.787	25.368	9.11E-15	***

Overall in RBC and Hemoglobin content recovery level of ethanolic extracts was better than acetone extracts while in WBC acetone extracts were found to be better. At last in the ethanolic extract, no correlation was found between RBC and HB in control group while there was positive correlation coefficient between RBC and HB in treated mice with radiation which was also observed in mice treated with only plant extract. However, when mice treated with radiation and plant extract, there

was a negative co-relation between RBC and HB. Further, we have represented their correlation coefficient also **Fig. 1**. In acetone extract, there was a slightly positive correlation between RBC and HB in the control group which was observed positive in treated mice with radiation while slightly negative in mice treated with plant extract alone. Finally, when mice treated with radiation and plant extract, there was a positive correlation between RBC and HB **Fig. 2**.

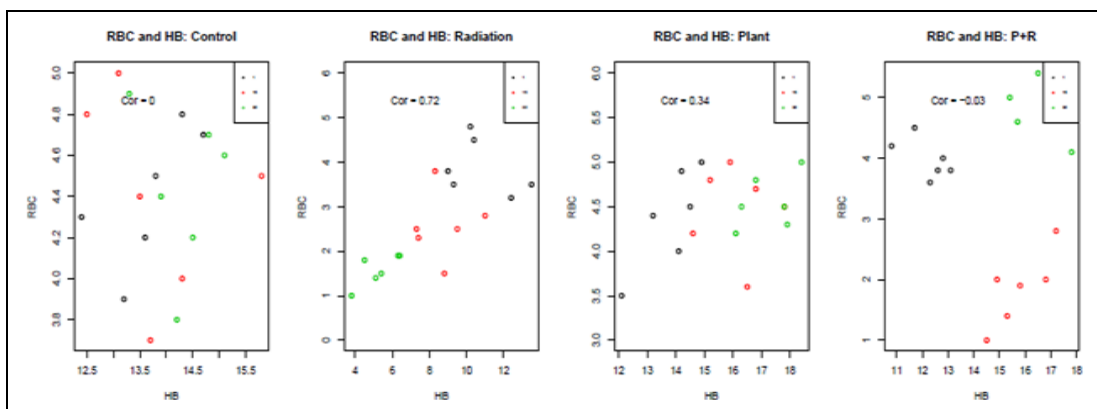


FIG. 1: SHOWING CORRELATION COEFFICIENT AMONG VARIOUS GROUP TREATED WITH ETHANOLIC EXTRACTS

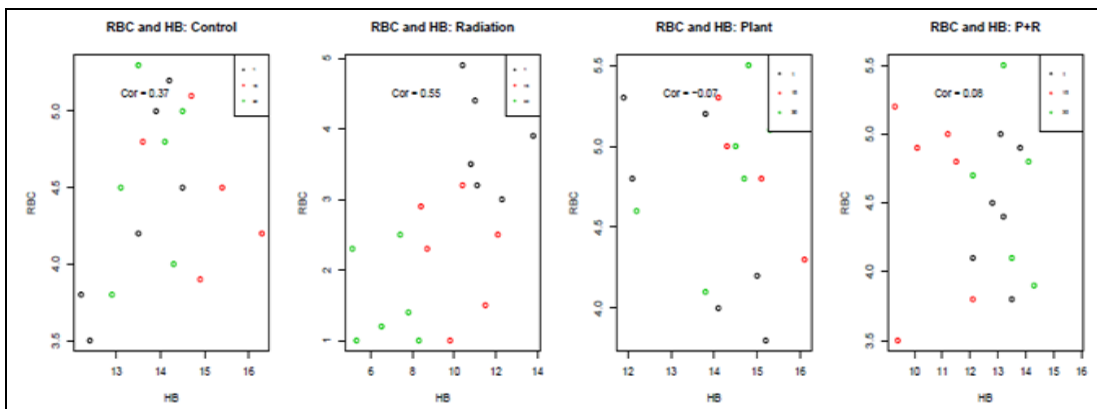


FIG. 2: SHOWING CORRELATION COEFFICIENT AMONG VARIOUS GROUP TREATED WITH ACETONE EXTRACTS

DISCUSSION: In the present investigation it was observed that due to the effect of radiation, the RBC count reduced which occurs due to loss through haemorrhage and radiation-induced damage¹⁷. Similar observations was made in hemoglobin level without returning to normal during experiment. The present investigation suggests that the hemoglobin level follows a same pattern to that of RBC in general. These observations are in agreement with Zeid *et al.*,

(2018)¹⁸ who reported significant reduction in hemoglobin concentration in Swiss albino mice exposed to 6 Gy gamma radiation. A depression in the hematocrit value can be related to total cell depletion in peripheral blood aided by confusion in steady state mechanisms in blood forming organs as well as an increase in plasma volume after irradiation. This view is supported by Shrikanth *et al.*, (2017)¹⁹.

The initial decrease in WBC count could be related to rapid decline of lymphocytes in peripheral blood which are the most radiosensitive as suggested by differential leucocyte count in the current investigation. This is also supported by (Kumar *et al.*, 2018)²⁰ who reported a reduction in the number of WBC of gamma irradiated mice. It has been observed that natural compounds from medicinal plants are effective in relation to blood circulation and to improve hemodynamics in various cardiovascular diseases²¹. The screening of these parameters including the red cells, white cells and the platelets and factors are correlated with inflammation, necrosis, various infections of visceral organs and due to stress related factors²². They have major role in the physiological, nutrition and pathological position of an organism²³. The vital role of the white blood cell and its differentials are to resist infections, defend the body by phagocytosis against invasion by foreign organisms and to carry and distribute antibodies for immune response²⁴.

The momentous elevation in WBC count and lymphocytic count caused by the plants extract reflect leucopoetic and possible immunomodulatory effects of the extract which amplified the production of increased amount of WBC²⁵. It will enhance the animal's potency of generating antibodies in the process of phagocytosis and have high notch of resistance to diseases and enhance acclimatization to local environmental and disease predominant conditions²⁶, the result of this study resembles with earlier research of Berinyuy *et al.*, (2015)²⁷, who observed similar things on *S. occidentalis*. Red blood cell and its related factors are key indicators for analysing circulatory erythrocytes and are playing vital role in the diagnosis of anaemia and also serve as useful indices of the bone marrow to initiate maturation of RBC as in mammals²⁸. The vital increase in RBC, WBC, Hemoglobin following administration of *T. occidentalis* is an indication of erythropoiesis incentive of the extract. The extract must have increase the rate of erythropoietin release in the kidney, which is the humoral regulator of RBC production²⁹.

Petruk *et al.*, (2017)³⁰ proved radio protective potency of *Opuntia ficus-indica* L. cladodes against UVA-induced oxidative stress in normal human

keratinocytes. They revealed the fact that when human keratinocytes were pre-treated with the extract pre irradiation to UVA radiations; a clear protective effect against UVA-induced stress was confirmed, as proved by inhibition of stress-induced processes, like free radicals production, lipid peroxidation and GSH depletion.

Further a potent protective effect against apoptosis in pre-treated irradiated cells was observed. It has been proved that economic and piscidic acids were accountable for the anti-oxidative stress action of cladode extract. In brief it was proved that a low-cost, bioactive, safe and high natural products bearing extract from *Opuntia* was recommended in skin protection. Kumar *et al.*, (2017)³¹ showed radioprotective of *Adhatoda vasica* Nees extracts against gamma irradiation in Swiss albino mice. This research was done at different post-irradiation intervals between 6 h to 30 days. Treatment dose of *A. vasica* leaf extract (800 mg/kg body weight) prior to whole body irradiation showed a important fortification in terms of survival percentage and hematological parameters.

Mice treated without plant extract exhibited signs of radiation sickness like anorexia, lethargicity, ruffled hairs and diarrhea and mortality was observed in animals within 25 days post-irradiation. The dose reduction factor (DRF $\frac{1}{4}$ 1.6) for *A. vasica* leaf extract was calculated from LD50/30 values. A significant reduction in hematological constituents (RBCs, WBCs, Hb and Hct) was proved till day 15 and no animal was able to revive after day 25. Contrariwise, animals pre-treated with *A. vasica* leaf extract showed 81.25% survival till 30 days after exposure and a steady salvage was noted in the hematological values. But, these hematological values were still below the normal even till day 30.

Mohamed and Ashour (2018)³² proved importance of ethanolic extract of *Morus alba* leaves on some biochemical and haematological alterations in irradiated male rats. The results proved that complete body irradiation of rats caused significant reduction ($P < 0.05$) in red blood cells (RBCs), haemoglobin (Hb), haematocrite percentage (HCT%), platelet, white blood cells (WBCs), lymphocytes, neutrophils, serum glucose-6-phosphate dehydrogenase (G-6-PD) and insulin.

CONCLUSION:

1. From the observations, concluded that in both extracts of *Opuntia elatior* cladode, acetone and ethenol, comparatively acetone extract was given best result for significantly recovered RBC count against gamma radiation.

2. From the observations, concluded that in both extracts of *Opuntia elatior* cladode, acetone and ethanol, comparatively acetone extract was given best result for significantly recovered WBC count against gamma radiation.

3. From the observations, concluded that in both extracts of *Opuntia elatior* cladode, acetone and ethanol, comparatively acetone extract was given best result for significantly recovered Heamoglobin content against gamma radiation.

Thus, the present study clearly suggests that this *Opuntia elatior* extract plant could be a promising protective agent against radiation exposure.

ACKNOWLEDGEMENT: The authors are thankful for Head, Department of Zoology and CAS (Center for advanced studies), University of Rajasthan for giving necessary facilities and also to UGC for giving me, non-net fellowship.

CONFLICT OF INTEREST: The authors had no conflict of interest in the present investigation.

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How to cite this article:

Poonia K and Sharma J: Radioprotective effect of *Opuntia elatior* on blood of Swiss albino mice. *Int J Pharm Sci & Res* 2019; 10(5): 2306-14. doi: 10.13040/IJPSR.0975-8232.10(5).2306-14.

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