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Effect of *Opuntia elatior* on Lipoprotein's alterations induced by gamma radiation in Swiss albino mice

Poonia Kavita¹ and Sharma jaimala^{2*}

¹Department of Zoology, University of Rajasthan, 302004, Jaipur, INDIA

²Department of Zoology, University of Rajasthan, 302004, Jaipur, INDIA

ABSTRACT

The modulatory effect of *Opuntia elatior* stem extract was studied in Swiss albino mice at dose 6 Gy of gamma radiation in the presence (experimental) or absence (control) of *Opuntia* extract (10 mg/kg body wt.) to observe lipoproteins alterations. These animals were sacrificed and their blood was collected at days 1, 15 and 30 post-irradiations. A constant level of all lipoproteins level was observed in the control group; whereas a recovery pattern was observed in experimental animals and a normal value was regained by day 30 post-treatment.

KEY WORDS:- *Opuntia elatior*; Gamma radiation; lipoproteins; Mouse; Plant extract.

***Corresponding author**

Professor Jaimala Sharma

Department of Zoology,

University of Rajasthan, 302004,

Jaipur, INDIA

Email.ID- jaimalauor@gmail.com Phone no. +91 9829321507

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INTRODUCTION

Radiation injury generally occurs due to oxidative stress¹. Reactive oxygen species (ROS) are known to play different roles in physiological and pathological metabolism and are constantly synthesized in living organisms². These may be toxic to cells by direct attack at the molecular level or indirectly by generating hydroxyl radical³. Radio-protectors has the target to control the initial damage induced in the biological system at the molecular level before other physiological or micro-anatomical changes occur. This research is based on the use of chemicals that prevent the development of radiation injury in exposed organisms by mitigation of radionuclide absorption through activation of the free radical scavenging system⁴. The efficiency of the radio protective agents is greatly dependent on their chemical properties, duration of treatment and post-irradiation time elapse after the application radioprotection. An ideal radio-protective agent should be active, rapidly absorbed and easily distributed in the body tissues. It must be without side effects or at least with minimal toxicity which gives insurance that no cumulative consequences could be expected from their repeated usage⁵. Therefore, screening of bioactive compounds represents a major source for the discovery of new radioprotective drugs.

Hyperlipidemia is defined as increased or abnormal levels of lipids, huge amount of cholesterol and triglycerides in the blood. These lipids are elated in a protein capsule, and the density of the lipids and the type of protein controls the fate of the particle and its effect on metabolism⁶. This fat-protein complexes in the blood is best-known as lipoproteins, and they are either LDL (low density lipoprotein), or HDL (high density lipoprotein). Excess LDL cholesterol elevate blockage of arteries, which are major cause of heart attack. So it is recommended as “bad” cholesterol. In contrast, HDL is recommended as “good” cholesterol inhibits atherosclerosis (Fessler, 2015)⁷. For a healthy person, the ideal LDL/HDL ratio is 3.5.⁸ Low HDL cholesterol levels are naturally escorted by an increase in blood triglyceride levels, while high HDL are major cause of coronary heart disease⁹.

Opuntia elatior is a member of cactaceae family. The fruit is an elongated. oval, berry, having profuse pericarp with a number of clefts of small prickles, dark reddish in colour, with a succulent sweet pulp melded with many hard seeds. The ripe fruits of *Opuntia* spp. are 30-220g in weight with pulp 40-69% ,seeds 10-15% and peel 31-51%¹⁰. *Opuntia elatior* has antihyperlipidemic¹¹, activity, so we used as herbal product to combat serum lipoprotein level.

MATERIAL AND METHODS

Male Swiss albino mice (*mus musculus norvegicus*) 6-8 weeks old, weighing 25±2 g each from an inbred colony at Department of Zoology, University of Rajasthan, Jaipur, were

selected for the experiments. They were maintained under controlled conditions of temperature $37\pm 5^{\circ}\text{C}$ and kept in natural day light and dark night cycles. The animals were provided 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.

Institutional animal ethics committee (IAEC) approval number of Department of Zoology, University of Rajasthan, Jaipur, is CPCSEA registration no. 1678/Go/Re/S/12/CPCSEA dated 16.6.2017.

Source of Irradiation

Animals were irradiated with Cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajasthan, India. Unanaesthetized animals were restrained in well ventilated Perspex boxes (30cm×30cm× 5cm) and exposed whole body to gamma radiation with external Co^{60} 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^{60} .

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 hours each. The extract was lyophilized 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 10mg/kg body weight.

Source of plant material

The plants were collected from Botanical garden (Smriti van) Jaipur, Rajasthan and voucher specimen was deposited in Department of Botany, 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 inbred colony maintained in the laboratory. Specific dose of *Opuntia elatior* (cladode) extract was selected on the basis of survival assay.

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 60 gamma 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 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.

Estimation of HDL Cholesterol:

Principal

High density lipoprotein-cholesterol (HDL-C) is extracted by a method described by Burstein, 1970. It was estimated by using Accurex kit method.

Estimation of VLDL and LDL Cholesterol:

Low and very low density lipoprotein cholesterol (LDL-C and VLDL-C) was estimated by Friedewald *et al.*, 1972 and used Accurex kit.

RESULTS

H.D.L.(HIGH DENSITY LIPOPROTEIN)

Acetone Extract

In this parameter it was observed that in acetone extract the values in control showed almost similar pattern from day 1 to 30. Further in group treated with only plant extract , it was observed that the value increased when registered by day 30th post treatment but maximum value was observed in day 15th (73.16 mg/dl). In group treated with radiation along with plant extract, it was observed that the values increased slightly registered by day 30th post treatment but it was maximum in day 30th (68.16 mg/dl) that is almost equal to the control group. Finally in group treated with only

6 Gy radiation it was observed that the level constantly decreased by day 30th post treatment from the day 1 (53.83 to 36.33 mg/dl). (FIGURE.1)

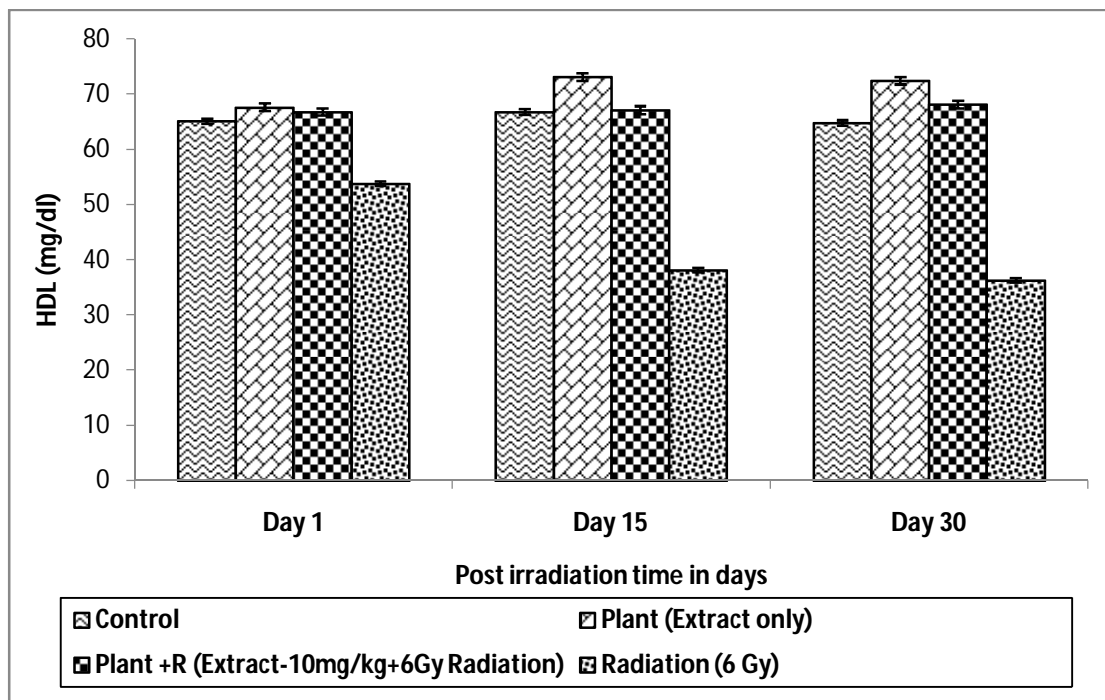


Fig. 1: Variations in the HDL level (mg/dl) of kidney in gamma irradiated Swiss albino mice, with and without *Opuntia elatior* pretreatment (Acetone extract)

TWO WAY ANOVA

When annova test was applied it was observed that in acetone extract, individually treatment and individually day were found to be moderate significant and treatment along day was less significant.(TABLE NO.1)

TWO WAY ANOVA

(TABLE NO. 1)FOR Effect of *Opuntia elatior* acetone extract on HDL level of serum against gamma radiation in Swiss Albino Mice

Two way anova's factor	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Significance
Treatment	3	1441.78	500.69	108.18	< 2.2e-54	**
Day	2	7.81	5.96	5.11	0.03567	**
Treatment : Day	6	211.13	30.28	20.889	1.15E-21	*

Ethanol Extract

Further in ethanolic extract the values in control showed almost similar pattern from day 1 to 30. Further in group treated with only plant extract, it was observed that the value increased when registered by day 30th post treatment (66.66 to 71.00 mg/dl). In group treated with radiation along with plant extract, it was observed that the values remain almost constant and no significant changes

were observed and the values were highly equal to the control group values. Finally in group treated with only 6 Gy radiation it was observed that the level heighly decreased by day 30th post treatment from the day 1 (37 to 38.66 mg/dl),as compared to the control group.(FIGURE .2)

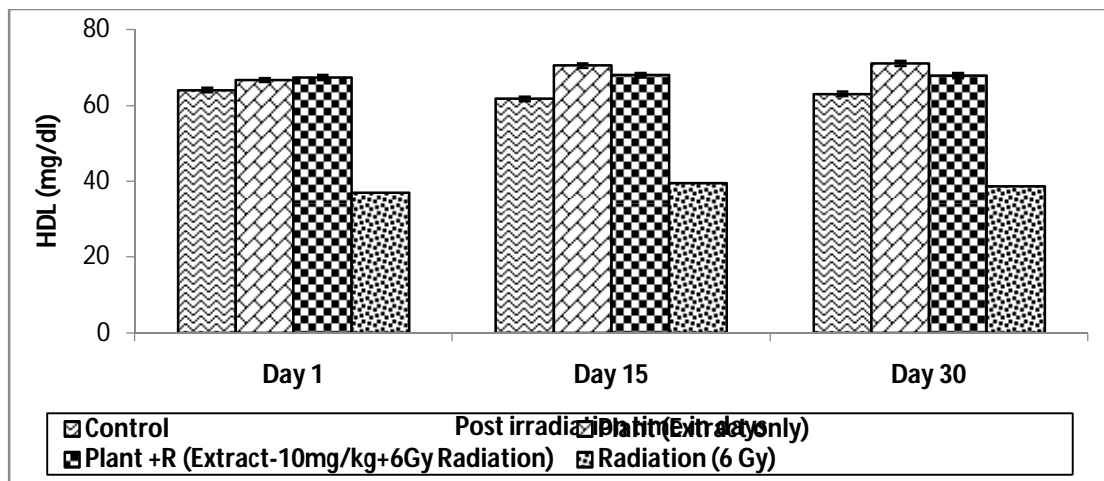


Fig. 2: Variations in the HDL level (mg/dl) of kidney in gamma irradiated *Swiss albino* mice, with and without *Opuntia elatior* pretreatment (Ethanol extract)

TWO WAY ANOVA

In ethanol extract treatment alone and with day were less significant while invidually day was moderate significant. In the below (TABLE NO.2)

TWO WAY ANOVA

(TABLE NO.2) FOR Effect of *Opuntia elatior* ethanol extract on HDL level of serum against gamma radiation in *Swiss Albino Mice*

Two way anova's factor	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Significance
Treatment	3	1429.34	502.23	106.56	< 2.2e-52	*
Day	2	7.67	6.01	5.09	0.03520	**
Treatment : Day	6	202.89	31.20	19.884	1.15E-10	*

L.D.L (LOW DENSITY LIPOPROTEIN)

Acetone extract

Here also it was observed that in acetone extract the values in control showed almost similar pattern from day 1 to 30. Further in group treated with only plant extract, it was observed that the

value increased when registered by day 30th post treatment but maximum value was observed in day 15th (71.16 mg/dl). In group treated with radiation along with plant extract, it was observed that the values decreased in day 15th but again increased in day 30th (97 to 132.5 mg/dl) that values were very heigh from the control group values. Finally in group treated with only 6 Gy radiation it was observed that the level constantly increased by day 30th post treatment from the day 1 (139.83 to 147.66 mg/dl). (FIGURE.3)

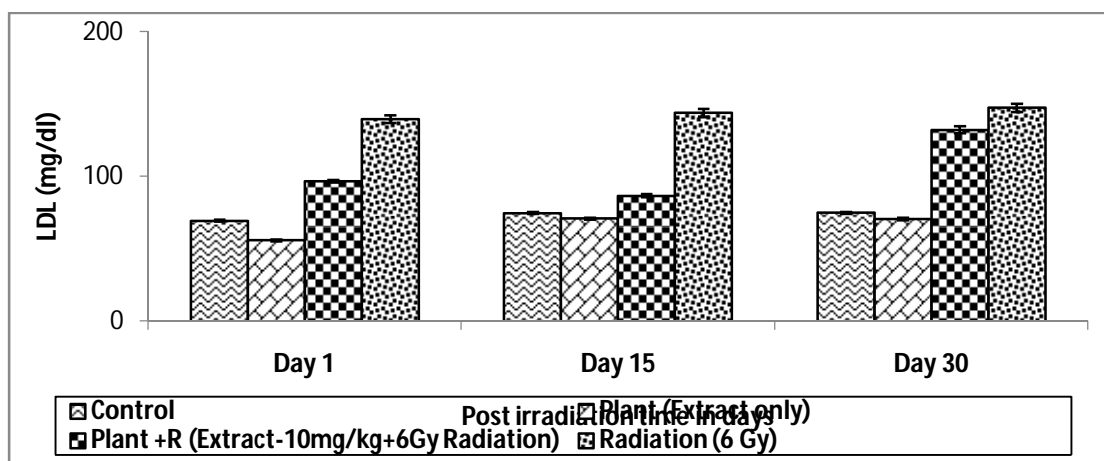


Fig. 3: Variations in the LDL level (mg/dl) of kidney in gamma irradiated Swiss albino mice, with and without *Opuntia elatior* pretreatment (Acetone extract)

TWO WAY ANOVA

When ANOVA test was applied it was observed that in acetone extract, treatment along with day and without day and individually day, were found to be moderate significant.(TABLE NO.3)

TWO WAY ANOVA

(TABLE NO. 3) FOR Effect of *Opuntia elatior* acetone extract on LDL level of serum against gamma radiation in Swiss Albino Mice

Two way anova's factor	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Significance
Treatment	3	1556.89	519.90	116.34	< 2.2e-61	**
Day	2	8.19	6.58	6.03	0.05691	**
Treatment: Day	6	215.78	35.48	24.667	1.15E-17	**

Ethanol extract

Again in ethanolic extract the values in control showed almost similar pattern from day 1 to 30. Further in group treated with only plant extract, it was observed that the value increased when registered by day 30th post treatment (73.16 to 93.83 mg/dl). In group treated with radiation along with plant extract, it was observed that the values increased constantly along with time interval and

significant changes were observed (78.16 to 112 mg/dl). Finally in group treated with only 6 Gy radiation it was observed that the level significantly increased by day 30th post treatment from the day 1 (96.33 to 153.16 mg/dl). (FIGURE. 4)

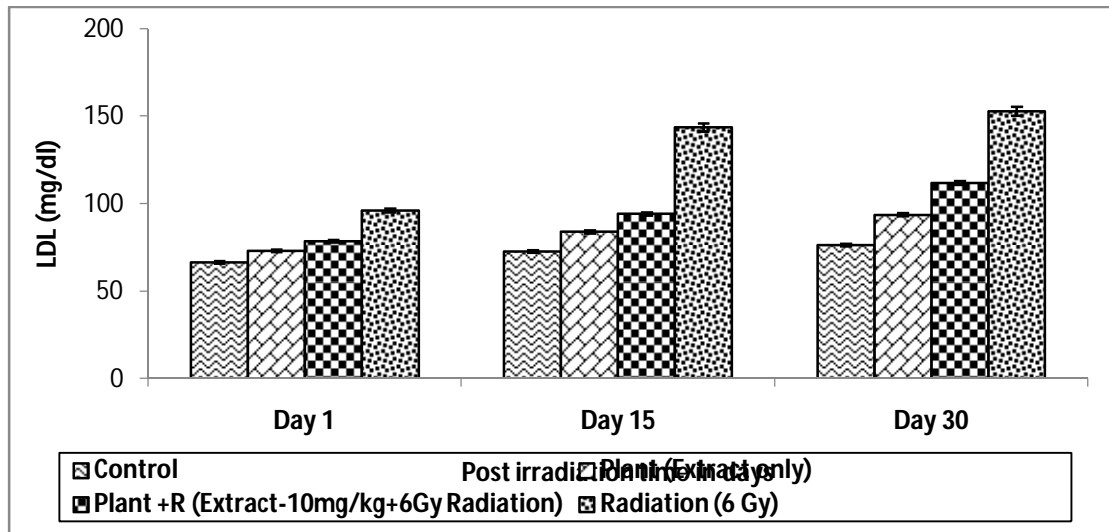


Fig. 4: Variations in the LDL level (mg/dl) of kidney in gamma irradiated Swiss albino mice, with and without Opuntia elatior pretreatment (Ethanol extract)

TWO WAY ANOVA

When annova test was applied it was observed that in ethanol extract, treatment along with day and without day and individually day, were found to be moderate significant. In ethanol extract same observations were made.(TABLE NO.4)

TWO WAY ANOVA

(TABLE NO.4) FOR Effect of Opuntia elatior ethanol extract on LDL level of serum against gamma radiation in Swiss Albino Mice

Two way anova's factor	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Significance
Treatment	3	1531.89	515.58	112.12	< 2.2e-57	**
Day	2	8.00	6.42	5.88	0.05456	**
Treatment : Day	6	210.45	33.35	23.776	1.15E-14	**

V.L.D.L.(VERY LOW DENSITY LIPOPROTEIN)

Acetone Extract

Here also it was observed that in acetone extract the values in control showed almost similar pattern from day 1 to 30. Further in group treated with only plant extract, it was observed that the value increased slightly when registered by day 30th post (40.16 to 44.16 mg/dl) but almost equal to the control group value. In group treated with radiation along with plant extract, it was observed that

the values increased constantly in day 30th (44 to 53 mg/dl) but these values were equal to the control group values. Finally in group treated with only 6 Gy radiation it was observed that the level constantly increased by day 30th post treatment from the day 1 (52.62 to 62.00 mg/dl). (FIGURE 5)

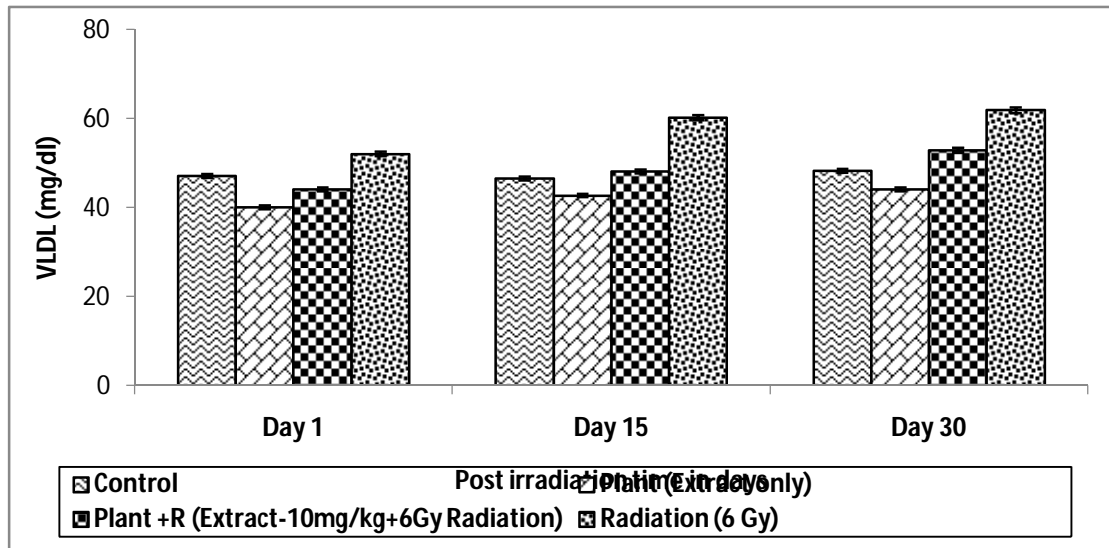


Fig. 5: Variations in the VLDL level (mg/dl) of kidney in gamma irradiated Swiss albino mice, with and without *Opuntia elatior* pretreatment (Acetone extract)

TWO WAY ANOVA

When annova test was applied it was observed that in acetone extract, treatment along with day and without day and only day, was less significant.(TABLE No. 5)

TWO WAY ANOVA

(TABLE NO.5) FOR Effect of *Opuntia elatior* acetone extract on VLDL level of serum against gamma radiation in Swiss Albino Mice

Two way anova's factor	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Significance
Treatment	3	1111.36	505.12	107.19	< 2.2e-44	*
Day	2	6.58	5.11	3.12	0.04419	*
Treatment : Day	6	200.11	30.12	24.778	1.15E-24	*

Ethanol extract

Again in ethanolic extract the values in control showed almost similar pattern from day 1 to 30. Further in group treated with only plant extract , it was observed that the value increased when registered by day 30th post treatment (44 to 52 mg/dl) but almost equal to the control group value. In group treated with radiation along with plant extract, it was observed that the values increased constantly along with time interval and significant changes were observed (42 to 56 mg/dl) these values were slightly increased from the control values. Finally in group treated with only 6 Gy radiation it was observed that the level significantly increased by day 30th post treatment from the day 1 (57 to 77 mg/dl).(FIGURE 6)

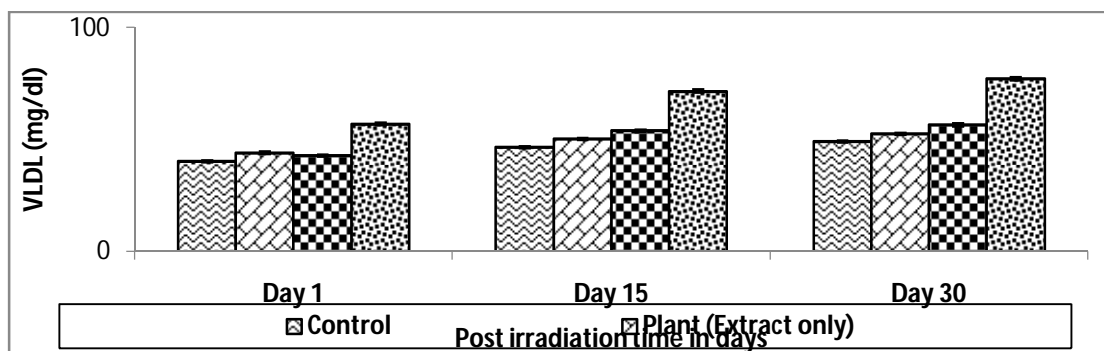


Fig. 6: Variations in the VLDL level (mg/dl) of kidney in gamma irradiated Swiss albino mice, with and without *Opuntia elatior* pretreatment (Ethanol extract)

TWO WAY ANOVA

When an nova test was applied it was observed that in Ethanol extract, treatment along with day and without day and only day, was less significant. (TABLE NO.6)

TWO WAY ANOVA

(TABLE NO.6) FOR Effect of *Opuntia elatior* ethanol extract on VLDL level of serum against gamma radiation in Swiss Albino Mice

Two way anova's factor	Df	Sum Sq	Mean Sq	F Value	Pr (>F)	Significance
Treatment	3	1008.45	490.48	100.32	< 2.2e-40	*
Day	2	5.76	4.97	2.98	0.04312	*
Treatment : Day	6	188.34	28.12	22.789	1.15E-20	*

DISCUSSION

When the metabolism of TG is sluggish, the increased TG and decreased HDL levels are not associated with elevated LDL. The liver transforms excess calories to TG, which is packed into VLDL and concealed into the circulation. In the fasting state, the VLDLs are the highest lipoproteins that carry a big load of buoyant TG through the blood stream. As the TG is removed by adipose tissue for storage of for muscle fuel, the VLDLs become successively smaller. They are converted first into intermediate lipoprotein (IDLs), and then LDLs, each lower in TG content and thus smaller and denser than its precursor. As VLDL is metabolized, HDL is formed in the circulation from the excess part of the VLDL, mainly cholesterol, phospholipid and certain lipoprotein. In the investigation it was observed that both acetone and ethanolic extract of plant sample reduced LDL, and HDL due to the suppression of LDL receptor activity or newly secreted lipoproteins enriched with cholesteryl ester at the expense of triglycerides.¹²

HDL lipoprotein is the main cholesterol carrier from the body cells to the liver, including those from the arterial walls. In the liver, cholesterol is transformed into bile acids and then excreted through the intestine .

Opuntia spp. have shown a variety of medicinal properties such as antiviral¹³, antihyperlipidemic and hypercholesterolemic¹⁴.

Opuntia elatior's cytotoxic evaluation showed a potent action against to K562(Human chronic myelocytic leukemia) cell line. It was found that hydroalcoholic extract of the fruits of *Opuntia elatior* Mill have antioxidant activity and promising antileukemic activity¹⁵.

Sinha and Ghosh reported studies of hypolipidemic effect of ethanolic leaf extract of *Aegle marmelos* in hyperlipidemic rat models. They revealed the fact that the hypolipidemic activity of *Aegle marmelos* induced hyperlipidemic effect at dose of 125 and 250mg/kg. Hyperlipidemia in experimental rats is proved by with ethanolic elevated serum cholesterol, TG, LDL and decreased HDL levels. Treatment with extract significantly reduced serum triglycerides, cholesterol, low density lipoprotein and significantly enhanced the high density lipoprotein in hyperlipidemic rats.¹⁶ So based on these studies, *Opuntia elatior* possibly effect our research work on radiation induced alteration in lipoprotein level.

CONCLUSION

Herbal drugs have been use in several traditional system of medicine for several hundred years for treating various human ailments since they often holistic treatment they are considered highly acceptable effective and non-toxic, thus treatment by *Opuntia elatior* may be useful.

CONFLICT OF INTEREST

The authors had no conflict of interest in the present investigation.

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