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

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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 in the presence (experimental) or absence (control) of *Opuntia* extract (10 mg/kg body wt.) to observe lipoproteins alterations. These animals were scarified 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.

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INTRODUCTION

Radiation injury generally occurs due to oxidative stress. Reactive oxygen species (ROS) are known to play different roles in physiological and pathological metabolism and are constantly synthesized in living organisms. These may be toxic to cells by direct attack at the molecular level or indirectly by generating hydroxyl radical. 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 system. 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 usage. 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 metabolism. 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). For a healthy person, the ideal LDL/HDL ratio is 3.5. Low HDL cholesterol levels are naturally escorted by an increase in blood triglyceride levels, while high HDL are major cause of coronary heart disease.

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 prickels, 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%. Opuntia elatior has antihyperlipidemic 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±5° 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 Co60 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 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 68ºC and 40 ºC 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 Co60 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 $30^{th}$ post treatment but maximum value was observed in day $15^{th}$ (73.16 mg/dl). In group treated with radiation along with plant extract, it was observed that the values increased slightly registered by day $30^{th}$ post treatment but it was maximum in day $30^{th}$ (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)

**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)

**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

**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)

**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 highly decreased by day 30th post treatment from the day 1 (37 to 38.66 mg/dl), as compared to the control group. (FIGURE 2.2)

**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)

<table>
<thead>
<tr>
<th>Two way anova's factor</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F Value</th>
<th>Pr (&gt;F)</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>1429.34</td>
<td>502.23</td>
<td>106.56</td>
<td>&lt; 2.2e-52</td>
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<td>Day</td>
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<td>7.67</td>
<td>6.01</td>
<td>5.09</td>
<td>0.0352</td>
<td>**</td>
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<tr>
<td>Treatment : Day</td>
<td>6</td>
<td>202.89</td>
<td>31.20</td>
<td>19.884</td>
<td>1.15E-10</td>
<td>*</td>
</tr>
</tbody>
</table>

**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 (71.16 mg/dl) that values were very high 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

<table>
<thead>
<tr>
<th>Two way anova's factor</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F Value</th>
<th>Pr (&gt;F)</th>
<th>Significance</th>
</tr>
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<td>116.34</td>
<td>&lt; 2.2e-61</td>
<td>**</td>
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<tr>
<td>Day</td>
<td>2</td>
<td>8.19</td>
<td>6.58</td>
<td>6.03</td>
<td>0.05691</td>
<td>**</td>
</tr>
<tr>
<td>Treatment: Day</td>
<td>6</td>
<td>215.78</td>
<td>35.48</td>
<td>24.667</td>
<td>1.15E-17</td>
<td>**</td>
</tr>
</tbody>
</table>

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 30\textsuperscript{th} post treatment from the day 1 (96.33 to 153.16 mg/dl). (FIGURE 4)

![Variations in the LDL level (mg/dl) of kidney in gamma irradiated Swiss albino mice, with and without Opuntia elatior pretreatment (Ethanol extract)](image)

**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

<table>
<thead>
<tr>
<th>Two way anova's factor</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F Value</th>
<th>Pr (&gt;F)</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Treatment</td>
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<td>1531.89</td>
<td>515.58</td>
<td>112.12</td>
<td>&lt; 2.2e-57</td>
<td>**</td>
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<tr>
<td>Day</td>
<td>2</td>
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<td>5.88</td>
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<td>Treatment : Day</td>
<td>6</td>
<td>210.45</td>
<td>33.35</td>
<td>23.776</td>
<td>1.15E-14</td>
<td>**</td>
</tr>
</tbody>
</table>

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 30\textsuperscript{th} 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 30\(^{th}\) (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 30\(^{th}\) 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)](image)

**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 30\(^{th}\) 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 30\(^{th}\) post treatment from the day 1 (57 to 77 mg/dl).(FIGURE 6)
**TWO WAY ANOVA**

When a 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

<table>
<thead>
<tr>
<th>Two way anova's factor</th>
<th>DF</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F Value</th>
<th>Pr (&gt;F)</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>3</td>
<td>1008.45</td>
<td>490.48</td>
<td>100.32</td>
<td>&lt; 2.2e-40</td>
<td>*</td>
</tr>
<tr>
<td>Day</td>
<td>2</td>
<td>5.76</td>
<td>4.97</td>
<td>2.98</td>
<td>0.04312</td>
<td>*</td>
</tr>
<tr>
<td>Treatment : Day</td>
<td>6</td>
<td>188.34</td>
<td>28.12</td>
<td>22.789</td>
<td>1.15E-20</td>
<td>*</td>
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</table>

**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 antiviral, antihyperlipidemic and hypercholesterolemic.

Opuntia elatior’s cytotoxic evaluation showed a potent action against 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 activity.

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.

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.

ACKNOWLEDGMENT

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REFERENCES