Summary and Conclusions

Humans are continuously exposed to radiation from environment and as society has evolved, the use of radiation has become an essential component of modern life, particularly in energy production, and has widespread applications in fields of medical and scientific research, industries and agriculture. These increasing applications of radiation and its inadvertent exposure during air and space travel, nuclear accidents and nuclear terror attacks require a safeguard against human exposures to minimize deleterious effects associated with radiation exposure.

Ionizing radiation causes injuries to living cells and tissues through a series of molecular events which are initiated by the generation of free radicals or reactive oxygen species (Paul et al., 2011). These reactive oxygen species causes oxidative stress and play a critical role in cell damage by causing DNA strand breaks, lipid peroxidation, protein modification and also initiate a variety of cellular signal transduction pathways (Kalpana et al., 2011). The net effect is the disruption of molecular structure and function which leads altered cell metabolism, cell death and if the dose is sufficiently high, mortality of the organism (Joshi et al., 2010).

Search for useful radioprotectors has been an important issue in the field of radiation biology. Patt et al. (1949) reported that the natural amino acid cysteine protected rats and mice against radiation-induced sickness and mortality. Since then, several compounds with varied chemical structures and pharmacologic properties have been screened for their radioprotective ability in mammals (Jagetia, 2007; Bhandari, 2013).

There is a long list of active chemicals like, 2-MPG (Ayene et al., 1988), AET (Garriott and Crowe, 1983), alpha-TMG (Satyamitra et al.,

2001), Edaravone (Anzai et al., 2004), diltiazem (Nunia et al., 2007), oltipraz (Johari et al., 2011), Nicaraven (Kawakatsu et al., 2013), vanadate (Wang et al., 2013), WR-2721 or amifostine (Li et al., 2014) etc. that have been shown to protect against radiation induced damage.

However, several synthetic chemical agents produce serious side effects and are toxic at the doses required for radioprotection. Alternatively, **natural plant extracts are being studied that can protect cells and tissues against ionizing radiation without obvious side-effects** (Bhandari, 2013).

Plants have played a significant role in maintaining health and herbs have been widely used by mankind for the treatment of various ailments since human history. There are abundant studies on herbal preparations such as Triphala (Jagetia et al., 2002), abana (Jagetia et al., 2003a), chyavanaprasha (Jagetia and Baliga, 2004b); plant extracts as *Amaranthus paniculatus* (Krishna and Kumar, 2005) *Brassica compestris* (Soni et al., 2006), *Mentha piperita* (Samarth et al., 2006), *Adhatoda vasica Nees* (Kumar et al., 2007), *Myristica fragrans* (Sharma and Kumar, 2007), *Podophyllum hexandrum* (Lata et al., 2009), *Nelumbo nucifera* (Duan et al., 2010), *Ocimum sanctum* (Joseph et al., 2011), *Xylopia aethiopica* (Adaramoye et al., 2011), *Aframomum melegueta* (Nwozo et al., 2013) and plant based products and phytochemicals such as mangiferin (Jagetia and Baliga, 2005), eckol (Park et al., 2008), Zingerone (Rao et al., 2009), diphlorethohydroxycarmalol (Ahn et al., 2011), hesperidin (Kalpana et al., 2011), tea polyphenols (Das et al., 2013) etc. for their radioprotective potential.

Prosopis cineraria (L) Druce commonly known as **khejri**, belongs to the family Mimosaceae. This plant is distributed mainly in arid to semi-

arid region of less rain fed area of all part of the country. It is a moderate sized, thorny, irregularly branched, evergreen tree and it is widely used in folk medicine. *Prosopis cineraria* (*P. cineraria*) has been found to contain numerous bioactive compounds such as flavonoids, alkaloids, diketones, phenolic contents, tannins, saponins, terpenoids, free amino acids, lipids, sugars and vitamins etc (George et al., 2012; Mohammad et al., 2013).

It has been reported *P. cineraria* has exhibited anti-pyretic activity (Manikandar et al., 2009), antihyperglycemic, antihyperlipidemic and antioxidative properties (Sharma et al., 2010), free radical scavenging potential (Dharani et al., 2011), antitumor activity (Robertson et al., 2011), antimicrobial properties (Napar et al., 2012) and analgesic activity (Muzammil et al., 2013).

The present study was planned to evaluate the radiomodulatory effects of *P. cineraria* (Linn) Druce in Swiss albino mice by calculating DRF evaluating hematological and biochemical and histopathological parameters in liver.

Materials and methods

Animals

Male Swiss albino mice, 6-8 weeks of age, weighing 25±2 gm from an inbred colony were used in this study and were maintained under optimal conditions of temperature (25±2°C) and light (14 hours of light and 10 hours of dark). Standard mice feed and tap water *ad libitum* was provided to them.

Irradiation

The Cobalt teletherapy unit (ATC-C9) at cancer treatment center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur was

used for irradiation. Whole-body exposure was performed at a dose rate of 1.54 Gy/min. with surface distance (SSD) of 80 cm.

Plant material and extract preparation

Plant material (*P. cineraria*) was collected locally and was identified and specimen was placed in Herbarium, Department of Botany, University of Rajasthan, Jaipur, bearing voucher/identification number is RUBL-20422. Fresh leaves of *P. cineraria* were washed, air dried and powdered. The extract was prepared by boiling of leaves powder in double distilled water (DDW) for 36 hours. The solution was cooled, filtered, concentrated and dried to make it in powder form. The extract was re-dissolved in DDW prior to the oral administration in mice.

Experimental design

Drug tolerance study

Mice were divided into four groups of 10 animals each and were given 200, 400, 800 and 1600 mg/kg body weight/day of *P. cineraria* leaves extract (PCLE) in double distilled water (DDW) for 7 consecutive days. These mice were observed regularly till 30 days for any sign of sickness, weight loss and mortality. Maximum tolerance dose of PCLE was determined accordingly.

Determination of optimum dose of PCLE against radiation

For the selection of optimum dose of PCLE, mice were divided into 3 groups of 10 animals each and were given 200, 400 and 800 (mg/kg body weight/day) for 7 consecutive days. Thirty minutes after the last administration, these were exposed whole body to 8 Gy gamma radiation. Animals were observed till 30 day post-irradiation for any sign of radiation sickness, weight loss and mortality.

Determination of dose reduction factor (DRF)

The efficacy of any radioprotective agent is evaluated by the determination of its dose reduction factor (DRF). The DRF of PCLE based on LD50/30 survivability experiment was calculated after irradiating a large number of Swiss albino mice to different doses (6, 8 and 10 Gy) of gamma rays in the presence (experimental) or absence (control) of PCLE. The percentage of mice surviving at each radiation dose till 30 days following exposure was used to construct survival-dose-response curves. Regression analysis was done to obtain $LD_{50/30}$, to determine dose reduction factor (DRF). DRF was computed by the formula:

$$\mathbf{DRF} = \frac{\mathbf{LD_{50/30}\ (Experimental\ animals)}}{\mathbf{LD_{50/30}\ (control\ animals)}}$$

Determination of radiomodulatory effects of PCLE

For determining the radiomodulatory effects of PCLE, mice were divided into following four groups:

- Group-I (Normal): Animals were given double distilled water (DDW) for seven consecutive days.
- Group-II (PCLE alone): Animals were given PCLE dissolved in DDW for seven consecutive days.
- Group-III (Control- irradiated alone): Animals were given double distilled water (DDW) for seven consecutive days and then exposed to 8 Gy of gamma radiation.
- Group-IV (Experimental PCLE + irradiation): Animals were given PCLE dissolved in DDW for seven consecutive days and then exposed to 8 Gy of gamma radiation after 30 minutes of last treatment of PCLE.

Animals from these groups were autopsied by cervical dislocation at 6 hours, 1st day, 3rd day, 7th day 15th day and 30th day post irradiation. Haematological, biochemical and histological parameters were assessed.

Parameters studied

Following parameters were studied to assess the modulation of radiation-induced alteration in Swiss albino mice by the *P. cineraria* leaves extract:

General parameters

(i) Radiation sickness

(ii) Body weight changes

(iii) Mortality

(iv) Abnormality, if any

Hematological parameters

(Blood sample was taken from the heart puncture)

(i) Total Erythrocyte Count (RBC)

(ii) Haemoglobin content (Hb)

(iii) Hematocrit (Hct)

(iv) Total Leucocytes Count (WBC)

(v) RBC - derived indices

   a. Mean corpuscular hemoglobin (MCH)

   b. Mean corpuscular volume (MCV)

   c. Mean corpuscular hemoglobin concentration (MCHC)

(vi) Differential Leucocytes Count (DLC)

Biochemical parameters

(i) Reduced Glutathione (GSH) Assay
   a. Blood GSH (Beutler et al., 1963)
   b. Liver GSH (Moron et al., 1979)

(ii) Lipid Peroxidation (LPO) assays in liver and blood (Ohkhawa et al., 1979)

(iii) Superoxide Dismutase (SOD) Assay in liver (Marklund and Marklund, 1974)

(iv) Catalase (CAT) Assay in liver (Aebi, 1984)

Histopathological study of liver

A minimum of 4 animals from 1 and 2 groups and each set of both control and experimental were sacrificed by cervical dislocation at different time intervals from 6 hours to 30 days (¼, 1, 3, 7, 15 and 30) liver was taken and fixed in Bouin's fluid for 24 hours. Paraffin sections were cut at five micrometer and stained with Harris hematoxylin and eosin for histopathological study.

Statistical analysis

Data are expressed as Mean±SEM and ANOVA was used for making statistical comparison between the groups. Significance level was set at $p<0.05$, $p<0.01$ and $p<0.001$. Regression analysis was done to calculate $LD_{50/30}$ values for DRF determination.

Results

PCLE tolerance study

Animals treated with different concentrations (200, 400, 800 and 1600 mg/kg body weight/day) of PCLE for seven consecutive days did not show any signs of sickness, toxicity and mortality till 30 days of

observation. These results indicate that PCLE was well tolerated upto the concentration of 1600 mg/kg body weight in Swiss albino mice.

Determination of optimum dose of PCLE against gamma radiation

Maximum protection was observed in animals those were treated with 400 mg/kg body weight/day PCLE for seven consecutive days before exposure to 8 Gy gamma radiation in terms of improved survival, body weight and increased hematological and biochemical protection. Therefore, a dose of 400 mg/kg body weight/day was selected for the further studies as optimum dose of PCLE.

Dose reduction factor

In control group, 100, 66.67 and 12.50% survival was reported at 4, 6 and 8 Gy doses of gamma rays respectively and no mice survived at 10 Gy after 30 days post-irradiation observations. Animals of experimental group showed decreased mortality at all exposure doses of gamma rays and survival percentage was reported as 100, 90, 62.5 and 33.33% at 4, 6, 8 and 10 Gy doses of gamma rays respectively after 30 days post-irradiation.

The $LD_{50/30}$ values were obtained as 6.70 Gy for control group and 8.88 Gy for experimental group using regression analysis and the dose reduction factor was calculated as 1.32.

Determination of radiomodulatory effects of PCLE

General observations

Radiation sickness

Irradiated alone animals showed signs of radiation sickness such as reduction in food and water intake, lethargy, diarrhea, ruffled hairs with hair loss, weight loss and mortality. Whereas, animals pre-treated with

PCLE and exposed to 8 Gy of gamma radiation showed less severe signs of radiation sickness.

Average body weight

Significant reduction in body weight of control group animals was observed. Maximum weight loss was observed at day 9 post-irradiation where it was recorded 73.18% of initial body weight and after that a gradual recovery was seen till day 30 and it reached up to 97.58% of initial body weight. Whereas, experimental group animals exhibited an almost consistent body weight from day1 (27.25±0.99 gm) to day 14 (27.60±0.81 gm) and thereafter regular weight gain was observed till day 30 post-irradiation.

Survival

Only 12.5% mice survived till 30 day post-irradiation in control group whereas, 62.50% survival was reported in experimental group on day 30 post-irradiation.

Hematological parameters

Oral administration of PCLE alone for seven consecutive days did not show significant variation in hematological parameters such as RBC, WBC, hemoglobin, hematocrit, DLC and RBC derived indices in comparison to normal.

RBC count- Significant decrease in RBC count was observed at all autopsy intervals in control group (irradiated alone) following irradiation when compared with normal. Maximum decline was observed at day 1 post-irradiation in both control (4.97 ±0.07 million/mm^3; $p<0.001$) and experimental (6.89±0.26 million/mm^3; $p<0.01$) groups. Thereafter a gradual increase was observed till day 7 and again on day 15, RBC count

showed a decrease. On day 30 post-irradiation maximum recovery was reported in both control (8.06±0.17 million/mm^3) and experimental (8.53±0.21 million/mm^3) groups. In experimental group (PCLE pre-treated and irradiated), significant increase in erythrocytes count was reported as compared to control group except at day 30 post-irradiation where the increase was statistically insignificant.

WBC count- Total leucocytes count was decreased significantly in control group in comparison to normal at all autopsy intervals. Minimum count was reported at day 1 post-irradiation in both control (2.77±0.12 thousand/mm^3; $p<0.001$) and experimental group (3.72±0.13 thousand/mm^3; $p<0.01$) and after that a gradual increase was observed till day 30 post-irradiation and reached to 5.12±0.23 and 6.08±0.22 thousand/mm^3 in control and experimental groups respectively. Oral administration of PCLE in experimental group before radiation exposure significantly increased the number of WBC as compared to control group animals.

Hemoglobin- A significant decline was reported in hemoglobin level at all autopsy intervals in control group with respect to normal. Maximum decrease was observed at day 1 and reported as 8.93±0.21 gm/dl ($p<0.001$), 11.72±0.25 gm/dl ($p<0.01$) in control and experimental groups respectively. Pattern in Hb level was similar as observed in RBC count. In experimental group, significant increase in hemoglobin level was reported as compared to irradiated alone (control) animals except at day 7 post-irradiation where the increase was statistically insignificant.

Hematocrit - Hematocrit values showed significant decline in control group at all autopsy intervals when it compared with normal value. Minimum hematocrit value was found at day 1 and estimated as 25.53±0.82% ($p<0.001$), 32.84±0.86% ($p<0.01$) in control and experimental groups

respectively. Values showed gradual increase from day 3 to day 30 post-irradiation. PCLE treatment prior irradiation improved hematocrit values in experimental group significantly at all autopsy intervals except day 30 post-irradiation where change was insignificant when compared with control group.

RBC derived indices

Mean Corpuscular Hemoglobin (MCH)- MCH values in irradiated alone mice were found higher than normal animals except day 30 post-irradiation where the MCH value was lower than normal value. Highest value of MCH was reported as 18.67±0.74 pg ($p<0.05$) at day 3 post-irradiation. MCH values in experimental group animals were calculated lower than control group animals at 6 hours, day 1 to day 15 post-irradiation. At day 30, the MCH value was higher than respective control group value. Highest value of MCH was reported as 17.49±0.51 pg at 6 hours post-irradiation. Difference between MCH Values of experimental group and control group was statistically insignificant.

Mean Corpuscular Volume (MCV)- MCV values in irradiated alone animals (control group) were higher than normal group animals at all intervals. In experimental group animals MCV values were lower than irradiated alone animals at all autopsy intervals except day 15 where value was higher than control group. Only at 6 hours autopsy interval MCV value of experimental group was statistically significant when compared with control group, rest were insignificant.

Mean Corpuscular Hemoglobin Concentration (MCHC)- MCHC values in irradiated alone animals were observed higher than normal animals except at day 30 post irradiation where the value was lower than normal value. MCHC values of control group were statistically significant at day 1

($p<0.05$), day 3 ($p<0.01$) and day 15 ($p<0.05$) when compared with normal. MCHC values in animals pre-treated with PCLE and exposed to radiation were measured higher than control group at 6 hours, day 1, day 3 and day 30 post-irradiation and lower at day 7 and day 15. Values were insignificant at all autopsy intervals in comparison to respective controls.

Differential leucocytes count - Significant reduction in lymphocytes and monocytes was observed following irradiation in control group. In experimental group, the lymphocyte and monocyte percentage remained higher than their respective controls. Minimum percentage of lymphocytes was noticed at day 1 in both control (42.60±1.36%; $p<0.001$) and experimental group (46.40±0.68; $p<0.05$) and thereafter, a continuous increase was observed till day 30 post-irradiation. Changes in monocyte count were statistically insignificant. Minimum monocyte count was observed at day 1 in both control (2.40±0.51) and experimental group (2.80±0.37).

Irradiated alone animals showed a significant increase ($p<0.001$) in neutrophil percentage in comparison to normal group. The percentage of neutrophils remained lower than irradiated alone animals at all autopsy intervals in experimental group. Maximum increase was observed at day 1 in both control (48.6±1.56) and experimental group (44.80±1.28) and thereafter, a continuous decrease was observed till day 30 post-irradiation.

Eosinophil count was higher in irradiated animals than normal animals, but it remained lower in experimental group than control group at all autopsy intervals. Maximum increase was observed at day 1 in both control (5.40±0.51) and experimental group (5.20±0.58). No noticeable change in basophil percentage was reported.

Biochemical study

Lipid peroxidation (LPO) - LPO level showed significant increase following irradiation in both liver and serum of mice. PCLE alone treatment did not exhibited any significant alteration in LPO level. However, PCLE pre treatment prior irradiation significantly lower the liver and serum LPO level in experimental group in comparison with respective controls. Maximum increase was reported at day 3 in serum and liver LPO in control group and serum LPO in experimental group, while liver LPO level in experimental group showed maximum increase at day 1 and after that LPO level was decreased gradually till day 30 post-irradiation.

Reduced glutathione (GSH)- The blood and liver GSH levels was decreased significantly ($p<0.001$) in irradiated alone animals than normal. Maximum decrease was observed at day 1 in liver (32.25±1.41 µmole/gm) and blood (1.57±0.07 µg/ml) GSH level in control group. PCLE alone treatment did not exhibited any significant variation in GSH level. However, PCLE pre treatment prior irradiation significantly increased the liver and blood GSH level in experimental group in comparison with respective controls. Maximum decrease was observed at day 1 in liver (44.23±1.48 µmole/gm) and blood (2.65±0.11µg/ml) GSH level in experimental group.

Superoxide dismutase (SOD) - The liver SOD activity was significantly lower than normal value at all autopsy intervals in irradiated alone animals and maximum decrease was reported at 6 hours (2.33±0.11 µmole/mg protein; $p<0.001$). PCLE alone treatment did not exhibited any significant variation in SOD activity in liver. A significant increase in liver SOD activity was observed in experimental group at all autopsy intervals when compared to control except at day 3 where increase was insignificant.

Minimum liver SOD activity in experimental group was observed at 6 hours (3.03±0.13; $p<0.01$).

Catalase - Liver catalase activity was decreased significantly ($p<0.001$) in irradiated alone animals when compared with normal animals and maximum decrease was observed at day 1 (18.50±0.70 µmole H_2O_2 consumed/min./ mg protein). PCLE alone treatment did not exhibited any significant variation in liver catalase activity. Oral administration of PCLE prior irradiation increased the catalase activity in comparison to control group and maximum decrease in catalase activity was reported at day 1 (25.54±0.98; $p<0.01$).

Histopathological study

Liver of PCLE alone treated animals did not show any significant histopathological changes, hepatic architecture was same as normal (DDW treated). Several histopathological changes were reported in the liver of mice after exposure to gamma radiation. Damage was reported as early as 6 hours post irradiation as expanded sinusoidal spaces and cytoplasmic vacuolization was clearly observed, hepatocytes showing degranulation of cytoplasm, karyolysis and karyorhexis. Enucleation, cytoplasmic vacuolization and expanded sinusoidal spaces were observed in varying degree on different autopsy intervals. Damage was more prominent from day 3 to day 15 post-irradiation. Histopathological damage in liver of experimental group animals was also observed, but this damage was milder than their respective controls. Prominent lymphocytic infiltration was also observed at day 15 post-irradiation. Less cytoplasmic degranulation, karyolysis, karyorhexis, enucleation was reported in experimental animals when compared with respective controls.

Conclusions

- Oral administration of aqueous extract of *Prosopis cineraria* leaves (PCLE) for seven consecutive days did not show any toxicity and mortality till 30 days of observation in mice up to 1600 mg/kg body weight.
- PCLE alone treatment did not show any variation in hematological and biochemical parameters studied in the present study.
- Optimum dose of PCLE for radioprotective studies was selected as 400 mg/kg body weight/day in terms of improved body weight and survival, increased hematological and biochemical parameters.
- PCLE treatment (400 mg/kg body weight/day) before irradiation for seven consecutive days increased the LD50/30 value from 6.70 Gy (control group) to 8.88 Gy (experimental group), resulted in the increase of $LD_{50/30}$ value by 2.18 Gy and the dose reduction factor was calculated as 1.32.
- PCLE treatment before irradiation for seven consecutive days improved average body weight and reduced severity of signs and symptoms of radiation sickness and enhanced survival.
- Oral administration of PCLE for seven consecutive days prior irradiation protected the hematological constituents like RBC, WBC, hemoglobin and hematocrit in peripheral blood of mice against gamma radiation and also maintained the values of RBC derived indices (MCV, MCH and MCHC).
- PCLE administration prior radiation exposure significantly reduced the lipid peroxidation in both liver and peripheral blood and also

protected against radiation induced depletion in endogenous GSH level.

- PCLE administration prior radiation exposure in mice maintained the activities of antioxidant enzymes like SOD and catalase, thus, found to protect and enhance the endogenous antioxidant defense system.
- PCLE pretreatment protected the liver of mice against radiation induced histopathological lesions in terms of reduced cytoplasmic degranulation, karyolysis, karyorhexis, enucleation and less widen sinusoidal spaces.
- PCLE scavenged DPPH in a dose dependent manner and also scavenged ABTS free radicals efficiently *in vitro.* It showed that PCLE has free radical scavenging potential.
- PCLE probably exerts its radioprotection by suppressing the initial radiation induced damage by its antioxidant and free radical scavenging activity, stimulating the antioxidant defence system of the body, protecting hematopoietic system and maintaining hepatic histo-architecture.

Significance of the work

The results of present study indicate that *Prosopis cineraria* possesses good potential to render protection against gamma radiation as exhibited by reduced radiation sickness, improved survival, almost normal hematological and biochemical parameters and modulation in histo-architecture of liver. Thus, the present study clearly suggests that *P. cineraria* leaves extract could be a promising protective agent against radiation exposure.