Discussion

In the present study, no adverse effects in terms of sickness and mortality were reported in mice treated with different concentrations of PCLE up to 1600 mg/kg body weight/day for seven consecutive days and also no significant variation in body weight was reported.

Oral administration of different concentrations (200, 400 and 800 mg/kg b.wt.) of PCLE before irradiation (8 Gy) showed improved survival, increased hematological (RBC, WBC, hemoglobin and hematocrit) and biochemical (lipid peroxidation, GSH, SOD and catalase) protection. Maximum protection was observed in animals those were treated with 400 mg/kg body weight/day PCLE. Therefore, **400 mg/kg body weight of PCLE was found most effective dose** and it was selected as optimum dose and used for further detailed investigation to evaluate radiomodulatory effects of *Prosopis cineraria* against gamma radiation induced hematological and biochemical alterations and histo-architecture of liver in Swiss albino mice.

5.1 Radiation sickness and body weight changes

In the present study, control group animals (irradiated alone) exhibited signs and symptoms of radiation sickness such as reduced food and water intake, diarrhea, ruffled hair and hair loss, facial edema, letharginess and weight loss. These findings of present study are in close agreement with findings of Jagetia et al. (2004), Yadav (2005), Soni et al. (2006), Saini and Saini (2011) and Gupta et al. (2013) who also reported similar radiation sickness symptoms after exposure to different doses of radiation.

Radiation sickness and mortality are dose-dependent and are caused due to various radiation syndromes and excessive loss of electrolytes (Sinha, 1990). Yadav (2005) noted that the signs and symptoms of radiation

sickness correspond to one or the other radiation syndrome i.e. (i) the hematopoietic, (ii) the gastrointestinal or (iii) the central nervous system (CNS) syndrome.

Exposure of the total body or a major part of the body to ionizing radiation can result in Acute Radiation Sickness (ARS), which can cause symptoms that range from mild to severe, occur according to the absorbed dose, the rate of cell proliferation and the duration of the exposure; and may include mortality (Heslet et al., 2012; Romero-Weaver et al., 2014).

In the present study, significant loss in **body weight** of irradiated alone animals was observed. Maximum weight loss was observed at day 9 post-irradiation (73.18% of initial body weight), thereafter a gradual recovery was seen till day 30 and it reached up to 97.58% of initial body weight.

Weight loss in the initial phase may be probably due to the gastrointestinal damage following irradiation (Quastler, 1956). One of the common features of radiation induced gastrointestinal syndrome is marked as loss of water and electrolytes, which may contribute to the weight loss (Griffiths et al., 1999). The weight loss during second phase is associated with the decrease in water intake by animals (Nakamura et al., 1968).

Reduction in body weight following radiation exposure was also reported in mice (Samarth and Kumar, 2003; Kumar et al., 2005; Saini and Saini, 2011; Fan et al., 2012) and rats (Mirjana et al., 2009; Adaramoye et al., 2011; Nwozo et al., 2013). In the present study, weight loss in irradiated animals may be attributed to the reduced food and water intake, fluid loss by diarrhea and the diminished absorption capacity of the gastro-intestinal tract.

In the present study, mice pretreated with PCLE and exposed to 8 Gy of gamma radiation showed less severe signs of radiation sickness and exhibited an almost consistent body weight from day1 to day 14 and thereafter regular weight gain was observed till day 30 post-irradiation.

Radiation induced sickness and damage can be minimized by radioprotective agents, which may be chemical compounds, plant extracts or phytochemicals if they are present in the body of the animals during radiation exposure. Animals treated with PCLE provided significant protection against radiation induced severe weight loss and it indicates that gastrointestinal tract and other vital organs of mice too have received significant protection against radiation by *Prosopis cineraria* leaves extract. This study is in agreement with the findings of Kumar et al. (2005), Lata et al. (2009), Hu et al. (2011), Nwozo et al. (2013) who also reported herbal extract and products provide protection against radiation induced weight loss.

5.2 Radiation induced mortality

In the present study, dose dependent mortality was reported in animals irradiated with 6, 8 and 10 Gy, 11.11, 62.50 and 55.56 percent animals died during first 10 days after irradiation and only 66.67 and 12.5 percent survival at 6 and 8 Gy was observed till day 30 post irradiation whereas no animals survived at 10 Gy till day 30 post irradiation. Similarly, dose dependent mortality in irradiated alone mice was reported by Xu et al. (2014).

Mortality occurred during first 10 days post-irradiation can be assigned to gastrointestinal syndrome (Jagetia and Baliga, 2005), whereas the later deaths may be due to hematopoietic syndrome and bacteremia (Sharma and Kumar, 2007). The gastrointestinal epithelium is less sensitive

than bone marrow progenitor cells but as the cell transit time is more rapid, gastrointestinal syndrome is appeared earlier than hematopoietic syndrome (Jagetia et al., 2004).

Death of animal suffering from gastrointestinal syndrome causes as a result of electrolyte loss, infection to nutritional impairment (Bond et al., 1965). The chief factors responsible for the fluid and electrolyte imbalance are severe diarrhea, failure of intestinal absorption and increased leakage into the bowl lumen (Sinha, 1990).

Radiation exposure above 7 Gy contributes to gastrointestinal syndrome by inhibiting the renewal of cells of lining the digestive tract, and it was also reported that at the doses of 3-8 Gy, tight junctions between the epithelial cells are disrupted, allowing increased fluid and electrolyte loss and permitting the movement of bacterial endotoxins in to the blood. At high doses (10-15 Gy), denudation of mucosa occurs and death results from dehydration, electrolyte imbalance and septicemia (Mettler and Morsely, 1985).

It is generally evident that damage in hematopoietic system plays a major role in radiation-induced death which is thought to be resulted primarily from the effects of radiation on hematopoietic cells (Zhang et al., 2011).

The primary cause of mortality during the early phases of radiation-induced hematopoietic syndrome is sepsis, resulting from opportunistic infection, due to low numbers of neutrophils and increased translocation of bacteria across the gastrointestinal mucosa. This is complicated by thrombocytopenia and concomitant hemorrhage and defects in the adaptive immune system resulting from apoptosis of lymphocytes and deficient lymphopoiesis (Whitnall et al., 2000).

Both epithelial cells of alimentary tract and the circulating leukocytes are relatively short lived and their systematic renewal depends on a population of constantly dividing stem cells. Irradiation inhibits the proliferation of stem cells and as a result replacements are not available when normal abrasion results in the progressive loss of senescent cells and therefore, any damage to these cells spoils normal physiological process with a drastic adverse impact on survival (Fajardo et al., 2001b; Sharma and Kumar, 2007).

Radiation has been found to induce myelo-suppression and loss of hematopoietic progenitor cells, and to contribute to changes in peripheral blood cells. The lower levels of circulating erythrocytes and hemoglobin result in decreased oxygen-carrying capacity, leading to hypoxia and death following radiation (Zhao et al., 2014).

The death due to irradiation in addition to various syndromes is being attributed to the inhibition of immune system i.e. immuno-suppression that increases the chances of infection (Krishna and Kumar, 2005). Decrease in the levels of antioxidant enzymes, a compromised immune system and consequent bacterial infections are the principal factors responsible for radiation induced death (Habib et al., 2007; Kalpana et al., 2011).

In the present investigation, significant increase in survival of mice was observed in experimental group (animals pre-treated with PCLE and irradiated) when compared with irradiated alone, no mortality was reported at 6 Gy within first 10 days and only 37.50 and 33.33 percent animals died during first 10 days at 8 and 10 Gy respectively after irradiation and 90, 62.50 and 33.33 percent survival was reported at 6, 8 and 10 Gy respectively on day 30 post-irradiation.

The dose reduction factor (DRF) is an expression of magnitude of protection against radiation damage and it is a reliable process to assess a radioprotective agent. **In the present investigation, oral administration of PCLE prior irradiation increased the $LD_{50/30}$ value form 6.70 Gy (for control group) to 8.88 Gy (for 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.**

Survival after irradiation actually results from the recovery of several target systems, such as the bone marrow, gastrointestinal tract, skin and haemostatic systems (Widel et al., 2003). Radiation survival is the result of many factors that include-

- The prevention of damage through the inhibition of free-radical generation, efficient scavenging of free radicals (Weiss and Landauer, 2000),
- Repair of damaged DNA (Chandrasekharan et al., 2009)
- Repair of membrane and other damaged target molecules (Belli et al., 2002)
- The replenishment of severely damaged or dead cells (Xu et al., 2014).

Treatment with PCLE prior irradiation delayed the time of onset and severity of radiation induced sickness and improved survival in mice. Administration of PCLE before irradiation showed only 25 percent mortality in comparison to 50 percent of irradiated alone animals within seven days post-irradiation. This observation indicates that PCLE rendered protection against radiation induced gastrointestinal injuries. Only 37.50 percent mortality was reported in experimental group as compared to 87.50 percent in irradiated alone control group and thus pretreatment of PCLE also

provided protection against hematopoietic death. **Overall, an enhanced survival was observed.**

This study is in agreement with the findings of Samarth and Kumar (2003), Krishna and Kumar (2005), Kumar et al. (2005), Sharma and Kumar (2007) and Xu et al. (2014).

In various studies it was reported that treatment of mice with different doses of herbal preparations, plant extracts and chemo-synthetic compounds (derived from plants) delayed the onset of mortality and reduced the symptoms of radiation sickness when compared with the non-drug treated irradiated controls and reduced mortality by providing protection against gastrointestinal and bone marrow death (Pahadiya and Sharma, 2003; Jagetia and Baliga, 2004b; Kalpana et al., 2011; Gupta et al., 2013) and it was also confirmed by the present study.

5.3 Hematological study

In the present investigation, significant reduction in hematological constituents was reported in control group (irradiated alone) animals. This reduction may be attributed to direct damage by radiation dose (Samarth et al., 2001). Hu et al. (2011) stated that after whole body irradiation, manifestations of damage to mammalian tissues are clearly seen in peripheral blood.

Radiation damage to the hematopoietic system affects the hematopoietic stem cells and reduces their cell proliferation capacity (Fan et al., 2012). Although 3 Gy total body dose is required to produce a detectable reduction in total red blood cells, whole body irradiation of moderate dose range (5–10 Gy) leads to a decreased concentration of all the cellular elements in blood (Kumar et al., 2005). This may be due to

direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage or leakage through capillary walls and loss of production of cells (Casarett, 1968). The sequence of changes in total number of various types of circulating blood cells demonstrates largely a reflection of radiation effect on their precursor cells in the hematopoietic organs and hematopoietic cell maturation kinetics as well as circulation and survival period of blood cells (Rubin and Casarett, 1968).

A significant reduction in **RBC count** was observed at all autopsy intervals till day 30 post- irradiation in irradiated alone animals, maximum decline was observed at day 1 post-irradiation after that a gradual increase was observed till day 7 and again decreased on day 15 and then an increase was observed.

Alteration in plasma volume may be an important factor in early decrease in erythrocyte numbers (Bond et al., 1965). Radiation induced depletion of hematopoietic stem cells may be a significant factor responsible for decline in erythrocytic population (Fred and Smith, 1968). Radiation has been shown to cause electrolyte imbalance, loss of thiol groups of erythrocyte membrane proteins, lipid peroxidation and structural changes in erythrocyte membrane proteins (Todo et al., 1982).

In different studies, it was reported that the decrease in erythrocyte count in the irradiated alone group may be assigned to the impairment of cell division, destruction of blood-forming organs, gastrointestinal tract injury, depletion of factors needed for erythroblast differentiation and reticulocyte release from the bone marrow, inhibition of new cells entering into the blood and the loss of cells from the circulation by hemorrhage or leakage through capillary walls, the direct destruction of mature circulating

cells and/or indirectly affecting them via vessel trauma (El-Habit et al., 2000; Gridley et al., 2001; Abouelella et al., 2007).

The findings of present study are in close agreement with those of Kumar et al. (2005), Soni et al. (2006), Nunia et al. (2007), Acharya and Goyal (2008), Duan et al. (2010), Hu et al. (2011) who also reported radiation induced reduction in erythrocytes.

A significant reduction in **hemoglobin** concentration was observed at all autopsy intervals till day 30 post- irradiation in irradiated alone animals, maximum decline was observed at day 1 post-irradiation after that a gradual increase was observed till day 7 and again decreased on day 15 and then increased. The change in hemoglobin content was followed similar pattern as observed in RBC count.

Radiation induced depletion in hemoglobin level in Swiss albino mice was also reported by Kumar et al. (2005), Soni et al. (2006), Duan et al. (2010), Hu et al. (2011), Begum et al. (2012) and Gowda et al. (2013).

A decrease in hemoglobin content may be assigned to the decreased erythropoiesis, and therefore, a reduction in RBC numbers and/or the leakage of RBC into lymphatic tissue and other tissue spaces due to increased capillary permeability and hemorrhage caused by radiation induced lesions in blood vessels. Decrease in hemoglobin content may also due to the inhibition of the synthesis of hemoglobin or increase in the rate of hemoglobin destruction after irradiation (Moss et al., 1964).

Hematocrit values showed significant decline in irradiated alone animals with maximum decline at day 1 post-irradiation. The depletion in the hematocrit value can be assigned to the failure of erythropoiesis,

obliteration of mature cells and internal bleeding. Lukin and Gregersen (1957) reported that there is a gradual and moderate decrease in the red cell volume after irradiation but the total blood volume is well maintained by compensatory increase in plasma volume up to the last day before death. Therefore, increase in plasma volume after exposure to irradiation may also significantly decrease the hematocrit value.

RBC derived indices such as MCH, MCHC and MCV values exhibited an increase in irradiated alone animals than normal values at almost all autopsy intervals. Increased MCH and MCV values show hemolytic anemic condition and could be developed due to diminished hemoglobin level. The increased MCHC values after irradiation shows no abnormality in hemoglobin synthesis, but its decline may be due to the reduced number of RBCs (Yadav, 2005). Sharma and Sharma (2010) reported that an increase in MCH may be due to the altered membrane permeability and survival of hemoglobin rich RBC. Higher values of MCV may be on account of swelling of erythrocytes (Malhotra et al., 1990). Similarly, Malhotra et al. (1990), Samarth (2001), Heikkinen et al. (2001), Sharma and Sharma (2010) have also reported a higher value of MCV in irradiated mice.

Total leucocytes count was decreased significantly in irradiated alone animals, minimum count was reported at day 1 post-irradiation and after that a gradual increase was observed till day 30 post-irradiation.

The decrease in WBC count indicates that radiation-induced injury has seriously weakened intrinsic hematological system and immuno-modulatory function (Yang et al., 2011). The rapid fall in total leukocytes count was probably due to a fast decrease in lymphocytes count in circulating blood as they are most sensitive radiation targets (Sanzari, 2014)

and the same was also demonstrated by the study of differential leucocytes count in the present investigation.

These findings also favor the results of earlier studies carried out by Samarth and Kumar (2003), Kumar et al. (2004), Kumar et al. (2005), Verma et al. (2006), Soni et al. (2006), Nunia et al. (2007), Guruvayoorappan and Kuttan (2008), Hu et al. (2011), Gupta et al. (2013), who also reported depletion in WBC count following radiation exposure.

Significant decline in **lymphocytes** was observed in irradiated alone group animals at all autopsy intervals and minimum percentage of lymphocytes was noticed at day 1 post-irradiation. In the present investigation, maximum decrease in lymphocyte at early intervals mainly may be due to direct destruction of such cells elucidating an early cell killing effect of radiations on this cell type. The decrease in lymphocytes also might result from oxidative damage in spleen tissues as spleen plays an important role in immune functions by proliferating lymphocytes (Witztum, 2002).

Studies have demonstrated that lymphocytes are considered to be the most sensitive type of blood cells and the earliest blood change following whole body irradiation is lymphopenia (Seddek et al., 2000; Ezz, 2011). Hughes and Walden (1988) reported that numbers of lymphocytes reduced in a dose dependent manner after exposure to radiation. In the present study, lymphocytes also behaved as most radiosensitive blood cells and lymphopenia was observed shortly after irradiation, which was either due to direct killing of circulating lymphocytes or due to destruction of stem cells.

The change in **neutrophil** count was inversed to that of lymphocytes. Significant increase in neutrophils was reported in irradiated alone animals at all autopsy intervals and minimum percentage was observed at day 1 post-irradiation. This abrupt rise in neutrophil percentage may be due to an abortive rise phenomenon as described earlier by Waghmare et al. (2011b).

Eosinophil count was higher in irradiated alone animals than normal group. No noticeable changes were recorded in monocyte and basophil count following irradiation in the present investigation. However, Jacobson (1954) reported that monocytes exhibit a similar pattern as of lymphocytes in peripheral blood after exposure to 100R or above dose of radiation but regained normalcy or showed increase between day 4 and day 6.

Exposure to ionizing radiation leads to develop a complex dose dependent cascade of alterations including injury to the lymphoid and hematopoietic system in mammals, which can result in septicemia and death (Prasad, 1999).

PCLE facilitates protection to hematological constituents in experimental group animals against irradiation, which were highly reduced in irradiated alone animals.

In the present study, animals treated with PCLE prior irradiation showed significant increase in erythrocytes count and in WBC count as compared to irradiated alone (control) animals. The recovery of blood cells appeared due to recovery of hematopoietic organs, which supply new cells for peripheral circulation.

In the present study, animals treated with PCLE prior irradiation were showed significant increase in hemoglobin level as compared to

control group and significant increase in hematocrit values was also observed.

In experimental group (treatment with PCLE before irradiation) animals, MCH and MCV values retained lower than in irradiated alone animals at almost all autopsy intervals, values of MCHC 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.

PCLE treatment prior irradiation facilitates protection to the hematological constituents possibly by stimulating and/or protecting hematopoietic stem cells from damaging effects of radiation, therefore PCLE pre-treated irradiated animals exhibited lower values of RBC derived indices as compared to irradiated alone animals.

Oral administration of PCLE prior to irradiation increased the lymphocyte percentage at all autopsy intervals and decreased in neutrophil and eosinophil percentage at all autopsy intervals. No noticeable changes were observed in monocyte and basophil count following irradiation in the present investigation.

These results indicated that the treatment of the Swiss albino mice with PCLE significantly modulated the bone marrow depression. This study indicates that the stimulatory effect of PCLE was not only limited to the erythropoiesis, but it also extended to the leukopoiesis and also it may suggest that PCLE has the immuno-modulating property.

This may be due to the presence of phyto-constituents that could interact and stimulate the formation and the secretion of erythropoietin and the hematopoietic growth factors/committed stem cells (Gowda et al., 2013).

5.4 Biochemical study

Most of radiation-induced damage to biological molecules in aqueous media, such as those prevailing in living systems, is caused by the formation of free radicals resulting from the radiolysis of water (Orsolic´ et al., 2007). Reactive oxygen species mediated cascading chain reactions and redox imbalances have been well documented in radiation toxicity studies. Radiation toxicity and repair mechanisms depend on the status of endogenous antioxidant enzymes (Sun et al., 1998; Duan et al., 2010). In the present study, PCLE reduced radiation induced damage in Swiss albino mice by increasing endogenous antioxidant enzyme levels (SOD, Catalase and GSH) and reducing lipid peroxidation.

Lipid peroxidation

Lipid peroxidation is a highly destructive process that affects cellular organelles and causes them to loose biochemical function and structural integrity which may lead to irreparable damage or cell death, thus the measurement of LPO is a convenient method to moniter oxidative cellular damage.

Lipid peroxidation (LPO) has been shown to induce disturbance of membrane organization and functional loss and modification of proteins and DNA bases, and it has been implicated in the pathogenesis of various diseases (Niki, 2009; El-Beltagi and Mohamed, 2013).

Free radicals generated by radiation attack the fatty acid component of membrane lipids, leading to lipid peroxidation and finally resulting in cell death (Kalpana et al., 2011). Lipid peroxidation can be initiated by lipid radiolytic products including hydroxyl and hydroperoxyl radical (Raleigh, 1987). The increases in plasma membrane permeability to organic substance

including enzymes are the direct concern of membrane damage due to the process of lipid peroxidation (Hamza and El-Shennawy, 2009).

In the present study, significant increase in the lipid peroxidation level in liver and serum in terms of TBARS or MDA was observed in irradiated alone animals at all autopsy intervals post-irradiation. Maximum increase was reported on day 3 post-irradiation in both hepatic and serum LPO levels.

The ability of ionizing radiation to induce lipid peroxidation in liver, serum and other tissues of irradiated animals has been reported by several researchers (Duan et al., 2010; Ahn et al., 2011; Adaramoye et al., 2011; Kucukkurt et al., 2011; Hu et al., 2011; Nwozo et al., 2013) and was confirmed in the present study.

High level of MDA is detrimental to cells and tissues, and leads to lose of their normal biological functions (Cheng et al., 2011; Du et al., 2012). This elevation in MDA level after irradiation as in present study is a clear indication of increased oxidative stress. Oxidative stree is a state of imbalances between generation of ROS and the level of antioxidant defense system. It has been reported that LPO starts to increase as soon as the endogenous GSH is exhausted, and the addition of GSH promptly stops further oxidations (Zhao et al., 1989).

The present study demonstrates that administration of PCLE alone did not influence LPO levels in liver and serum; **PCLE treatment prior irradiation considerably decreased the radiation induced lipid peroxidation in terms of malondialdehyde production.** A number of Plant extracts and phytochemicals has been showed to inhibit radiation induced lipid peroxidation (Goel et al., 1999; Uma Devi et al., 2000;

Kumar et al., 2003; Samarth and Kumar, 2003; Kumar et al., 2005; Adaramoye et al., 2011).

Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants (Konings and drijver, 1979). It has been shown that α-tocopherol in the membranes protect polyunsaturated fatty acids (PUFA) against radiation induced lipid peroxidation when low dose rates are applied (Konings et al., 1979). Shimoi et al. (1996) proposed that plant flavonoids that exhibit antioxidant activity *in vitro*, also act as antioxidants *in vivo* and their radioprotective potential may be assigned to their radical scavenging activity. The decrease in lipid peroxidation in experimental group could be due to the ability of PCLE to scavenge secondary reactive radicals or to prevent formation of superoxide and/or hydrogen peroxide in response to the irradiation.

Reduced glutathione (GSH)

GSH is the major component of cellular antioxidant system with the following characteristics-

- GSH in diet can be partly absorbed from the small intestine and can be synthesized *de novo*, so that GSH is both an exogenous and endogenous antioxidant.
- The multiple physiological and metabolic functions of GSH include thiol transfer reactions that protect all membrane and proteins.
- GSH is essential for maintaining the reducing capacity of cells, the loss of this capacity may cause the cell to die.
- It plays a key role in the liver in detoxification reactions.

In the present study, significant decrease in the reduced glutathione level in liver and blood was observed in irradiated alone animals at all autopsy intervals post-irradiation. Maximum decrease was reported on day 1 post-irradiation in both hepatic and blood GSH levels.

Decrease in the tissue GSH levels following radiation exposure was reported by several workers (Kumar et al., 2004; Uma Devi, 2001; Kumar et al., 2005; Samarth et al., 2006; Sandeep and Nair, 2012; Kucukkurt et al., 2011; Nwozo et al., 2013; Das et al., 2013) and similar results were reported in the present study.

Decreased level of GSH is generally considered as an index of increased oxidative stress. It may indicate inability of the cells to generate enough GSH, due to severe cellular damage or due to greater utility in combating the oxidative stress (Uma Devi, 2001; Bhartiya et al., 2008) and also elevated reactive species levels leading to a depletion of GSH pool (Das et al., 2014), which overwhelms the cellular defense and lead to membrane lipid peroxidation and loss of protective thiols (Konings, 1987). Depletion in GSH level following irradiation in this study could be due to an increased utilization of the antioxidant system during detoxification of the free radicals produced by radiation. Excessive LPO can also cause increased glutathione consumption (Comporti, 1987).

In a study, Savoure et al. (1996) reported that the reduction in GSH contents may be due to the inactivation of glutathione reductase and peroxidase activities with subsequent production of GSSG. Moreover, normal synthesis/repair of GSH could be impaired due to damage to DNA and membranes (Koiram et al., 2007). The decrease in GSH may be also attributed to its diffusion through impaired cellular membrane and/or

inhibition in GSH synthetase and glutathione reductase enzymes (Zahran et al., 2006; El-Missiry et al., 2007; Hussein, 2008; Farag et al., 2009).

The present study demonstrates that administration of PCLE alone did not influence significantly the endogenous GSH level in both liver and blood of mice, but **PCLE pretreatment following irradiation protects the endogenous GSH depletion in experimental group animals, GSH level was significantly higher in PCLE pretreated and irradiated animals at all autopsy intervals in comparison with irradiated alone group.**

The comparatively less decrease in GSH level in PCLE pretreated irradiated animals could be due to higher availability of GSH, which enhances the ability to cope up with free radicals generated by irradiation. The increase in GSH levels may be due to the activation of protective response in these tissues to regulate physiological mechanisms within the cells through redox balancing reactions (Pathak et al., 2007; Adaramoye et al., 2011). It suggests that radioprotection by PCLE may be mediated through the modulation of cellular antioxidant levels.

Free radical generation during radiolysis of water plays significant role in the indirect biological damage caused by ionizing radiation. The GSH/GST detoxification system is an important part of cellular defense against a large array of damaging agents. GSH protects against oxygen derived free radicals and cellular lethality followed by radiation exposure (Biaglow et al., 1987). Evidences suggest that intracellular non protein sulfhydryl compounds has an important role in the cellular response to ionizing radiation (Bump and Brown, 1990), and glutathione accounts for 90 percent of non protein sulfhydryls in mammalian cells (Biaglow et al., 1987).

GSH executes its radioprotective function through free radical scavenging, restoration of damaged molecules by donating hydrogen, reduction of peroxides and maintenance of protein thiols in the reduced state (Bump and Brown, 1990). Exposure of cells to radioprotective agents increases the intracellular concentrations of non protein sulfhydryl groups (Revesz et al., 1972) and it is evident that cells rich in non protein sulfhydryls are radio-resistant. Therefore, in the present study, it is supposed that the release of non protein sulfhydryls after PCLE administration might responsible for radioprotection of cells. It was also reported that administration of plant extracts increases the GSH levels in irradiated animals (Kumar et al., 2005; Adaramoye et al., 2011).

Thus, increased GSH level represents an important line of defense in radiotoxicity, which detoxifies and eliminates free radicals and electrophilic compounds from the cell.

Superoxide dismutase and catalase activity

Superoxide dismutase (SOD) and catalase (CAT) are metalloproteins that employ dismutation reactions to detoxify ROS. The metal ions in these enzymes undergo redox (oxidation/reduction) changes to bring about dismutation reactions that are energy independent (Patel and Day, 1999)

In the present study, significant decrease in the superoxide dismutase and catalase activity in liver was observed in irradiated alone animals at all autopsy intervals post-irradiation. Maximum decrease was reported on 6 hours post-irradiation. Reduction in super oxide dismutase and catalase activity after irradiation was reported by several workers (Samarth et al., 2006; Mansour et al., 2008a; Hamza and El-Shennawy, 2009; El-Fatih and El-Tawil, 2009; Adaramoye et al., 2011; Nwozo et al., 2013) and also confirmed in the present investigation.

The decreased activities of SOD and catalase in the tissues of the animals could be due to the organ's response to an increased production of ROS as a result of irradiation or response to other noxious metabolites generated (Adaramoye et al., 2011). The findings of decreased SOD and catalase activities in the liver and blood of Swiss albino mice as a result of irradiation is not unexpected as both SOD and catalase are co-regulated in tissues in response to toxic assaults (Lew and Quintanilha, 1991). This observed decrease of enzymatic activities in irradiated rats may be due to the increased consumption of these antioxidants to counteract lipid peroxidation (Kalpana and Menon, 2004; El-Fatih and El-Tawil, 2009).

The reported reduction in SOD activity also suggests inactivation of the enzyme possibly due to increased superoxide radical production or an inhibition by the H_2O_2 as a result of corresponding decrease in the activity of catalase which selectively degrades H_2O_2 (El Shahat, 2013). Further, the decrease in CAT activity might be interpreted by the prospect of oxidative modifications of various protein types which leads to functional alteration which can have substantial physiological impact, as oxidative damage to enzymes associated with a modification of their activity (Prasad et al., 2005; El Shahat, 2013).

SOD and catalase are important anti-oxidative enzymes which can scavenge the superfluous free radicals, thereby protecting cells from damage and decreasing the deleterious species or lipid peroxides, such as MDA (Curello et al., 1987; Anscher et al., 2005; Du et al., 2012). SOD catalyses the breakdown of $O_2^{\bullet}$ to O_2 and H_2O_2, prevents formation of $OH^{\bullet}$ and thereby has been implicated as an essential defense against the potential toxicity of oxygen. The ROS scavenging activity of SOD is effective only when it is followed by the actions of other enzymes, because

the dismutase activity of SOD generates hydrogen peroxide, which needs to be further scavenged by CAT and GPX (Rao et al., 2009).

In the present investigation, PCLE treatment prior irradiation improved super oxide dismutase activity as well as catalase activity in liver and it indicates antioxidant potential of PCLE. Both super oxide dismutase and catalase activity was significantly higher in PCLE pretreated and irradiated animals at all autopsy intervals in comparison to irradiated alone group.

Several studies on other plant extracts using various animal models have been carried out, where improved SOD and catalase activities have been reported (Sinha et al., 2011; Du et al., 2012; Osman et al., 2013; Verma et al., 2014).

In the present study, administration of PCLE alone did not influence significantly the LPO and endogenous GSH levels in both liver and peripheral blood of mice, and also no significant variations was observed in liver SOD and catalase activity in PCLE alone treated animals. Whereas when PCLE administered prior irradiation it influences these parameters significantly, increase in GSH level, SOD and catalase activities and also decrease in LPO level were reported in comparison to irradiated alone values. Therefore, the results of present study indicate that PCLE provides significant protection against gamma radiation.

5.5 Histopathology of liver

Radiation induced severe damage in hepatic tissues was observed in the present study. Karyolysis, karyorhexis, pyknosis and enucleation of hepatocytes, cytoplasmic vacuolization, lymphocytic infiltration, de-granulation of cytoplasm, dilation of sinusoidal spaces and central vein

were reported in irradiated alone animals. **Oral administration of PCLE prior irradiation in experimental group reduced the severity of these histopathological changes in liver with slight recovery of normal histoarchitecture, however, till day 30 post-irradiation normalcy was not achieved in both control and experimental animals.**

These observed changes in the liver tissue showed similarity and conforms those reported by several workers, with various experimental animals exposed to radiation (Nassar et al., 2008; Purohit et al., 2009; Alam et al., 2010; Sharma and Sisodia, 2010). Mansour et al. (2008b) also reported that liver of rat treated with 6 Gy γ-radiations displayed fragmentation of the hepatic cells in addition to the presence of many of the hepatocytes manifested pyknotic nuclei and also many of aggregated inflammatory cells were detected. Jeong et al. (2007) studied fast neutron mediated liver injuries and they reported various damages as loss of hepatocytes, necrotic foci and vacuolar changes in the dose dependent manner.

Maurya and Devasagayam (2013) observed that radiation exposure resulted in significant morphological damages in hepatocytes which contains pyknotic, multilobed, dense and haematoxylin rich nuclei and with the increase of time intervals hepatocytes found swelled and membranes appeared severely disrupted, sinusoidal spaces increased and disrupted nuclei of hepatocytes.

The hepato-toxicity of gamma irradiation has been reported to be due to production and release of free radicals in the aqueous medium of cells which damage the all cellular structures and vital molecules inside the cell (Nassar et al., 2008) and/or the direct harmful effect of irradiation on the biological system (Hagen, 1989). Different radio-lesions observed in

the present study may be produced due to increased lipid peroxidation and also due to destruction of DNA, protein, cytoskeleton and organelles (Soyal et al., 2007b).

Although, binucleated cells are present in normal liver, but in the present study, an elevation in these cells was observed in irradiated alone animals. Binucleated cells may be formed as a result of failure of cell separation after completion of mitosis or due to the fusion of cells (Sharma and Sharma, 2005). Grizzi and Chirira-Internati (2007) hypothesised that increase in binucleated cells may be an index of liver illness rather than the result of errors occurring during course of the cell cycle. Thus, there may be a possibility that increase in bionucleated cells in early phase post-irradiation is due to fusion of mononucleated cells induced by radiation generated free radicals; in later stages and in PCLE pre-treated irradiated animals it may be due to recovery of hepatic tissue through regeneration (Maharwal et al., 2005).

Lymphocytic infiltration was reported in the present study and similar inflammatory cells infiltration was observed by Rashed et al. (2014). The appearance of inflammatory cells in hepatic tissue may suggest that radiation induced ROS may imitate an inflammatory response (Johar et al., 2004). Disturbed cellular metabolism related to protein fat and enzymes might be responsible to produce degeneration of hepatocytes with cytoplasmic degranulation and vacuolization and dilation of sinusoidal spaces after irradiation in present study and also confirmed by Roudkenar et al. (2008) and Sharma and Sisodia (2010).

In the present study, it was found that the radiation induced damage to hepatic tissues was reduced by PCLE pre-treatment. The protective effect was manifested in an early recovery as indicated by

the less severe damage in experimental animals. Although the exact mechanism is not fully understood; the antioxidant activity is found to be involved in this process as seen in terms of increase in GSH content, SOD and catalase activities and decrease in LPO.

5.6 Mechanism of radioprotection by *Prosopis cineraria*

In the present study, **PCLE has been shown to scavenge the free radicals in both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays *in vitro*,** suggesting that plant extract contain such compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. It is also reported that *P. cineraria* have been shown to exhibit *in vitro* antioxidant and free radical scavenging potential (Dharani et al., 2011; Napar et al., 2012). Thus, free radical scavenging seems to be an important mechanism of radioprotection by PCLE.

As irradiation causes gastrointestinal syndrome and hematopoietic syndrome, in the present study, **PCLE might have protected from these syndromes as it reduced the severity of radiation sickness and not only delayed the onset of mortality but also enhanced survival of irradiated mice.** Survival studies revealed that oral administration of PCLE prior irradiation increased the $LD_{50/30}$ value from 6.70 Gy (for control group) to 8.88 Gy (for 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.

Protective agents that are capable of enhancing survival in the radiation dose inducing hematopoietic syndrome have typically been associated with accelerated hematopoietic regeneration (Hosseinimehr et al., 2006). An enhanced ability to regenerate new hematopoietic elements, especially those that are important in controlling microbial infections, such

as granulocytes, allow the host to resist opportunistic infections better and, therefore, improved survival.

Velmurugan et al. (2010) and Napar et al. (2012) reported that *P. cineraria* exhibits antimicrobial and antibacterial properties and therefore, it can protect against opportunistic infections of microbes.

The results of present study showed that the oral administration of PCLE for seven consecutive days prior irradiation protects from radiation induced hematological and biochemical changes in Swiss albino mice.

The results of present study indicates that PCLE administration prior irradiation improved blood cells count (RBC and WBC) as compared with irradiated alone animals. The results of this study also demonstrated that PCLE can reduce radiation induced damage in Swiss albino mice by increasing endogenous antioxidant enzyme levels (SOD, Catalase and GSH) and reducing lipid peroxidation. Therefore, it seems that stimulation of the antioxidant defense system of the body is one of major mechanism behind the radioprotective effect of PCLE.

The present study showed that PCLE exerts its radioprotective potential in two ways, first, it suppresses the initial radiation induced damage by antioxidant activity and second, it stimulates the cellular regeneration, particularly, hematopoietic cells, in the post irradiation period and improve the production of bone marrow progenitor cells. Compounds with antioxidant potential have been shown to prevent the harmful effects of ionizing radiation in living systems due to their efficacy to scavenge free radicals (Agarwala and Goel, 2002; Mathew et al., 2007; Menon et al., 2011).

P. cineraria has also been shown to exhibit antitumor properties (Robertson et al., 2011) and a plant with chemopreventive properties is known to possess radioprotective properties (Jo et al., 2004, Saini and Saini, 2011).

***P. cineraria* contains alkaloids, flavonoids, glycosides, tannins, phenolic compounds, saponins, steroids, terpenoids, free amino acids and vitamins etc (George et al., 2012; Mohammad et al., 2013) and thus, radioprotective activity of PCLE may be a synergistic contribution of these constituents as many of these constituents independently showed antioxidant and radioprotective efficacy.**

Saponins have been known to exhibit immuno-stimulant properties (Francis et al., 2002). Yalinkilic and Enginar (2008) concluded that the supplementation with saponin-containing extracts may serve to reinforce the antioxidant systems, thus having protective effect against cell damage by X radiation. It has been reported that saponins derived from Tribulus terrestris showed protection against UVB radiation induced damages (Sisto et al., 2012).

Many flavonoids are already known for their antioxidant action and anti-apoptotic potential, and thus contribute towards radioprotection; by providing the recovery of damaged cells after radiation exposure and minimization of cell death by inhibition of apoptosis (Zakaria and Ibrahim, 2014). Thus, in the present investigation, hepato-architecture was more or less maintained with modulation in number of hepatocytes. Wang et al. (2012) reported that administration of flavonoids might be efficacious in the prevention of radiation-induced lung damage. Flavonoids such as rutin and luteolin, also reported in *P. cineraria*, have been reported to be

radioprotective due to their free radical scavenging properties (Shimoi et al., 1996; Patil et al., 2013).

Phenolic compounds such as ferulic acid and gallic acid possess radioprotective activity as they enhance recovery of hematopoietic cells (Ma et al., 2011), decrease DNA damage (Gandhi and Nair, 2005; Maurya and Devasagayam, 2013), and also found to ameliorate radiation induced inflammation (Das et al., 2014). Thus, the presence of these compounds in PCLE may responsible for its protective potential against gamma radiation in mice.

Various mechanism such as prevention of damage through inhibition of generation of free radicals, scavenging of free radicals, protection of cellular and sub-cellular entities especially against oxidative damage, enhancement of repair of target molecules like DNA, protein, etc., restoration of cell proliferation and stimulation of immune cell activity are considered important for radioprotection (Zhou et al., 2005; Madhu and Kumari, 2014) and this was proved by PCLE.

The results of the present investigation demonstrate that PCLE probably exerts its radioprotection by suppressing the initial radiation induced damage by its antioxidant and free radical scavenging activity, stimulating the antioxidant defense system of the body, protecting hematopoietic system and stimulating production of hematopoietic cells.