

Incessant natural and anthropogenic activities like use of automobiles, pesticides, waste disposal, industries, radiation etc. are immensely contributing to environmental pollution (Tiwari and Gupta, 2016). Humans are at much more risk through natural and occupational exposure to radiation in nuclear power plants, nuclear laboratories, industries and those involved in transportation of radioactive wastes etc. (Uosif et al., 2016). Ionizing radiations lead to oxidizing events through spontaneous generation of ROS such as superoxide anion ( $O^{2-}$ ), the hydroxyl radical ( $OH\cdot$ ) and hydrogen peroxides ( $H_2O_2$ ), resulting in depletion of antioxidants in the cells culminating in cell injury (Srinivasan et al., 2007; Li et al., 2016; Ozougwu, 2016). One of the basic mechanisms of radiation injury is the production of free radicals leading to the formation of peroxides and reactive oxidative species (Poljsak et al., 2013; Ozougwu, 2016). These peroxides, via lipid peroxidation, damage the cell membrane and other cell components (Bhattacharjee, 2014; Greenwald et al., 2016). Membrane damage caused by these excessive ROS may allow sequential biochemical and micro-anatomical cellular degranulation which leads to necrosis.

Strengthening of inbuilt protective mechanisms or exogenous administration of antioxidants may be useful in the protection of tissue damage from ROS (Flora et al., 2006; Hewawasam et al., 2016). Mechanism of action of phyto preparations differ in many respects from those of the synthetic drugs or single

substances (Jagetia and Baliga, 2004; Kumar et al., 2016). Several phytochemicals have the advantage of low toxicity and are protective when administered at pharmacological doses (Weiss and Landauer, 2003; Lee and Kim, 2016). Several pathways of radioprotection have been suggested for protection against the deleterious effects of ionizing radiation (Bala et al., 2014; Kumar and Tiku; 2016).

*Aloe vera* (syn. *Aloe barbadensis* Miller) is a traditional medicinal plant belonging to family of Liliaceae (Rajeswari et al., 2012; Singh et al., 2016). This plant contains glucomannan, acemannan, anthraquinone, glycoproteins, glucose, linolenic acid, saponins, lignin, sterols, cholesterol, amino acids, vitamins, minerals etc. (Moghaddasi and Verma, 2011; Pandey and Singh, 2016). The bioactive constituents of *Aloe vera* have been reported to have antioxidant, antitumor, anti-diabetic, anti-aging, wound-healing, anti-inflammatory properties etc. (Zhang et al., 2006; Sahu et al., 2013; Kumbhar et al., 2015). *Aloe vera* has a long history of its use for medicinal, cosmetic and many other purposes (Sahu et al., 2013; Mahor and Ali, 2016). Recently, many authors have focused the radioprotective research towards *Aloe vera*. By the early 1800s, *Aloe vera* was used as a laxative in the United States. But in 1930's, it was successfully used to cure chronic and severe radiation dermatitis (Atherton, 1998; Surjushe et al., 2008). Many investigators have shown that *Aloe vera* extract has damage resistant properties against whole body radiation induced biochemical alterations in mice liver (Goyal and Gehlot, 2009; Nada et al., 2013). Radioprotective effect of *Aloe vera* was also studied against X-ray

induced distorted histoarchitecture in hepatic tissue of mice (Dadupanthi and Saini, 2015). *Aloe vera* conferred significant amelioration against ionizing radiation induced renotoxicity in murine models (Chakrawarti et al., 2013). Furthermore, experimental studies have demonstrated the ability of *Aloe vera* to inhibit neuro, hepatic and renal toxicity induced by cadmium and gamma radiation exposed mice (Purohit et al., 2014). Considering various reports on curative effect of *Aloe vera* against ionizing radiation, the present investigation was an endeavour to determine the radioprotective potential of *Aloe vera* gel extract against X-ray induced oxidative stress in various organs of mice.

Various reports are available on *Aloe vera* that demonstrate its beneficial as well as deleterious effects depending upon the dose and duration of consumption (Pandiri et al., 2011; Boudreau et al., 2013; Sharma et al., 2014). Despite *Aloe vera*'s long history of use, several studies have reported contrasting results. Some studies suggested that *Aloe vera* possesses toxic potential while as others claimed that it has no adverse effects which may be an effect of the dose of *Aloe vera* employed (Williams et al., 2010). Aloin and aloe-emodin may act as either pro-oxidant and antioxidants depending on their concentration being used (Tian and Hua, 2005). It has been reported that *Aloe vera* extract at the dose of 100 mg/ kg b.w. and 70 mg/ kg b.w. resulted in decrease in sperm motility and sperm counts while 30 mg/ kg b.w. of extract administration caused no change in the same in male rats (Oyewopo et al., 2011). Administration of *Aloe vera* (100 and 300 mg/ kg b.w.) with paracetamol induced toxicity in liver of rats (Samuhasaneetoa and

Kajornvuthidej, 2014). Adverse effects of *Aloe vera* whole-leaf powder have been reported at concentrations of 2 g/ kg b.w. (Zhou et al., 2003). Moreover, consumption of Aloe latex during pregnancy is restricted because it may cause severe uterine contractions and increase the risk of miscarriage. Ingestion of Aloe latex should also be avoided by nursing mothers because of the possibility of causing severe cramps and diarrhoea in the infant (Brinker, 1998). Excessive consumption of *Aloe vera* has been reported to cause renal problems in humans (Pawar et al., 2011). In mice, higher doses of *Aloe vera* could result insignificant decrease in red blood cell count and sperm damage (Shah et al., 1989). The present study was conducted to establish the toxicity profile of *Aloe vera* in experimental animals which render strong evidence for its safety against various diseases. Thus, keeping in view, the complexities inherent in *Aloe vera* pharmacology and the inconsistencies reported in literature regarding its safety and effectiveness, a dose of *Aloe vera* was standardized for possible medicinal effects.

### **6.1. Phytochemical Analysis of Aqueous *Aloe vera* Gel Extract**

Phytochemicals in *Aloe vera* extract were qualitatively analysed based on the presence or absence of color change indicated as positive or negative results. *Aloe vera* contains chemical constituents include tannin, phlobatannins, saponin, flavonoids, steroids, terpenoids and glycosides anthroquinones, which exhibit medicinal effects (Meenakshi and Kalavathy, 2015). The phytochemical analysis of aqueous *Aloe vera* gel extract used in the present study revealed the presence of anthraquinone glycosides, carbohydrates, tannins and absence of

alkaloids. Presence of phytoconstituents (phenols, flavonoids, tannins) in the *Aloe vera* extracts may be responsible for its antibacterial and antioxidant activity of the plant (Marjorie, 1999). Phytochemical screening of alcoholic *Aloe vera* gel extract showed absence of tannins and anthraquinones (Mariappan and Shanthi, 2012). Anthraquinones and tannins may act as antioxidants and free radical scavengers (Soni and Sosa, 2013; Adeosun et al., 2016). Various phytoconstituents have been reported to possess strong antioxidant activities due to their ability to absorb, quench free radicals and decompose peroxides generated in the system (Paulpriya and Mohan, 2013). Many studies reported that *Aloe vera* gel contain polysaccharides which possesses immunomodulatory property in mice (Im et al., 2010; Bal et al., 2013; Sahu et al., 2013). Anthraquinone glycosides are powerful purgative and potent anti-microbial agents (Joseph and Raj, 2010). *Aloe-emodin* is an anthraquinone, which has the ability to suppress or inhibit the growth of malignant cancer cells (Urch and David, 1999). *Aloe vera* has wound healing property due to the presence of anthraquinone (Nandal and Bhardwaj, 2012). Anthraquinones present in *Aloe vera* has previously been shown to have anti-microbial property (Wu et al., 2006). Tannins are phenolic compounds known to exhibit analgesic, anti-oxidant, anti-inflammatory and anti-microbial activities (Raphael, 2012). It is known to be useful in the treatment of inflamed or ulcerated tissues, cancer. Tannins are known to have therapeutic applications in wound and burn healing because of their antioxidant, anti-inflammatory and antifungal properties (Fahimi et al., 2015).

### 6.1.2. Components of Aqueous *Aloe vera* Gel Extract

Flavonoids are the most common polyphenolic constituents found ubiquitously in natural compounds (Rathanavel and Arasu, 2014). In the present study, total flavonoid concentration was found to be 4.28 µg/ mg wet weight of *Aloe vera* gel extract. It has been reported that the gel of *Aloe vera* possesses significant amount of phenols and flavonoids which contributes to its antioxidant activities (Taukoorah and Mahomoodally, 2016). As previously reported, total phenol and flavonoid content was estimated in different fractions of *Aloe vera* extract which contribute to the antioxidant activities (Sultana et al., 2009; Ibe et al., 2014; Vastrad et al., 2015).

The presence of polysaccharides in *Aloe vera* contributes to its antioxidant and free radical scavenging activity (Chun-hui et al., 2007; El-Shemy et al., 2010). Polysaccharide and flavonoid in *Aloe vera* extract plays important role for its antioxidant property (Hu et al., 2003). In the present study, total carbohydrate concentration was found to be 7.33 µg/ mg wet weight of *Aloe vera* gel extract. Polysaccharides in *Aloe vera* gel extract were suggested to be most effective in anti-genotoxic and anti- promoting activities (Kim et al., 1999). Polysaccharides from *Aloe vera* conferred protection against X-ray induced skin damage in mice (Wang et al., 2004). Carbohydrates present in *Aloe vera* have exhibited immunomodulatory activity which may play important role in radioprotection (Im et al., 2010).

### 6.1.3. Analysis of Trace Elements in Aqueous *Aloe vera* Gel Extract

Minerals, the nutritive elements present in biological system, have decisive role to maintain certain physicochemical processes and serve as cofactors in many metabolic pathways (Soetan et al., 2010). Quality of many medicinal plants depends upon minerals present in it (Bahadur et al., 2011). Excessive uptake of trace elements by plants may affect the metabolic pathways in the body (Jabeen et al., 2010; Rai et al., 2011). Trace elements like arsenic, cadmium, chromium, lead, mercury etc. have the potential to accumulate in different organs for a long period of time and cause toxic effects in the body (Ata et al., 2009). Prolonged exposure to heavy elements like lead and cadmium can cause deleterious effects in the body. Various researchers are trying to link the contents of trace elements and medicinal values of the plants (Zafar et al., 2010; Sharma et al., 2011). In the present study, quantitative analysis of various trace elements in *Aloe vera* extract was determined using WD-XRF technique. *Aloe vera* gel extract contains significant quantity of sodium, potassium, calcium, magnesium, iron and zinc (Rajendran et al., 2007). Potassium and sodium are essential trace elements which play crucial role in maintaining cellular homeostasis (Sharma et al., 2011; Pohl et al., 2013). Calcium is main component in bone which is helpful for regulating skeletal and cardiac muscles contractions (Toyoshima et al., 2000). Manganese is essential for normal functioning of central nervous system and has antioxidant property (Bibi et al., 2006). Zinc helps in wound healing, regulation of systolic blood pressure and also functions as an antioxidant (Chow and Barbul, 2014). Zinc and Copper

play major roles to regulate cardiovascular homeostasis including pathogenesis of hypertension (Carpenter et al., 2013). Zinc plays an important role in the structure of proteins as well as in catalytic process (Auld, 2001). The pro-oxidant or antioxidant activity depends on the concentration of phyto-antioxidants. Many of the phytochemicals display pro-oxidant activity at higher doses or in the presence of transition metals (Bouayed and Bohn, 2010). Copper and iron are the most redox active metal ions present in the living system (Poljsak, 2011). *Aloe vera* may possess pro-oxidant property leading to DNA degradation due to the presence of metal ions like copper (Naqvi et al., 2010).

#### **6.1.4. Antioxidant Capacity of Aqueous *Aloe vera* Gel Extract**

Various methods are used to evaluate antioxidant activities of natural compounds, plant extracts and commercial products (Alam et al., 2013). ABTS and DPPH antioxidant assays are commonly used to assess *in vitro* antioxidant activity (Badarinath et al., 2010). ABTS• radical scavenging assay is one of the popular indirect methods of determining the antioxidant capacity of compounds (Roginsky and Lissi, 2005). The observations of present study are consistent with previous reports in which ABTS and DPPH free radical scavenging properties of *Aloe vera* whole leaf and gel extracts were demonstrated (Masaldan and Iyer, 2011; Wintola and Afolayan 2011; Khanam and Sharma, 2015).

DPPH radical assay is widely used to measure the efficacy of pure antioxidants, natural compounds and crude mixture of plants and herbs. This

colorimetric assay is popular owing to the relative stability of the DPPH radical, its sensitivity and technical simplicity (Huang et al., 2005). The results indicated that the free radical scavenging activity increases with increase in concentration of *Aloe vera* extract. ABTS and DPPH activities of *Aloe vera* extract were similar and comparable to that of tannic acid or BHT. In the present study IC<sub>50</sub> value of extract with ABTS and DPPH assays was found to be 20µg/µl and 50µg/µl respectively. Many researchers summarily proved that different fractions of *Aloe vera* leaves and gel extracts has adequate antioxidant potential (Khanam and Sharma, 2015). Previously reported studies revealed *Aloe vera* gel extract inhibited the generation of DPPH radical in a dose dependent manner (Saritha et al., 2010; Mazzulla et al., 2012; Bawankar et al., 2013).

## **6.2. Toxicity Profile of Aqueous *Aloe vera* Gel Extract**

### **6.2.1. To Standardize the Safe and Effective Dose of Aqueous *Aloe vera* Gel Extract in Various Organs Based on Histopathological and Biochemical alterations**

After the completion of treatment with various doses of *Aloe vera* gel extract (250, 100 and 50 mg/ kg b.w.), the tissues were analysed for histoarchitectural alterations. Control animals exhibited normal histoarchitecture of liver, spleen, kidney and testes. Treatment with higher dose of *Aloe vera* extract (250 and 100 mg/ kg b.w.) caused detrimental changes in the histoarchitecture of various tissues when compared to control and animals treated with low dose (50 mg/ kg b.w.) of *Aloe vera* extract.

### **6.3. Histopathological Alterations**

#### **6.3.1. Effect of Different Doses of Aqueous *Aloe vera* Gel Extract in Liver**

Liver section of animals administered with high dose of aqueous *Aloe vera* extract (250 and 100 mg/ kg b.w.) revealed enlargement of central vein with congested sinusoids (Kosif et al., 2010) when compared to animals of control group and those treated with low dose of *Aloe vera* extract. These changes may be due to cytotoxicity and hepatotoxicity caused after consumption of higher doses of *Aloe vera* gel extract (Yang et al., 2010; Guo and Mei, 2016). Previously reported study showed similar results that intake of higher doses of *Aloe vera* extract could lead to impairment of liver histoarchitecture (Sodani, 2016).

#### **6.3.2. Effect of Different Doses of Aqueous *Aloe vera* Gel Extract in Spleen**

Histological examination of splenic tissue of mice treated with aqueous *Aloe vera* gel extract at higher doses (250 and 100 mg/ kg b.w.) revealed some histopathological changes when compared to control animals. These changes were characterized by decreased white pulp with infiltration, increased red pulp and dilated sinusoids. However, lower doses of *Aloe vera* extract consumption caused no change in histoarchitecture of splenic tissue. To the best of our knowledge, no report is available regarding the effect *Aloe vera* administration on splenic tissue of mice.

#### **6.3.3. Effect of Different Doses of Aqueous *Aloe vera* Gel Extract in Kidney**

The animals treated with higher concentration of *Aloe vera* extract showed atrophy of renal corpuscle, decrease in number of Bowman's capsule, increase

in shrinkage of Bowman's capsule, inflammatory cells infiltration in cortex region, attenuation of glomeruli and glomerular congestion with increased Bowman's spaces (Paul and Didia, 2012). These alterations were reduced after administration of lower doses of *Aloe vera* gel extract.

#### **5.3.4. Effect of Different Doses of Aqueous *Aloe vera* Gel Extract in Testes**

A wide spectrum of damage in testes like shrunken tubules, disorganized and distorted seminiferous tubules, depletion in cell population, inflammation, abnormal widening of interstitial spaces were observed in animals treated with high concentration of *Aloe vera* extract as compared to control mice and animals treated with lower dose of *Aloe vera* extract. Similar kind of histoarchitectural alterations indicating negative effects have been reported with metal and drug induced toxicities (Brzoska et al., 2003; Lim et al., 2010; Modaresi and Khodadadi, 2014).

### **6.4. Biochemical Alterations**

#### **6.4.1. Effect of Various Doses of Aqueous *Aloe vera* Gel Extract on LDH activity in Serum and Different Organs**

Lactate dehydrogenase (LDH) is a marker of cell damage and has been employed in various toxicity studies (Joshi et al., 2012; Arika et al., 2016; Valvona et al., 2016). Subsequent to tissue or cell damage leakage of LDH enzyme out of the cell and into the serum raises LDH activity. Rise in serum LDH activity has been associated with lung tissue injury (Wu et al., 2013; Koul et al., 2015). LDH as tissue injury marker was found to be elevated in serum

caused by Acute Myocardial Infarction (AMI) in human study (Khan et al., 2013). LDH activity was significantly elevated in rat liver when treated with paracetamol (Muthulingam et al., 2010). Doxorubicin administered to rats resulted in significant increase in LDH activity in testes (Mohan and Bhandare, 2012). NDEA induced hepatotoxicity showed increased LDH activity in mice (Koul et al., 2006). In the present study increased serum LDH activity was observed upon treatment with high dose of *Aloe vera* extract (250 mg/ kg b.w.) when compared to control group. No significant alteration was observed in serum LDH activity of low dose group (50 mg/ kg b.w.) when compared to its control counterparts. The increase in LDH activity in animals of high dose *Aloe vera* group (250 and 100 mg/ kg b.w.) may be related to the histoarchitecture damage observed in the various tissues studies.

#### **6.4.2. Effect of Various Doses of Aqueous *Aloe vera* Gel Extract on LPO Level in Whole Blood and Different Organs**

Lipid peroxidation is a process which involves the formation and propagation of lipid radicals which causes rearrangement of double bonds in the unsaturated lipids. The eventual disruption of intracellular membranes leads to cellular damage (Sharma et al., 2012). It is a process generated naturally in small amounts in the body, mainly due to the effect of several ROS (Devasagayam et al., 2004). These ROS readily attack the polyunsaturated fatty acids of fatty acid membrane and initiating a self-propagating chain reaction (Dauqan et al., 2011). The destruction of membrane lipids and the end-products of such lipid

peroxidation reactions alter the viability of cells or even tissues (Guerin et al., 2001). *Aloe vera* (250 and 100 mg/ kg body weight) treated animals revealed significantly increased LPO levels in liver, testes and blood as compared to the control and animals administered with low dose of *Aloe vera* extract.

### **6.5. Standardization of X-ray Doses for Whole Body Exposure**

In the present study, an optimal method for standardization of X-ray irradiation to mice was developed which was then used to determine the biological effects of irradiation. For this purpose, a well ventilated perspex box was designed so that radiation could distributed and absorbed more accurately in various organs. Animals were exposed to (0.258Gy twice a day) for two days in a week and (0.258Gy twice a day) for four days in a week. LDH activity and LPO levels were estimated after two days to check any alterations found in blood and to standardize the radiation dose near to accidental. Significantly 2 folds increased activity of LDH and LPO level were observed after two days of X-ray exposure when compared to control animals. However, 3 folds increase for four days of exposure was more significant as compared to two days of exposure. Therefore, four days of X-ray exposure (2Gy) was selected which was considered as near to the accidental exposure (Haines et al., 2002; Morita et al., 2003; Focea et al., 2012; Casciati et al., 2016).

After standardization of the toxicity profile of aqueous *Aloe vera* gel extract at the dose level (50 mg/ kg b.w.) against whole body X-ray exposure of 2Gy, the following studies were carried out in blood and different organs.

### **6.5.1. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Histopathology of Liver**

Control animals depicted normal architecture of hepatic tissue. Liver section of animals exposed with X-ray irradiation showed widening and dilated central vein and ruptured endothelial lining (E) with congested sinusoidal spaces (Nada et al., 2013) when compared to control and *Aloe vera* extract treated animals. Histopathological lesions such as dilation of sinusoids and enucleated hepatocytes were reported after gamma ray exposure in murine liver (Dadupanthi, 2016). Alterations in histopathological architecture of hepatocytes were observed after X-ray exposure and lycopene showed effective radioprotection in mice (Srinivasan et al., 2014). *Aloe vera* administration to X-ray irradiated mice showed improved hepatic architecture with normal central vein and cell membrane. *Aloe vera* conferred protection against ionizing radiation induced histopathological alterations in different tissues of rats (Rezk, 2005).

### **6.5.2. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Liver Injury Markers**

It is proposed that oxidative stress is linked to the organ or tissue damage following exposure to ionizing radiation. Liver is an organ responsible for metabolism and play a major role in the detoxification of harmful substances (Hu et al., 2016). Some investigators have documented significant elevation in the activity of liver function enzymes (ALT and AST) after gamma-irradiation

(Bhatia and Manda, 2004; El-Deeb et al., 2006; Makhlouf and Makhlouf, 2012), while other investigators have reported the opposite (Abdelhalim and Moussa, 2013). The increase or decrease in the activity of hepatic enzymes might indicate liver impairment. Elevation of these enzymes in serum gives strong indication of tissue injury. Increase in serum aminotransferase activities could be due to liver damage induced by ROS generated after radiation exposure. Albumin/Globulin ratio may be a helpful indicator for early diagnosis of liver damage (Delcourt et al., 2005). Increase in enzymatic activity of these markers in serum indicates enhanced cell permeability or rupture (Pari and Arumugam, 2008).

Exposure to fractionated dose of gamma radiation (1Gy x 5 times each day) led to a significant increase in plasma creatinine, urea, uric acid, AST and ALT activity due to the production of ROS and oxidative stress (Hanafi, 2010). Rats irradiated with 5Gy of gamma radiation produced a significant increase in the activity of the liver enzymes (ALT, AST, ALP, A/G ratio), kidney enzymes (urea, creatinine) (Moussa et al., 2015). Liver stress markers like SGPT, SGOT, bilirubin, A/ G ratio were raised after X-ray exposure to mice indicating hepatic injury and leakage of these enzymes (Ali et al., 2012; Makhlouf and Makhlouf, 2012). *Polyalthia longifolia* extract treatment showed significant reduction in elevated levels of ALT, AST and bilirubin caused by X- ray exposure to mice (Jothy et al., 2016). *Azadirachta indica* extract significantly reduced these elevated levels of AST, ALT against paracetamol induced

hepatotoxicity in rats (Bhanwra et al., 2000). Treatment of rats with *Cichorium Intybus* reduced the radiation induced increased level of ALT, AST enzymes which are associated with improvement in liver enzymes (Osman et al., 2011). Pre-treatment of *Hippophae rhamnoides* extract ameliorated the liver enzyme alterations induced by ionizing radiation in mice (Khan et al., 2014). Similarly, *Aloe vera* extract treatment before irradiation displayed amelioration in the elevation of these enzyme activities.

### **6.5.3. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Chromosomal Aberration Analysis in Liver**

Chromosome aberrations have been widely accepted for many years as a biological marker for exposure to ionizing radiation (Tucker, 2008). X-ray exposure induced free radicals in liver tissue which are responsible for the disruption of chromosomal integrity. *Aloe vera* being an antioxidant had reduced the number of chromosomal aberrant cells demonstrating the positive correlation between ROS production and DNA damage. Augmentation in chromosomal aberrations was reported in the bone marrow of gamma irradiated mice (Ramachandran and Nair, 2012). Chromosomal aberration frequency increased significantly in bone marrow of gamma irradiated animals (Tawfik et al., 2013). The frequency of chromosomal aberrated cells were increased significantly in X-ray irradiated group, which were decreased significantly on treatment with *Aloe vera* extract administration. Similarly, *Adhatoda vasicia* extract reduced the chromosomal abnormalities induced by radiation in bone

marrow cells of mice (Kumar et al., 2007). Protective action of *Alstonia scholaris* extract against ionizing radiation was evident by decreased chromosomal aberrations in bone marrow cells of mice (Jahan and Goyal, 2010).

#### **6.6. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Histopathology of Spleen**

X-ray irradiation caused decrease in the amount of white pulp, increased red pulp, large number of macrophages and reduction in number of lymphocytes in spleen. Lymphocytes appear to be more susceptible to radiation injury (Dainiak, 2002). Infiltration in white pulp and thickened trabeculae were also observed in response to X-ray irradiation. Poor germinal center with marginal zones and decreased lymphocytes were observed after X-ray irradiation in murine model (Xu et al., 2014). Various pathological alterations in splenic tissue were found after exposure to gamma radiation in rats (Jin et al., 2015). Spleen contains many types of immune cells, thus explaining the widespread and varying pathological alterations. These changes could be due to overproduction of ROS induced by ionizing radiation leading to generation of apoptotic cells. Spleen of X-ray irradiated animals pretreated with *Aloe vera* gel extract revealed considerably less macrophages when compared to X-ray irradiated group. Histopathological alterations induced by gamma radiation in mice spleen were suppressed when *Adhatoda vasica* extract administered orally before irradiation (Sharma and Singh, 2013). *Pinus koraiensis* pretreatment

caused decrease in histopathological damage induced by radiation exposure in mice spleen (Li et al., 2016). Similarly, improvement in histoarchitecture of splenic tissue in *Aloe vera* treated X-ray irradiated animals was also observed as compared with the X-ray irradiated group.

### **6.6.1. Modulatory Effect of *Aloe vera* Gel Extract on Clastogenic Damage Induced by X-ray in Spleen**

Ionizing irradiation can directly/ indirectly cause DNA damage such as base loss, formation of pyrimidine, single or double strand breaks etc. (Borrego-Soto et al., 2015). The unrepaired or misrepaired DNA fragments induced by X-ray irradiation leads to enhanced number of micronucleus formation or chromosomal aberration (Kaspler et al., 2009). Micronucleus assay on human lymphocytes is widely accepted cytogenetic biomarker to assess ionizing radiation induced chromosomal damage in the case of occupational, medical and accidental exposed population (Bouraoui et al., 2013). It has been reported that elevated levels of micronucleus frequency could be explained by the clastogenic ability of gamma ray irradiation which in turn leads to structural chromosomal abnormality. DNA damage induced by ionizing radiation was determined by increase in micronuclei formation in human peripheral blood lymphocytes (Tiwari et al., 2009). An increase in the number of micronuclei in mice bone marrow was observed after exposure to ionizing radiation (Jindal et al., 2006). In the present study, whole body X-ray exposure revealed increased number of micronuclei in mice splenocyte. Ionizing radiation increased

micronucleus frequency (Konopacka and Wolny, 2001; Hosseinimehr et al., 2003). *Zingiber officinale* extract reduced the numbers of micronuclei in bone marrow polychromatic erythrocytes induced by ionizing radiation in mice (Du et al., 2010). A significant decline in micronuclei number in *Aloe vera* treated X-ray irradiated group suggests the radioprotective ability of *Aloe vera*.

### **6.7. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Histopathology of Kidney**

Histoarchitecture of kidney section from X-ray irradiated animals showed decrease in number of Bowman's capsule, shrinkage of Bowman's capsule, glomerular attenuation and glomerular congestion. The thick and thin parts of loop of Henle and greater part of collecting ducts present in inner medulla revealed normal histoarchitecture. These results were consistent with the reports of histoarchitectural changes caused by gamma radiation in renal tissue of rats (Mansoub and Sarvestani, 2011; Saini et al., 2014). X-ray irradiated animals pretreated with *Aloe vera* gel extract revealed normal histoarchitecture of cortex and medulla as observed in control group. Previously, *Averrhoa carambola* extract had shown recovery of the distorted renal tissue after gamma radiation in rats (Kumar et al., 2014).

#### **6.7.1. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Renal Injury Markers**

In the present study, plasma urea and creatinine levels were significantly elevated after exposure to X-ray irradiation indicating renal impairment in

animals. The most commonly used molecular biomarker for assessment of renal function is serum creatinine. Increased serum creatinine levels in the irradiated rats indicate the development of nephritis and renal dysfunction (Borg et al., 2002). These alterations may be attributed to impairment of glomerular selective properties caused by exposure to ionizing radiation. A significant increment in the concentration of serum urea in rats by gamma irradiation could be due to radiation induced changes in amino acids metabolism (Nada et al., 2011; Muhammad et al., 2015). The increase in blood creatinine, urea and BUN levels leading to renal damage has been reported after exposure to irradiation (Gaurav et al., 2010). In addition, the elevation in urea and BUN may be attributed to an increase in nitrogen retention or excessive protein breakdown (Nunia and Goyal, 2004). The present results corroborate with Abou-Safi and Ashry, (2004) who suggested that the significant increase in serum urea was attributed to the increase in glutamate dehydrogenase enzyme levels, which might increase carbamyl phosphate synthetase activity. Kafafy (2004) reported that the increase in urea could be an indication for the elevation of protein catabolic rate. Elevated levels of urea and creatinine induced by gamma radiation were reduced after administration of *Ginseng* extract in rats (Mansour, 2013). *Rubab* extract preserve the hepatic and renal enzymes disturbed by gamma ray irradiation in rats (Nada and Hawas, 2012). *Ziziphus* leaves extract provide protection against gamma ray irradiation induced alterations in hepatic and renal enzymes in rats (El-Desouky et al., 2014). In the present study, levels of urea, creatinine and BUN were significantly reduced in *Aloe vera* treated X-ray irradiated mice.

### **6.7.2. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on GFR of Kidney**

Alterations in creatinine and urea levels might be used as biomarkers of renal dysfunction (Vaidya et al., 2008). The measurement of GFR is used as an index of renal function in clinical practice (Stevens and Levey, 2009). It represents the plasma volume presented to the nephrons per unit time during urine formation (Lopez-Giacoman and Madero, 2015). Radionuclide based markers allow for the rapid and reliable measurement of GFR in plasma (Ferguson and Waikar, 2012). Single bolus administration of radioactive agents like  $^{51}\text{Cr}$ -EDTA and  $^{99\text{m}}\text{Tc}$ -DTPA has been used as surrogate markers of GFR measurement (Sandilands et al., 2013). Renal inulin clearance and total plasma clearance of  $^{51}\text{Cr}$ -EDTA are the widely accepted methods to measure GFR (Medeiros et al., 2009). However, these tests are time consuming and repeated urine and blood sampling is cumbersome on mice. Thus,  $^{99\text{m}}\text{Tc}$ -DTPA clearance is considered an acceptable alternative to  $^{51}\text{Cr}$ -EDTA clearance because it has the advantages of being inexpensive, widely available and urine sample is not required (Fleming et al., 2004). Intraperitoneal injection of  $^{99\text{m}}\text{Tc}$ -DTPA has been documented for the assessment of GFR in small animals (Nankivell et al., 1992). To the best of our knowledge, no report is available regarding the effect of ionizing radiation on GFR in the animal models. In the present study, X-ray irradiation caused reduction in GFR levels when compared to control animals. This might be due to the improper clearance of  $^{99\text{m}}\text{Tc}$ -DTPA from glomeruli indicating renal damage. However, *Aloe vera* treated irradiated group revealed increased levels of GFR when compared to X-

ray irradiated group. It has been documented that *Momordica charantia* extract alleviate renal damage to rats as indicated by improved GFR levels (Abdollahi et al., 2011).

### **6.8. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Histopathology of Testes**

In the present study, X- ray exposure caused histological damage in testes as evident by shrunken tubules, disorganized/distorted seminiferous tubules and depletion in germinal cell population, disrupted basement membrane and empty tubules. Lumen was found to be full of cellular and spermatogenic debris along with thinning of seminiferous epithelium with loosely arranged cells as compared to control animals (Gong et al., 2014). X-ray exposure to testes revealed presence of marked disorganization of cells and depletion of the spermatogenic cells, depleted spermatozoa and reduced spaces among the seminiferous tubules (Hussein et al., 2006). Animals of X-ray exposed group pretreated with *Aloe vera* revealed organized histoarchitecture of seminiferous tubules but exhibited debris in lumen of testes. However, histoarchitectural damage appeared to be reduced in *Aloe vera* treated X-ray irradiated animals in comparison to X-ray irradiated group. *Adhatoda vasica* extract suggested lesser degree of damage to testes tissue and various cell populations including spermatogonia, spermatids and Leydig cells after exposure to ionizing radiation in mice (Kumar et al., 2007). Disappearance of the lymphocytic infiltration and desquamation in the epithelium of renal tubules was observed in gamma irradiated *Nigella Sativa* treated animals (Saleh et al., 2013).

### **6.8.1. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Testicular Function Parameters**

Male reproductive disorders have become an important health issue that may cause abnormal outcomes in the offspring. These disorders may be the consequence of environmental or occupational exposure to ionizing radiation (Jensen et al., 2006; Mansour, 2014). Testicular tissue remains vulnerable to oxidative stress induced pathologies due to the inherent abundance of highly unsaturated fatty acids, high metabolic activity, high mitotic activity and the presence of potential ROS generating systems (Sheweita et al., 2005; Sisodia et al., 2008). The high rates of cell division inherent in this process imply correspondingly high amount of mitochondrial oxygen consumption by the germinal epithelium. Ionizing radiation can disturb normal spermatogenic metabolism, proliferation and differentiation, which result in mutagenesis or apoptosis of radiosensitive cells, low sperm counts and defective sperm function (Ibrahim and Ghoneim, 2014). Epididymal sperm count and motility is widely accepted, simple and sensitive biological marker for assessing the effects of toxicants on male reproductive system at workplace (Migliore et al., 2002). Sperm count and motility are important indicators for reflecting spermatogenic capacity and predicting male reproductive function. X-ray exposure to mice decreased the sperm count and motility in comparison to the control counterparts.

Some investigational studies in humans have suggested decrease in sperm count after X-ray irradiation. Ionizing radiation has been shown to reduce the

total number of sperm and the number of motile sperm in humans (Clifton and Bremner, 1983; Fischbein et al., 1997; Gong et al., 2014). In this study, it was found that when animals were exposed to X-ray irradiation the sperm counts and the motility significantly decreased. Low dose (0.05-0.2Gy) of X-ray exposure diminished sperm count and sperm morphology in mice (Dobrzynska, 2011). Gamma rays showed reduction in spermatozoa concentration on rabbits (Georgieva et al., 2006). Reduction in sperm count and sperm motility was observed after gamma radiation in mice (Khan et al., 2015). *Aloe vera* treatment to X-ray irradiated animals significantly improved sperm count/motility. Previous studies reported that *Aloe vera* has the damage resistant properties in mice testes exposed to gamma irradiation (Gehlot et al., 2007). Radioprotective potential of *Podophyllum hexandrum* extract was shown from improved spermatogenesis of mice (Samanta and Goel, 2002). Administration of *Telfairia occidentalis* extract to gamma irradiated mice revealed increased sperm counts and motility (Adejuwon et al., 2014).

The testicular tissue consists of seminiferous tubules that form the sperm and the interstitial leydig cells, which secrete testosterone (Li et al., 2012). Testosterone is a key hormone secreted by the testes that promotes spermatogenesis and normal sperm development (Amory and Bremner, 2003; Walker, 2011). Decrease in testosterone levels could be the reason for sexual disinterest and decreased sperm count. Various forms of radiation exposure can cause sex hormonal disorders (Hajiuon et al., 2013). X-ray irradiation caused

significant decrease in testosterone levels in mice. Osman, (2011) observed testicular damage after gamma ray irradiation in rats. In this study, a marked decrease in serum testosterone level was observed in X-ray irradiated mice. Previously reported studies also documented decrease in testosterone levels of irradiated rats (Kim et al., 2003; El-Dawy and Ali, 2004; SivaKumar et al., 2006; Abdel-Magied and Ahmed, 2011; Jiang et al., 2013). The decrease in testosterone levels after exposure to ionizing radiation might be attributed to the production of free radicals and increase in LPO levels which attack the testicular parenchyma causing damage to the tissue (Nada et al., 2011). The present study exhibited a significant decrease in testosterone levels in X-ray exposed group when compared to control and *Aloe vera* group. Pretreatment with *Aloe vera* resulted in a significant increase in testosterone levels when compared to the animals exposed to X-ray irradiation only.

### **6.9. Effect of Aqueous *Aloe vera* Gel Extract Against DNA Damage Caused by X-ray Irradiation in Various Organs**

Apoptosis, a process of programmed cell death, is characterised by morphological criteria associated with cytoplasmic shrinkage, blebbing of plasma membrane, specific chromatin condensation and nuclear fragmentation (Orrenius et al., 2011). Ionizing radiation induces damage through direct or indirect interaction with DNA and also by excessive production of free radicals which leads to oxidative stress (Kryston et al., 2011). Oxidative stress is associated with increased oxidative DNA damage and contributes to apoptosis

(Nita and Grzybowski, 2016). It has been reported that oxidative stress is involved in apoptosis induction by radiation exposure (Azzam et al., 2012). Ionizing radiation induced DNA double stranded breaks is the most important type of damage which leads to cell damage (Vignard et al., 2013). DNA is always susceptible to damage caused by ionizing radiation and this can produce cell death or mutation (Abouelella et al., 2007). Ionizing radiation induced DNA damage in irradiated cells is most likely through the production of ROS. Occurrence of apoptosis after irradiation may be a better indicator of tissue injury/ damage (Chi et al., 2005). TUNEL assay is used to detect free 3'-OH terminals of both single and double-strand DNA breaks, thus potentially labelling both apoptotic and necrotic derived DNA strand breaks (Ito et al., 2006). In this study, X-ray irradiated mice revealed numerous brown stained apoptotic cells when compared to control mice. This observation is in agreement with previous reports (Chi et al., 2005; Jeong et al., 2008; Kim et al., 2016). However, significant reduction in the number of TUNEL positive apoptotic cells were found in *Aloe vera* gel extract treated X-ray exposed mice when compared to only X-ray irradiated mice.

DNA ladder gel has been used to indicate radiation induced genetic damage (Eshak and Osman, 2013). We observed diffused band in X-ray irradiated animals indicating DNA damage accompanied by unresolved tail of high molecular weight DNA fragmentation when compared to control and *Aloe vera* extract treated group in hepatic, renal and testes tissues (Gangar and Koul, 2008). This pattern showed an intact as well as higher molecular weight

fragmented DNA. An intact genomic DNA band was observed in control and *Aloe vera* treated group. Increased DNA fragmentation was observed in liver after gamma ray radiation exposure to rats (Alam et al., 2010; Madhu and Kumari, 2014). However, *Aloe vera* treated X-ray irradiated group revealed decreased smear formation when compared to X-ray irradiated group. Similarly, Kumar et al., (2016) reported the beneficial effect of *Psidium guajava* extract against X-ray irradiation induced apoptosis in experimental animals. Furthermore, Grapevine fruit extract served as a potential radioprotector which attenuated ionizing radiation induced ROS and apoptosis in human lymphocytes (Singha and Das, 2015).

#### **6.10. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extracton LDH**

##### **Activity in Serum and Various Organs**

LDH leaks out from the damaged tissue into the blood stream when the cell membrane becomes permeable or is ruptured (Cho and Kim, 2012). The amount of cellular enzymes in the serum reflects alterations in the plasma membrane integrity and/ or permeability. It has been reported by various authors that elevated activity of LDH was observed after irradiation in serum, thymus, liver, spleen, kidney and testis of mice (Hori et al., 1968; Mishra et al., 2002, Mohamed, 2011; Freitas et al., 2013). Elevated LDH activity as an indicator of heart damage was also observed in mice (Goo et al., 2013). In the present study, an increase in the activity of LDH observed in the serum is a clear indication of damage caused by exposure to X-ray radiation (Barshishat-

Kupper et al., 2014). This observation is in concordance with *Podophyllum hexandrum* administration to gamma irradiated animals exhibiting significantly decreased LDH activity in BALF of mice lung (Saini et al., 2013). LDH activity in serum was found to be decreased in *Ginseng* pretreated irradiated mice (Mansour, 2013). X-ray irradiated and *Aloe vera* pretreated mice showed ameliorative effect of *Aloe vera* when compared to X-ray exposed group. Therefore, *Aloe vera* conferred the protection by preserving the membrane integrity and restraining the leakage of these enzymes from different tissues like liver, spleen, kidney and testes induced by X-ray.

### **6.11. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on ROS Formation and LPO Levels in Blood and Various Organs**

Ionizing radiation leads to ROS formation resulting in cellular damage either directly or indirectly by water radiolysis mechanism (Azzam et al., 2012; Rappole et al., 2012). ROS affects various cellular functions by damaging nucleic acids, oxidizing proteins and causing lipid peroxidation (Jyothi et al., 2012). Excessive production of ROS causes testicular injury in mice (Adejuwon et al., 2014). Ionizing radiation leads to oxidative imbalance with the production of excessive ROS in testosterone levels of experimental rats (Michael, 2011). The deleterious effects of ionizing radiation in the biological systems are mainly mediated through the generation of ROS by a process called oxidative stress, in a variety of cells as a result of water radiolysis (Kamat et al., 2000). Very low dose of gamma irradiation causes testicular

damage in rat model due to excessive formation of ROS (Ibrahim and Ghoneim, 2014). The oxidative status of cell is the primary factor for regulating gene expression and activities of these enzymes (Rodriguez et al., 2004). Both, endogenous and exogenous agents act as oxidants which alters cellular oxidative equilibrium and consequently, antioxidant enzyme expression (Nicotera et al., 1989; Yoo et al., 1999).

Lipid peroxidation within the membrane has a devastating effect on functional state of the membrane because it alters membrane fluidity, typically reduce it and thereby allowing ions such as  $\text{Ca}^{+2}$  to leak into the cell. It is a highly destructive process causing cellular organelles and whole organism to lose biochemical function and/ or structural architecture which may lead to cell damage (Kale and Sitaswad, 1990). LPO has been suggested as one of the main causes of ionizing radiation induced membrane damage. Free radicals induce oxidative deterioration of lipids by lipid peroxidation process which results into the formation of fatty acid radicals (Sinha et al., 2012). Elevated levels of LPO are indicative of the oxidative damage caused by gamma radiation in testicular tissue (Sharma et al., 2011). LPO levels have been significantly increased after X-ray exposure to experimental rats (Kucukkurt et al., 2011). Increased level of LPO was observed after X-ray exposure in rabbits (Deger et al., 2003). The present study revealed significantly increased LPO levels in X-ray irradiation as compared with control and *Aloe vera* treated animals in blood and various organs. These results were consistent with the reports of increased LPO levels after X-ray exposure to rats (Marina et al., 2015; Kumar et al., 2016). *Aloe vera*

pretreated X-ray irradiated animals exhibited a significant decrease in LPO level and ROS production when compared with X-ray irradiated animals. The basic effects of radiation on cellular membrane are believed to be the membrane lipids peroxidation. Radiolytic products can initiate LPO including hydroxyl and hydroperoxy radicals (Konings and Drijver, 1979). Generation of lipoperoxides due to interaction of excessive formation of ROS induced by ionizing radiation with unsaturated fatty acids of membrane resulting in cellular disruption. These ROS and lipid radicals promote damage to DNA and are known to induce apoptosis (Barrera, 2012; Kumar et al., 2016). Various previous reports also proved enhanced levels of LPO when gamma ray irradiation induced hepatic and testes tissues damage in animals which was improved by ingestion of *Aloe vera* (Gehlot et al., 2007; 2010). A significant increase in MDA and a significant decrease in GSH levels were observed in brain, liver and kidney tissues due to generation of ROS resulting in imbalance of pro-oxidants and antioxidants in the cells, which is suggested to culminate in cell damage (Ramachandran and Nair, 2012).

#### **6.12. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Antioxidant Defense System in Blood Plasma and Various Organs**

Against oxidative damage, cells are equipped with several natural enzymatic and non-enzymatic antioxidant defences (Gargouri et al., 2011). To control flux of ROS, aerobic cells have developed their own antioxidant defense system which includes the superoxide dismutase (SOD) and catalysis the dismutation of superoxide anion ( $O_2^{\cdot-}$ ) into  $H_2O_2$ . Glutathione peroxidase (GSH-Px) reduces

lipidic or non- lipidic hydroperoxides as well as  $H_2O_2$  (Taysiet al.,2002). The exposure to ionizing radiation could lead to depletion of these endogenous antioxidants. GSH is a tri-peptide naturally occurring antioxidant synthesized in all mammalian cells in substantial concentrations and functions to maintain the cellular redox state (Lushchak, 2012). GSH is major mechanism for regulating intracellular free radical concentration (Rose et al., 2012). GSH is regarded as an indigenous protective agent against drugs (Nurrochmad et al., 2010; Jaswal and Shukla, 2015). It represents an important defense against oxygen derived free radicals and cellular lethality from ionizing radiation exposure. GSH exhibit antioxidant effect by reducing superoxide and hydroxyl radicals following the formation of oxidized glutathione and reduces peroxides in the non-enzymatic reaction (Lu et al., 2010). GSH-Px catalyzes the destruction of  $H_2O_2$  and hydroxyl radical ( $OH^\cdot$ ). GSH-Px activity increases for the elimination of  $O_2^\cdot$  and  $H_2O_2$  formed by radiolysis of water. In individuals undergoing radiotherapy, the increased inflammation results in the generation of free radical species, thus inducing an increase of the activity of GSH-Px and other antioxidant enzymes. Previous studies showed increased GSH-Px activity after X-ray exposure in rabbits (Deger et al., 2003). However, GR catalyse the regeneration of GSH from GSSG. Thus, GR and GSH-Px are enzymes in the glutathione regeneration pathway.

Some studies reported decrease in enzymatic antioxidant activities and GSH in X-ray irradiated mice (Srinivasan et al., 2014). A significant reduction in GSH concentration was observed after X-ray exposure when compared to control group in liver, which could be due to enhanced utilization of antioxidant

system as an attempt to detoxify the ROS generated by ionizing radiation. Nada et al., (2013) have reported decrease in GSH concentration of liver in gamma-irradiated group when compared to control group. Reduction in concentration of GSH was also observed after radiation exposure in blood, splenic, renal and testicular tissues of mice (Mohamed and Farghaly, 2009; Sharma et al., 2011; Verma et al., 2011; Sharma et al., 2014). Significantly increased levels of GSH based enzymes were observed after whole body X-ray to rats (Ishikawa et al., 2013). GST is a soluble protein located in cytosols which play an important role in detoxification and excretion of xenobiotics. In the present investigations, a significant increase in the GST activity was observed in the hepatic, splenic, renal and the testicular tissues of X-ray exposed animals when compared to the animals of control group. This might be attributed to the reduction in the concentration of GSH indicating insufficient detoxification system. However, GST activity in *Aloe vera* treated irradiated group was observed to be decreased when compared to X-ray irradiated group.

Catalase (CAT) is an endogenous antioxidant enzyme that neutralizes ROS by converting  $H_2O_2$  into  $H_2O$  and  $O_2$ , and can be upregulated by oxidative stress (Hunt et al., 1998; Racchi, 2013). Thus, catalase provides protection from radiation by detoxifying  $H_2O_2$ . Increased catalase activity was observed in brain, liver and kidney of animals at higher (2Gy) and lower (0.5Gy) doses of X-ray radiation (Focea et al., 2012). SOD detoxifies superoxide anion ( $O_2^{\cdot-}$ ) to hydrogen peroxide ( $H_2O_2$ ) and then catalase converts  $H_2O_2$  into  $H_2O$  and  $O_2$ . It has been reported that low (0.5Gy) and high (2.5 Gy) doses of radiation caused

SOD activity to be increased in order to eliminate superoxide radicals present in the liver (Yamaoka et al., 1991). The resulting H<sub>2</sub>O<sub>2</sub> has a higher reactivity which caused the increased activity of GSH-Px and catalase that eliminates H<sub>2</sub>O<sub>2</sub>. In the present study, enhanced GSH-Px, GR, GST and SOD activities was observed in X-ray irradiated group when compared to control group, could be due to excessive production of free radicals. Significantly increased levels of GSH related enzymes were observed after whole body X-ray to rats (Ishikawa et al., 2013). Alterations in antioxidant defense system were also documented after consumption of *Aloe vera* extract against cigarette smoke inhalation induced lung injury in mice (Koul et al., 2015). *Panax ginseng* extract significantly improves endogenous antioxidant enzymes induced by radiation exposure in mice (Verma et al., 2011). *Aloe vera* showed ameliorative role against ionizing radiation induced hepatic and renal toxicity through improvement in antioxidant defense mechanism (Saada et al., 2003).

### **6.13. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Inflammatory Markers in Serum**

Ionizing radiation induces enhanced ROS production which leads to inflammatory response as indicated by the production of inflammatory cytokines (Azzam et al., 2012). Pro-inflammatory mediators are released through local and systemic reaction simultaneously when triggered by insults (Cavaillon and Annane, 2006). Pro inflammatory cytokines that participate in acute phase response are interleukin- 6 (IL-6) and Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Playfair and Chain, 2005). TNF- $\alpha$  and IL-6 are one of the pro

inflammatory cytokines that promotes upregulation of inflammatory reactions (Dinarello, 2000; Zhang and An, 2007). Inflammatory mediators like cytokines and chemokines are released from stressed or inflamed tissue (Schulte et al., 2013). However, their release results in the activation and recruitment of leucocytes to the respective tissue from the blood circulation. This is directly linked to more tissue damage and an increased release of inflammatory mediators, leading to uncontrolled inflammation and reduced wound healing. Production of inflammatory mediators is essential for host defence and neutrophils are the first line of immune defense (Muralidharan and Mandrekar, 2013). In response to ionizing radiation, inflammatory cells such as neutrophils are recruited to the ionizing radiation derived inflamed lesion via release of pro-inflammatory cytokines (Malik et al., 2010). Neutrophils are the first WBC population generally thought to be an initiator of inflammation (Butterfield et al., 2006). It was previously reported that transient inflammatory response is induced by neutrophil infiltration, excessive ROS and inhibition of antioxidant pathways leading to apoptosis after whole body X-ray irradiation (Uchimura et al., 2000).

An unrestricted release of inflammatory mediators together with an increased inflammatory infiltrate could lead to tissue damage or severe inflammation. TNF- $\alpha$  and IL-6 are important pro-inflammatory cytokine in radiation mediated tissue damage (Sultani et al., 2012). TNF- $\alpha$  exerts a variety of effects that are mediated by TNFR-1 which leads to the activation of multiple apoptotic pathways such as extrinsic and intrinsic (Blaser et al., 2016). In the present

investigation, it was observed that the levels of the pro-inflammatory cytokines IL-6 and TNF- $\alpha$  significantly increased in serum after X-ray exposure to mice. In a previous study, TNF- $\alpha$  level and other cytokines are up regulated in plasma and various organs following radiation exposure (Fedorocko et al., 2002; Huang et al., 2006; Li et al., 2010). Numerous studies have shown that excessive amount of TNF- $\alpha$  can cause cell apoptosis, resulting in injury of liver tissue (Leist et al., 1995; Ding et al., 2004). Increased levels of TNF- $\alpha$  was observed after treatment with X-ray in human sarcoma cells (Hallahan et al., 1989). The expression of pro-inflammatory cytokines IL-6 and TNF- $\alpha$  was also upregulated after X-ray exposure leading to liver and lung injury in animals (Christiansen et al., 2007; Jang et al., 2013). The ability of *Aloe vera* extract to impressively scavenge free radicals may reflect its ability to reduce ROS and hence, decrease inflammation. Pro-inflammatory cytokines were also elevated after X-ray exposure induced liver injury in animals and was attenuated by treatment of *Panax ginseng* (Kim et al., 2016).

#### **6.14. Effect of X-ray and/ or Aqueous *Aloe vera* Gel Extract on Haematological Parameters**

Blood is a circulating fluid that delivers nutrients and oxygen to the cells and transports metabolic wastes away from the cells. It also regulates body temperature, carries immune cells to the site of infection or injury (Karsheva, et al., 2009). The RBCs carry oxygen from the respiratory organs to rest of the body (Etim et al., 2013). WBCs are the cells of the immune system which are

derived from bone marrow a hematopoietic stem cell. Platelets are fragments of cytoplasm which are derived from the megakaryocytes of bone marrow (Machlus et al, 2014). The hematopoietic progenitor cells (HPCs) and hematopoietic stem cells (HSCs) undergo apoptosis after exposure to ionizing radiation which causes bone marrow suppression (Yu et al., 2010). The rapidly dividing cells of the blood system especially leukocytes, erythrocytes, the immune organs and cells are highly sensitive to ionizing radiation (Widel et al., 2003). The hematopoietic system is radiosensitive organ causes a disturbance in the function of red blood cells including intravascular hemolysis and decrease in the erythrocytes (Kotbet al., 1990; John and Gray, 1992). Among these the hematopoietic tissue is the most sensitive to radiation (Flidner et al., 2012). Exposure of animals to ionizing radiation could induce long term hematopoietic cells damage, mainly the self-renewal damage to hematopoietic stem cells (HSCs) (Chua et al., 2012). The haemopoietin syndrome is induced by low doses of irradiation which is manifested by depletion of haemopoietin stem cell and ultimately by the depletion of mature haemopoietin cells (Jagetia et al., 2003).

Haematological studies in the field of radiation have played an active role to estimate the exposure to ionizing radiation, as it increases the frequency of chromosomal aberrations in human blood lymphocytes (Anderson et al., 2000). Wang et al., (2014) found that RBC counts and Hb levels were not altered after gamma radiation exposure to mice. Contrarily, Nunia and Goyal, (2004) reported that total RBC counts showed significant decrease at different

radiation dose levels. Several authors showed significant decrease in RBC counts were observed after ionizing radiation exposure in animals (Jankeer, 2014; El-Shanshoury et al, 2016). Low RBCs count or low Hb levels may suggest anaemia which can be attributed to many causes. The damaging effect of gamma radiation on RBCs count may be attributed to the cessation of erythrocytic production in bone marrow, the loss of cells from the circulation by leakage through capillary walls or haemorrhage and/or direct destruction of mature circulatory cells (Chlebovsky et al., 1983; El-Deebet et al., 2006; Manisha et al., 2011; Sharma et al., 2012). After whole body exposure to gamma radiation, injury to animal tissues were well reflected in the peripheral blood (Mihandoost et al., 2014; Joiya and Purohit, 2015; El-Shanshoury, 2016). Some studies showed that RBC count decreased that could be attributed to the leakage on account of haemorrhage caused by radiation induced lesions in blood vessels (Sancheti and Goyal, 2007).

A decrease in the value of Hb level was observed in various studies after radiation exposure in mice (Sancheti and Goyal, 2007; Gupta and Agrawal, 2013). The decrease in Hb content is an indication of the loss of progenitor cells to form new red blood cells. It can be related to either direct destruction of the RBCs or loss of RBCs in circulation due to haemorrhage or leakage through capillary walls and loss of production of cells because of direct cytopathic effects of radiation on the dividing cells of the hemopoietic system. Previously documented studies revealed that the decreased RBC count may be due to defective haemopoiesis as well as intravascular red cell damage

(Ramakant and Rajendra, 2015). However, in the present study RBC count and Hb levels remained unaltered after X-ray exposure when compared with the control group. This may be due to the remarkable regenerative capacity of blood cells with recovery in short periods of time. Davoudi et al., (2012) showed no significant difference in RBC and WBC count between control and radiation field workers. The increase in WBCs count in rats exposed to X- rays compared with the control group might be due to oxidative stress induced by ROS generation and release of pro-inflammatory cytokines

Ionizing radiation is known to have a detrimental effect on lymphoid and hematopoietic tissues (Park et al., 2008). Depletion of the cellular elements of the blood was reported after exposure to gamma radiation (Azab et al., 2011). Lymphocytes were found to be particularly radiosensitive and their depletion is associated with a reduced immune response (Kumar and Umadevi, 1982; Manda et al., 2012). It has been documented that gamma radiation decreased lymphocytes that might result from ROS induced oxidative stress. TNF-  $\alpha$  induces neutrophil apoptosis via death receptor signalling through TNF receptors, leading to caspase activation. Increased neutrophil count, caspase activation and enhanced ROS generation acts as mediators of apoptosis (Mittal et al., 2014). It has been reported that rapid and massive apoptosis may induce neutrophil accumulation (Uchimura et al., 2000). In the present study, neutrophil counts increased in X-ray exposed group which may be due to increased levels of ROS which leads to apoptosis when compared to control group. However, in *Aloe vera* treated irradiated group revealed decrease in

neutrophil counts when compared to X-ray irradiated group. Lymphocyte counts remained unaltered in X-ray irradiated, *Aloe vera* treated and *Aloe vera* treated X-ray irradiated groups when compared to control animals.

Thrall et al., (2013) also reported that the platelet number did not change in a significant manner after gamma radiation exposure within 48 hours. However, other studies suggest that decreased platelet counts were observed after radiation exposure to animals. Seed et al., (2002) demonstrated that the platelet count was transiently depressed after exposure to whole body gamma radiation. Platelets are a rich source of cytokines and platelet derived growth factor (PDGF). They play an essential role in blood clotting and wound healing (Semple et al., 2011). Therefore, it is conceivable that a reduction in platelet numbers could affect these biological processes (Billings et al., 2014). In the present study, blood platelet counts were significantly decreased in the X-ray exposed group. These results are in agreement with Eshak and Osman, (2013), Gharib et al., (2013). However, *Aloe vera* showed protective effect against X-ray induced depletion in platelet counts. The present study demonstrated that *Aloe vera* could boost haematological parameters and useful for immune suppression induced by X-ray irradiation. It has been reported that alterations in haematology induced by ionizing radiation was improved by consumption of *Aloe vera* extract in mice (Singariya et al., 2015). These observations are also supported by earlier investigations in which *Alstonia scholaris* extracts mitigated haematological alterations induced by ionizing radiations in mice (Gupta and Agrawal, 2013). It has been reported that alterations in

haematology induced by ionizing radiation was improved by consumption of *Aloe vera* extract in mice (Singariya et al., 2015).

*The results of the present investigation demonstrate that X-ray irradiation induced damage by depleting GSH, enhancing ROS/LPO levels and altering haematological contents. Irradiation resulted in disruption of hepatic, renal and testicular functions and caused clastogenic damage in various organs. The present study suggests prophylactic effect of aqueous Aloe vera gel extract against X-ray induced alterations in blood and various organs by virtue of its antioxidant, anti-inflammatory and anti-apoptotic potential.*