Section 8

DISCUSSION
Ionizing radiations generates reactive oxygen species (ROS) in form of OH\(^-\), H\(^-\), O\(_2\)\(^{•-}\), singlet oxygen and peroxyl radicals that follows a cascade of events leading to damages to biomolecules such as DNA, proteins, lipids and carbohydrates (Jagetia, 2007; Hosseinimehr, 2007). Increased interaction of ROS with critical macromolecules can induce cell dysfunction and death. When ROS generation is massive, as it is during gamma irradiation exposure, the cytotoxic effect is not merely local but may result in intracellular and extracellular propagation. Damage to DNA such as single or double strand breaks, base damage, DNA-DNA or DNA-protein cross links may be the most important factor in cell death. Oxidation of proteins by ROS can generate a range of stable as well as reactive products such as protein hydroperoxides that can generate additional radicals particularly upon interaction with transition metal ions (Devasagayam et al., 2004). Similarly, lipids when reacted with free radicals can undergo the highly damaging chain reaction of lipid peroxidation (LPO), which is highly detrimental to the functioning of the cell (Devasagayam et al., 2004). Some of the earlier studies also summarized the mechanisms of cellular damage by free radicals in oxidative stress and their implications in pathology of several diseases including cancer, artherosclerosis, rheumatoid arthritis, neurodegenerative disorders etc. (Arouma, 1998; Valko et al., 2006).

The elimination of the excessive free radical species from the cellular and extracellular environment can inhibit the side effects induced by irradiation. Therefore, presence of the molecules capable of scavenging the free radicals arising from irradiation can confer protection to radiation and could be useful as radioprotector. Many synthetic and natural chemicals have been investigated in the recent past for their efficacy to protect against radiation induced damage in biological systems (Nair et al., 2001). Unfortunately, most of them have toxic side effects, which limit their therapeutic use. Investigations for effective and non toxic compounds with radioprotection capability led to increasing interest in naturally occurring antioxidants. Usually these compounds were isolated from herbal and natural products such as medicinal plants, fruits, vegetables or propolis and included into dietary supplements or formulated as part of medicines to treat various diseases (Lima et al., 2009). Radioprotective efficacy of certain plant extracts such as Tinospora cordifolia, Ocimum sanctum, Aegle marmelos, Podophyllum hexandrum etc., has been evaluated earlier (Ganasoundari et al., 1998; Goel et al., 2004; Jagetia et al., 2004; Samarth et al., 2008; Lata et al., 2009). In the present study, the medicinal plants such as Terminalia chebula, Withania somnifera, Asparagus racemosus and another source
of natural antioxidants propolis has also been taken into consideration for various antioxidant and radioprotective studies. The radioprotective efficacy of the extracts derived from plants and other natural sources seems to be largely due to phenolic and flavonoid compounds with strong antioxidant activities (Jagetia, 2007; Samarth et al., 2008).

The focus of the present study was to elucidate the antioxidant and radioprotective potential of the herbal and natural products against gamma irradiation induced oxidative stress. It has been often observed that the crude extract of herbal and natural products elicit greater biological activity than individual constituents, at an equivalent dose (Singh et al., 2009; Rasoanaivo et al., 2011). Thus, the study was designed to explore this property of whole extracts from the perspective of antioxidant and radioprotection. The various properties of TCE, PE, WSE and ARE in relation to antioxidant activity were tested in vitro. The extracts showed high levels of the antioxidant constituents such as phenols, flavonoids and triterpenoids. Several in vitro studies demonstrated that the polyphenolic compounds contributed significantly to the antioxidant activities of medicinal herbs, fruits etc. (Prakash et al., 2011; Zhang et al., 2011). The antioxidant and radical scavenging ability of flavonoids, phenols and triterpenoids in medicinal plants and propolis was reported (Prakash et al., 2007; Lima et al., 2009). It is suggested that the ability of the extracts to scavenge free radical is due to the presence of polyphenols i.e., phenols, flavonoids, and triterpenoids etc. by which they act as antioxidant. These phenolic antioxidants are good scavengers of lipid hydroxyl radical and could be responsible for the inhibition of iron (Fe$^{3+}$) induced LPO. Flavonoids, as one of the most diverse and wide spread groups of natural compounds, are probably the most natural phenolics (Shimoi et al., 1996). Flavonoids may directly scavenge the radicals and are oxidized to a less reactive and stable radical. Flavonoids and other phenols are likely to have multiple potential biological activities, such as inhibiting cyclooxygenases and decreasing xanthine oxidase activity (Van Hoorn et al., 2002). Among the principal properties that may account for the potential health benefits of flavonoids is their antioxidant activity (Rice-Evans et al., 1996). Several in vitro studies have demonstrated that flavonoids can scavenge O$_2^\cdot$ (Afanas’ev et al., 1989), OH$^\cdot$ (Husain et al., 1987) and peroxyl radicals (Lotito and Fraga, 1998) and inhibit LPO in various systems. Several mechanisms may account for the antioxidant activity of flavonoids, in addition to free radical scavenging viz., chelation of transition metal ions (Morel et al., 1994), inhibition of oxidant enzymes (Ueno et al., 1984) or by regeneration of α-tocopherol from α-tocopheryloxyl radical (Salah et al., 1995).
addition to the flavonoids and phenolic compounds, some of the alkaloids, saponins and triterpenoids are reported to possess antioxidant activity (Rai et al., 2006). The extracts TCE, PE, WSE and ARE also markedly inhibited iron induced LPO in vitro. It is suggested that the presence of the active constituents as phenols, flavonoids and terpenoids may contribute for the inhibition of LPO.

Further the antiradical activity of extracts was measured by DPPH radical scavenging activity and FRAP assay. Extracts with high polyphenolic compounds showed markedly high percent radical scavenging and FRAP values as in case of TCE and PE. The reaction between antioxidant molecule and DPPH radical, results in the scavenging of the radical by hydrogen donation. The total antioxidant activity of herbal and natural product cannot be evaluated by using one single method due to their complex composition and oxidative processes (Gulcin et al., 2005). Therefore along with FRSA and FRAP various other antioxidant methods such as RP, MCA, SASA, HRSA, BDDD, etc. were used, to evaluate the complete in vitro antioxidative profile of the test extract. For the measurements of the reductive ability of the extracts, the transformation of Fe$^{+3}$ - Fe$^{+2}$ was studied by reducing power assay. The extracts showed significant increase in reducing power with the increase in concentration of the extracts. This reducing capacity of the extracts may serve as significant indicator for their potential antioxidant activity. In view of the role of Fe$^{+2}$ in accelerating ROS generation and provoking peroxidation reactions (Halliwell and Gutteridge, 1984), the chelation of transition metal ion attributes to the antioxidant activity of an antioxidant compound. It was reported that chelating agents form bonds with a metal and are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Gulcin et al., 2003). The data obtained from the results reveals that extracts demonstrated a marked capacity for iron binding, suggesting their action as protector of peroxidation. SASA and HRSA of the extracts showed that extracts were able to scavenge O$_2^•$ and OH$^•$ relatively at all concentrations. The extracts also reduced bleomycin-Fe$^{+3}$ to bleomycin-Fe$^{+2}$ effectively in a concentration dependent manner. The tendency to reduce bleomycin-Fe$^{+3}$ to bleomycin-Fe$^{+2}$ was due to their high reducing power. TCE and PE due to their higher reduction potential in presence of bleomycin sulphate showed increased DNA oxidation, while WSE and ARE due to their lower reducing potential showed lesser DNA oxidation in vitro. The results of the invitro antioxidant parameters suggest that TCE, PE, WSE and ARE have higher antioxidant activities due to the presence of polyphenolic constituents. The results
for the in vitro studies are in consonance with the earlier studies elucidating that presence of high levels of phenolic compounds in extracts may have contributed to the observed antioxidant activities (Wojdylo et al., 2007; Kuete et al., 2011).

The antiradical and DNA protecting ability of herbal and natural products was further evidenced by the protection of plasmid DNA (pBR322) against oxidative DNA strand breakage induced by Fenton’s reactants in vitro. Plasmid in native form is predominantly in supercoiled form. DNA when exposed to ROS generating system suffers strand breakage (Gandhi and Nair, 2005). H$_2$O$_2$ is a prominent ROS that causes DNA damage in cells (Halliwell and Arouma, 1991). The generated OH’ from H$_2$O$_2$ by Fenton’s reaction can also induce the DNA fragmentation (Imlay et al., 1998). The conversion of supercoiled plasmid DNA to linear or nicked circular form takes place as a consequence of OH’ generation from H$_2$O$_2$ by Fenton’s reaction. Incubation of DNA samples with TCE, PE, WSE and ARE partially protects H$_2$O$_2$ induced conversion of pBR322 DNA to linear or nicked circular form in concentration dependent manner i.e., reduced the intensity of the band corresponding to the linear or nicked circular form in the reaction mixture. The extracts may render protection either by neutralizing the H$_2$O$_2$ or by scavenging the OH’ generated from the Fenton’s reaction.

The antiradical activity of antioxidants was observed to be positively correlated with the number of hydroxyl groups bonded to the aromatic ring. The ortho and para hydroxy group substitution showed stronger antioxidant and antiradical activity, while meta position display lower activities. These are probably the most important but not the only factors influencing the antiradical and antioxidative activities of phenolic acids (Sroka and Cisowski, 2003). The various antioxidant properties shown by the extracts such as DPPH, FRAP, RP, MCA, SASA, HRSA and bleomycin dependent DNA damage may be the function of hydroxyl groups of polyphenols including flavonoids present in the extracts. These properties may be because of the hydroxyl groups but there may also be other factors responsible for these properties. A strong negative correlation of these properties has been shown with invitro iron induced LPO.

Endogenous antioxidant system is equipped with antioxidant enzymes such as SOD, CAT, GST, GPx and non enzymatic entities such as GSH, lipoic acid, uric acid, vitamins, carotenoids etc. capable of detoxification and removal of ROS. However, when the system is exposed to adverse physicochemical, environmental or pathological agents such as
radiations, the delicately maintained balance between ROS and antioxidants is shifted in favour of ROS resulting in oxidative stress. Since exposure to the radiations in radiotherapy or accidental exposure to radiation can produce significant unwanted side effects, it is important to ameliorate such effects by the use of radioprotective drugs. Investigations for effective and non toxic compounds with radioprotection capability led to increase in medicinal plants and naturally occurring antioxidant source such as propolis. These natural products are rich in phenolic compounds, nitrogen compounds, vitamins, terpenoids, flavonoids that are known for their antioxidant activity (Cai et al., 2004; Benkovic et al., 2008). Shimoi et al., (1996) concluded that plant flavonoids, which show antioxidant activity in vitro also function as antioxidants in vivo, and their radioprotective effect may be attributed to their radical scavenging activity.

LPO and antioxidant enzyme assays were performed to ascertain the antioxidative effects of natural extracts against gamma irradiation induced oxidative stress in vivo. The animals were administered with TCE, WSE, ARE or PE intraperitonially (i.p) for five days prior to gamma irradiation exposure. The pretreatment with TCE, WSE, ARE or PE in gamma irradiated animal has been observed to be an effective antioxidants as well as radioprotective agents. The significant increase in liver and erythrocytes LPO in gamma irradiated animals as compared to control was a consequence of increased oxidative stress and cellular damage. Elevated levels of LPO have been linked to injurious effects such as loss of fluidity, inactivation of membrane enzymes, increase in permeability of ions and eventually disruption of cell membrane leading to the release of cell organelles (Halliwell and Gutteridge, 1990). However, a decrease in LPO was observed in animals pretreated with extracts, prior to gamma irradiation exposure suggesting that pretreatment strengthen the antioxidant defense system and prepared the animals to withstand the damaging effects of irradiation. The pretreatment with TCE, WSE, ARE or PE provides an extra supplementation of antioxidants to the animals, to cope with the increased oxidative stress induced by gamma irradiation. The results indicate that the various properties as shown in in vitro studies may lower oxidative stress and may be also responsible for the radioprotective effect in vivo. The decrease in LPO may be due to the changes in antioxidant system in erythrocytes and tissues of the animals.

Antioxidant enzymes i.e., SOD, CAT and GST activity in liver and erythrocytes showed significant decrease in gamma irradiated group, probably due to the excessive
generation of free radicals and increased oxidative stress. SOD, one of the first antioxidant enzymes in the line of defence against the deleterious effects of oxygen radicals in the cells, scavenges ROS by catalyzing the dismutation of superoxide to hydrogen peroxide ($\text{H}_2\text{O}_2$), which is decomposed by CAT (Fridovich, 1978). Decrease in liver and erythrocytes antioxidant enzymes activity in gamma irradiated animals may be due to the alteration in balance between oxidant and antioxidant systems (Weiss and Kumar, 1998). Moreover, decrease in CAT activity in animals exposed with gamma irradiation was probably due to the inactivation of CAT, as a flux of superoxide anions have been shown to reduce CAT activity (Kono and Fridovich, 1982). TCE, WSE, ARE and PE treatment prior to gamma irradiation reversed the SOD and CAT activity to near normal levels.

GSH, a multifunctional non-enzymatic and a tripeptide antioxidant molecule in the cell, is synthesized intracellularly from its constituent amino acids ie., glutamic acid, cysteine and glycine. It is considered to be the major thiol redox buffer of the cell (Masella et al., 2005). GSH plays an important role in the detoxification mechanism and in the protection of cellular constituents against ROS. A number of hepatotoxins are converted to electrophilic metabolites, which readily conjugate with GSH and generally increase the demand of antioxidants (Bray and Taylor, 1993). Gamma irradiation resulted in a significant increase in GSH content in erythrocytes, while no significant change in GSH content of liver was observed in gamma irradiated animals as compared to control. The increase in the supply of GSH from the blood pool can enhance the ability of extra-hepatic tissues to cope with a generalized increase in tissue oxidative stress (Akerboom and Sies, 1981; Yadav and Bhatnagar, 2007b).

GST detoxifies a variety of electrophiles generated during oxy-radical detoxication by conjugating them with GSH (Hayes and Pulford, 1995). The increase in liver and erythrocytes GST activity was observed to be maximum in rats pretreated with PE followed by gamma irradiation than that of rats pretreated with TCE, WSE or ARE as compared to rats gamma irradiated alone. The restoration of antioxidant enzyme activities after pretreatment with extracts shows the antioxidant property of the extracts against free radicals. Over all, the liver and erythrocytes GST activity was significantly increased in TCE, WSE, ARE and PE pretreatment as compared to gamma irradiated rats, suggesting that pretreatment with these extracts facilitates the removal of free radicals and enhanced the post irradiation repair of endogenous antioxidant system.
The histological studies revealed the effectiveness of pretreatment with the extracts prior to gamma irradiation in reducing the extent of hepatocellular and gastrointestinal damage. This accelerated the recovery process against gamma irradiation induced oxidative damages depending on the susceptibility of the tissues towards radiations. The results suggest that pretreatment with extracts prior to gamma irradiation reduced the extent of intestinal cellular radiation damage and accelerated the recovery process of endogenous antioxidant system of the animal to sustain oxidative assault. Overall, reduced lipid peroxidation, enhanced antioxidant enzyme activities, decrease in DNA damage in vitro as well as recovery of cellular architecture of damaged tissue suggest that the extracts TCE, WSE, ARE and PE mitigates the oxidative stress and have a radioprotective function against gamma irradiation exposure. The results of the present study suggested that the high antioxidant activities exhibited by the extracts might be responsible for the possible protective effects against radiation induced alterations in gamma irradiated animals. The possible antioxidant potential and probable radioprotection by these natural products can be due to the active constituents present in the extracts.

Radioprotective efficacy was further evaluated by 30 days post irradiation survival of rats at 10.0 Gy, the ability to protect the hematopoietic stem cells as well as immunostimulating activity through endogenous spleen colony forming unit assay (CFU), bone marrow stem cell protection ability with micronucleus assay and DNA damage in peripheral blood leukocytes by comet assay were performed. The induction of symptoms of radiation sickness like reduction in food and water intake, irritability, epilation, weight loss, emaciation, lethargy and ruffling of hairs within 3–5 days by 10.0 Gy of gamma irradiation is in agreement with the earlier studies (Jagetia and Baliga, 2003; Jagetia et al., 2003b; 2004; Mantena et al., 2008). The death of the animals exposed to 10 Gy of radiation within 10 days is because of the functional failure of the gastrointestinal tract (Jagetia and Baliga, 2002; Jagetia et al., 2002). The survival after exposure to high doses of irradiation, i.e., 10 Gy depends on the survival of a critical number of haemopoietic stem cells (HSC) and the ability of these cells to generate an effective level of mature cells of multiple lineages to repopulate the depleted haematopoietic compartment (MacVittie et al., 1990). The bone marrow stem cells are more sensitive to radiation damage than the intestinal crypt but the peripheral blood cells have a longer transit time than the intestinal cells and hence the gastrointestinal syndrome appears earlier and the bone marrow syndrome appears later (Jagetia and Baliga, 2002; Jagetia et al., 2003a; 2004). The main
cause of bone marrow syndrome is the severe depletion of the HSCs, since these being more sensitive to radiation than the committed and mature peripheral blood cells (Devi et al., 2000).

The 30-day time period after lethal whole body gamma irradiation for survival of rats indicates the capacity of the drug in test to modulate the recovery and regeneration of the gastrointestinal (GI) epithelium and the hemopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for sustaining the life (Guruvayoorappan and Kuttan, 2008). Most of the earlier studies have also taken 10.0 Gy of gamma irradiation as a lethal dose for the survival studies on rats (Jagetia et al., 2004; Singh et al., 2005; Lata et al., 2009). The pretreatment of rats with TCE, WSE, ARE and PE prior to whole body irradiation resulted in overall decrease in radiation induced mortality and reduced the severity of radiation sickness as compared with the concurrent irradiated group. Increase in survival in TCE and ARE pretreated rats prior to gamma irradiation was higher than WSE and PE. The survival of rats against irradiation is a result of several factors, such as prevention of damage by free radicals, efficient scavenging of free radicals, and repair of DNA or replenishment of severely damaged or dead cells (Goel et al., 2004). A number of other factors may also influence the survival of irradiated mice such as irradiation dose, drug form and dose, vehicle, time, schedule of treatment, mouse strain, and/or composition of control diets in dietary studies (Srinivasan et al., 1983). The increased survival of animals might be due to the various repair mechanisms afforded by the extracts.

Radioprotection in terms of endogenous spleen colony forming units assay was studied at lethal and sub lethal doses i.e., 6, 8 and 10 Gy respectively. The effect of whole body irradiation is mainly felt by the highly proliferating germinal epithelium, gastrointestinal epithelium and the bone marrow progenitor cells (Weiss and Kumar, 1998). The haematopoietic protective ability was confirmed by the increase in the spleen weight and endogenous colonies on 13th day of post irradiation at 6, 8 and 10 Gy in rats pretreated with extracts. Protection of bone marrow stem cells was found to be more significant in rats exposed to lower and medium doses of gamma radiation (6 and 8 Gy) as compared to those exposed at lethal dose (10 Gy). Haematopoietic regeneration and release of humoral factors that stimulates and regulates CFU formation has been suggested to be more significant at lower irradiation doses as compared to the irradiation at higher
radiation dose (Grande et al., 1990). Variations in spleen weight showed similar pattern as that of CFU counts in animals of different experimental groups, which was in accordance with the recent studies (Mantena et al., 2008). These results suggest that TCE, WSE, ARE and PE were very effective in stimulating the regeneration of hemopoietic cells as evidenced by the increase in spleen colonies indicating the immunostimulating and hematoprotective activity. The mechanisms by which extracts stimulate the regeneration of hematopoietic cells are not known. One possible mechanism may operate through the drug induced suppression of prostaglandin production as prostaglandins are reported to block proliferation of hematopoietic cell (Kurland et al., 1978; Gentile et al., 1983).

As recovery progresses in irradiated animals, colonies of hemopoietic cells become grossly visible on the spleen, the number of colonies being inversely related to radiation dose. Mortality as a basis of DRF estimation has the obvious disadvantage that death is a remote effect of irradiation, subjects to conditions which may be difficult or impossible to control. Moreover, the period of approximately 30 days needed for completion of a test is considerably longer than the time required for protection to become apparent. Reduction in the number of CFU appears to be a more direct effect of irradiation than death of the animal and hence a preferable end point (Smith et al., 1966). The estimation of DRF from CFU counts have advantage of being completed within 12 days after irradiation. The estimation of DRF by this method has essentially same precision as that obtained with the other methods (Smith et al., 1966). The results suggest that PE, TCE, ARE and WSE has considerable DRF with a range of 1.48 to 1.60 indicating the radioprotective efficacy of the extracts at lethal radiation doses.

Ionizing radiation generates free radical damage in DNA and induces genotoxic effects and death of cells (Reily, 1994; Pietta, 2000). A broad spectrum of DNA lesions, including damage to nucleotide bases, cross-linkage, DNA single and double strand breaks (DSBs) are induced (Maurya et al., 2007). This is followed by altered cell division, cell death, depletion of stem cells pool and even death if the radiation dose is high. Among all these, DNA DSBs have been considered the critical lesion for the radiation induced chromosome break and cell death (Iliakis, 1991). The cytogenetic damage induced by radiation and clastogenic agents on the mitotic cells is expressed as an increased in number of micronuclei. Moreover, the non-repair or misrepair DNA breaks contributes to the principle lesions in the induction of chromosomal aberrations, quantitatively analysed by
micronuclei (Paul et al., 2011; Little, 2000). Hence, micronucleus assay was used to examine the gamma irradiation induced genotoxicity in bone marrow polychromatic erythrocytes of rats pretreated with extracts without or with gamma irradiation. Bone marrow micronucleated erythrocytes provide a simple and rapid method for the detection of chromosomal damage by chemical and physical agents. An antimutagenic effect of Calendula officinalis in terms of micronucleus test after the treatment with mutagen has recently been reported (Leffa et al., 2012). This protective effect of C. officinalis has been proposed due to its chemical constituents including steroids, terpenoids, phenolics, flavonoids and other compounds found in this extract (Re et al., 2009).

The antioxidant effect is associated with the anti-genotoxic and anti-mutagenic properties that can be exploited for its use against a number of disorders including cardiovascular diseases, inflammation and cancer (Preethi et al., 2006). Prevention of radiation induced micronucleus formation and DNA damage in human lymphocytes by antioxidant thiols (GSH, N-acetyl-l-cysteine, thioproline) has also been reported (Tiwari et al., 2009). Citrus fruits containing flavonoids have also been reported to show protective effect on mouse bone marrow cells against gamma radiation (Hosseinimehr et al., 2003). The results for micronucleus assay were in consonance with earlier studies (Jagetia et al., 2003b; Shimoi et al., 1996) where an enhancement of MnCs in the bone marrow of gamma irradiated mice was reported. Pretreatment with extracts prior to gamma irradiation significantly reduced the formation of micronuclei indicating the anticlastogenic effect of TCE, WSE, ARE and PE with respect to the gamma irradiated group.

Genotoxic effect of gamma radiations is mediated through the formation of ROS that cause DNA damages as mentioned earlier, ultimately leading to chromosomal damage (Rao and Rao, 2010). The increased levels of DNA lesions such as single and double strand breaks due to gamma irradiation could be detected by alkaline comet assay. The alkaline comet assay is an effective technique to monitor the extent of DNA damage including both single and double strand breaks, base modifications, oxidative damage and alkali labile lesions (Tice et al., 2000). Using the comet assay, it was aimed to study the levels of primary DNA damage in white blood cells of gamma irradiated rats pretreated with extracts. The damages by ionizing radiation to DNA can cause the loss of viability of the cells exposed to radiation. The alkaline single cell gel electrophoresis of peripheral blood leucocytes of the whole body gamma irradiated rats displayed the increased comet
parameters such as tail length, percent DNA in tail, tail moment and olive moment indicating radiation induced DNA damage. The results suggested that pretreatment of rats with the extracts prior to gamma irradiation effectively inhibited the radiation induced oxidative lesions in peripheral blood leucocytes resulting in the lowering of comet parameters. This reduction in the comet parameters in extracts pretreated gamma irradiated rats to that of gamma irradiated animals indicated the in vivo DNA protective ability of all the extracts. DNA protecting properties of natural antioxidants in peripheral blood leukocytes with comet assay against gamma radiations has been reported (Maurya and Nair, 2006; Maurya et al., 2007).

The results obtained in the present study were in consonance with other studies against gamma radiations mediated oxidative assaults (Ganasoundari et al., 1998; Bhatia and Jain, 2004). Herbal and natural products can efficiently restore the disturbed equilibrium during radiation injury, in a collective and holistic manner due to their various bioactive constituents (Arora et al., 2005a; Hosseinimehr, 2007; Kumar et al., 2007; Prabhakar et al., 2007; Veerapur et al., 2010). The radioprotective activity of these natural antioxidant sources may be mediated through several mechanisms, since they are complex mixtures of many chemicals. The polyphenols present in the extract may upregulate mRNA of antioxidant enzymes such as SOD, CAT, GST etc. and thus may counteract the oxidative stress induced by gamma irradiation. Upregulation of DNA repairing genes may also protect against radiation induced damages by bringing error free repair of DNA damage as suggested by Jagetia (2007). Reduction in lipid peroxidation and elevation in non protein sulphahydroly groups may also contribute to some extent to their radioprotective activity. The extracts may also inhibit activation of protein kinases C (PKC), mitogen activated protein kinases (MAPK), cytochrome P-450, nitric oxide and several other genes that may be responsible for inducing damage after gamma irradiation (Jagetia, 2007).

In conclusion, analysis of the principle constituents confirms the presence of the potent antioxidants such as phenolics, flavonoids and triterpenoids in the extracts, which may be responsible for their overall antiradical and antioxidant activity. Extracts showed significantly high levels of FRSA, TAC, RP, MCA, SASA, BDDD and HRSA at different concentrations. Moreover, the extracts provide protection to DNA from oxidative damage by radical generating system, which further confers the antioxidant potential. TCE, WSE,
ARE and PE pretreatment delayed or reduced the severity of irradiation sickness and also delayed the onset of irradiation induced mortality when compared with the concurrent irradiation group. The inhibition of lipid peroxidation, increase in antioxidant enzyme activities, recovery of hepatocellular and gastrointestinal damage as well as decrease in DNA damage in vivo suggest that these herbal and natural extracts, mitigates the oxidative stress and have a radioprotective function against gamma irradiation exposure. The study establishes that the selected herbal and natural products have strong antioxidant and radioprotective potential. However, the overall activity of these extracts is a result of several active constituents functioning via multifarious mechanisms in a synergistic manner. These natural antioxidants are lower in toxicity, easy availability and inexpensive that makes them a better choice as radioprotectors. It is therefore suggested that these extracts possess antioxidant activity and ability to counteract gamma irradiation induced oxidative stress, they can be prospective agents as antioxidants and radioprotectors.