Introduction
Radiation is all around us and naturally present in our environment since the birth of this planet. Radiation is a process in which energy is transmitted in the form of waves or particles through a medium or space. There are two distinct types of radiation; ionizing and non-ionizing. Non-ionizing radiation has low energy level and it is incapable of removing electron from an atom or molecule. Ionizing radiation has a high energy level. Ionizing radiation comes from outer space (cosmic), the ground (terrestrial) and even from within our own bodies. It is also present in the construction materials used to build our homes. Man made sources of radiation are medical, commercial and industrial activities. Some consumer products such as tobacco, fertilizer, welding rods, exit signs, luminous watch dials and smoke detectors also contribute to radiation exposure at low levels.

Rays of alpha and beta particles are relatively non-penetrating, external exposure to them causes only localized damage for example burns on the skin. Gamma rays and neutrons are more penetrating, causing diffuse damage throughout the body (e.g. radiation sickness) and increased incidence of cancer.

Primary source of gamma exposure is naturally occurring radionuclides, particularly Potassium-40, which is found in soil and water. Some man made radionuclides that have been released in the environment, emit gamma rays. Radium is also a source of gamma radiation. Small quantities of radioactive materials occur naturally in the air we breathe, the water we drink, the food we eat, and in our own bodies. People receive some background radiation each day from the sun, from
radioactive elements in soil and rocks and from household appliances (such as television sets and microwave ovens), and from medical and dental X-rays.

The most damaging forms of gamma radiation occur in the gamma ray window, between 3 and 10MeV. High energy gamma rays being less harmful because the body is relatively transparent to them. Positive application of gamma rays include cancer treatment through radiation, measurement and tracking of fluid flows, resource exploration, sterilization of medical equipment, pasteurization and geodesic surveys.

Gamma rays travel very fast and they pass through the entire body very quickly, affecting all the organs. Radiation exposure can occur without any prior warning through radiography, nuclear medicine, radiotherapy, radiological imaging, radionuclide production, biomedical research, military, public domain, transportation, nuclear reactors, space flights etc. Patients may be exposed to ionizing radiation during radiotherapy or following exposure to radionuclides in nuclear medicine.

The level of radiation that we constantly exposed to have risen dramatically over the last half century. Ambient fallout from nuclear catastrophes is impacting our environment and ultimately our health as well as come in contact with radioactive materials. The bombing of Hiroshima and Nagasaki in 1945, the Chernobyl nuclear disaster in 1986 and the most recent 2011 Japan, Tsunami Calamity at Fukushima nuclear power plant, each had devastating consequences for the environment, damaging the ecosystem and the quality of our air, water and soil.

Exposure of biological system to ionizing radiation results in the form of reactive species i.e. reactive oxygen species (ROS), reactive nitrogen species (RNS)
and also the generation of free radicals. Free radicals can be defined as atoms or a group of atoms having an unpaired electron. They are highly reactive and are capable of altering all biological molecules including lipids, DNA and proteins. Living organisms contain 60%-70% water. Reactive species include hydrated electron (e$_{aq}$), hydrogen radical (H), hydroxyl radical (OH), H$_2$O$_2$, peroxyl radical (ROO.), O$_2$-, singlet oxygen (^{1}O$_2$) etc resulting from radiolysis of aqueous solutions.

Acute effects of intense and sudden radioactive exposure are known as non-stochastic effects and are mostly related to skin damage. However, several other symptoms that may occur are nausea and vomiting, tiredness, fatigue, diarrhoea, headache, hair loss, tissue swelling, skin erythema, cough, difficulty in swallowing, difficulty in breathing and organ failure. In the event of acute exposure that causes these symptoms, treatment is almost impossible and death is very likely. These effects are associated with very close proximity to a gamma ray emitting source such as handling of raw materials or a nuclear device.

The primary concern of stochastic effects is cancer as a result of cell ionization and mutation. These cancers may appear in months and years after exposure has occurred. For women, birth defects when carrying to term are also a major concern, and a particularly long lasting manifestation of radiation exposure that can take place even in subsequent generations. Genetic mutation can arise as a result of gamma ray poisoning, affecting people years after exposure.

Due to the increased use of ionizing radiation in various aspects of human life, there is a need to develop an effective and non-toxic radioprotector. Radioprotectors are compounds that have ability to reduce the biological effects of
ionizing radiation on normal tissues, including lethality, mutagenicity and carcinogenicity and have applications in clinical oncology, space travel, radiation site clean up, radiological terrorism and military scenarios. The Radioprotectors can elicit their action by various mechanisms such as: suppressing the formation of free radicals, inducing the cellular radioprotectors such as superoxide dismutase (SOD), enhancing the DNA repair and inducting hypoxia in the tissues. Different radioprotectors offer protection to cellular molecules by different mechanisms (Maurya et al., 2006). Some of these compounds protect the target molecules because of their antioxidant mechanism by neutralizing the free radical, some enhances the cellular DNA repair (Maurya et al., 2005a; Maurya et al., 2005b), some modified the signaling pathways, some modulate the immune system and some contribute to a combination of all of these mechanisms.

Chemical radiation protection has a history of about six decades, when the first report that the natural amino acid Cysteine protected mice against the radiation induced sickness and mortality appeared (Patt et al., 1949). Numerous drugs of both synthetic and natural origin eg. antioxidants (Tempace, Vitamin A,C and E (α-tocopherol), Tocopherol monoglucoside (TMG), melatonin), cytoprotective agents (Mesna, Dexrazoxane), sulphhydryl compounds (cysteine, cysteamine, glutathione, Amino-ethyl-isothio-uronium bromide hydrobromide (AET), WR-2127 and other WR-compounds), Angiotensin converting enzyme (ACE) inhibitors (Captopril, Elanopril, Pencillamine, Pentoxyfylline, L-158, 809), metalloelements (Manganese Chloride, Cadmium salts, Bismuth Subnitrate), Immunomodulators (Gamma-interferon, Polysaccharides AMS, AM218, AS101), lipopolysaccharides and prostaglandins, DNA binding ligands have been tested in both in vitro and in vivo
models. The synthesis of WR-2721 or Amifostine was a major breakthrough in the development of radioprotective drugs which is currently used as an adjuvant in radiotherapy.

The high toxicity of synthetic compounds necessitated search for alternative agents, which could be less toxic and highly effective at non-toxic dose levels. The products or compounds isolated from natural sources could be of substantial use as non-toxic radioprotectors. Therefore, investigators diverted their attention towards the plants and natural products during the last two decades.

The plants have been the companion of man since times immemorial and formed the basis of several useful drugs for treatment of various ailments. The use of plants and natural products may be beneficial in protecting against the radiation induced damage, as they are less toxic or non-toxic as compared to the synthetic compounds. In the view of low cost, easy accessibility and less toxic effects, there is a growing interest towards ethno-medicines (Chatterjee and Pakrashi, 1995). Plants exhibit a diverse array of biological activities that may be relevant to the mitigation of ionizing radiation induced damage in mammalian system, as no ideal safe synthetic remedies are available till date. In Ayurveda several plants have been mentioned which are used to treat free radical mediated ailments. Some plants exhibit pharmacological activities like antiemetic, antioxidant, anti-inflammatory, wound healing, cell proliferation, metal chelation, anti-microbial, immuno-stimulant and haemopoietic protection.

From the last one decade various plants have been utilized for their radioprotective properties. Plants may contain certain compounds that can protect
against radiation-induced reactive oxygen species (ROS)-mediated damage. Plant extracts eliciting radioprotective efficacy contain a plethora of compounds including antioxidants, immunostimulants, cell proliferation stimulators, antiinflammatory, antimicrobial agents and some of which may act in isolation as well as in combination with other constituents from the same plant. (Arora et al., 2005). Published reports are available for radioprotective properties of various plants like *Moringa oleifera* (Katoch and Yonezawa, 1991), *Hericium Erinaceus* (Benzie and Strain, 1996), *Ocimum sanctum* (Devi PU, 2001), *Ginkgo biloba* (Weiss and Landauer, 2003), *Morus bombycis* (Weiss and Landeuer, 2003), *Centella asiatica* (Shobi and Goel, 2001; Sharma and Jaimala, 2003), *Hippophae rhamnoides* (Shukla et al., 2006), *Ginseng* (Ivanova et al., 2006), *Podophyllum hexandrum* (Mittal et al., 2001; Shukla et al., 2006; Gupta et al., 2008; Sankhwar et al., 2011), *Tinospora cordifolia* (Goel et al., 2004; Sharma and Pahadiya, 2010), *Zingiber officinale* (Arora et al., 2005), *Allium sativum* (Jagetia and Baliga, 2003), *Rhodiola imbricate* (Arora et al., 2005), *Adhatoda vasica* (Kumar et al., 2005), and *Piper betel* (Bhattacharya et al., 2005; Verma et al., 2010).

At this time, an increasing proportion of people around the world are more health conscious than ever before. This is probably due to the increased awareness of what seems to be mostly preventable diseases (i.e., obesity, heart disease, cancers, osteoporosis, arthritis and type 2 diabetes mellitus) in part related to an expansion in educational vehicles and the greater collective health consciousness of the continuous shift of the population towards a more advanced age. Along with age comes an increased incidence of disease and thus individual focus upon prevention and treatment of such disorders. The notion that food may possess the ability to prevent
disease and/or be used as treatment of ailments dates back a couple millennia. Hippocrates proclaimed, some 2500 years ago, “Let food be thy medicine and medicine be thy food.”

The pomegranate, *Punica granatum* L., an ancient, mystical and highly distinctive fruit, is the predominant member of two species comprising the Punicaceae family. It was lauded in ancient times in the old Testament of the Bible, the Jewish Torah and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance and good luck. It also features prominently in the ceremonies, art and mythology of the Egyptians and Greeks and was the personal emblem of the Holy Roman Emperor, Maximilian. Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain from which the city gets its name. The genus name Punica, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as Granada, and literally translates to seeded (“granatus”) apple (“pomum”).

The pomegranate is native to the Iranian plateau and the Himalayas of northern India. It has been cultivated throughout in Iran, Azerbaijan, Afghanistan, India, Pakistan, Bangladesh, Iraq, Egypt, China, Burma, Saudi Arabia, Israel, the drier parts of Southeast Asia and the Mediterranean region of Southern Europe and tropical Africa.

The fruit is consumed fresh, or processed in the form of juice, jam, or syrup. The arils are also used as an ingredient in main dishes. Dried seeds are used (whole or powdered) as a spice for several main dishes in Indian cuisine. Pomegranate fruit juice has long been a popular drink in Persian and Indian cuisine. Wild pomegranate
Figure 1: (a) and (b) Pomegranate fruit, (c) Fruit rind and (d) Seeds of *Punica granatum*
seeds are used as a spice known as ‘anardana’ most notably in Indian and Pakistani cuisine. Dried arils can be used in several culinary applications, such as trail mix, granola bars or as a topping for salad, yogurt or ice cream. It is a sacred fruit for several religions, and is deeply rooted in the symbolism of mythology and legends, as well as in religious rites and traditions (Figure 1).

The open pomegranate was considered a symbol of the mercy of god. Several very famous paintings depict the Madonna with child offering a pomegranate fruit, including (i) “Maria, dem Kind einen Granatapfel reichend [Mary Offering a Pomegranate to the Child]” by Hans Holbein the Elder (approximately 1465), (ii) Botticelli’s “Madonna della Melagrana [Madonna of the Pomegranate]” (1487). Another very famous painting, (iii) Lorenzo Di Credi’s so-called “Madonna and Child with a Pomegranate (Dreyfus Madonna)” (which is displayed at the National Gallery in Washington D.C., USA), depicts a pomegranate offered to the child by Mary. The pomegranate was also a sign of power. (iv) Albrecht Duerer (1519) painted the emperor Maximilian I of Habsburg with a pomegranate (Figure 2).

In the Indian subcontinent ancient Ayurvedic system of medicine, the pomegranate has extensively been used as food and source of traditional remedies for thousands of years. Pomegranate’s roots, leaves, fruit, rind and seeds all are used for medicinal purposes. Pomegranate has been used for centuries as therapeutic agents for the treatment of inflammatory diseases and disorders of the digestive tract.

Pomegranate is loaded with tannins, anthocyanins, polyphenolics, antioxidant and vitamins A, E and C all of which have a good effect on the body. Pomegranate is digestive, carminative, enhances semen formation, activates memory, destroys
Figure 2: Famous paintings depict the Madonna with child offering a pomegranate fruit, including (i) “Mary offering a pomegranate to the child” by Hans Holbein the Elder (1465) (ii) Botticelli’s “Madonna of the Pomegranate” (1487). (iii) Lorenzo Di Credi’s so-called “Madonna and Child with a Pomegranate”, (iv) Albrecht Duerer (1519) painted the emperor Maximilian I of Habsburg with a pomegranate.
disturbances caused by wind, bile, phlegm, improves formation of hemoglobin and is a blood purifier.

The dry fruit rind powder is used as a tooth powder and also as a medicine in cosmetic industries. It is an excellent source of beta-carotene, potassium, phosphorous and calcium. The rind of the fruit and the tree bark is used against diarrhoea, dysentery and intestinal parasites. Pomegranate contains many phytochemicals with antioxidant action, such as ellagic acid. Ellagic acid has anticarcinogenic, antiatherogenic and antifibrosis activity.

The seed and juice are considered a tonic for the heart and throat and classified as a bitter astringent under the Ayurvedic system. The astringent qualities of the flower juice, fruit rind and tree bark are valuable for stopping nose bleeds and gum bleeds, toning skin, firming-up sagging breasts and treating hemorrhoids. The whole or complex pomegranate fractions possess antiproliferative, pro-apoptotic and/or anti-angiogenic effects superior to those observed with their isolated key active compounds, suggesting therapeutic strategies that may depart from the orthodox preference for pure single agents.

In the present study Punica granatum rind and seed extract were used separately to test their radioprotective efficacy against Co\(^{60}\) gamma radiation. The animal model chosen for the experiment was Swiss albino mouse.

Liver has a wide range of functions including detoxification, protein synthesis and production of biochemicals necessary for digestion. It is the internal organ capable of natural regeneration of lost parts. The liver is regarded as moderately
radiosensitive organ and affected adversely by gamma radiation. Due to high dose of radiation exposure severe damage in hepatic tissue occurs.

A cell depends on its DNA for coding information to synthesize various types of proteins that include enzymes, certain hormones, carrier and structural proteins, which support life. DNA is the primary cellular target of radiation damage. Mainly two types of changes are observed in DNA at the molecular level, altered bases sequences and strand breaks. Both types of changes, if not repaired affect the cell function. Proteins are large biological molecules, or macromolecules, consisting of one or more chains of amino acid residues. Proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, responding to stimuli, and transporting molecules from one location to another. Proteins are a major target for radiation as a result of their abundance in biological systems, and their high rate constants for reaction. Lipid peroxidation refers to the oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. Membrane protein and lipids can be damaged by radiation. In proteins it can lead to formation of protein carbonyls and loss of protein thiols besides loss of activity of membrane bound enzymes. Membrane lipids are highly susceptible for radiation damage mainly due to the presence of polyunsaturated fatty acids. The resulting damage results in lipid peroxidation. Unchecked peroxidative decomposition of membrane lipids has severe consequences for the cell and the organism. Since many cellular reactions are membrane based they are affected by lipid peroxidation. The products formed during this phenomenon also have effects at other targets away from the site of generation.
Glutathione (GSH) is an important natural protector that prevents damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side-chain and the amine group of cysteine. Superoxide dismutase and Catalase are the most important antioxidants in the body that play an important role in scavenging reactive oxygen species.

The aim of the present study was to find out the protective effects of *Punica granatum* fruit rind and seed extract against gamma radiation and assess the role of the extract against radiation induced changes in liver.