Modulation of Radiation and Cadmium Induced Changes in the Hepatic Nucleic Acid Content of Mice by *Emblica officinalis* (Amla)

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Abstract— Amla is well – known for its rich vitamin c (ascorbic acid) and polyphenol contents. The present study was to assess the protective efficacy of amla fruit extract against gamma radiation and cadmium induced changes in Nucleic acid content in mice liver. For this mice were exposed to different dose of gamma radiation (3.5 Gy and 7.0 Gy) separately, with and without cadmium chloride treatment in control groups. While in experimental groups, the animals were given amla fruit extract orally seven days prior to radiation or cadmium chloride treatment and continue to last autopsy. It was found that DNA content decreased while the RNA content increased in both control as well as *Emblica* treated groups. Such alteration in nucleic acid content showed a dose-dependent and synergistic action. An early recovery observed in *Emblica* pre treated animals showing protective action of *Emblica*.

Index Terms— Cadmium chloride, DNA, *Emblica*, Gamma radiation, mice, liver, RNA.

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1 INTRODUCTION

In recent times, attention has been focused on the physiological importance of a wide variety of naturally occurring polyphenol compounds that act as antioxidants. These compounds are found abundantly in plants including ginger, garlic, *Aloe vera*, and cabbage. They exert profound chemo-Radio preventive activities due to their ability to scavenge and reduce the production of free radicals and act as transition metal inhibitors, DNA cleavage protector [1-3]. Exposure to ionizing radiation represents a genuine, increasing threat to mankind and our environment. The steadily increasing applications of radiation in clinical practice, industrial and agricultural activities, on top of residual radio-activity resulting from nuclear test explosions, have a measurable impact contributing to possible radiation hazards in humans. Control of radiation hazards is considered as one of the most important challenges in order to protect our lives from radiation damage [4]. A number of preventive and therapeutic chemical and biological agents can combat radiation injury. However, some can also result in a series of side effects [5] and have therefore the anti-radiation effects of natural products have been investigated [6].

Metals, when concentrated can be quite toxic and can result in death of organisms. Numerous hazardous heavy metals are inhaled and absorbed by humans and animals every day [7]. Some of heavy metals such as the alkaline earth metals and

particularly trace elements are essential for survival because they help build molecules that sustain life. Other metals such as lead (Pb), mercury (Hg) and cadmium (Cd), which are examples of heavy metals are very toxic at even minutes quantities and serve no purpose of sustaining life [8]. Radiation and Cd toxicity has been proposed to involve the generation of reactive oxygen species [9, 10]. Antioxidant nutrients such and as vitamin C, E and Selenium have been found to counter free radical generation by Cd and Radiation [11,12].

In this context chemo - radioprotective potential of *Emblica officinalis* was studied. According to believe in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to family Euphorbiaceae. It is also named as Amla, *Phyllanthus Emblica* or Indian gooseberry. Amla is extremely nutritious and might be a chief dietary source of vitamin C, amino acids, and minerals. Entire parts of the plant are used for medicinal purposes, particularly the fruit, the fermented liquor prepared from the fruits is used in jaundice, dyspepsia and cough. Exudation from incision on the fruit is used as external application for the inflammation of eye. *E. officinalis* fruit contains ellagic acid, gallic acid, micic acid, phyllembic acid, lipid, quercetin, kaempferol, emblicanin, flavonoids, glycosides and proanthocyanidins. Vitamin C (ascorbic acid or ascorbate), tannins (eg emblicanin A and B) and flavonoids present in amla have very powerful immunomudulatory, antioxidant and anticancer activities. Due to rich vitamin C, amla is successfully used in the treatment of human scurvy. Quercetin present in amla has hepatoprotective effect [13-15]. Administration of *Phyllanthus emblica* extract prior to irradiating mice prevented body weight loss significantly [16]. Administration of *Emblica officinalis* aqueous extract (2 mg/animal/day) and ochratoxin for a period of 45 days caused a significant amelioration in the ochratoxin-induced lipid peroxidation in mouse liver and kidney [17]. The wide use of *Emblica officinalis* fruits for various purposes prompted us to select for screening of chemo-radioprotective activity of Amla against radiation and cadmium induced changes in nucleic acid content of mice liver.

2. MATERIALS AND METHODS

2.1 Animal care and handling

The adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The Govt. Dungar College, Bikaner is registered under CPCSEA, Chennai (registration no. 1066/ac/07/ CPCSEA) and has its own Institutional Animal Ethics Committee (IAEC). In the view the present experiments were conducted under the supervision of IAEC of the College. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*. They were acclimatized to laboratory conditions before use. The temperature of the room was maintained between 22-27^{o}C.

2.2 Source of irradiation

The animals were exposed by the Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate ranging from 0.85 Gy/min to 1.65 Gy/min.

2.3 Cadmium

Cadmium in the form of inorganic cadmium chloride, obtained from S. D. Fine Chemicals Ltd. Boisar, Mumbai (India) was used for present study. The dose 20 ppm was selected for the present experiment. For this aqueous solution of cadmium chloride was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water and administered orally as drinking water [1].

2.4 *Emblica officinalis* Aqueous Extract (EOE)

Fresh fruits of the *Emblica officinalis* were cleaned, cut into small pieces, air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hrs.(12 hrs. x3). The extract thus obtained was vacuum evaporated so as to make it in powder form. The extract was redissolved in DDW just before oral administration. An approximate thirty eight per cent yield of the extract was obtained. The drug was given orally at the dose rate of 1000mg/kg body wt/ animal / day from seven days prior to cadmium chloride treatment or irradiation. The EOE dose was selected on the basis of dose survival essay as described previously [18].

2.5 Experimental design

The animals for the experiments were divided into the following groups:

Group – I (Sham-irradiated animals)
Group– II (Cadmium chloride treated animals)
Group- III (Irradiated animals)
Sub-group III a: 3.5 Gy
Sub-group III b: 7.0 Gy
Group- IV (Animals treated with radiation and Cadmium chloride)
Sub-group IV a: 3.5 Gy + Cadmium chloride

Sub-group IV b: 7.0 Gy + Cadmium chloride
Group – V (Cadmium chloride and *Emblica* treated animals)
Group- VI (Radiation and *Emblica* treated animals)
Sub-group VI a: 3.5 Gy + *Emblica*
Sub-group VI b: 7.0 Gy + *Emblica*
Group-VII (Radiation, Cadmium chloride and *Emblica* treated animals)
Sub-group VII a: 3.5 Gy + Cadmium chloride + *Emblica*
Sub-group VII b: 7.0 Gy + Cadmium chloride + *Emblica*

Group II, III and IV: served as control groups and group V, VI and VII as experimental groups.

2.6 Autopsy of animals

A minimum of three animals from each group was sacrificed after 1, 2, 4, 7, 14 and 28 days of treatment. The animals were sacrificed by cervical dislocation. After sacrificing the animals the liver was taken out, it was blotted, weighed and kept at -20^{o}C for biochemical studies.

2.7 Biochemical studies

Nucleic acid content of the liver was determined as
(1) DNA Ceriotti [19]
(2) RNA Ceriotti [20]

2.8 Statistical analysis

All the values are expressed as mean ± standard error (S.E.) in the tabular form. The S.E. was calculated by Fischer's formula [21]. The data were subjected to students 't' test for comparison between control and experimental groups [22]. The significance of the results was computed at different levels as $p<0.05$, $p<0.01$, and $p<0.001$.

3 RESULTS

It was found that DNA content decreased while the RNA content increased in all the control as well as *Emblica* treated experimental groups (fig. 1, 2). Such alteration in nucleic acid content was observed with higher dose (7.0 Gy) at early intervals. Recovery started at day 14 but standard value could not attained up to the last intervals. A similar pattern of nucleic acid alteration was observed with 3.5 Gy, but it was found comparatively less than 7.0 Gy group. Results indicated that the infliction of radiation insults and subsequent repair in the liver nucleic acid of mice to be dose dependent. In the cadmium treated group a similar decreasing and increasing pattern of nucleic acid content was observed as noted in ionizing radiation groups. In combined treatment of radiation and cadmium the pattern of biochemical changes was similar to individual treatment but the magnitude of their occurrence was statistically higher and the normal value could not be attained even at the last autopsy intervals (28 days). Thus the damage and recovery pattern in combined treatment (radiation and cadmium chloride) indicated the synergistic effect of these two agents. In the *Emblica* treated groups V, VI and VII the DNA content decreased and RNA content increased up to day-7 then DNA content increased and RNA content decreased on day-14 which continued up to day-28. In the *Emblica* treated groups less severe alteration in nucleic acid was observed showing protection by the EO fruit extract.

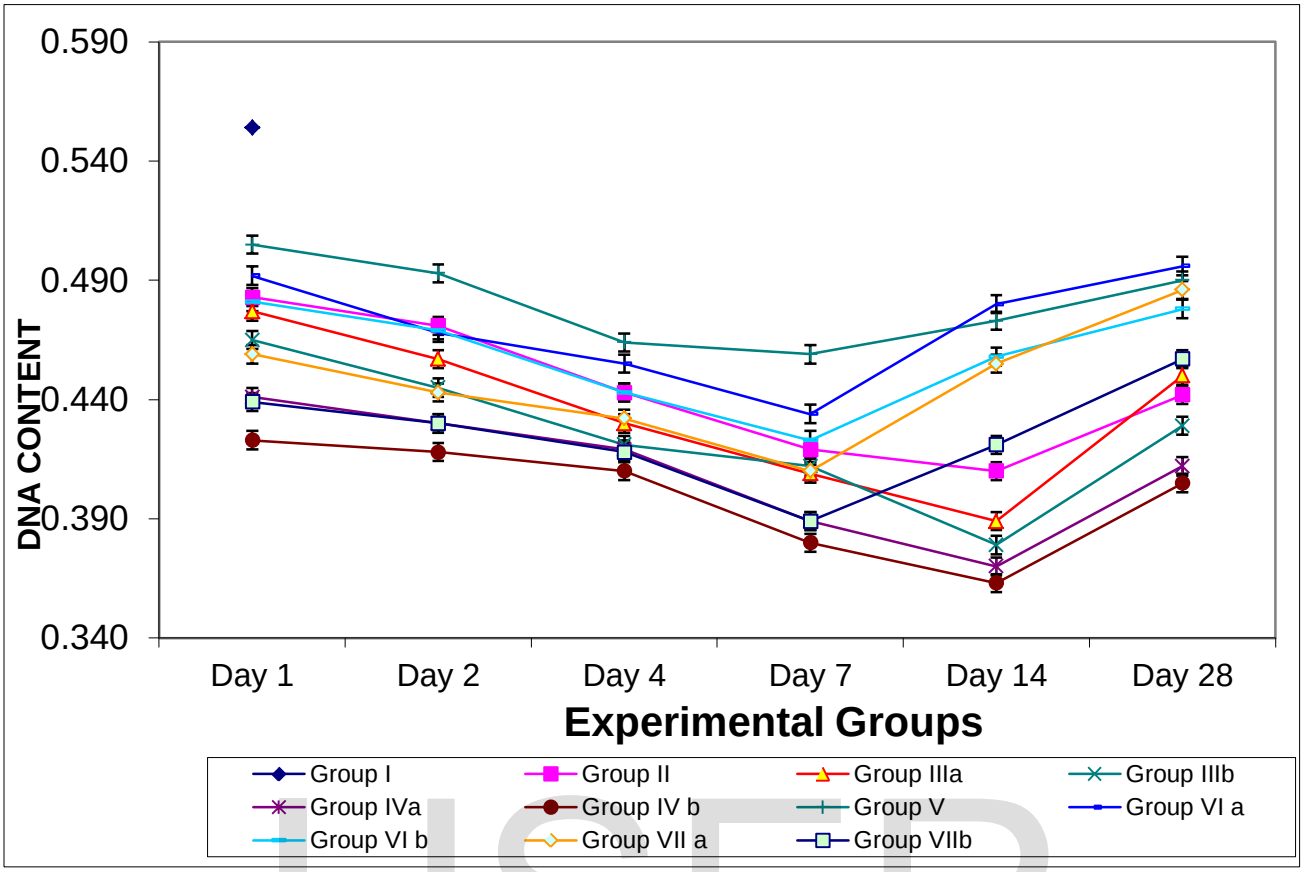

Fig.1. Variations in the DNA in Liver of mice in various groups (mg/gm of tissue weight)

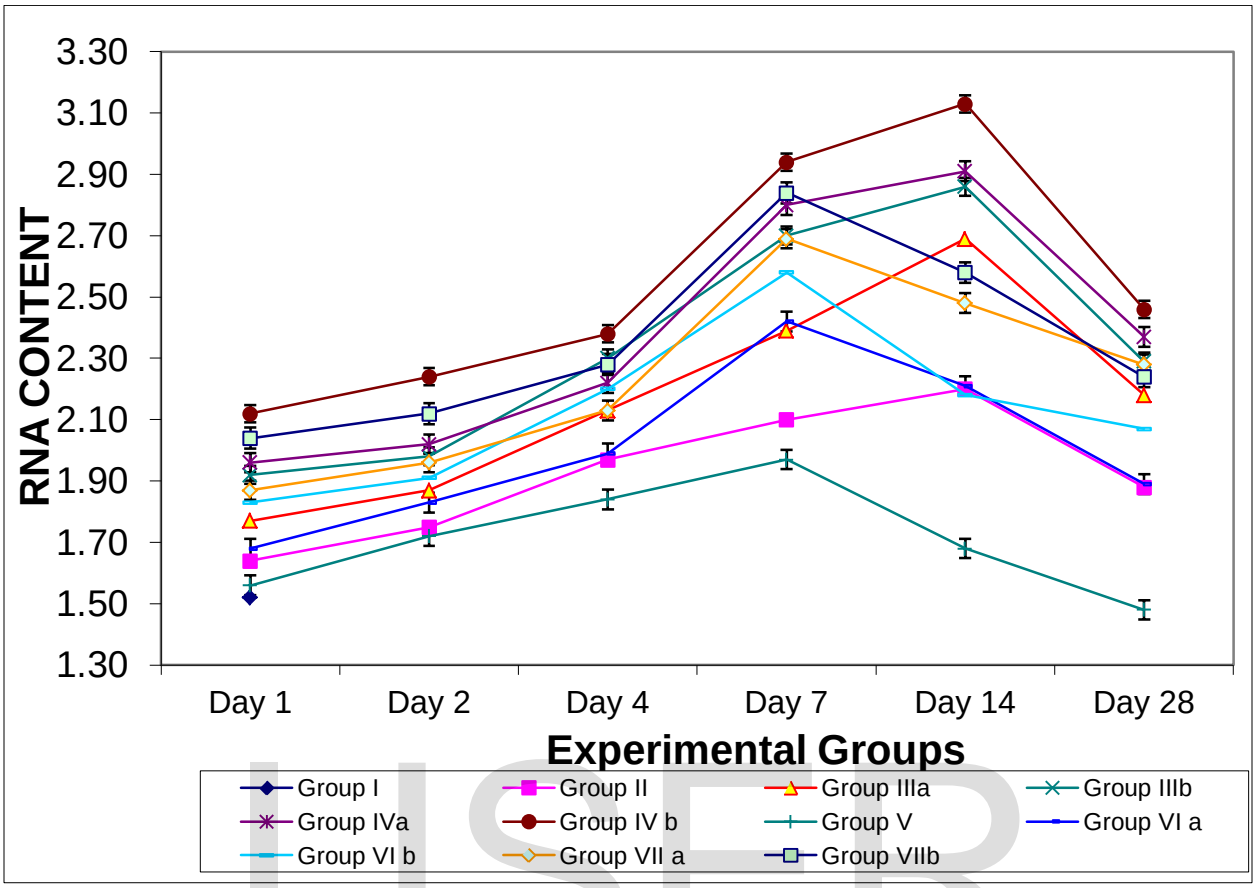

Fig. 2.Variations in the RNA in Liver of mice in various groups (mg/gm of tissue weight)

4 DISCUSSION

In the present study administration of cd in group II cause significantly (P<0.001) decrease in DNA up to day-14 in Swiss albino mice liver. It might be due to direct effect of Cd on hepatocyte DNA [23]. Cd is rendered inert in liver by being complexed to metallothionein, a low- molecular- weight scavenger protein, rich in cysteine residues. Excess free cd in the cytoplasm binds to cellular organelles, including the nucleus, and disrupts their functions. Cd is genotoxic *in vitro*, causisng single- strand breaks in DNA [24], frame- shift mutation [25], chromosomal aberrations [26] and inhibition of DNA repair [27]

Cadmium induced deficit of zinc can lead to changes in the activity of many enzymes, including thymidinkinase and hence to inhibition of DNA synthesis in the course of some days [28]. Histochemically, cadmium treated rats exhibited decrease of DNA contents in the tissues of liver, kidney and testis while the lung tissue exhibited an increase in DNA contents. Cd induced random fragmentation of genomic DNA [29]. The enzyme thymidine kinase (TK), responsible for the phosphorylation of deoxythymidine and its subsequent incorporation into DNA, has been involved in the inhibition of DNA synthesis in Cd-treated cell cultures [30].The enzyme TK is inhibited in the liver of Cd-treated rats [31] and the increase of DNA content in lung tissue may be due to the high increase of inflammatory cells.

DNA content showed a continuous decline significantly (P<0.001) after radiation exposure up to day-14 in the non-drug treated group III, It has been shown that post-irradiation acute cell death could lead to loss of DNA in excess that normally eliminated from the tissue. The prolonged interphase or delayed onset of DNA synthesis after irradiation also could lead to decreased content of DNA [32]. It was reported that depletion in the DNA content after irradiation of a tissue *in vivo* is due to reduction in or absence of the essential factors controlling the DNA synthesis [33]. The relative incorporation of DNA precursors into a given tissue is depressed because fewer cells are in S phase. Although the amount of DNA synthesized per cell in the 'S' period is normal, the total amount of DNA synthesis is depressed.

DNA is considered to be the primary target for cell killing by ionizing radiation. Ionizing radiation induces reactive oxygen species in the form of hydroxyl ion, hydrogen ion, singlet oxygen and peroxyl radicals that follow as cascade of events leading to DNA damage such as single or double strand breaks (DSB), damage to DNA base and sugar, and DNA-DNA or DNA-protein cross-links, and these lesions cluster as complex local multiply damaged sites. The DNA-DSBs are considered the most

lethal events following ionizing radiation which are likely to lead to cell death [34]. It was reported that the modification of primary and secondary DNA structure in irradiated animals demonstrate serious disturbance of hepatocytes genetic apparatus. This is responsible for morphologic and ultra structural changes in rat liver tissue after exposure to ionizing radiation [35].

The scientist carried out cytochemical and biochemical studies on nucleic acids in the liver, kidney and pectoral muscle of guinea pig, rat and mouse after 240 X irradiation and observed significant depletion in the DNA content of mouse tissues [36]. These results are in conformation with our present findings of the amount of DNA in 3.5 Gy and 7.0 Gy irradiated groups.

Administration of cadmium in present study influence RNA content in mice liver due to activation of RNA metabolism which may be partly explained by the increased expression of genes involved in detoxication and adaptation (e.g., metallothionein, stress response proteins, etc.) [37]. A significant increase in RNA synthesis in liver, observed in present study, suggests a generalized metabolic effect of Cd, same result also found in various organs (tongue, buccal mucosa, parotid gland, kidney, testes, intestine and brain). The effect of cadmium on RNA metabolism, exerted mainly by stimulation of tRNA and mRNA, especially related to MT metabolism has been reported [38-41].

The influence of Cd on RNA and protein synthesis was reported earlier in relation to individual organs, mostly liver and kidneys [42-44]. A transient decrease with secondary increase in pancreatic RNA level was demonstrated in rats exposed to Cd [45]. A secondary regeneration (cell renewal) after primary lesion was suggested. Inhibition of RNA synthesis in *Physarum polycephalum* was ascribed to the damage to nucleoli structure and function caused by Cd [46]. Thus, the nucleolus (aside from nucleus itself) was supposed to be the main cellular target for Cd toxicity. A slowdown in the transport and processing of nucleolar RNA, seen in HeLa cells [47], probably reflects these alterations. In present experiment, Cd effect was found to be generally much stronger on the RNA.

An increase in the RNA content in mice liver is also observed soon after the irradiation which could be due to an increase in the RNA concentration of the surviving cells after radiation insult. The biological effects of total body gamma -irradiation on nucleic acids of birds have also been investigated [48]. They found that the synthesis of RNA increased in liver up to 72 hours of exposure to 4 Gy of gamma irradiation. They found that the RNA content was increased in four tissues (liver, muscle, kidney and spleen) of birds. Hidvegi *et al.*[49] have shown that an increased amount of r-RNA and m-RNA in the cytoplasm are the consequences of enhanced synthesis of both RNA and Proteins in liver after whole body irradiation. They have also reported that higher activity of RNA polymerase in nuclei can also be correlated with increased RNA synthesis [50].They concluded that the sub-lethal dose (4.0 Gy) of gamma irradiation caused significant changes in the nucleic acids. These results are in close agreement with the present findings.

In combined treatment of radiation and cadmium in group IV the pattern of nucleic acid content changes was similar to individual treatment but the magnitude of their occurrence was statistically higher because cadmium has high affinity for –SH and disulphide groups of proteins [51] which are mainly responsible for protecting repair system against damage caused by radiation induced free radicals [52]. Due to this, DNA is exposed to these free radicals and the risk increases during the combined exposure and the normal value of nucleic acid content could not be attained even at the last autopsy interval (28 days).

In the present study prior administration of *Emblica* in groups V, VI and VII treated animals less severe alteration and pronounced recovery significantly ($p<0.001$) observed on day 7 in nucleic acid content due to multiple protective mechanisms showing by *Emblica* (fig.3). This may be due to antioxidants present in EOE which help in decreasing the genotoxicity created by toxicant and also inhibit mutagenesis and carcinogenesis [53,54].These are in accordance with studies which come to the fact that the imbalance in antioxidant status in the cells as a result of ionizing may be ameliorated as result of administration of EOE [55,56]. EOE significantly restored the antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GSHPx), and Glutathione Reductase (GR); Reduced Glutathione (GSH); and decreased levels of the lipid peroxide Malondialdehyde (MDA) in hypertensive patients [57,58].

GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense processes and detoxification. Glutathione is a component of a pathway that uses NADPH to provide cells with their reducing milieu. This is essential for (a) maintenance of the thiols of proteins and of antioxidants (e.g. ascorbate, alpha-tocopherol), (b) reduction of ribonucleotides to form the deoxyribonucleotide precursors of DNA, and (c) protection against oxidative damage, free radical damage, and other types of toxicity. Perturbation of GSH status of a biological system reflects defunct oxidant defense system and has been reported to lead to serious consequences [59]. It was reported that prior administration of *Emblica* fruit extract has significantly ($P<0.001$) elevated the level of GSH in irradiated mice liver [60]. The *Emblica* fruit extract also reduce oxidative stress due to cadmium administration by increasing activity of SOD, CAT and decreasing level of MDA significantly [61]. Similar protective mechanism also show other herbal plant like spirulina to protect nucleic acid content in mice tissues [62].

Liver RNA, an interferon inducer, is able to offer significant cytogenetic protection from radiation, implying indirectly that the induction of interferon by low dose radiation may also play a protective role as one of the mechanism in the induction of the cytogenetic adaptive response [63]. Mentat, herbal formulation inhibited radiation induced damage by free radical scavenging [64]. Upregulation of DNA repair genes may also protect against radiation induced damage by bringing error free repair of DNA damage. It is also claimed that protection of nucleic acid is due to the hydrogen atom donation by the protector as vitamin C (Ascorbic acid), present in *Emblica officinalis*, is good H-atom donor [65,66]. Chemical repair of the radiation induced DNA radicals by H-atom donation may be an important mechanism of protection against radiation and cadmium induced DNA, RNA damages in liver of mice.

Another mechanism of protection of nucleic acid by *Emblica* show due to presence of polyphenols in Amla. Polyphenols exhibit antioxidant activities by their hydroxyl group (–OH) in aromatic ring, which helps in mediating redox reaction, and thereby scavenges free radicals [67]. The reducing property of a compound signifies its potent antioxidant activity. The transformation of Fe3+ to Fe2+ has been looked for, on adding different extract concentrations. The poly phenols present in extract served as electron donors resulting in reducing ferricyanide complex to ferrous form [68]. The results obtained coincided to the fact that reducing power of amla extract increases with increase in concentration [69]. In our experiment such a higher dose (1000 mg/kg body wt.) of EO fruit extract significantly protect alteration of nucleic acid content in mice liver.

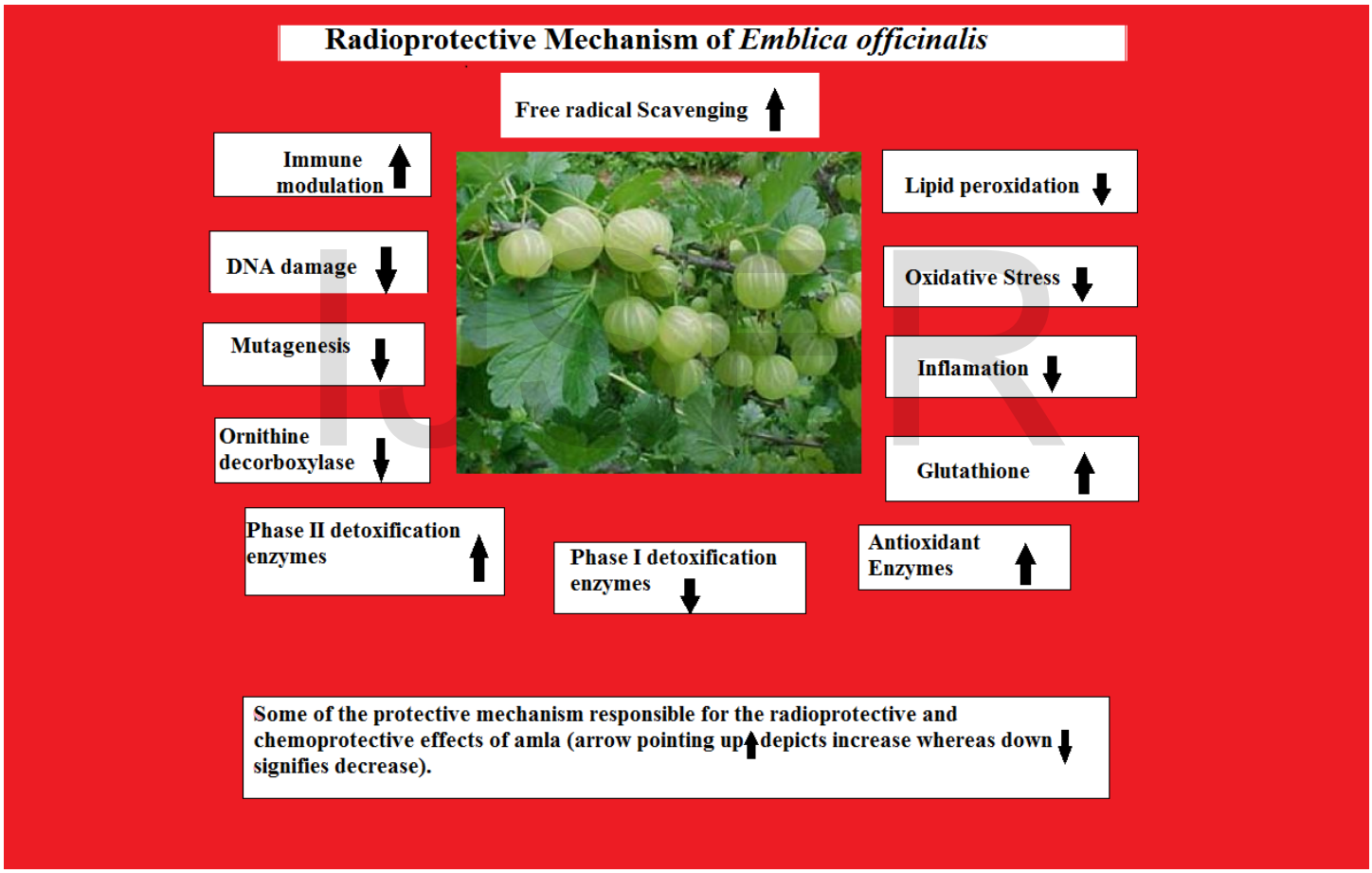

Fig. 3. Protective mechanism of *Emblica officinalis*

4 CONCLUSION

These observations show that pre treatment with EOE protects from gamma radiation and cadmium induced nucleic acid alteration in mice liver. It may be concluded that natural antioxidants and free radicals scavenging agents in *Emblica* might be effective during therapeutic implications and can be given to cancer patients during chemo as well as radiotherapy to minimize the side effect of such a hazardous exposure.

ACKNOWLEDGMENT

Authors gratefully acknowledge the Head, Department of Zoology and the Principal, Govt. Dungar College, Bikaner (India) for providing necessary facilities in the department. The irradiation facility provided by the Radiotherapy Department, Acharya Tulsi Cancer Hospital and Research Centre, PBM Hospital, Bikaner (India) is also gratefully acknowledged.

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