CHAPTER-5-

PROTECTION OF MEMBRANE LIPIDS AND CELLULAR DNA AGAINST $\gamma$-RADIATION INDUCED DAMAGES BY THE EXTRACTS OF MEDICINAL PLANTS - A.CALAMUS AND H.INDICUS AND ALPHA-ASARONE
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5.4. DISCUSSION
1. INTRODUCTION

Exposures to ionizing radiation occur from natural as well as from man-made sources - cosmic rays, radioactive minerals, nuclear weapons, nuclear reactors, aircrafts, medical diagnostics and cancer treatment. The toxic effect of radiation results mostly from oxidative damage through the generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radicals etc, and the most important sub cellular target for radiation inactivation is genomic DNA (Wallace, 1988).

The damaging effect of ionizing radiation is brought about by both direct and indirect mechanisms. The direct action produces disruption of sensitive molecule in the cells where the radiation induced ions physically break one or both of the sugar phosphate backbones or break the base pairs of the DNA. Indirect action of ionizing radiation occur when it interacts with water molecules in the cell, resulting in the production of highly reactive free radicals such as \( \text{OH}^\cdot, \text{H}^\cdot, \cdot\text{O}_2, \cdot\text{e}_{\text{aq}}^-, \cdot\text{HO}_2, \cdot\text{H}_3\text{O}^+, \) etc. (Nair et al., 2001, Scholes, 1983). Radiolysis of water in the cellular system causes an imbalance in the prooxidant/antioxidant status, which ultimately leads to a condition called “oxidative stress” with ROS overload exceeding the cellular antioxidant capacity, affecting critical biological macromolecules and triggering oxidative damage (Guelman et al., 2005; Halliwell, 1992).

DNA forms the primary cellular target of radiation damage and membrane the alternative target. Mainly two types of changes are observed in DNA at molecular levels namely altered bases and strand breaks. Both type of this damage if not repaired affect the cell structure and function. Apart from DNA damage lipid peroxidation is another critical event of ionizing radiation effect (Nair et al., 2001). Membrane lipids are highly susceptible to radiation damage mainly due to the presence of polyunsaturated fatty acids. The resulting damage causes lipid peroxidation. Lipid radicals (L·) are believed to be formed by the reaction of OH· radicals generated by ionizing radiation with polyunsaturated fatty acids (LH) which subsequently reacts with oxygen to form lipid peroxyl radical (LOO·) after undergoing molecular rearrangement of conjugation in double bonds and eventually a chain reaction is initiated on irradiation in oxygenated condition (Pandey and Mishra, 2000). Further lipid peroxidation products such as malondialdehyde (MDA)
forms adduct with cellular DNA. Many compounds, natural and synthetic, have been evaluated for the radioprotective potential but their human application and clinical utility is limited by toxicity and side effects (Weiss and Landauer, 2003). Compounds with antioxidant properties have been shown to prevent the deleterious effect of ionizing radiation in living systems and bio-molecules, and the radioprotecting ability of these compounds has been attributed to their ability to scavenge free radicals (Weiss and Landauer, 2003).

Many natural and synthetic compounds were found to protect against radiation induced damage in biological systems (Nair et al., 2001). However most of them exhibit inherent toxicity and side effects at the radioprotective concentrations. Hence the interest in search of effective and non-toxic compounds with radioprotection capability leads to investigations on naturally occurring antioxidants and phytoceuticals. Moreover pharmacological effects of several medicinal plants are related to its free-radical scavenging properties which include inhibition of lipid peroxidation, maintaining integrity and permeability of cell wall (Kavitha and Manoharan, 2006).

5.2. MATERIALS AND METHODS

5.2.1. Preparation of the extract

Aqueous-ethanol extract of *Acorus calamus* was prepared as described in section 2.2.1.

5.2.2. Animals

Male Swiss albino mice of 6 weeks old weighing 25 ± 2 g were employed for the studies.

5.2.3. Exposure to γ-radiation

Irradiation of cells was carried out using a ⁶⁰Co-Theratron Phoenix Teletherapy unit (Atomic Energy Ltd, Ottawa, Canada) at a dose rate of 1.88 Gy/minute.

5.2.4. EFFECT OF A.CALAMUS EXTRACT, ALPHA-ASARONE AND H.INDICUS EXTRACT ON γ-RADIATION INDUCED MEMBRANE DAMAGE - AN IN VITRO STUDY
Damage to membrane, in cells and tissue, by $\gamma$-radiation can be assessed in terms of peroxidation of membrane lipid according to the method of Buege and Aust (1978). 25% liver homogenate were prepared in ice cold PBS (pH 7.4). The homogenates were centrifuged at 1000 x g for 10 minutes at 4°C and the supernatant was exposed to 25 Gy radiations with and without the presence of varying concentrations of A.calamus extract, $\alpha$-asarone and H.indicus extract. After irradiation the samples were analyzed for the presence of thiobarbituric acid reacting substance (TBARS). In brief the reaction mixture contained 100 µl of liver homogenate (25%) with different concentration of the extracts or $\alpha$-asarone, 0.375% TBA, 0.003% N HCL, 0.015% trichloro acetic acid and 0.224% EDTA. The reaction mixture was heated at 90°C for 30 minutes, cooled and centrifuged at 1000 x g for 10 minutes. The amount of TBARS in the supernatant was estimated by measuring the absorption at 532 nm. The lipid peroxidation values are expressed as nano moles of MDA (using 1, 1, 3, 3-tetraethoxy propane as standard) per mg protein. Protein was estimated by Lowry’s method.

5.2.5. EFFECT OF A.CALAMUS EXTRACT, ALPHA-ASARONE AND H.INDICUS EXTRACT ON $\gamma$-RADIATION INDUCED DNA DAMAGE.

5.2.5.1. Protection against $\gamma$-radiation (25 Gy) induced DNA damage in pBR322 by A.calamus and H.indicus extracts - In vitro

The plasmid pBR322 DNA (100 ng) in phosphate buffer (0.1 M, pH 7.4) was exposed to 25 Gy $\gamma$-radiation in the presence and absence of different concentrations of the extracts of A.calamus (0 - 2.5 mg/ml) and H.indicus at (0 - 7.5 mg/ml). After irradiation the DNA was electrophoresed in 0.8% of agarose gel in 0.08 M Tris borate/0.2mM EDTA buffer (pH 8.3) and DNA damage was analyzed by Digital Gel Documentation and Analysis Software, Biotech R&D Laboratories, Yercaud.

5.2.5.2. Protection of plasmid pBR 322 DNA by $\alpha$-asarone against different doses of $\gamma$-radiation (0-25Gy) - In vitro

Here plasmid pBR 322 DNA (100 ng) in phosphate buffer (0.1 M, pH 7.4) was exposed to $\gamma$-radiation (0-25 Gy) in the presence and absence of different concentrations of $\alpha$-asarone (0-5mM). After irradiation the DNA was electrophoresed as mentioned above.
5.2.5.3. **Protection against γ-radiation induced DNA damage in human peripheral blood leucocytes by A.calamus- Ex vivo**

To monitor radiation induced damages in cellular DNA, human blood was collected from healthy nonsmoking volunteers aged between 25 and 30 years by vein puncture into heparinised tubes. The blood samples were exposed to 6 Gy γ-radiation at an ambient temperature immediately following the addition of the extract (0.05 – 0.5 mg/ml) and extent of DNA damage was measured by comet assay.

5.2.5.4. **Protection against γ-radiation induced DNA damage in peripheral blood leucocytes and bone marrow cells of mice- Ex vivo**

To study the effects of *A.calamus* extract, α-asarone and *H.indicus* extract on radiation induced cellular DNA damage under *ex vivo* condition comet assay was performed in murine peripheral blood leucocytes and bone marrow cells. Bone marrow cells were collected from femur bones by flushing out the cells into PBS (Phosphate Buffered Saline) containing 10% FBS (Fetal Bovine serum). Blood was collected from tail vein in heparinised tubes. The cell suspensions (10⁶cells/ml) and the blood were exposed to different dose of γ-radiation (2Gy, 4Gy, and 8Gy) in presence or absence of 1mg/ml of *A.calamus* extract, 5mM of α-asarone or 1mg/ml of *H.indicus* extract and the extent of DNA damage was measured by comet assay as mentioned in section 2.2.19.

5.3. **RESULTS**

5.3.1. **EFFECT OF A.CALAMUS EXTRACT, ALPHA-ASARONE AND H.INDICUS EXTRACT ON γ-RADIATION INDUCED MEMBRANE DAMAGE**

Mouse liver homogenate when exposed to γ-irradiation (25 Gy) showed a significant increase in lipid peroxidation. However when the tissue samples were exposed to γ-rays in presence of *A.calamus* extract of various concentrations (0.25 mg/ml to 1 mg/ml) there was a linear increase in the inhibition of lipid peroxide formation measured as TBARS with increasing concentration of the extract. As illustrated in fig. 5.1.(A) at 0.17 mg/ml of the extract there was 50% inhibition of lipid peroxidation.
Fig.5.1.(A). Effect of *A.calamus* extract on 25 Gy γ-radiation induced lipid peroxidation in mouse liver homogenate. The lipid peroxidation values are expressed as nano moles of MDA per mg protein. Each point represents the mean ± SD.

Similarly when the homogenate were exposed to γ-radiation in presence of α-asarone at different concentrations (0.0 mM to 4.0 mM) there was a significant reduction in the peroxidation of membrane lipid, measured as TBARS formation. From fig. 5.1.(B) it was clear that there was a linear decrease in the formation of TBARS with the increase concentration of asarone up to 4 mM indicating the membrane protecting property of α- asarone against radiation induced damage.

The *H.indicus* extract also showed significant reduction in the TBARS formation when the liver homogenate was irradiated in presence of different concentrations (0.25 mg/ml to 1 mg/ml) of this extract and the reduction was concentration dependent. From fig.5.1.(C) it was clear that 50% inhibition of lipid peroxidation was occur at a concentration of 0.325 mg/ml of *H.indicus* extract.
**Fig. 5.1. (B).** Effect of α-asarone (0.0- 4.0 mM) on γ-radiation (25 Gy) induced lipid peroxidation in mouse liver homogenate. The lipid peroxidation values are expressed as nano moles of MDA. Each point represents the mean ± SD.

Treatments-1- 0 Gy, 2- 0 Gy+4 mM, 3- 25 Gy, 4- 25 Gy+0.5 mM, 5- 25 Gy+1 mM, 6- 25 Gy+1.5 mM, 7- 25 Gy+2 mM, 8- 25 Gy+2.5 mM, 9- 25 Gy+3 mM, 10- 25 Gy+3.5 mM, 11- 25 Gy+4 mM.

**Fig. 5.1. (C).** Effect of *H.indicus* extract on 25 Gy γ-radiation induced lipid peroxidation in mouse liver homogenate. The lipid peroxidation values are expressed as nano moles of MDA per mg protein. Each point represents the mean ± SD.
5.3.2. **EFFECT OF A.CALAMUS, H.INDICUS AND ALPHA-ASARONE ON γ-RADIATION INDUCED DNA DAMAGE**

5.3.2.1. **Protection against γ-radiation induced DNA damage - in pBR322**

Exposure of plasmid pBR322 DNA to γ-radiation resulted in the production of strand breaks as a result of which the super coiled covalently closed circular (ccc) form of DNA was converted to open circular (oc) and linear forms. The disappearance of ccc form of DNA could be taken as an index of DNA damage induced by the radiation exposure. Figure 5.2. (A) presents the data on the effect of different concentrations of *A.calamus* extract on plasmid pBR322 DNA against 25 Gy γ-radiation induced strand breaks. It could be seen that the amount of ccc form decreased due to strand breaks when plasmid DNA was exposed to radiation and, as a result of which the intensity of the ccc band was found reduced. The presence of *A.calamus* extract (0.5, 1.5 and 2.5 mg/ml) along with the DNA during radiation exposure helped to prevent the decrease in the intensity of ccc form. The data on the percentage of ccc form of plasmid DNA remaining following radiation exposure under various conditions is presented in fig. 5.2(B). The % ccc form of DNA remaining after radiation exposure increased with the increasing concentrations of the extract. The results thus revealed that the plasmid DNA was protected from radiation induced damages by *A.calamus* extract in a concentration dependent manner.
Fig.5.2. (A and B): Effect of *A. calamus* extract on γ-irradiation induced strand break in plasmid pBR322 DNA.

(A) - Agarose gel electrophoresis pattern of pBR322 DNA exposed to 25 Gy. (Lane 1- 0 Gy, Lane 2- 0 Gy + 0.5 mg/ml Ac, Lane 3- 0 Gy + 1.5 mg/ml Ac, Lane 4- 0 Gy + 2.5 mg/ml Ac, Lane 5- 25 Gy, Lane 6- 25 Gy + 0.5 mg/ml Ac, Lane 7- 25 Gy + 1.5 mg/ml Ac, Lane 8- 25 Gy + 2.5 mg/ml Ac).

(B) - Graphical representation of the % CCC form of pBR322 DNA remaining after radiation exposure (25 Gy) along in presence or absence of different concentrations of *A. calamus* extract. Each point represents the mean ± SD.
Fig. 5.3(A) presents the data on the effect of different concentrations of *H.indicus* extract on plasmid pBR322 DNA against 25 Gy γ-radiation induced strand breaks. Presence of *H.indicus* extract (0.5-7.5 mg/ml) along with the DNA during radiation exposure helped to prevent the decrease in the intensity of ccc form. The data on the percentage of ccc form of plasmid DNA remaining following radiation exposure under various conditions is presented in Fig. 8.3(B). The % ccc form of DNA remaining after radiation exposure increased with the increasing concentrations of the extract. The results thus revealed the radio protecting ability of *H.indicus* extract.

![Fig.5.3. (A and B)](image)

**Fig.5.3. (A and B) -** Effect of *H.indicus* extract (0.5-7.5 mg/ml) on 25 Gy γ-irradiation induced strand break in plasmid pBR 322 DNA.

(A) - Agarose gel electrophoresis pattern of pBR322 DNA exposed to 25 Gy. Lane1-0GY, Lane 2-0Gy+7.5mg, Lane 3-25Gy, Lane 4-25Gy+0.5 mg, Lane 5-25Gy+1mg, Lane 6-25Gy+2.5mg, Lane 7-25Gy+5 mg, Lane 8- 25Gy+7.5 mg  
(B) - % of CCC form of pBR 322 DNA remaining after radiation exposure (25 Gy) along with the presence of *H.indicus* extract (0.5-7.5 mg/ml). Each point represents the mean ±SD.
5.3.2.2. Protection of plasmid pBR 322 DNA by α-asarone against different doses of γ-radiation (0-25Gy)

Figure 5.4. (A) represents the data on the effect of different concentrations of α-asarone (0-5 mM) on plasmid pBR 322 DNA against 25 Gy γ-radiation induced strand breaks and it was observed that the presence of α-asarone prevent the decrease in the intensity of ccc form in a concentration dependent manner under the experimental condition Figure 5.4. (B). Here maximum protection occurred at a concentration of 5 Mm.

Fig.5.4. (A) - Effect of different concentrations of α-asarone (0-5 mM) on 25 Gy γ-irradiation induced strand break in plasmid pBR 322 DNA (100 µg)

Fig.5.4. (B) - % of CCC form of pBR 322 DNA remaining after radiation exposure (25 Gy) along with the presence of α-asarone at different concentration. Each point represents the mean ±SD

(A &B) - Lane1- 0 Gy, 2- 0 Gy+5 mM As, 3- 25 Gy, 4- 25 Gy+0.5 mM As, 5- 25 Gy+1 mM As, 6- 25 Gy+2 mM As, 7-25 Gy+3 mM As 8- 25 Gy+4 mM As, 9- 25 Gy+5 mM As.
The plasmid DNA was then exposed to different dose of γ-radiation (0 – 25 Gy) in the presence or absence of 5 mM α-asarone. The data presented in fig. 5.5.(A) revealed that presence of 5 mM α-asarone partially prevented the disappearance of ccc form of plasmid DNA following exposure to 5 to 25 Gy γ-radiation. The reduction in the super coiled form of plasmid DNA was directly related to the radiation dose as can be seen in Lanes 1, 3, 5, 7 and 9 of fig.5.5.(A). Thus the results indicated that under *in vitro* condition α-asarone help to protect DNA from γ-radiation induced strand breaks.

![Fig. 5.5. (A and B) - Effect of α-asarone (5 mM) on different dose of γ-radiation (0-25 Gy) induced strand break in plasmid pBR 322 DNA (100 µg).](image)

(A) Lane 1- 0 Gy ; Lane 2- 0 Gy+5mM As; Lane 3- 5 Gy ; Lane 4- 5 Gy+5 mM As; Lane 5 - 10 Gy; Lane 6- 10 Gy+5mM As; Lane 7 - 15 Gy; Lane 8- 15 Gy+5 mM As; Lane 9 - 25 Gy; Lane 10- 25 Gy+5 mM As.

(B) % of CCC form of pBR 322 DNA remaining after radiation exposure (0-25 Gy) along with the presence of 5mM α-asarone. Each point represents the mean ±SD.
5.3.2.3. Protection against γ-radiation induced DNA damage - in human peripheral blood leucocytes by A.calamus

The effect of A.calamus extract against radiation induced DNA damage on human peripheral blood leukocytes were analysed to find out its effect on human system. Figure 5.6.ddepicts the results of comet assay performed on human blood leukocytes irradiated *ex vivo* in the presence and absence of different concentration of A.calamus extract (0.05-0.5 mg/ml). Gamma-radiation (6 Gy) resulted in increase in the comet parameters (such as % DNA in tail, tail length, tail moment and olive tail moment) of blood cell due to damage to cellular DNA. Presence of 0.05, 0.1, 0.25 and 0.5 mg/ml of A.calamus extract during the irradiation decreased the comet parameters from 5.38 ± 0.91 to 2.03 ± 0.46, 1.83 ± 0.7, 1.58 ± 0.5 and 1.24 ± 0.22 (Tail DNA %), from 10.62 ± 1.53 to 4.32 ± 0.61, 3.88 ± 0.1, 4.3 ± 0.5 and 3.22 ± 0.9 (Tail length), from 1.29 ± 0.31 to 0.22 ± 0.12, 0.21 ± 0.05, 0.22 ± 0.04 and 0.31 ± 0.03 (Tail moment) and from 1.45 ± 0.22 to 0.47 ± 0.2, 0.47 ± 0.07, 0.49 ± 0.04 and 0.52 ± 0.2 (olive tail moment), respectively as can be seen in fig.5.6. indicating protection of human cellular DNA from radiation induced lesions by this extract in a concentration dependent manner.
Fig. 5.6. Comet parameters of genomic DNA from human blood leukocytes exposed to 6 Gy γ-radiations, presenting the effect of different concentrations of *A. calamus* extract on the radiation induced DNA strand-breaks. Each point represents the mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test.

Treatments: 1- 0 Gy, 2- 6 Gy, 3- 6 Gy + 0.05 mg/ml, 4- 6 Gy + 0.1 mg/ml, 5- 6 Gy + 0.25 mg/ml, 6- 6 Gy + 0.5 mg/ml (a- indicate p <0.001 when compared with irradiated control).
5.3.2.4. Protection against γ-radiation induced DNA damage - in mouse peripheral blood leucocytes and bone marrow cells.

Exposure to γ-radiation (2, 4 and 8 Gy) induced damage to the cellular DNA of various tissues such as blood leucocytes and bone marrow cells was examined by alkaline single cell gel electrophoresis or comet assay. The representative photo micrographs of the comets from the bone marrow cells exposed to 4 Gy γ-radiation in presence and absence of A.calamus extract, at 40 X magnification is presented in fig.5.7. The parameters of the comets from the cells of various tissues treated with A.calamus extract, α-asarone and H.indicus extract were analysed using CASP software and the data are presented in fig.5.8 to fig.5.13. The comet parameters are found to be increased in irradiated cells. In blood leucocytes, % DNA in tail was increased from 2.96 ± 0.47 to 5.67 ± 1.12, tail length from 3.45 ± 0.52 to 6.18 ± 1.63, tail moment from 0.301 ± 0.11 to 1.02± 0.75 and olive tail moment from 0.54 ± 0.18 to 2.45 ± 1.298 in 2 Gy irradiated cells, where as in 4 Gy irradiated cells the parameters were increase to 6.49 ± 1.79, 7.69± 2.36, 1.64± 0.069 and 2.89± 0.91 respectively in case of tail DNA %, tail length, tail moment and Olive tail moment. In 8 Gy irradiated cells the values are found to be increased to 18.45±2.928, 19.94±4.78, 4.40±0.66 and 4.75±0.93 respectively in case of tail DNA %, tail length, tail moment and Olive tail moment. But when the cells were irradiated in presence of the extracts or asarone there was a significant reduction (P<0.001) in the comet parameters as seen in fig.5.8, fig 5.10 and fig.5.12.

In bone marrow cells, % DNA in tail was increased from 2.76 ± 0.78 to 5.23± 1.11, tail length from 3.15 ± 1.11 to 5.97± 1.63, tail moment from 0.37 ± 0.15 to 1.03 ± 0.41 and olive tail moment from 0.67 ± 0.35 to 3.21± 0.98 in 2 Gy irradiated cells. Similarly in 4Gy and 8Gy irradiated cells also dose dependent increase in comet parameters were observed. The tail length was increased from 3.15 ± 1.11 to 8.67±1.18 and 14.46±3.99 in 4 Gy and 8 Gy irradiated cells. But in the presence of A.calamus extract, α-asarone or H.indicus extract there was significant reduction in all the comet parameters of irradiated bone marrow cells. The parameters such as % DNA in tail, tail length, tail moment and olive tail moment were brought down significantly (fig.5.9, fig.5.11 and fig.5.13). Thus the results clearly indicated the ability of both the extracts and pure compound to offer protection to cellular DNA against γ-radiation under ex vivo condition.
Fig. 5.7. (A, B, C and D): Representative photographs of silver-stained mouse bone marrow cells following 4 Gy γ-radiation in presence or absence of *A.calamus* extract, assayed by alkaline single cell gel electrophoresis. (A) - 0 Gy; (B) - 0 Gy + *A.calamus*; (C) - 4 Gy; (D) - 4 Gy + *A.calamus*. 
Fig. 5.8. Effect of *A. calamus* extract on DNA damage in murine blood leucocytes induced by γ-radiation (2, 4, 8 Gy) exposure assayed by comet assay. Mean of percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test. (d-indicate not significant a- indicate p <0.001 when compared with respective control).
Fig. 5.9. Effect of *A. calamus* extract on DNA damage in murine bone marrow cells induced by γ-radiation (2, 4, 8 Gy) exposure assayed by comet assay. Mean of percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test. (d-indicate not significant a- indicate p <0.001 when compared with respective control).
Fig. 5.10. Effect of α-asarone on DNA damage in murine blood leucocytes induced by γ-radiation (2, 4, 8 Gy) exposure assayed by comet assay. Mean of percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test. (d-indicate not significant a- indicate p <0.001 when compared with respective control).
Fig. 5.11. Effect of α-asarone on DNA damage in murine bone marrow cells induced by γ-radiation (2, 4, 8 Gy) exposure assayed by comet assay. Mean of percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test. (d- indicate not significant a-indicate p < 0.001 when compared with respective control).
**Fig. 5.12.** Effect of *H.indicus* extract on DNA damage in murine blood leucocytes induced by γ-radiation (2, 4, 8 Gy) exposure assayed by comet assay. Mean of percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test. (d-indicate not significant a- indicate p <0.001 b-indicate p<0.01 when compared with respective control).
**Fig. 5.13.** Effect of *H.indicus* extract on DNA damage in murine bone marrow cells induced by γ-radiation (2, 4, 8 Gy) exposure assayed by comet assay. Mean of percentage DNA in tail, tail length, tail moment and olive tail moment are presented as mean ± SD. Statistical analyses of the results were performed using ANOVA with Tukey-Kramer multiple comparisons test. (d-indicate not significant a- indicate p <0.001 b-indicate p<0.01 when compared with respective control).
5.4. DISCUSSION

It is well known that most of the damages induced by radiation to living cells are due to the generation of aqueous free radicals. The body’s innate mechanism has many enzymes and non protein compounds that protect it from the free radicals and reactive oxygen species produced inside the body during normal metabolism and also due to external stimuli. These include superoxide dismutase, glutathione reductase, catalase, glutathione peroxidase, and glutathione. Thus, any compound capable of reducing the free radical activity could be useful as radioprotector. In the present experiment the membrane and DNA protecting ability of *A. calamus* extract, α-asarone and *H. indicus* extract against the lethal and sub-lethal dose of (0-25 Gy) γ-radiation under *in vitro* and *ex vivo* conditions were examined.

The basic effect of radiation on cellular membrane is believed to be the peroxidation of membrane lipids. Lipid peroxidation can be initiated by radiolytic products, including hydroxyl and hydroperoxyl radicals (Konings and Osterloo, 1980). In addition to that the free radicals generated during the radiolysis of water play the most significant role in the biological damage induced by ionizing radiation (Hall, 1978). *A. calamus* extract has been found to possess antioxidant activities against stable free radicals like DPPH, ABTS and other free radicals like superoxide and hydroxyl radicals (Sandeep and Nair, 2010). In the present study it was clearly observed that there was a reduction of radiation induced MDA formation measured as TBARS by *A. calamus* extract in a concentration dependent manner. Similarly presence of α-asarone or *H. indicus* extract they also found to be posses strong antioxidant activity, prevented the increase in TBARS synthesis showing the protective effect of α-asarone or *H. indicus* extract against radiation induced membrane damage. MDA is known to be mutagenic in bacterial and mammalian cells and carcinogenic in rats (Gandhi and Nair, 2005). Hence the prevention of TBARS formation is desirable.

The damages by ionizing radiation to DNA can cause the loss of viability of cells exposed to radiation. The present study shows that DNA is protected from the deleterious effect of γ-radiation by *A. calamus* extract α-asarone and *H. indicus* extract both *in vitro* and *ex vivo*. The major damage to DNA inflicted by free radicals
is strand breakage. The majority of free radicals may react with DNA by adding to the
double bonds of the bases, forming base radicals. A small percentage of them will
react directly with the deoxy ribose moiety by abstracting hydrogen atoms, leading to
the formation of deoxy ribose radicals. Both of these events would lead to strand
breaks (Ross, 1999). This damage when present in sub lethal quantity can produce
carcinogenic effects. So the evaluation of these lesions is an important step. In the
present study the method used for evaluating the DNA damage under \textit{in vitro}
condition is based on the disappearance of the ccc (covalently closed circular) form of
plasmid DNA pBR 322 when irradiated. In the \textit{in vitro} studies it was found that
when plasmid pBR322 was exposed to $\gamma$-radiation, the ccc form of the molecule was
converted the oc (open circular) form, with a difference in the mobility in the agarose
gel because of the induction of strand breaks in the DNA, but the presence of both
the extracts and $\alpha$-asarone help to reduce the formation of oc form which indicates
its ability to protect the plasmid DNA from the radiation induced damages.

Several phytochemicals have been shown to radioprotectors (Umadevi et al., 1999;
Nair et al., 2001; Jagetia et al., 2003; Hosseinemehr et al., 2003). Compounds that
can protect DNA against ionisation radiation have considerable potential as
radioprotectors and could be used in preventing disease like cancer and degenerative
disease arising from gene mutation. All these compounds used for the present study
does not induce DNA damage by itself, but it inhibits the induction of single strand
breaks in DNA by $\gamma$-radiation.

The damage by ionizing radiation to DNA can cause the loss of viability of cells
exposed to radiation. Alkaline comet assay is an elegant and effective technique to
monitor the extent of the DNA damage and its protection. When the human
leucocytes were exposed to $\gamma$-radiation \textit{ex vivo}, the cellular DNA undergoes damage,
as reflected in the increase in comet parameters (tail length, % DNA in tail, tail
moment and olive tail moment). Presence of \textit{A.calamus} extract during irradiation
(6Gy) of the cells decreased the comet parameters in a concentration dependent
manner indicative of its radioprotecting property. Thus the present study reveals
that the ethanolic extract of \textit{A.calamus} has the ability to protect the DNA when
exposed to $\gamma$-radiation in mammalian system.
The repairable and non-repairable DNA lesions and alkali labile sites that are converted into single strand breaks, are being monitored as DNA lesions in alkaline comet assay which could be used for the estimation of DNA strand breaks (Kassie, et al., 2000). In murine cells, following lethal and sublethal γ-irradiation (2, 4 and 8Gy), increased comet parameters were discernible indicating radiation induced DNA damage and the decrease in comet parameters in the A.calamus extract α-asarone and H.indicus extract treated cells indicated reduction of radiation induced damage in cellular DNA due to their radio protecting property. When compared together the A.calamus extract exhibits higher rate of protection than that of its pure component α-asarone which may be due to the presence of other components present in the extract. The H.indicus extract showed almost similar effect with that of α-asarone.

Thus the present work showed the protecting ability of A.calamus extract α-asarone and H.indicus extract against ionizing radiation induced damages in DNA and membrane both under in vitro and ex vivo conditions. The mechanism of radioprotection by the extracts and phytocutical could be ascribed to their antioxidant and free radiacal scavenging activities. The present study suggests the possibility of using the extract of the medicinal plant A.calamus and H.indicus and also the pure component α-asarone to prevent deleterious effect of ionizing radiation in situations of radiation exposure.