Radioprotection of Swiss Albino Mice by *Myristica fragrans* houtt

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Nutmeg, the dried seed kernel of *Myristica fragrans*, MF (Family: Myristicaceae) possesses antifungal, hepatoprotective and antioxidant properties. Its radioprotective effect against 6, 8 and 10 Gy gamma radiation was evaluated by 30 day survival assay. Regression analysis yielded LD$_{50/30}$ as 6.83 Gy and 8.89 Gy for irradiated only and (MF + radiation) groups, respectively. The dose reduction factor was computed as 1.3. Administration of MF significantly enhanced hepatic glutathione (GSH) and decreased testicular lipid peroxidation (LPO) level whereas acid phosphatase (ACP) and alkaline phosphatase (ALP) activity did not show any significant alteration. Irradiation resulted in significant elevation in LPO level and ACP activity, and decreased the GSH content and ALP activity. MF pretreatment effectively protected against radiation induced biochemical alteration as reflected by a decrease in LPO level and ACP activity, and an increase in GSH and ALP activity. The present study has implications for the potential use of MF as a radioprotector.

INTRODUCTION

The development of effective radioprotectors and radiorecovery drugs is of great importance in view of their potential application during both planned (e.g. radiotherapy) and unplanned (e.g. in nuclear industry, natural background radiation emanating from the earth or other sources) radiation exposure. Over the past 50 years, research in the development of radioprotectors has focused on screening a plethora of chemical and biological compounds.

Several synthetic compounds such as lipoic acid, deoxy-spergualin$^3$ have been tested for protection against radiation. But they have limited use due to inherent toxicity. The natural products offer an alternative to their synthetic counterparts due to low toxicity with no side effects.$^4,5$ The plant extracts such as *Panax ginseng,*$^4$ *Amaranthus paniculatus* $^5$ were reported to have radioprotective ability in animal model systems. Spices and herbs are well known potent sources of natural antioxidants.$^6$ They have been widely used for medicinal and antiseptic properties. They are also reported as powerful inhibitors of lipid peroxidation (LPO).$^7$

Nutmeg, the dried seed kernel of the aromatic tree, *Myristica fragrans*, MF (Family: Myristicaceae) has long been used as a spice and folk remedy. It shows antifungal, anti-dysenteric, anti-inflammatory, analgesic, hepatoprotective and memory enhancing property.$^8-12$ Its component such as Myristicin,$^{13}$ Lignan$^{14}$ and Eugenol$^{15}$ has been reported to maintain level of antioxidants in the cell.

Testis is one of the highly radiosensitive organ due to cell renewal system.$^{16}$ Radiation injury to living cells is, to large extent, due to oxidative stress induced by reactive oxygen species. The formation of lipid peroxides is a major biomarker of oxidative damage.$^{17}$ Enzymes such as acid (ACP) and alkaline phosphatase (ALP) are widely distributed in testis and are important in physiology of sperm.$^{18}$ Changes in activity of these enzymes may be indicative of spermatogenic suppression and extenstive lytic activity. Glutathione (GSH) and its dependent enzymes are one of the protective mechanisms vs oxidative damage, both in circulation and in various tissues. Liver is the most active tissue in production and use of GSH.$^{19}$ Hence, in the present study, radiation effects were assessed through biochemical estimation of LPO, ACP, ALP in the testis and GSH in the liver. Also, dose reduction factor (DRF) was calculated as it clearly gives the drug quantitative capacity to enhance the tolerance of tissue to radiation.

Therefore, the present study was undertaken to investigate the radioprotective efficacy of *Myristica fragrans* by biochemical studies and animal survivability.

MATERIAL AND METHODS

Animals

Healthy young Male Swiss albino mice (*Mus musculus,* 6–
8 weeks old) with an average body weight (22 ± 2 gms) were procured from IVRI Izatnagar, India. They were maintained in animal house under control condition of temperature (25 ± 2°C) and light (14-hrs. light and 10-hrs. darkness). These animals were given pelleted standard mice feed (obtained from Hindustan Lever Ltd., Delhi) and water ad libitum.

**Irradiation source**

Cobalt teletherapy unit (ATC-C9; Canada) at Cancer Treatment Centre, Radiotherapy Department, S.M.S. Medical College and Hospital, Jaipur was used for irradiation. Unanesthetized animals were kept in a well ventilated wooden box at a distance of 77.5 cms from the source to deliver radiation at the dose rate of 0.93 Gy/min.

**Plant material and Extraction procedure**

The nutmeg seeds were purchased from commercial source. They were soaked in 50% alcohol. Then, the seeds were crushed and semi-solid paste thus obtained was sieved through a sterilized cloth. The alcoholic extract obtained was dried at 37°C to make it into powder form. It was redissolved in saline (0.9%NaCl) at a concentration of 10mg/kg body weight and administered to each mouse by oral gavage.

**Experimental Design**

**Experiment 1: Radioprotective effect of MF** - The protective capacity of any agent (chemical or plant extract) is expressed as dose reduction factor (DRF). For this, animals were divided into 2 groups of 12 animals each.
1) Irradiated group - The animals were administered 0.9% NaCl (volume equal to MF) before exposure to gamma irradiation at different doses (6, 8 and 10 Gy).
2) Experimental group (MF + radiation) - Animals were administered MF seed extract (10 mg/kg body weight/day) once orally for three consecutive days and then on the 3rd day, they were exposed to gamma irradiation at the dose of 6, 8 and 10 Gy at the time of 30 minutes after the MF administration. The animals of both the groups were monitored daily for mortality. The percentage of mice surviving at each radiation dose till 30 days following exposure was used to obtain the dose response curves of survival. Regression analysis was performed to obtain LD50/30 values and to determine DRF.

DRF was calculated using the following formula.

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DRF = \frac{LD_{50/30}(\text{Experimental animals})}{LD_{50/30}(\text{Control animals})}
\]

**Fig. 1.** 30 Day % survival of Swiss albino mice pretreated with or without MF seed extract at 6, 8 and 10 Gy of gamma-ray irradiation.

**Experiment 2: Biochemical study:** To examine the mechanism of radioprotection, animals were divided into 4 groups.

Group-I (Control): Animals were administered 0.9% NaCl orally.

Group-II (MF treated group): The animals were administered 10 mg/kg body weight MF seed extract once orally in 0.9% saline for three days.

Group-III (Irradiated group): The animals were given 0.9% NaCl (volume equal to MF) before gamma irradiation at the dose of 8 Gy.

Group-IV (Combination group): Animals were administered MF seed extract (10 mg/kg body weight) once orally for three consecutive days, and then on the 3rd day, they were irradiated at the dose of 8 Gy with gamma rays.

6 animals from each group were autopsied on the 1st, 3rd, 7th, 15th and 30th day after each treatment. Testes were dissected out; 10% (w/v) homogenate was prepared in 1.15% KCl to estimate LPO level and in distilled water for ACP and ALP activity. Liver was perfused immediately with 0.9% sodium chloride and its 10% (w/v) homogenate was prepared in tris KCl buffer to estimate GSH content.

LPO was measured by the method of Okhawa et al. in

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The concentration of TBARS was expressed as n moles of malondialdehyde (MDA) per mg of tissue. The 1, 1, 3, 3-tetramethoxypropane was used as standard. The absorbance was read at 532 nm using Ultraviolet-visible Systronic spectrophotometer.

ACP and ALP activity were estimated by the method of Fiske and Subbarow\(^1\) for the determination of phosphate liberation. ACP and ALP activity is the difference between inorganic phosphate content of the incubated and control samples expressed as Bodansky unit. One Bodansky unit corresponds to the liberation of 1 mg of inorganic phosphorus from the tissue in 1 mg gm\(^{-1}\) hr\(^{-1}\). The absorbance was read at 410 nm.

GSH was determined by the method of Moron et al.\(^2\). The reduced GSH reacts with dithionitrobenzoic acid to form a yellow coloured complex that absorbs at 412 nm.

**Statistical analysis**

The results obtained were expressed as mean ± SE. Student’s t test was used to make statistical comparison between the groups. A statistical comparison was completed with the control vs MF treated group, control vs irradiated group and irradiated group vs combination group (MF+radiation). The significance level was set at \(p < 0.05\), \(p < 0.01\) and \(p < 0.001\).

**RESULTS**

**Experiment 1**

In the present study, a dose dependent survivality was observed in both the groups. However, the survival percentage was higher at all doses in the experimental group. In irradiated group, 33.34 & 75 percent mortality was observed at 6 and 8 Gy respectively while no animal could survive till day 30 at 10 Gy gamma-ray irradiation (Fig. 1). Pretreat-

**Fig. 2.** 30 Day % survival of Swiss albino mice after exposure to 6, 8 and 10 Gy gamma-ray irradiation with and without pretreatment of MF seed extract (after regression).

**Fig. 3.** Variation in LPO level (n mol of MDA / mg of tissue) in the testis of male Swiss albino mice in different experimental groups. These data represent the mean ± SD of three independent experiments.
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ment with MF before irradiation enhanced the survival percentage of mice at all doses of radiation. Mortality was completely inhibited at 6 Gy whereas it was reduced to 25 and 75 percent at 8 and 10 Gy respectively. Regression analysis yielded LD_{50/30} values as 6.83 and 8.89 Gy for irradiation alone and experimental group, respectively, and produced DRF of 1.3 (Fig. 2).

**Experiment 2**

The level of lipid peroxides (TBARS) reduced significantly in MF treated group as compared to control. A marked elevation was observed in irradiated group (Group III) which reduced significantly by MF pretreatment in combination group (Group IV) (Fig. 3). Both ACP and ALP activity did not show any significant alteration in group treated with MF alone as compared to control. A marked increase in ACP activity (Fig. 4) was noticed in irradiated group (Group III) which was significantly reduced by MF pretreatment in combination group. On the contrary, ALP activity (Fig. 5) showed a significant decline in irradiated animals, which was sup-

![Fig. 4. Variation in the ACP activity (mg Pi/gm/hr) in the testis of Swiss albino mice in different experimental groups. These data represent the mean ± SD of three independent experiments.](image)

![Fig. 5. Variation in ALP activity (mgPi/gm/hr) in the testis of Male Swiss albino mice in different experimental groups. These data represent the mean ± SD of three independent experiments.](image)
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pressed by MF pretreatment before irradiation. In comparison to control, GSH content (Fig. 6) significantly increased in the animals administered with MF alone (Group II). Whereas, irradiated animals (Group III) showed a highly significant reduction as compared to control (Group I). Pretreatment with MF in the combination group resulted in significantly elevated levels of GSH as compared to irradiated group.

**DISCUSSION**

In the present study, significant radioprotection was achieved when MF was given orally (10mg/kg bodyweight/day) for three consecutive days before irradiation. Its radioprotective effect was demonstrated by determining LD<sub>50/30</sub> (DRF = 1.3) and evaluation of parameters biochemically in testis and liver.

A dose dependent mortality was observed on exposure to 6, 8 and 10 Gy gamma-ray irradiation in 30 day survival assay. Death observed at this dose range of radiation is mainly attributed to gastrointestinal (GI) and haematopoietic syndrome. Both epithelial cell lining of the alimentary tract and the circulating leukocytes are relatively short lived and their orderly renewal depends on a population of constantly dividing stem cells. Irradiation inhibits the proliferation of stem cells and, as a consequence, replacements are not available when normal attrition results in the progressive loss of senescent cells. Therefore, any damage to these cells impairs normal physiological process, with a drastically adverse impact on survival. Also, 16.66%, 25% and 50% animals died within seven days at 6, 8 and 10 Gy respectively, in irradiated group. The death during this period could be attributed to GI syndrome. Though GI epithelium is less sensitive than the bone marrow progenitor cells, but as the cell transit time is more rapid, it is expressed earlier than the haematopoietic syndrome. The later death after day 7 may be due to hematopoietic syndrome as restoration of GI epithelium is completed by this time. This is in agreement with the findings of Samarth and Kumar. Pretreatment with MF in combination group showed no mortality at 6 and 8 Gy within seven days after irradiation. This indicates that MF showed protection against radiation-induced gastrointestinal damage. Mortality was completely inhibited at 6 Gy whereas only 25 and 75 percent mortality occurred in combination group as compared to 75 and 100 percent at 8 and 10 Gy, respectively, in irradiated group. Thus MF pretreatment also provided protection against hematopoietic death; thereby an enhanced survival was observed. The mortality after irradiation could also be due to immunosuppression that increases the chances of infection. Nutmeg has also been reported to exhibit antimicrobial activity. Thus MF extract might have protected irradiated animals against secondary infection and stimulated their fast recovery. MF has also been reported to have curative effect on digestive organs. It was frequently used to cure stomachache, intestinal weakness, vomiting and to regulate the movement of bowels in Indian & Arabian system of medicine. These properties of MF might be responsible for reducing the severity of GI damage, which in turn resulted in less mortality in the experimental group.

Radiation inflicts its adverse effect through the generation of reactive oxygen species (ROS). The presence of polyunsaturated fatty acid (PUFA) in cell membranes makes it highly susceptible to oxidative attack, leading to a chain reaction, called as lipid peroxidation. Significant amount of PUFA has been reported in testis. This has been related...
to their possible effect on fluidity of sperm membrane and its motility. This explains our present results as significantly increased LPO level was observed in irradiated testis (Group III) as compared to control (Group I).

In the present investigation, the activity of ACP was found to increase significantly in radiation treated group as compared to control. Szeinfeld and Villiers also observed a marked augmentation in ACP activity in irradiated testis. ACP has been detected in the acrosome of spermatozoon. Its presence has also been reported in the lysosome of Sertoli cells, spermatocytes and spermatids. Irradiation causes lipid peroxidation of lysosomal membrane. Thus, increased activity of ACP may be attributed due to breakdown of lysosomal membrane and liberation of the enzyme. These studies are in agreement with the findings of Samarth et al. A significant decline in ALP activity was also noticed in irradiated group as compared to control. ALP is known to be associated with germinal cells. It has been detected in the seminiferous tubule, basement membrane and interstitial cells. It plays vital role in transport of material from Sertoli cells to various germinal cells, differentiation and proliferation of the germinal epithelium and in the testicular metabolism. Radiation depletes germ cell population. So, decrease in ALP activity is correlated with the state of germ cell population. ALP also plays an important role in maintaining membrane permeability. Radiation damages the cell membrane, which also might be responsible for decline in ALP activity.

To counteract the harmful effect of ROS, living organism is equipped with a protective network referred to as antioxidant defense, which includes antioxidative enzymes, non-enzymatic antioxidant molecules, and enzymes reversing the cellular damage induced by oxidants. GSH is one of major components of cellular antioxidant system. It is the principal non-protein thiol functioning as an antioxidant and as a cofactor for enzymes involved in detoxification of xenobiotics. A significant decline in GSH content was noticed in irradiated group compared to control. This could be due to its enhanced utilization as an attempt to detoxify the free radicals generated by radiation.

Pretreatment with MF (Combination group) was found to effectively protect against radiation induced biochemical alteration as reflected by a decrease in LPO level and ACP activity, and an increase in GSH and ALP activity. This could be attributed to its antioxidant property, due to which MF might have scavenged free radicals generated by radiation. The components of MF such as Myristicin, Lignan and Eugenol has been reported to maintain the level of antioxidants in the cell. Eugenol, a major constituent, is reported to maintain the activities of enzymes such as superoxide dismutase, catalase, glutamine transerase, glucose-6-phosphate dehydrogenase and glutathione peroxidase. GSH acts as a substrate for glutathione peroxidase mediated reduction reaction of $\text{H}_2\text{O}_2$ into non-toxic products. Thus, the eugenol component of MF might have prevented the radiation induced GSH depletion from liver, as observed in our present investigation.

In our present study, LPO level significantly declined in MF pretreated animals (Combination group) as compared to irradiated animals. Eugenol has been reported to inhibit LPO in irradiated animals. This has been reported to occur in two steps: 1) it interferes with the chain reactions by trapping the active oxygen, such as superoxide anion and hydroxyl radicals. 2) it is metabolized to a dimer (dieugenol) that inhibits LPO at the level of propagation of free radical chain reaction. This also explains the decline in ACP activity in MF pretreated animals as lysosomal membrane must have been protected against radiation induced LPO.

Thus, the present study suggests that deleterious effects of radiation may be reduced by MF seed extract, which in turn reflected in increased survival, significant decline in LPO level and ACP activity and significant enhancement in GSH content and ALP activity in combination group as compared to irradiated group in Swiss albino mice. This radioprotective property of MF may be attributed to its components such as myristicin and eugenol, which shows strong free radical scavenging activity. This study also suggests potential use of MF against other ROS mediated disorders such as Alzheimer’s disease, rheumatoid arthritis and cancer. Further studies are required to unravel the underlying mechanism of MF against ROS mediated damage for improving its efficiency better.

ACKNOWLEDGEMENT

Authors are greatly thankful to In charge of Department of Radiotherapy, S.M.S. Medical College Hospitals, and Jaipur for radiation facility and dosimetry.

REFERENCES


