CHAPTER 5
RADIOPROTECTION BY 
\( \alpha \)-LIPOIC ACID PALLADIUM COMPLEX 
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5.5. DISCUSSION
5.1. ABSTRACT

This chapter outlines the studies on radioprotective properties of the dietary supplement POLY-MVA. Initially, the effect of the formulation on radiation induced membrane lipid peroxidation was evaluated under in vitro conditions. Subsequently, POLY-MVA was administered to animals prior to sub lethal dose of whole body radiation exposure and their protective effect on endogenous antioxidant, hematopoietic and gastrointestinal systems from radiation induced damages were evaluated. Its ability to prevent radiation induced body weight alterations and mortality was also determined. Plasmid relaxation assay and comet assay were employed to explore the effect of this supplement in preventing radiation induced genotoxicity.

5.2. INTRODUCTION

POLY-MVA is a dietary supplement containing the active ingredient, palladium- $\alpha$-lipoic acid (LAPd) complex. LAPd polymer exists as a trimer of lipoic acid–palladium complex joined to thiamine (Garnett, 1995). Besides the LAPd core unit, POLY-MVA also contains free LA (Corduneanu et al., 2007), minerals (molybdenum, rhodium, ruthenium), vitamins (B1, B2, B12) and amino acids (N-acetyl cysteine and formyl methionine). LAPd in POLY-MVA was shown to regulate ischemic cell death and is a potent neuroprotective agent (Antonawich et al., 2004). Palladium $\alpha$-lipoic acid complex’s unique electronic and redox properties appear to be the key to its physiological effectiveness (Garnett, 1997; 1998; Krishnan and Garnett, 2006). The toxicological studies indicated that the $LD_{50}$ of POLY-MVA exceeded 5000 mg/kg, and no mutagenic effect of the combination was observed in the Ames test (Riklis et al., 1990).

In the present chapter, we have tried to explore the radioprotective properties of POLY-MVA by assessing their influence on antioxidant, hematopoietic and gastrointestinal systems. Its effectiveness in protecting mammalian systems from radiation induced alterations in body weight and survival rates was also monitored. Role of POLY-MVA in protecting the DNA from radiation induced genotoxicity was also investigated.
5.3. MATERIALS AND METHODS

5.3.1. Determination of effect of POLY-MVA on radiation induced membrane lipid peroxidation in vitro

Exposure to gamma radiation causes peroxidation of the membrane lipids which results in formation of malondialdehyde (MDA). 10% Mouse liver homogenate was prepared in ice cold phosphate buffered saline (PBS), pH 7.4 and centrifuged at 6,000 x g for 10 minutes and the supernatant (1 ml) was exposed to 10 Gy gamma radiation in presence or absence of different concentrations of POLY-MVA (0–2.5% v/v). The extent of damage to membranes was assessed in terms of measurement of MDA according to the method of Buege and Aust (1978). Protein content was estimated by the method of Lowry et al., (1951). Detailed procedures are given in section 2.2.7.6.

5.3.2. Determination of effect of POLY-MVA on endogenous antioxidant status, bone marrow cellularity and gastrointestinal system toxicity in whole body gamma irradiated mice

Mice were divided into groups of five each and administered with POLY-MVA as detailed below. Dosage was such that the animals received POLY-MVA (1 ml/kg body weight) for seven days prior to radiation exposure. On the 7th day, one hour after drug administration, animals were exposed to sub lethal dose of 4 Gy whole body gamma radiation.

Group 1- Distilled water + Sham irradiation
Group 2- POLY-MVA (1 ml/kg body weight) + Sham irradiation
Group 3- Distilled water + 4 Gy $^{60}$Co γ rays
Group 4- POLY-MVA (1 ml/kg body weight) + 4 Gy $^{60}$Co γ rays

After 24 hours of radiation exposure, 3 animals from each group were sacrificed by cervical dislocation and liver and brain were excised. Blood was collected by heart puncture into heparinised tubes and analyzed for hemoglobin content. Femurs of the animals were dissected out and bone marrow cellularity determined (Sredni et al., 1992).
10% (w/v) homogenates of liver tissues were prepared in ice cold PBS. Glutathione peroxidase (GPx) (Hafeman et al., 1974), GSH (Moron et al., 1979), SOD (McCord and Fridovich, 1969) levels were determined. The concentrations of malondialdehyde (MDA) as indices of lipid peroxidation were assessed (Buege and Aust, 1978). Tissue protein was estimated (Lowry et al., 1951). Detailed procedures are given in section 2.2.7.

At 72\textsuperscript{nd} hour post radiation exposure, remaining 2 animals from each group were sacrificed. A portion of the small intestine was removed, washed in PBS, and fixed in 10 % formaldehyde solution and embedded in wax. Sections were taken and stained with hematoxylin-eosin. The slides were observed in light microscope and photographed.

5.3.3.Determination of effect of POLY-MVA on endogenous spleen colony formation in whole body gamma irradiated mice

Mice were divided into groups of three each and administered with POLY-MVA as detailed below. Dosage was such that the animals received POLY-MVA (1 ml/kg body weight) for seven days prior to radiation exposure. On the 7\textsuperscript{th} day, one hour after drug administration, animals were exposed to sub lethal dose of 6 Gy whole body gamma radiation.

Group 1- Distilled water + 6 Gy \textsuperscript{\textit{60}}Co \gamma rays
Group 2- POLY-MVA (1 ml/kg body weight) + 6 Gy \textsuperscript{\textit{60}}Co \gamma rays

POLY-MVA administration was continued for 5 more days post radiation exposure. The animals were sacrificed on the 12\textsuperscript{th} day post irradiation by cervical dislocation and the spleens were excised out, weighed and fixed in Bouin’s solution and analyzed for colony formations (Till and Culloch, 1961; 1963) as described in section 2.2.6.3.
5.3.4. Determination of effect of POLY-MVA on radiation induced body weight loss and mortality in whole body gamma irradiated mice

Mice were divided into groups of ten each and administered with POLY-MVA and exposed to whole body gamma radiation as detailed below. Dosage was such that the animals received POLY-MVA (1 ml or 8 ml/kg body weight) for seven days prior to radiation exposure. On the 7th day, one hour after drug administration, animals were exposed to a lethal dose of 8 Gy whole body gamma radiation.

Group 1- Distilled water + Sham irradiation  
Group 2- POLY-MVA (1 ml/kg body weight) + Sham irradiation  
Group 3- POLY-MVA (8 ml/kg body weight) + Sham irradiation  
Group 4- Distilled water + 8 Gy $^{60}$Co $\gamma$ rays  
Group 5- POLY-MVA (1 ml/kg body weight) + 8 Gy $^{60}$Co $\gamma$ rays  
Group 6- POLY-MVA (8 ml/kg body weight) + 8 Gy $^{60}$Co $\gamma$ rays

The administration of POLY-MVA was continued for 5 more days post radiation exposure. The animals were checked on a daily basis to record mortalities, if any, and the body weights of the survivors were recorded every alternate day.

5.3.5. Determination of effect of POLY-MVA on radiation induced genotoxicity

5.3.5.1. Determination of effect of POLY-MVA on gamma radiation induced DNA strand breaks in plasmid pBR322 by Plasmid relaxation assay

Plasmid pBR322 DNA (100 ng), in phosphate buffer (0.1 M, pH 7.4) was exposed to gamma irradiation of 0- 15 Gy in the presence or absence of POLY-MVA (5% v/v). After irradiation, DNA was electrophoresed in 1% agarose gel by using Tris borate/ EDTA buffer pH 8.3 (Sambrook et al., 1989), at 55 V for 2 hours and DNA damage analyzed by a digital gel documentation and analysis system (Biotech R&D Laboratories, Yercaud, India) as described in section 2.2.10.1.
5.3.5.2. Determination of effect of POLY-MVA on radiation induced cellular DNA damage in mice exposed to whole body gamma radiation

Mice were divided into groups of three each and administered with POLY-MVA and exposed to whole body gamma radiation as detailed below. Dosage was such that the animals received POLY-MVA (1 ml/kg body weight) for seven days prior to radiation exposure. On the 7th day, one hour after drug administration, animals were exposed to a whole body gamma radiation (8 Gy).

Group 1- Distilled water + Sham irradiation
Group 2- POLY-MVA (1 ml/kg body weight) + Sham irradiation
Group 3- Distilled water + 8 Gy $^{60}$Co γ rays
Group 4- POLY-MVA (1 ml/kg body weight) + 8 Gy $^{60}$Co γ rays

Immediately after radiation exposure, the animals were sacrificed. The femurs of animals were dissected out and bone marrow cells flushed into ice cold phosphate buffer (pH 7.4) containing 10 % fetal bovine serum. Spleens were dissected out and made into single cells in ice cold phosphate buffer (pH 7.4). Radiation- induced DNA damage in bone marrow cells and splenocytes were assessed by Comet assay (Section 2.2.10.2.).

5.4. RESULTS

5.4.1. Protective effect of POLY-MVA on radiation induced membrane lipid peroxidation in vitro

Exposure of mouse liver homogenate to gamma radiation resulted in peroxidative damage to membranous lipids and subsequent TBARS formation. Figure 5.1. depicts the effect of varying concentrations of POLY-MVA on radiation induced membrane lipid peroxidation. The presence of POLY-MVA significantly prevented the extent of lipid peroxidation in liver tissues irradiated in vitro, at both the concentrations studied in a concentration dependent manner.
Figure 5.1. Protective effect of POLY-MVA on gamma radiation induced lipid peroxidation in mouse liver homogenate. Values are expressed as mean ±SD. (‘a’- p < 0.001 when compared to sham irradiated control group; ‘b’- p < 0.001 when compared to irradiated control group; ‘h’- p < 0.05 when compared to POLY-MVA 1.25% treated group).

5.4.2. Protective effect of POLY-MVA on endogenous antioxidant status, bone marrow cellularity and gastrointestinal system toxicity in whole body gamma irradiated mice

Administration of POLY-MVA had a significant effect on rescue from radiation induced deterioration in levels of endogenous antioxidants and various hematological parameters. The changes in antioxidant levels in liver and brain tissues of different treatment groups are presented in Figures 5.2 and 5.3, respectively. POLY-MVA administration aided to maintain the levels of SOD, CAT and GPx and of reduced glutathione (GSH) to near normal levels in POLY-MVA treated radiation exposed animals. The protective effect of POLY-MVA on the hematopoietic system against deleterious effects of ionizing radiation is evident from the data on bone marrow cellularity presented in Figure 5.4.(A). Sham irradiated control animals had bone marrow cellularity of 15.19x10^6 cells/ml whereas in the irradiated group, this dropped drastically to 6.25x10^6 cells/ml. POLY-MVA administration protected the animals from this radiation induced depletion in bone marrow cellularity. Sham irradiated control animals exhibited
hemoglobin content of 22.30 gm/dl and radiation exposure lowered these levels to 16.98 gm/dl. Administration of POLY-MVA prior to radiation exposure helped to maintain the hemoglobin levels at 20.37 gm/dl (Figure 5.4. B).

Figure 5.2. Effect of POLY-MVA administration on (A) GPx, CAT and SOD (B) GSH and MDA levels in liver tissues of whole body radiation exposed mice. Values are expressed as mean ±SD. (‘a’- p<0.001 when compared to respective sham irradiated control groups; ‘b’- p<0.001, ‘c’- p<0.01, and ‘d’- p<0.05 when compared to respective irradiated control groups).
Figure 5.3. Effect of POLY-MVA administration on (A) GPx, CAT and SOD (B) GSH and MDA levels in brain tissues of whole body radiation exposed mice. Values are expressed as mean ±SD. (‘a’- p<0.001 when compared to respective sham irradiated control groups; ‘b’- p<0.001, ‘c’- p<0.01, and ‘d’- p<0.05 when compared to respective irradiated control groups).
Figure 5.4. Effect of POLY-MVA administration on (A) Bone marrow cellularity and (B) Blood hemoglobin content in mice exposed to whole body gamma radiation. Values are expressed as mean ±SD. (‘a’- p<0.001 when compared to respective sham irradiated control groups; ‘c’- p<0.01 when compared to respective irradiated control groups).

Figure 5.5. Effect of POLY-MVA administration on gastrointestinal injury in mice upon whole body gamma radiation exposure.
Microscopic examination of stained sections of the intestine of radiation-exposed animals revealed alterations in structures of mucosa and sub-mucosa layers. Irradiated mice exhibited gastrointestinal damage as crypt epithelial cell necrosis, blunting of villi as well as diffused lymphatic and plasmacellular infiltration. The administration of mice with POLY-MVA (1 ml/kg body weight) prior to irradiation protected the intestinal epithelial cells from radiation induced structural alterations (Figure 5.5).

5.4.3. Protective effect of POLY-MVA on endogenous spleen colony formation in whole body gamma irradiated mice

Formation of endogenous spleen colonies is an index of hematopoietic stem cell proliferation. From the results presented in Figure 5.6, it can be inferred that POLY-MVA (1 ml/kg body weight) administration enhanced spleen colony formation in whole body irradiated mice. A significant loss in spleen weight was also observed in the animals of radiation exposed group. On the contrary, the spleen weights were higher in animals of POLY-MVA administered radiation exposed groups.

![Figure 5.6. Effect of POLY-MVA administration on spleen colony formation and recovery of spleen weight in mice exposed to whole body gamma radiation. Values are expressed as mean ±SD. (‘b’- p<0.001 and ‘c’- p<0.01 when compared to respective irradiated control groups).](image)
5.4.4. Protective effect of POLY-MVA on radiation induced body weight loss and mortality in whole body gamma irradiated mice

Data from survival study of the animals exposed to the lethal 8 Gy gamma radiation is presented in Figure 5.7.a. Mortality of the irradiated animals commenced on the 16th day in the irradiated control group and on 17th day in POLY-MVA (8 ml/kg body weight) treated group. There was complete mortality of the animals by 24th day in the irradiated control group. POLY-MVA, when administered at a lower dose (1 ml/kg body weight) was not much effective in preventing the radiation induced mortality and loss of body weight. However a higher dose of 8 ml/kg body weight was found advantageous. In the group of animals administered with POLY-MVA (8 ml/kg body weight), the percentage of survival was higher than the irradiated control group and showed 80% survival even on 22nd day of study followed by survival rates of 70% on 23rd day, 60% on 24th day, 40% on 27th day and 20% from 28th day. All the sham irradiated groups exhibited 100% survival throughout the course of study.
From the data presented in Figure 5.7.b, it can be inferred that upon exposure to a lethal dose of 8 Gy gamma radiation, there was a profound loss in the body weight of the survivors. In the control irradiated group, all animals died by the 24th day. In the group administered with POLY-MVA (8 ml/kg body weight), the loss of body weight continued till 23rd day and thereafter, there was recovery and increase in the bodyweight. Thus the results reveal that POLY-MVA (8 ml/kg body weight) administration significantly improved the body weight of the surviving animals. The administration of POLY-MVA at the lower dose (1 ml/kg body weight) did not have any effect on enhancing the recovery from body weight loss though it helped to improve the survival rates of the radiation exposed animals to a minor extent. All the un-irradiated control groups showed normal body weight range throughout the course of study.

Figure 5.7.b. Effect of POLY-MVA administration on whole body gamma radiation induced alterations in body weight of surviving animals. POLY-MVA 1 and POLY-MVA 2 represents POLY-MVA administered at 1 ml/kg body weight and 8 ml/kg body weight respectively. Statistics of irradiated groups is provided as an inset. ***p<0.001 and ‘ns’- p>0.05 when compared with the irradiated control group.
5.4.5. Protective effect of POLY-MVA on radiation induced genotoxicity

5.4.5.1. Protective effect of POLY-MVA on gamma radiation induced DNA strand breaks in plasmid pBR322

The effect of POLY-MVA on DNA strand breaks formation in plasmid pBR322 by different doses of gamma radiation (0-15 Gy) is depicted in Figure 5.8. Presence of POLY-MVA (5% v/v) inhibited the radiation induced disappearance of ccc form. The dose modifying factor (DMF), defined as the ratio of the radiation doses to bring down the percentage of ccc DNA to the same extent in presence and absence of the radioprotector was found to be 1.99, at doses causing 10% DNA damage.

Figure 5.8. Protection of plasmid pBR322 by POLY-MVA against radiation induced DNA strand breaks.

(A) Agarose gel electrophoresis pattern of pBR322 DNA exposed to radiation doses of 0 to 15 Gy. Lane 1- 0Gy, Lane 2- 5Gy, Lane 3- 10Gy, Lane 4- 15Gy, Lane 5- POLY-MVA + 0Gy, Lane 6- POLY-MVA + 5Gy, Lane 7- POLY-MVA + 10Gy, Lane 8- POLY-MVA + 15Gy.

(B) Graph representing the effect of POLY-MVA on % of ccc form of pBR322 DNA remaining after radiation exposure (0-15 Gy). Values are expressed as mean ±SD.
5.4.5.2. Protective effect of POLY-MVA on radiation induced cellular DNA damage in different tissues of mice exposed to whole body gamma radiation

Whole body exposure of mice to 8 Gy gamma radiation caused cellular DNA damage in splenocytes and bone marrow cells as evident from the increase in the comet parameters. The administration of POLY-MVA (1 ml/kg body weight) prevented this increase (Figure 5.9.a and 5.9.b). The results clearly indicate the ability of POLY-MVA to offer protection to cellular DNA against gamma radiation \textit{in vivo}.

Figure 5.9.a. Comet parameters indicating the effect of POLY-MVA administration on gamma radiation induced DNA strand breaks in splenocytes of whole body radiation exposed mice. Values are expressed as mean ±SD. (‘a’- p<0.001 when compared to respective sham irradiated control groups; ‘b’- p<0.001 when compared to respective irradiated control groups).
Figure 5.9.b. Comet parameters indicating the effect of POLY-MVA administration on gamma radiation induced DNA strand breaks in bone marrow cells of whole body radiation exposed mice. Values are expressed as mean ±SD. (‘a’- p<0.001 when compared to respective sham irradiated control groups; ‘b’- p<0.001 when compared to respective irradiated control groups).

5.5.DISCUSION

The toxic effect of radiation results mostly from oxidative damages through the generation of several ROS such as superoxide, hydrogen peroxide, hydroxyl radicals etc. The ORAC value reported for POLY-MVA was 5.65 (µmoles of trolox equivalent/g), which is very high when compared to other well-known antioxidants (Antonawich and Valane, 2007). MDA is the major reactive aldehyde resulting from peroxidation of biological membrane PUFAs and is used as an indicator of tissue damage by a series of chain reactions (Ohkawa et al., 1979). POLY-MVA inhibited radiation induced lipid peroxidation in irradiated liver tissues in vitro.

Whole body exposure of mammals to ionizing radiation results in a complex set of syndromes; the onset, nature and severity of which are a function of total radiation dose, radiation quality, the stage of the cell in the cell cycle, the levels of cellular
antioxidant defense system and the availability of oxygen in the tissues. As proliferating cells are highly sensitive to radiation, gastrointestinal tract and hematopoietic systems are more prone to whole body γ-radiation induced damages, which, in turn, may cause the death of animals at higher doses of radiation.

Administration of mice with POLY-MVA prior to radiation exposure effectively helped to maintain the different endogenous antioxidants to near normal levels and also lowered the extent of membrane lipid peroxidation in vivo. The gastrointestinal system is one of the major targets for the somatic injuries associated with radiation exposure. Whole body γ- irradiation is known to result in the depletion of hematopoietic organs owing to the intensive destruction of irradiated cells as well as impede their ability to proliferate (Dainiak, 2002). The so called hematopoietic syndrome death is often lethal for the organism as a result of infection due to the impairment of the immune system. The decrease in hematological constituents may be attributed to a direct damage by radiation dose and hematopoietic recovery after whole body irradiation is dependent on the presence of spared hematopoietic stem and progenitor cells in the bone marrow (Herodinan and Drouet, 2005). POLY-MVA bestowed significant protective effects against radiation induced depletion in different hematological parameters such as bone marrow cellularity, hemoglobin count, spleen weight and endogenous spleen colony formation. The side effects and the sequelae of severe intestinal radiation injury include varying degrees of intestinal inflammation, mucosal thickening, collagen deposition, and fibrosis as well as impairment of mucosal and motor functions (Coia et al., 1995; Hauer-Jensen, 1990; Zimmerer et al., 2008). POLY-MVA administration protected the intestinal epithelial cells from radiation induced structural lesions to a considerable extent.

Irradiation induces several types of damage to DNA, including double and single-strand breaks, base and sugar damage as well as DNA–DNA and DNA–protein cross-links. Misjoined or unrepaired DNA double strand breaks causes deletions, translocations, and acentric or dicentric chromosomes, thereby results in cellular damage or cell death (Barker et al., 2005; Belli et al., 2002; De Flora et al., 2001). Presence of POLY-MVA prevented the formation of radiation induced strand breaks in plasmid pBR322 as evidenced by the conservation of supercoiled form of DNA.
Thus POLY-MVA effectively protected DNA against ionizing radiation in a system devoid of repair and replication machinery. Alkaline comet assay is an elegant and effective technique to monitor the extent of DNA damage and its protection. Administration of POLY-MVA protected cellular DNA in different hematopoietic tissues (spleen and bone marrow) of whole body irradiated mice.

The radiation induced damages to cells and various tissues, unless repaired, results in organ failures, loss of body weight, and ultimately death. Although POLY–MVA at a dose of 1ml/kg body weight was found effective for all other studies conducted, the same dose was not found to offer much protection to the radiation induced survival and body weight alterations. Perhaps, the lower dose did offer protection to endogenous antioxidants, hematopoietic and gastrointestinal systems but not to other radiation target sites like central nervous system. However, a higher dose of 8 ml/kg body weight POLY- MVA provided significant protection from body weight loss and mortality in animals exposed to a lethal dose of whole body γ- radiation.

POLY-MVA has been used as a safe dietary supplement to improve the health conditions and vitality. The results obtained from the study reveals the usefulness of the formulation as an efficient radioprotector during radiation exposure situations. However, detailed studies need to be carried out to understand the proper dosage in order to achieve maximum protection levels.