ORIGINAL ARTICLE

Preparatory Liquid Blend, Poly-MVA and Rejeneril-A Protect the Radiation-Induced DNA Damage and Lesions of Ileum

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ABSTRACT

Background: Antioxidants have a significant effect against the ionizing radiation-induced damages. Protective effect of Poly-MVA and Rejeneril A against radiation-induced hematological changes and damage to the ileum part of the intestine in mice was studied. **Methods**: Mice exposed to a lethal dose of 8 Gy radiation were observed for survival following administration of Poly MVA or Rejeneril-A. The effect of these substances against 5 Gy radiation-induced hematological changes and damage to the intestinal ileum was also studied. The treatment was given seven days before and seven days after the irradiation, or only seven days following the radiation. Lesions in the ileum were measured using a microscopic scoring system, and the level of reduced glutathione (GSH) was measured in the mucosa on 8th day. DL-α- Lipoic acid was used as standard drug. **Results**: Poly-MVA, Rejeneril-A and lipoic acid were effective to sustain the survival of animals when treated 7-days before and after the irradiation. The level of GSH in the control group on the 8th day (11.20 ± 2.50 μ mol/mg protein) was lower than that of normal (11.64 ± 4.23 μ mol/mg protein). The GSH was improved in all the treated groups ($p>0.05$). Similarly, DNA and lesion to ileum also demonstrated statistically significant improved by the treatment. **Conclusion**: While each treatment demonstrated statistically enhanced protection, the hematological order of protection was Rejeneril-A>Lipoic- acid>Poly-MVA, while protection against DNA damage or lesions of ileum was in the order of LA>Poly-MVA>Rejeneril-A. Variations in the protection can be ascribed to the antioxidant activity.

Key words: Lipoic acid; radiation-induced toxicity; reactive oxygen species; antioxidants; ionizing radiation

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INTRODUCTION

Acute and long-term gastrointestinal symptoms had long been demonstrated for radiotherapy of lower gastrointestinal (GI) tumors. The adverse effect was mainly explained to the physical disruption of the sensitive regenerative epithelium of the intestinal mucosa.[1] Therefore, limiting toxicity of ionizing radiation without compromising its antitumor efficacy remains the major challenge to Oncologists. Agents that can attenuate the radiation-induced toxicity, while enhancing the antitumor effect, would be a welcome addition to cancer treatment. At the same time protecting the acute radiation-induced gastrointestinal and bone marrow damages is important.[2] The toxicity to gastrointestinal cells will be more in patients who receive radiotherapy in the abdominal area to treat an abdominal malignancy. Furthermore, the development of an effective treatment for radiation-induced GI damage is crucial for the intensive care of victims with high-dose radiation exposure.

Though the exact pathological mechanism has not yet been elucidated completely, the role of inflammation and oxidative stress (OS) mediated by cytokines, eicosanoids and reactive oxygen species (ROS) had been described as well.[3] ROS were associated indirectly with the toxic effect of ionizing radiation (IR). Therefore, antioxidants have a significant role in the alleviation of OS as they may protect the IR-induced damages.[4] Pretreatment with ascorbic acid was found to prevent the radiation-induced GI syndrome.[5] The previous study had demonstrated that Poly MVA was effective to protect the lethal dose of gamma rays induced damages in the peripheral lymphocytes.[6] Poly MVA is a scientifically validated vitamin, mineral and amino acid supplement with palladium-lipoic acid as the major ingredients. This preparatory liquid blend consists of covalently linked palladium with alpha-lipoic acid, molybdenum, rhodium, ruthenium, thiamine, riboflavin, cyanocobalamin, N-acetyl cysteine and N-formyl methionine. While Rejeneril–A contains all these ingredients in addition to vitamin A (100 IU/mL in the form of retinyl acetate). We have recently reported the role of Poly MVA as adjunct to radiotherapy in tumor-bearing mice.[7] Many vitamins and minerals were found to be effective to improve health in subjects undergoing cancer therapy, this study was aimed to evaluate the therapeutic effect of Poly-MVA, Rejeneril–A and lipoic acid against radiation-induced hematological changes and damage to the ileum part of the intestine of mice.

MATERIALS AND METHODS

Chemicals

Poly-MVA, Rejeneril-A and DL-α- Lipoic acid was gift from Garnett McKeen Laboratory, Inc., New York, USA. Dinitro phenyl hydrazine, reduced glutathione, and all other reagents were purchased from were purchased from Merck India Ltd. Mumbai, India. All the chemicals and reagents used were of analytical reagent grade.

Animals

Male Swiss albino mice (20-25 g) were purchased from the small animal breeding centre, Kerala Agriculture University, Thrissur, Kerala, India. Animals were kept in environmentally controlled condition (12 hours light-dark cycle, the temperature of 26-28°C and 60-70% relative humidity) with free access to food and water. The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government

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of India and after approval from the Institutional Animal Ethics Committee, Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, India.

Determination of protective effect of Poly-MVA, Rejeneril-A and lipoic acid against the lethal dose of radiation

Animals were divided into 4 groups with 6 animals each. Group 1: Animals treated with distilled water was kept as control. Group 2: Poly-MVA (2 ml/kg, p.o), 24 hr after 8 Gy irradiation; Group 3: Rejeneril-A (2 ml/kg, p.o), 24 hr after 8 Gy irradiation and Group 4: Lipoic acid (16 mg/kg, p.o, as negative control), 24 hr after 8 Gy irradiation. Whole body irradiation was carried out using high-energy x-rays (8 Gy) from a photon 6-MeV linear accelerator (21EX, Varian, Palo Alto, CA, USA) at a dose rate of 1.88 Gy per minute. The agents were administered using an oral gavage needle (22 gauge stainless steel needle of 1-inch size with 1.25 mm ball size) once daily for 7 days and were observed daily for the mortality.

The experiment was repeated with each group of animals treated with Poly-MVA (2 ml/kg), Rejeneril-A (2 ml/kg) or Lipoic acid (16 mg/kg), orally, once daily for 7 days prior to and 7 days post to irradiation (8 Gy).

Effect of Poly-MVA and Rejeneril-A against the radiation-induced ileum damage

Animals were divided into 5 groups of six animals each. Group I: treated with distilled water was kept as normal. Group II animals were exposed to 5 Gy radiation from X-ray was kept as positive control. Group III: treated with Poly-MVA (2 ml/ Kg, orally) Group IV: Rejeneril-A (2 ml/ Kg, orally) and group V were treated with DL-α- Lipoic acid (16 mg/ Kg, orally). Group III to V animals was treated 7 days before and 7 days after the 5 Gy irradiation.

Animals were euthanized by cervical decapitation on the 8th day. Weights of the animals were recorded before sacrifice. The organ weights noted and blood samples were collected after sacrificing for the determination of hematological parameters. Specimens were collected from the proximal part of ileum for the histopathological analysis and intestinal mucosa was collected for the determination of reduced glutathione (GSH) level. GSH level was estimated photo colorimetrically using dinitro phenyl hydrazine reagent by the method of Moron *et al.*[8] Microscopic scoring of the damage to ileum was done according to the method described previously.[9] Briefly, the score for normal morphology: 0; slight cellular desquamation at the villus tip or sub-epithelial congestion: 1; loss of less than half of the villus and congestion in the mucosa: 2; Loss of more than half of the villus: 3; and degeneration of the existing sub-mucosa: 4. Protein was estimated by the method of Lowry *et al.*[10] Comet assay was also performed in the peripheral blood sample according to the procedure described previously.[7]

Statistical analysis

All data were represented as mean ± SD. The mean values were statistically analyzed using one-way analysis of variance (ANOVA) (using the demo version of Graph Pad Instat Software package, CA, USA). The significant differences between the groups were further analyzed by Bonferroni's t-test. Dunnett multiple comparison tests were done for comparison of treated groups with the control group. P-value less than 0.05 were considered as significant.

RESULTS

Effect against the lethal dose of radiation

Poly MVA was not effective to sustain the survival of animals when treated 24 hrs after the irradiation. Rejeneril-A and lipoic acid could render the protection as evidenced from the slightly increased survival [Figure 1]. However, pretreatment of Poly-MVA, Rejeneril-A or lipoic acid once daily for 7 days prior to and 7 days post to irradiation could improve the survival of animals [Figure 2]. The order of survival benefit was found to be Rejeneril-A<lipoic acid<Poly-MVA.

Effect against the radiation-induced ileum damage

No significant change in hemoglobin (Hb) and total erythrocytes (RBC) count was found in the radiation alone treated animals compared to the normal group. Therefore, the effect of treatment with Rejeneril-A, lipoic acid or Poly-MVA before and after the radiation was non-significantly different. However, Rejeneril-A was effective in enhancing the Hb and RBC while comparing with the normal or control group. The platelet count was significantly ($p<0.001$) lowered in the radiation alone treated animals when compared to the normal group of animals [Table 1]. Treatment using Rejeneril-A or lipoic acid could improve

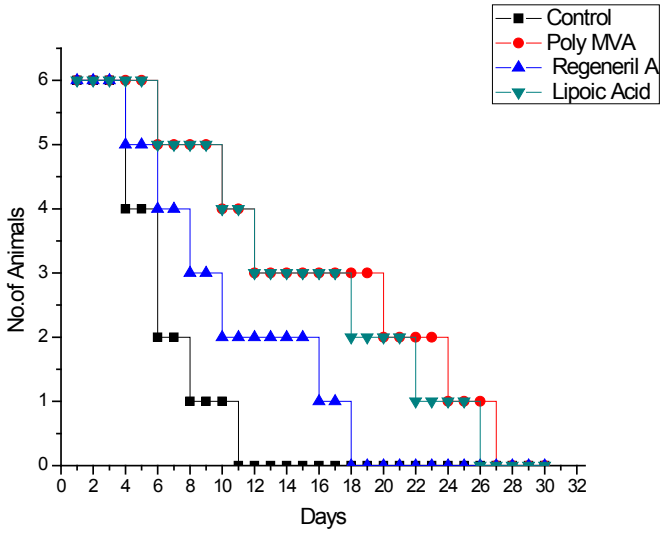

Figure 1: Effect of Poly MVA, Rejeneril-A or lipoic acid on the survival of 8 Gy irradiated animals. N=6 animals per group. The Poly-MVA (2 ml/kg), Rejeneril-A (2 ml/kg) or lipoic acid (16 mg/kg) treated 24 hr after the irradiation and continued once daily for 7 days

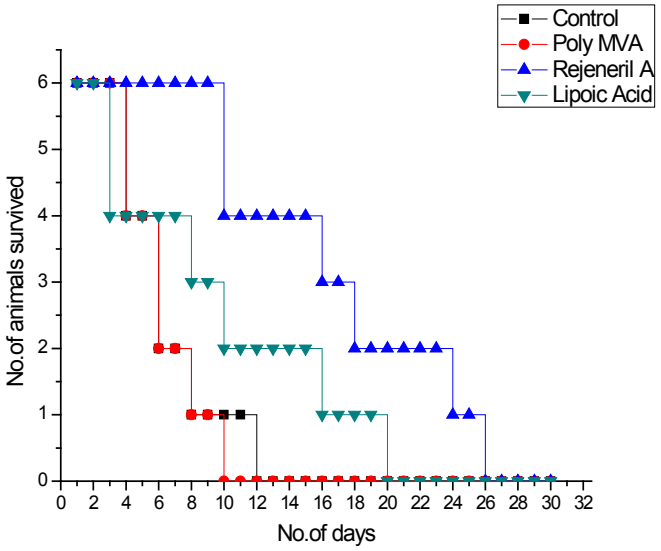

Figure 2: Effect of Poly MVA, Rejeneril-A or lipoic acid on the survival of 8 Gy irradiated animals. N=6 animals per group. The Poly-MVA (2 ml/kg), Rejeneril-A (2 ml/kg) or lipoic acid (16 mg/kg) treated 7 days prior to the irradiation and continued once daily for 7 days

the count than that of the control group. A similar effect of lowering of total WBC count was found in all the radiation exposed animals. Treatment with Rejeneril-A was effective to attenuate the radiation-induced lowering of the WBC count. LA or Poly-MVA was not effective to maintain the total WBC count. Results of the differential leukocytes count showed that neutrophils and lymphocytes were not significantly changed between normal and control group. But a significant effect of lowering the lymphocytes from the normal as well as from the control group was evidenced in the Poly-MVA treated group.

Effect of Rejeneril, LA and Poly-MVA on the change in the body weight of animals before and after radiation (5 Gy) exposure is presented in Table 2. No significant change in body weight was found in any of the treated group from normal or control animals. However, an increase in the relative kidney weight of radiation treated animals from that of normal animals was observed [Table 3]. All the three compounds were significantly effective to attenuate this effect. Analyzing the effect of treatment on the GSH level in the ileum of animals exposed to radiation, Rejeneril-A and Poly-MVA treated groups showed a non-significant ($p>0.05$) increase in the mean level. Effect of Rejeneril-A, LA and Poly MVA on GSH level in the ileum of animals exposed to radiation is given in Table 4.

Histopathological analysis of the ileum part of intestine showed a higher number of lesion score in the radiation alone treated animals than that of the LA, Rejeneril-A or Poly-MVA treated groups [Figure 3]. Radiation alone exposed group showed a high tail, tail moment, tail DNA content as well as the olive tail moment ($p<0.001$) which indicated the DNA damage in the blood cells [Table 5]. The treated groups with LA, Poly-MVA or Rejeneril-A showed in the descending order of protection [Figure 4].

DISCUSSION

Poly-MVA was not effective to sustain the survival of animals when treated 24 hrs after the irradiation. However, it could render protection when administered 7 days prior to the irradiation. Both Rejeneril-A and lipoic acid were effective to render the protection when administered before and after the irradiation. This may probably due to the prevention of ROS-mediated damage to bone marrow and the intestinal cells. The half-life of the radicals evoked by radiation is considered to be in nanoseconds and hence these radicals can interact with various biological molecules in their close vicinity and

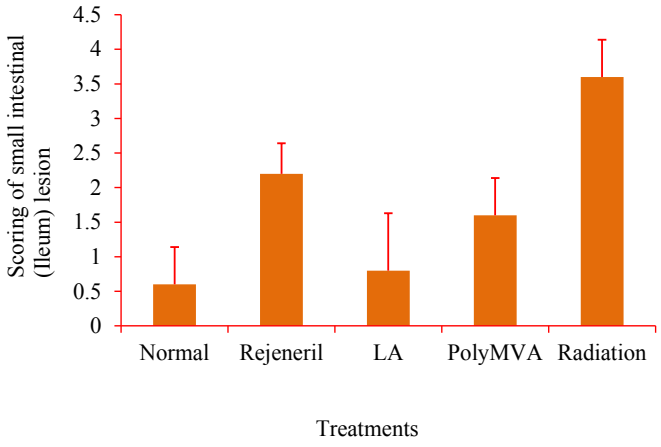

Figure 3: Histopathological scoring of the ileum section of 5 Gy treated animals. N=6 animals per group. The Poly-MVA (2 ml/kg), Rejeneril-A (2 ml/kg) or lipoic acid (16 mg/kg) treated 7 days prior to the irradiation and continued once daily for 7 days. a $p<0.001$ (Bonferroni multiple comparison test) significantly different from the normal group and all treated groups are significantly ($p<0.01$, (Dunnett multiple comparison test) different from the control group.

induce the radiation-induced biological effects on the tissue and cells including apoptotic cell death.[11] This result suggested that Poly-MVA, Rejeneril-A and lipoic acid can render the radiation protection in subjects with prior knowledge of radiation exposure such as patients undergoing radiotherapy or rescue team members in radiation-contaminated areas. Since the better protection was obtained from the 7-day pretreatment plan, the protective effect on the ileum against the sub-lethal dose of radiation at. 5 Gy was evaluated.

Poly-MVA, Rejeneril-A and lipoid acid were effective to protect the hematological changes and damage to the ileum by the sub-lethal dose of radiation. Radiation alone treated animals showed statistically significant lowering of platelet count and total WBC count. Treatment with Rejeneril or lipoic acid could attenuate the damage as evidenced by the statistically significant increase in the number of platelets and total WBC count. Despite the mean value of platelet, no statistically significant difference could evidence in the Poly-MVA treated group.

No change in body weight was observed in any of the treated group with respect to the control. This may probably due to the body's compensatory mechanism against the radiation. A similar observation was found in the relative organ weight among the treated group with respect to the control. However, relative organ weight of kidneys in all the treated groups was significantly different from that of the control. Relatively higher kidney weight in the control group indicated higher organ damage, probably due to the higher abdominal exposure to radiation as well as the lower antioxidant status.

The reduced GSH level in the cell was correlated with the generated ROS.[12] As the major non-protein thiol molecule present in the cell, GSH can scavenge the free radicals generated from the radiation and render protection against DNA and lipid peroxidation. Though the Rejeneril, lipoic acid and Poly-MVA could attenuate the radiation-induced decline of GSH level in the ileum, the effect was non-significantly different from the control group. Potten *et al.* demonstrated that low dose radiation can induce the apoptosis of the intestinal epithelial cells.[13] Since GSH has a role in the apoptosis,[14] the significant decline in the villus height, crypt number, an increase in the goblet and dead cells that are induced by radiation can be ascribed to the lowering of GSH. A single dose of IR (7 Gy) was found to enhance the expression pro-apoptotic molecules such as p53, Bax, and Bak in the small intestine of mice.[15] The maximum change in GSH was observed on day one of post-irradiation and signs of recovery at day seven of post irradiation.[16] Therefore, the non-significant difference of GSH level between the control and the treated group in this study may probably due to the recovery of toxicity.

The direct or an indirect effect of IR had been explained for their toxic effect. Among the molecules affected, damage to DNA remains the immediate effect[17] which can be ascribed to the hydroxyl radicals generated from the radiolysis of water in the cytosol.[18] The DNA protective effect of Poly-MVA, Rejeneril and lipoid acid was evidenced from the comet assay. All the three agents could alleviate the DNA damage in the peripheral blood.

The exact mechanism of protection has not yet been elucidated from this study. Our previous study demonstrated the antioxidant activity of the major ingredient-palladium lipoic acid present in the Poly-MVA, which can be ascribed as the possible protective mechanism.[19,20] Further, the B-complex vitamins present in the preparation can augment the radical scavenging effect and, thereby, the adverse effect of radiation. A recent study demonstrated the protective effect of lipoic acid (100 mg/kg, i.p) against 15 Gy radiation- induced enteritis in mice.[21] The mechanism was mediated through reducing the inflammation and oxidative stress. The levels of phosphorylated nuclear factor kappa

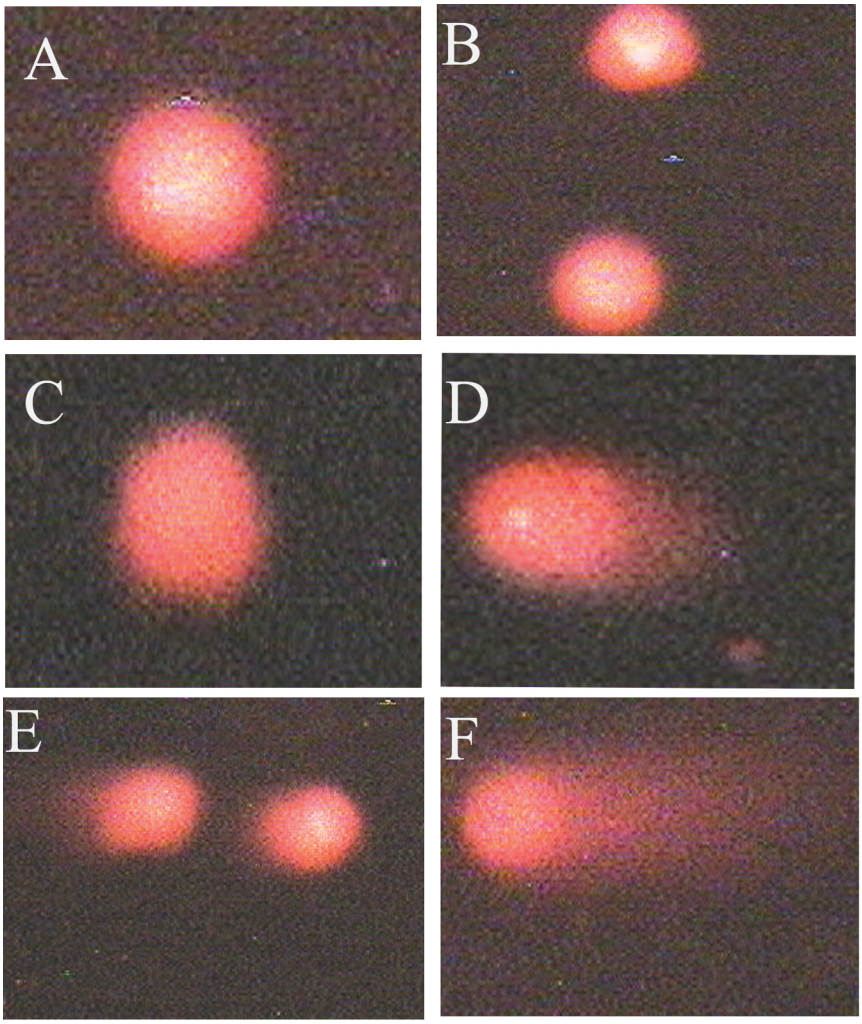

Figure 4: Photograph of comet assay using the peripheral blood of A and B) normal; C) Lipoic acid+5 Gy radiation; D) Rejeneril-A+5 Gy radiation; and E) Poly-MVA+5 Gy radiation and F) 5 Gy radiation

Table 1: Effect of Rejeneril-A, lipoic acid (LA) and Poly MVA on hematological parameters in radiation (5 Gy) exposed animals

Group	Hemoglobin g/dl	Total RBC Count million/cu mm	Platelet count Lakh/cu	Total WBC count Cells/cu mm	Neutrophils %	Lymphocytes %	Eosinophils %
Normal	14.50 ± 0.56	5.95 ± 0.35	5.33 ± 0.75	4833.3 ± 251.6	41.6 ± 0.5	51.3 ± 0.5	7.0 ± 1.0
Rejeneril+5 Gy	16.00 ± 0.84^{**,b}	6.30 ± 0.28^{a}	3.95 ± 0.07^{***,b}	3650 ± 212.13^{***,b}	43.0 ± 2.0	50.0 ± 2.0***	6.3 ± 0.5^{a}
LA+5 Gy	14.10 ± 0.56	5.50 ± 0.36	2.36± 0.15^{***,b}	2800 ± 200***	47.33 ± 3.05^{**,a}	47.0 ± 2.6^{*,b}	5.6 ± 0.5
Poly MVA+5 Gy	14.30 ± 0.28	5.60 ± 0.26	2.27 ± 0.09***	2575 ± 170.78***	50.6 ± 2.3^{***,b}	43.3 ± 1.5^{***,b}	6.5 ± 0.7^{a}
Radiation+5 Gy	13.73 ± 0.40	5.60 ± 0.14	2.15 ± 0.07***	2700 ± 141.42***	43.0 ± 3.0	52.0 ± 2.6	5.0 ± 1.0**

*P<0.05, **P<0.01 and ***P<0.001 (Bonferroni multiple comparison test) significantly different from the normal group. Others are non-significant (p>0.05); a p<0.05, b p<0.01 (Dunnett multiple comparison test) significantly different from the control (radiation) group

Table 2 Effect of Rejeneril-A, lipoic acid (LA) and Poly MVA on change in either initial or final body weight of animals before and after irradiation (5 Gy)

Group	Initial(g)	Final(g)
Normal	25.60 ± 1.91	28.00 ± 1.68
Poly MVA + 5Gy	27.28 ± 1.17	26.87 ± 3.14
Rejeneril + 5Gy	27.62 ± 1.64	27.14 ± 3.17
LA + 5Gy	27.56 ± 1.28	25.52 ± 5.32
Radiation (5Gy)	27.38 ± 1.25	25.88 ± 2.55

***P<0.001 (Bonferroni multiple comparison test) significantly different from the normal group bp<0.01 (Dunnett multiple comparison test) significantly different from the control group (radiation)

Table 3: Effect of Rejeneril-A, lipoic acid (LA) and Poly MVA on relative organ weight of animals after exposed to radiation (5 Gy)

Groups	Heart (g)	Liver (g)	Kidney (g)
Normal	0.360 ± 0.017	3.44 ± 2.98	1.139 ± 0.052
Rejeneril+5 Gy	0.366 ± 0.066	4.92 ± 0.53	1.090 ± 0.107[b]
LA+5 Gy	0.318 ± 0.064	4.45 ± 0.27	0.753 ± 0.060[b]
Poly MVA+5 Gy	0.317 ± 0.016	4.49 ± 0.69	1.200 ± 0.037[b]
Radiation (5 Gy)	0.364 ± 0.025	5.01 ± 0.16	3.460 ± 0.124[***]

***P<0.001 (Bonferroni multiple comparison test) significantly different from the normal group b p<0.01 (Dunnett multiple comparison test) significantly different from the control group (radiation)

Table 4: Effect of Rejeneril-A, lipoic acid (LA) and Poly MVA on reduced glutathione level in the ileum of animals exposed to radiation (5 Gy)

Groups	µ mol/mg protein
Normal	11.64 ± 4.23
Rejeneril+5 Gy	13.72 ± 3.57
LA+5Gy	9.59 ± 4.38
Poly MVA+5Gy	13.28 ± 2.24
Radiation (5 Gy)	11.20 ± 2.50

Values are mean ± SD, n=6. Not significantly (p>0.05) different from either normal or control (radiation)

Table 5: Effect of Rejeneril A, lipoic acid (LA) and Poly MVA on radiation (5 Gy) induced DNA damage in the peripheral blood

Groups	Tail DNA %	Tail Length (µm)	Tail Moment	Olive Tail Moment
Normal	4.0 ± 0.21	4.50 ± 0.87	1.20 ± 0.21	0.68 ± 0.08
Rejeneril A+5 Gy	24.87 ± 5.25	46.21 ± 15.69	10.25 ± 3.70	10 .00 ± 2.20
LA+5 Gy	20. 40 ± 3.40	30.80 ± 12.40	8.06 ± 0.99	6.13 ± 1.54
Poly MVA+5 Gy	25 .87 ± 4.77	40.11 ± 14.6	8.47 ± 1.40	13.50 ± 3.30
Radiation (5 Gy)	35.36 ± 5.5	50.25 ± 12.78	15.78 ± 2.44	18.00 ± 5.69

P<0.001 (Bonferroni multiple comparison test) radiation group was significantly different from the normal group. P<0.05, treated groups were significantly different from the control group (radiation) (Dunnett multiple comparison test)

B, matrix metalloproteinase-9 and mitogen-activated protein kinases were significantly decreased in the lipoic acid treated group than control group. Pretreatment with vitamin A (15,000 IU/kg body weight, i.p for 7 days) prior to irradiation was effective to protect the changes in intestinal enzymes.[22] Furthermore, it was effective against the radiation-induced an alteration in the polymorphonuclear leukocyte.[23] Intestinal villi damage results from radiotherapy found to be associated with malabsorption of nutrients; therefore, administration of nutritional supplement may be an appropriate approach to prevent the nutritional deficiency.[24]

CONCLUSION

The results of the study suggest that among the tested compounds, the descending order of protection against the whole body irradiation-induced hematological parameters was Rejeneril-A>Lipoic acid>Poly-MVA. The protection against DNA damage and lesions of ileum was LA>Poly-MVA>Rejeneril-A. The protection can be ascribed to the antioxidant activity of constituents present in the preparation.

Research funding

None.

Conflict of interest

None.

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