



Original Research Article

Radiotherapy Radio protective effect of 2- deoxy D-glucose with combination of selective antioxidant medicinal plant *Vitis vinifera*, *Camellia sinensis*, and *Zingiber officinale* extracts in mice

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ABSTRACT

High doses of radiation therapy are used to destroy cancer cells, these high doses of radiations also damage healthy cells and tissues near the treatment area or whole body. At present, major advances of radiation therapy have made it more precise, leading to fewer side effects. The side effects often start during second week of radiation treatment So it is necessary to minimize these side effects with natural available medication. Previous studies shown that, 2- deoxy D- glucose, an analogue of glucose and glycolytic inhibitor, enhances radiation induced damage, selectively in tumour cells while protecting normal cells suggest that 2- deoxy D- glucose can be used as a differential radio modifier to improve the efficacy of radiotherapy and also it is shown that antioxidant medicinal plants like *Vitis Vinifera*, *Camellia Sinensis*, and *zingiber officinale* have shown protective effect against radiation induced damage in previous studies. So in the present study, we have selected these antioxidant medicinal plant extracts combines with 2- deoxy D- glucose as a treatment, before and after radiation therapy and measured the levels of nitric oxide, lipid peroxidation and antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase in tissues like blood, liver, kidney and brain tissues of mice after completion of study. In the present study, it is found that the highest effect is found in a group of animals which are on combination of normal diet and feeding of deoxy – D glucose with grape seed extract, green tea extract and ginger extract in proportion of 1:1:1, in this group of animal's radiation induced micronucleus formation, formation of metamyelocytes and dysplastic myeloid series cells formation is also found significantly decreased.

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1. Introduction

Radiation therapy treats many types of cancer effectively. But like other treatments, it often causes side effects. The side effect varies from person to person, depend on type of cancer, its location, the dosage of radiation therapy and immunity of the person.

High doses of radiation therapy are used to destroy cancer cells, these high doses of radiations also damage

healthy cells and tissues near the treatment area or whole body. At present, major advances of radiation therapy have made it more precise, leading to fewer side effects. Reactions often starts during second week of treatment. Some of the common side effects include, hair loss, dryness of skin, itching, blistering or peeling. Fatigue around head and neck including dry mouth, mouth and gum sores, difficulty in swallowing, stiffness in jaw, nausea, shortness of breath, breast or nipple soreness, shoulder stiffness, cough, fever and fullness of the chest also known as radiation pneumonitis, radiation fibrosis, diarrhea, rectal

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bleeding, bladder irritation, sexual problems, lower sperm counts, reduced sperm activity, changes in menstruation, symptoms of menopause and infertility.¹

High rate of glucose utilization directly relates with poor prognosis of several types of malignant tumours. Previous studies shown that 2- deoxy D- glucose, an analogue of glucose and glycolytic inhibitor, enhances radiation induced damage, selectively in tumour cells while protecting normal cells suggest that 2- deoxy D- glucose can be used as a differential radio modifier to improve the efficacy of radiotherapy.²

Selected antioxidant medicinal plants like vitis Vinifera , camellia Sinensis and zingiber officinale have shown protective effect against radiation induced damage in previous studies³⁻⁵

Vitis vinifera, is one of largest growing crop, especially in and around western Maharashtra. Commonly called grapes. Grape seeds are rich source of monomeric phenolic compounds, such as (+) catechin, (-) epicatechin and (-) epicatechin-o-gallate and dimeric, trimeric, tetrameric procyanidines.⁶ These compounds act as antioxidants,³ antimutagenic and antiviral agents.⁷ Phenolics in grape seeds have been reported to inhibit low density lipoproteins in humans and oxidation in vitro.⁸ Studies have reported possible use of phenolics in grapes in preventing atherosclerosis.⁹ By considering these health benefits the grape seeds have been used as dietary suppliments.

Green tea (*Camellia sinensis* L.) contains large amount of polyphenolic compounds with antioxidant properties and may prevent oxidative damage of DNA.^{10(10,11)} Due to large content of flavanoids and other polyphenols, green tea shows anticarcinogenic, antioxidative and hypolipidaemic properties,^{4,11} anti-inflammatory,¹² cholesterol lowering,¹³ antiviral and antibacterial properties,¹⁴ antifilarial¹⁵ and antioxidant.⁽¹¹⁾ By considering these properties, the green tea can be used as dietary suppliment and ingradient in cosmetics, shampoos, sweet waters and antiageing emulsions¹⁶⁻²⁰

Ginger (*Zingiber officinale*) is used as carminative, diaphoric , antispasmodic, expectorant, circulatory stimulant, astringent, appetite stimulant, diuretic, digestive and anti-inflammatory agent.^{21,22} The dried rhizomes of the herb are used traditionally to cure human ailments. It is used to cure diarrhea, dysentery, fever, cough, ulcer, boils and wounds,²³ protective effect against alcohol induced renal damage.²⁴ and anticancer and antioxidant⁵

Chemicals- All chemicals used in the present study were Analar grade (AR), and obtained from Mark (Mumbai , India), Ranbaxy (New Delhi , India) and HI Media (Mumbai, India).

1.1. Preparation of extract

1.2. Preparation of Grape Seed (*Vitis vinifera*) extract

The grape seeds were procured from Sula vineyards, Nasik. The seeds were dried in shade. They were griend and powdered mechanically with the help of porcelain mortar and pestle. 100 gm. of powder was extracted in soxhlet extractor with hexane for 6 hours for removal of fatty material. The defated seed powder was extracted in soxhlet extractor for 5 hours with 300 ml. mixture of ethyl acetate: water, having the ratio of 17:3. The extract was pooled, dried with anhydrous sodium sulphate and concentrated under vacuum to yield a viscous liquid. Procyanidins were precipitated by adding double volme of hexane to viscous liquid. The precipitate was collected by filtration under vacuum. The extract obtained was weighed and stored in dessicator.

1.3. Preparation of Green tea (*Camellia sinensis* L.) extract

100 gm. packaged long leaf green tea was purchased from local super market of Tately brand with 12 month expiry date of batch code- 24TT118. 50 gm. of green tea leaves were mechanically powdered in a porcelain crucible. The powder then mixed with chloroform and petroluium ether (1:1) and kept in extraction thimble for about 2 hours to remove chlorophyll and hydrophobic substances. After separating the eluent and drying the plant material, the proper extraction was done in a soxhlet extractor by mixing the powder with 95% ethanol at a constant temperature of 70°C for 5 hours. Then the extract was pooled , dried under vacuum to yield a dark brown extract, the yield was weighed and stored in a desiccator.

1.4. Preparation of Ginger (*Zingiber officinale*) extract

Fresh rhizomes of ginger were purchased from local market Loni. They were washed with water to make them free of soil and air dried at room temperature. Air dried rhizomes of the herb (1 kg) were milled into fine powder mechanically with the help of porcelain crucible, then 100 gm powder was extracted with 95% ethanol for 24 hours. The extract was recovered and 300 ml. of 95% ethanol was further added to the ginger powder and the extraction was continued for 5 hours at constant temperature of 70 C. The process was repeated three times, the three extracts were pooled together, mixed, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resultant ethanolic extract was air dried, weighed and stored in a desiccator at room temp.

All the three selected plants are authenticated by Professor of Botany, PVP College, Loni.

1.5. Why mixture of plants?

It is found that mixture of two or more medicinal plants have synergistic effect in providing antioxidants and reductants like flavanols, flavonoids, catechin, epigallocatechin, epigallocatechin gallate etc.

It favours the study carried out by Khandu Hotkar, Wu SG et al.²⁵ Gordon, M.F.²⁶ Chan E.W.C et al.²⁷ and Sneha Hande et al.²⁸

1.6. Experimental design of animals

200 Swiss male mice weighing 30-40 gms. of 6 weeks' age was purchased from National Institute of Biosciences Pune, and were divided in 10 groups.

1. Group 1 – 20 mice- with normal diet (without any supplement) and no radiation exposure
2. Group 2 – 20 mice- with normal diet (without any supplement) and radiation exposure (after 45 days of dietary treatment)
3. Group 3 – 20 mice- with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) and radiation exposure (after 45 days of dietary treatment)
4. Group 4- 20 mice- with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Vitis vinifera extract (100 mg/kg body weight) and radiation exposure (after 45 days of dietary treatment)
5. Group 5 -20 mice - with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Camellia sinensis L. extract (100 mg/kg body weight) and radiation exposure (after 45 days of dietary treatment)
6. Group 6 - 20 mice - with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Zingiber officinale (100 mg/kg body weight) and radiation exposure (after 45 days of dietary treatment)
7. Group 7 - 20 mice- with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Vitis vinifera extract (100 mg/kg body weight) + Camellia sinensis L. extract (100 mg/kg body weight) and radiation exposure (after 45 days of dietary treatment)
8. Group 8 -20 mice- with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Vitis vinifera extract (100 mg/kg body weight) + Zingiber officinale (100 mg/kg body weight) and radiation exposure (after 45 days of dietary treatment)
9. Group 9 - 20 mice- with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Camellia sinensis L. extract (100 mg/kg body weight) + Zingiber officinale (100 mg/kg body weight) and radiation exposure (after 45 days of dietary treatment)
10. Group 10 - 20 mice- with normal diet + 2 deoxy D glucose (200 mg / kg of body weight) + Vitis vinifera extract (100 mg/kg body weight) + Camellia sinensis L. extract (100 mg/kg body weight) + Zingiber officinale (100 mg/kg body weight) and radiation

exposure. (after 45 days of dietary treatment)

The mice were on the above mentioned extracts daily once orally for 45 days, along with normal pellet diet. After 45 days of dietary treatment, the animals were irradiated with Linear Accelerator 6 mv telepathy (5 Gy / fraction) and kept on their respective diet again for 15 days. After 15 days, blood samples are to be collected and all animals are scarified (euthanized) by cervical dislocation and histopathological samples were collected and the remains of animal carcasses sent for incineration. Brain, liver and kidney tissues are collected in physiological saline, blotted to dry, weighed and homogenized in tris-HCL buffer of 0.1M with pH 7.4. 10% homogenate is used for biochemical assays.

Femur bones were excised to collect bone marrow; bone marrow is flushed with saline with the help of syringe in a tube containing 5% bovine serum albumin in buffered saline. The micronucleus test in bone marrow cells is performed according to Schmid²⁹ and Heddle.³⁰ Briefly, bone marrow containing tube is centrifuged at 1000 rpm for 5 mins. and the supernatant is removed. The sediment is mixed thoroughly and used for making bone marrow smears and allowed to dry overnight. The staining of slides is done using 0.2% May- Grunwald in ethanol and 2% Giemsa stain as described by Schmid.²⁹ For each animal, minimum 2000 polychromatic erythrocytes (PCEs) and 2000 normochromatic erythrocytes (NCEs) were to be analyzed and number of micronucleated PCE and NCE is scored.

1.7. Biochemical assays

1. Nitric oxide (NO) free radical scavenging activity by the method of Granger et al.³¹
2. Estimation of lipid peroxide in terms of Thiobarbituric acid reactive substances by colorimetric method by using TCA-TBA reagent³²
3. Superoxide dismutase in mammalian tissue homogenates by Spitz DR, Oberly³³
4. Catalase by Beers and Sizer³⁴
5. Glutathione peroxidase by Paglia Valentine WN³⁵

1.8.

All the data were expressed as mean \pm SD (n=20).

Statistically One Way Analysis of Variance (ANOVA) is applied to compare the nitric oxide level, lipid peroxidation level, and level of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxide in selected group of animals. If the p value is < 0.0001, it considered extremely significant and Tukey-Kramer multiple comparisons test is applied to compare the lipid peroxidation level, nitric oxide level and level of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxide in selected group of animals.

Table 1: Blood parameters

Blood	NO units/ gm tissue	TBARS nmol/mg tissue protein	SOD units/mg tissue protein	Catalase μ moles of H ₂ O ₂ utilized/min/mg tissue protein	GPx μ moles of GSH consumed/min/mg tissue protein
1 Control (no radiation) n=20	3.69 \pm 0.608	1.559 \pm 0.887	7.781 \pm 0.499	73.265 \pm 6.260	1.252 \pm 0.531
2 Expt. Cont (radiation) n=20	8.194*** \pm 0.84	2.336*** \pm 0.82	5.192*** \pm 0.87	53.360 *** \pm 0.22	0.7535*** \pm 0.51
3 deoxy D- Glu (radiation) n=20	7.285** \pm 1.201	1.967** \pm 0.803	6.622** \pm 0.40	61.70** \pm 6.577	0.771** \pm 0.536
4 deoxy D-Glu+GS (radiation) n=20	6.825** \pm 1.108	1.873** \pm 0.682	6.731** \pm 0.569	62.49** \pm 7.698	0.814** \pm 0.882
5deoxy D-Glu+GT (radiation) n=20	6.749** \pm 1.244	1.712** \pm 0.957	6.685** \pm 1.064	60.88** \pm 5.140	0.904** \pm 0.616
6deoxy D-Glu+Gin (radiation) n=20	7.296** \pm 1.306	1.911** \pm 0.914	6.954** \pm 0.925	58.225** \pm 5.042	0.766** \pm 0.786
7 deoxy D-Glu+GS+GT (radiation) n=20	5.778* \pm 0.9044	1.656* \pm 0.461	7.121* \pm 0.8049	63.46* \pm 3.218	0.819* \pm 0.345
8 deoxy D-Glu+GS+Gin (radiation) n=20	5.215* \pm 1.127	1.639* \pm 0.841	6.836* \pm 0.890	64.27* \pm 6.276	0.930* \pm 0.385
9 deoxy D-Glu+GT+Gin (radiation) n=20	5.032* \pm 1.302	1.594* \pm 0.421	6.940* \pm 0.710	68.710* \pm 4.217	0.999* \pm 0.409
10 deoxy D- Glu+GS+GT+Gin(radiation) n=20 (radiation) n=20	4.448 \pm 0.926	1.401 \pm 0.331	8.318 \pm 0.803	82.465 \pm 6.635	1.324 \pm 0.535

* - significant (p<0.05) , ** - Highly significant (p<0.005) ,

*** - - Highly significant (p<0.001)

2. Results and discussion

From Tables 1, 2, 3 and 4 , it is shown that the data were expressed as mean \pm SD (n=20). Statistically One Way Analysis of Variance (ANOVA) is applied to compare the nitric oxide level, lipid peroxidation level and the levels of antioxidant enzymes viz. superoxide dismutase, catalase and glutathione peroxidase in different group of animals. If the p value is < 0.001, it is considered extremely significant and Tukey- Kramer multiple comparisons test is applied to compare the nitric oxide level in different group of animals.

Highly significant increased level of blood, liver, kidney and brain nitric oxide (p<0.001), significantly increased level of blood , liver, kidney and brain lipid peroxidation (p<0.001), and significantly decreased level of antioxidant enzymes viz. superoxide dismutase, catalase and glutathione peroxidase (p <0.001) in blood , liver, kidney and brain of group 2 animals which are on normal diet and exposure of radiation are found when compared against group 1 animals, which are of control group, on normal diet but they are not exposed to radiation. which suggests radiation induces formation of nitric oxide,

formation of thiobarbituric acid reactive substances in the blood of group 2 animals, while the antioxidant enzyme activity suppresses in the blood , liver, kidney and brain tissues on radiation exposure. Highly significant increased level of blood , liver, kidney and brain nitric oxide (p<0.001), significantly increased level of blood lipid peroxidation (p<0.001), and significantly decreased level of antioxidant enzymes viz. superoxide dismutase, catalase and glutathione peroxidase (p <0.001) in blood of group 3 animals which are on normal diet with 2- deoxy D- glucose treatment, in blood of group 4 animals, which are on normal diet combines with 2- deoxy D- glucose and Vitis vinifera extract, group of 5 animals which are on normal diet combines with 2- deoxy D- glucose and Camellia sinensis extract and group of 6 animals which are on normal diet combines with 2- deoxy D- glucose and Zingiber officinale extract, group 7 animals, which are on normal diet combines with 2- deoxy D- glucose + Vitis vinifera extract + Camellia sinensis extract, group 8 animals, which are on normal diet combines with 2- deoxy D- glucose+ Vitis vinifera extract + Camellia sinensis extract, group 9 animals with normal diet

Table 2: Liver parameters

Liver	NO units/ gm tissue	TBARS nmol/mg tissue protein	SOD units/mg tissue protein	Catalase μ moles of H ₂ O ₂ utilized/min/mg tissue protein	GPx μ moles of GSH consumed/min/mg tissue protein
1 Control (no radiation) n=20	2.798 \pm 0.639	1.348 \pm 0.341	5.728 \pm 0.495	50.665 \pm 7.569	0.902 0.577
2 Expt.Cont (radiation) n=20	7.590*** \pm 0.999	2.10*** \pm 0.426	3.772*** \pm 0.551	40.650*** \pm 3.395	0.658*** \pm 0.0386
3 deoxy D- Glu (radiation) n=20	6.539** \pm 1.928	1.808** \pm 0.305	5.525** \pm 1.121	51.465** \pm 5.081	0.727** \pm 0.08705
4 deoxy D-Glu+GS (radiation) n=20	5.826** \pm 0.848	1.585** \pm 0.629	5.706** \pm 1.498	52.750** \pm 4.942	0.7990** \pm 0.090
5deoxy D-Glu+GT (radiation) n=20	5.688** \pm 0.812	1.690** \pm 0.775	5.658** \pm 1.185	52.055** \pm 6.263	0.8555** \pm 0.092
6deoxy D-Glu+Gin (radiation) n=20	6.227** \pm 0.665	1.734** \pm 0.091	4.875** \pm 1.681	50.910** \pm 5.088	0.743** \pm 0.077
7 deoxy D-Glu+GS+GT (radiation) n=20	4.601* \pm 0.834	1.444* \pm 0.735	5.908* \pm 0.844	53.150* \pm 4.818	0.851* \pm 0.042
8deoxy D-Glu+GS+Gin (radiation) n=20	4.812* \pm 0.777	1.548* \pm 0.141	5.889* \pm 0.655	52.860* \pm 4.795	0.9245* \pm 0.037
9deoxy D-Glu+GT+Gin (radiation) n=20	4.731* \pm 1.137	1.628* \pm 0.314	6.174* \pm 0.843	54.570* \pm 1.161	0.834* \pm 0.301
10 deoxy D-Glu+GS+GT+Gin (radiation) n=20	3.514 \pm 0.6159	1.383 \pm 0.6146	6.874 \pm 0.5325	62.025 \pm 1.8711	1.231 \pm 0.6091

* - significant (p<0.05) , ** - Highly significant (p<0.005) ,

*** - - Highly significant (p<0.001)

Table 3: Kidney Parameters

Kidney	NO units/ gm tissue	TBARS Nmol/mg tissue protein	SOD units/mg tissue protein	Catalase μ moles of H ₂ O ₂ utilized/min/mg tissue protein	GPx μ moles of GSH consumed/min/mg tissue protein
1 Control (no radiation) n=20	1.718 \pm 0.4957	1.212 \pm 0.06902	4.679 \pm 0.9936	48.550 \pm 3.120	0.7640 \pm 0.08810
2 Expt.Cont (radiation) n=20	5.828*** \pm 1.102	1.686*** \pm 0.608	3.217*** \pm 0.720	38.90*** \pm 4.039	0.402*** \pm 0.081
3 deoxy D- Glu (radiation) n=20	4.543** \pm 0.662	1.592** \pm 0.627	4.723** \pm 0.791	41.65** \pm 2.743	0.685** \pm 0.084
4 deoxy D-Glu+GS (radiation) n=20	3.790** \pm 0.9348	1.518** \pm 0.7636	4.834** \pm 0.9906	42.57** \pm 4.390	0.712** \pm 0.795
5deoxy D-Glu+GT (radiation) n=20	3.621** \pm 0.685	1.503** \pm 0.416	4.724** \pm 0.702	41.79** \pm 2.732	0.717** \pm 0.046
6deoxy D-Glu+Gin (radiation) n=20	4.197** \pm 0.882	1.512** \pm 0.752	4.349** \pm 0.64	40.65** \pm 3.441	0.643** \pm 0.072
7 deoxy D-Glu+GS+GT (radiation) n=20	2.548* \pm 0.216	1.307* \pm 0.423	5.407* \pm 0.662	43.37* \pm 2.940	0.662* \pm 0.047
8deoxy D-Glu+GS+Gin (radiation) n=20	2.78* \pm 0.1693	1.334* \pm 0.294	5.55* \pm 0.868	42.415* \pm 2.673	0.692* \pm 0.056
9deoxy D-Glu+GT+Gin (radiation) n=20	3.171* \pm 0.929	1.434* \pm 0.423	5.677* \pm 1.13	44.36* \pm 1.799	0.710* \pm 0.076
10 deoxy D-Glu+GS+GT+Gin (radiation) n=20	2.338 \pm 0.8184	1.241 \pm 0.4936	5.752 \pm 0.6437	52.545 \pm 3.5558	0.9795 \pm 0.05643

* - significant (p<0.05) , ** - Highly significant (p<0.005) ,

*** - - Highly significant (p<0.001)

Table 4: Brain Parameters

Brain	NO units/ gm tissue	TBARS Nmol/mg tissue protein	SOD units/mg tissue protein	Catalase μ moles of H ₂ O ₂ utilized/min/mg tissue protein	GPx μ moles of GSH consumed/min/mg tissue protein
1 Control (no radiation) n=20	1.848 \pm 0.414	0.9630 \pm 0.032	3.670 \pm 0.477	42.499 \pm 2.506	0.6420 \pm 0.082
2 Expt.Cont (radiation) n=20	2.901*** \pm 0.604	1.357*** \pm 0.306	2.731*** \pm 0.412	41.39*** \pm 0.725	0.547*** \pm 0.026
3 deoxy D- Glu (radiation) n=20	2.827*** \pm 0.343	1.235*** \pm 0.258	3.037*** \pm 0.441	41.43*** \pm 1.373	0.604*** \pm 0.043
4 deoxy D-Glu+GS (radiation) n=20	2.638*** \pm 0.339	1.175*** \pm 0.452	3.347*** \pm 0.517	41.542*** \pm 1.238	0.541*** \pm 0.021
5deoxy D-Glu+GT (radiation)n=20	2.74*** \pm 0.607	1.128*** \pm 0.339	3.148*** \pm 0.334	41.568*** \pm 1.580	0.547*** \pm 0.023
6deoxy D-Glu+Gin (radiation) n=20	2.651*** \pm 0.711	1.142*** \pm 0.260	3.344*** \pm 0.321	42.07*** \pm 1.567	0.611*** \pm 0.292
7 deoxy D-Glu+GS+GT (radiation) n=20	2.651*** \pm 0.304	1.168*** \pm 0.431	3.357*** \pm 0.60	42.22*** \pm 1.63	0.556*** \pm 0.028
8deoxy D-Glu+GS+Gin (radiation) n=20	2.534*** \pm 0.344	1.135*** \pm 0.214	3.477*** \pm 0.632	42.394*** \pm 1.943	0.634*** \pm 0.025
9deoxy D-Glu+GT+Gin (radiation) n=20	2.533*** \pm 0.767	1.143*** \pm 0.284	3.59 \pm 0.503	43.033 \pm 1.783	0.646 \pm 0.036
10 deoxy D-Glu+GS+GT+Gin (radiation) n=20	2.174 \pm 0.915	1.100 \pm 0.648	4.872 \pm 0.623	45.587 \pm 1.986	0.7460 \pm 0.037

* - significant (p<0.05) , ** - Highly significant (p<0.005) ,

*** - - Highly significant (p<0.001)

Table 5: Micronucleus formation

	1Control (no radiation) n=20	2Expt. Control (radiation) n=20	3deoxy D- Glu (radiation) n=20	4deoxy D-Glu + GS (radiation) n=20	5deoxy D-Glu + GT (radiation) n=20	6deoxy D-Glu + Gin (radiation) n=20	7deoxy D-Glu + GS + GT (radiation) n=20	8deoxy D-Glu + GS + Gin (radiation) n=20	9deoxy D-Glu + GT + Gin (radiation) n=20	10deoxy D-Glu + GS + GT + Gin (radiation) n=20
No. of Micronucleated cells / 1000 cells	0.85	14.19**** \pm 5.5	4.5** \pm 3.0	2.18* \pm 1.0	2.1* \pm 1.88	1.8 * \pm 1.1	2.55* \pm 1.80	2.35* \pm 1.62	3.45* \pm 2.1	1.55 \pm 1.23

* - significant (p<0.05) , ** - Highly significant (p<0.005) ,

*** - - Highly significant (p<0.001)

combines with 2- deoxy D- glucose + Camellia sinensis + extract+ Zingiber officinale extract on exposure of radiation is found in the present study.

But the concentration of blood, liver, kidney and brain nitric oxide, activity of blood lipid peroxidation is found significantly non-significant (p>0.05) in group 3 animals which are on normal diet + 2- deoxy D- glucose treatment when it compares with group 4, group 5, group 6, group 7, group 8, and group 9 animals which are on their respective diet and exposure of radiation and also there is no significant increased (p>0.05) or decreased levels of antioxidant enzymes is found in the present study when it

compares with other group of animals from group 4 to group 9, except group number 10 animals.

But the concentration of blood, liver, kidney and brain nitric oxide, activity of blood lipid peroxidation is found significantly decreases (p<0.001) in group 10 animals which are on normal diet combines with 2- deoxy D- glucose + Vitis vinifera extract + Camellia sinensis extract+ Zingiber officinale extract , as compared to other group of animals. The activities of antioxidant enzymes viz. superoxide dismutase, catalase and glutathione peroxidase in group 10 animals is found significantly increased (p<0.001) as compared to normal control group 1 animals

, and other group of animals which are on their respective diet and exposure to radiation, which suggests the radio protective effect of these antioxidant medicinal plants when all these three plants combines with 2 – deoxy D- glucose and fed orally. Reduced activities of the antioxidant enzymes may result in deleterious effects due to the accumulation of superoxide radical and hydrogen peroxide.

Treatment with the combination of selected antioxidant medicinal plant extracts provides recovery of the superoxide dismutase, catalase and glutathione peroxidase levels, indicates radio protective effects of these selected antioxidant medicinal plants.

From Table 5, the radiation induced micronucleus formation, formation of metamyelocytes and dysplastic myeloid series cells formation is found highly increased ($p < 0.001$) in group 2 animals, which are on normal diet and radiation exposure as compared to animals of group 1 which are on normal diet but not exposed on radiation.

The micronucleus formation, formation of metamyelocytes and dysplastic myeloid series cells formation is also found increased ($p < 0.01$) in group 3 animals which are on a diet having 2 - deoxy-D glucose and exposure of radiation as compared to animals of group 1 which are on normal diet but not exposed on radiation.

But the micronucleus formation is found not significantly increased ($p > 0.05$) in all groups, which are on normal diet with feeding of deoxy- D glucose and combination of one or more selected antioxidant medicinal plant extracts

Significantly low level of ($p < 0.001$) radiation induced micronucleus formation, formation of metamyelocytes and dysplastic myeloid series cells formation is found in group 10 animals, which are on normal diet combines with 2- deoxy D- glucose (200 mg / kg of body weight)+ Vitis vinifera extract (100 mg/kg body weight)+ Camellia sinensis L. extract (100 mg/kg body weight) + Zingiber officinale (100 mg/kg body weight) extracts. It suggests the radioprotective efficacy of these selected medicinal antioxidant plant extracts in combination of 2 – deoxy D- glucose against radiation induced damage to the tissues.

The present study favors the study carried out by M.Mohanasaundari, M. Sabesan & S. Sethupathy³⁶ Indrani Singha & Subir kumar Das³⁷ for grape seed extract, Dwarkanath B.S. et al² for diet having deoxy D- glucose, Lu QY et.al,³⁸ Chan E.W.C²⁷ and Sneha Hande et. Al.²⁸ for green tea extract and Jamal Akhtar Ansari et al⁵ ginger extract alone. The present study also favours the study of Wu SG (39) and Gordon , M.F et al.²⁶ High rate of glucose utilization by cells correlates with poor prognosis of several types of malignant tumours. 2- deoxy -D glucose, a glucose analog and glycolysis inhibitor enhances radiation induced damage selectively in tumour cells while protecting normal cells (2) favours present study.

It can be experimented on humans as placebo.

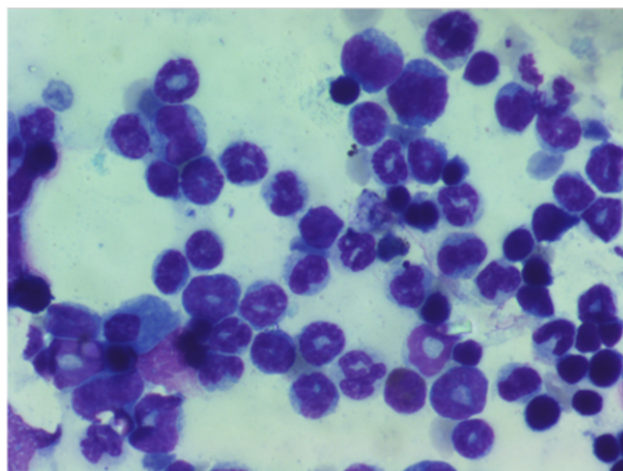


Fig. 1: Dysplastic myeloid series cells

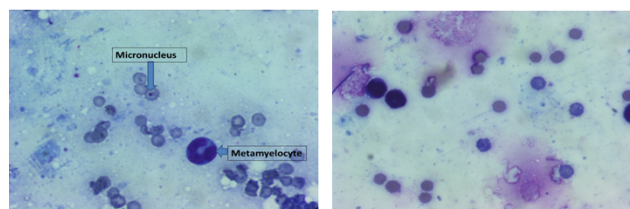


Fig. 2: Micronucleus Formation

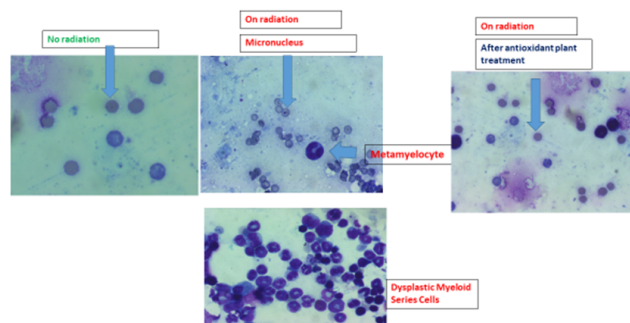


Fig. 3:

3. Conclusion

In the present study, it is found that after whole body exposure of gamma radiation, the lipid peroxidation and nitric oxide concentration significantly increases and concentration of antioxidant enzymes significantly decreases in a group of animals which are on normal diet and it is also seen that radiation induced micronucleus formation, formation of metamyelocyte and dysplastic myeloid series cell formation is also significantly increased in this group of animals in the present study when it compared with control group 1 animals, which are on normal diet and they are not exposed on radiation. But by feeding deoxy – D glucose with combination of

selected antioxidant medicinal plant extracts significantly decreases lipid peroxidation and nitric oxide level while the activities of antioxidant enzymes significantly increases. The highest effect is found in a group of animals which are on combination of normal diet and feeding of deoxy-D glucose with grape seed extract, green tea extract and ginger extract in proportion of 1:1:1, in this group of animal's radiation induced micronucleus formation, formation of metamyelocytes and dysplastic myeloid series cells formation is also found significantly decreased.

In the present study, it is found that there is strong correlation between the antioxidant activity of the plants, reducing power of the plants, content of total flavanols in individual plant and their combination as reported by Khandu Hotkar³⁸ and radioprotective effect.

From these observations, it is concluded that before and during radiation therapy, if the regular diet combines with deoxy-D glucose and mixture of selected medicinal plants like grape seed, green tea and ginger extracts have great beneficial effect in relieving the side effects of radiation therapy.

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3.2. Compliance with ethical standards

3.3. Informed consent

Present study does not relate to human studies.

3.4. Ethical approval

For the present study, all the applicable international, national and/or institutional guidelines for the care and use of animals were followed with the help of veterinarian, animal house, PIMS, Loni. The study protocol was approved by Institutional Animal Ethical Committee, Rural Medical College, Loni.

4. Source of funding

None.

5. Conflict of interest

None.

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