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RADIOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF SEEDS OF *DRYPETES ROXBURGHII* WALL. IN SWISS ALBINO MICE EXPOSED TO ELECTRON BEAM RADIATION

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ABSTRACT

Ionizing radiation acts as an important source for the generation of free radicals in the biological system which results in several pathological conditions. Ayurveda, traditional Indian system of medicine, has described several medicinal plants for the protective action against radiation induced damage. The present study was aimed at assessing the radioprotective activity of ethanolic extract of seeds of *Drypetes roxburghii* Wall. (Family: Euphorbiaceae), against electron beam irradiation on mice which was evaluated by 30 days survival study followed by antioxidant biochemical assays. Pretreatment with 400 mg/kg of ethanolic extract of *Drypetes roxburghii* Wall. delayed the onset of mortality and increased the survival rate (upto 40%) compared to irradiation control group. There was an increase in lipid peroxidation (LPO) activity *in-vivo* in irradiation control group which was attenuated by the extract treatment.. The glutathione-S-transferase (GST), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and glutathione reductase (GR) activities were significantly increased in extract group compared to irradiation control group. There was significant reduction in oxidative stress due to radiation exposure *in-vitro* to mitochondrial fraction with extract treated group was observed. The treatment with extract also showed *in-vitro* free-radical scavenging activity in a concentration dependent manner in DPPH, ABTS, Total Antioxidant Capacity and H_2O_2 assays. Thus our finding shows that 400

mg/kg of ethanolic extract of seeds of *Drypetes roxburghii* Wall. has potent radioprotective activity which may be due to antioxidant and free-radical scavenging property of the extract.

Keywords: Electron beam radiation, *Drypetes roxburghii* Wall., radioprotective, antioxidant, free-radical scavenging.

INTRODUCTION

In the present electronic era, Ionizing radiation has a wide spread application in medicine, agriculture, energy, food storage, power generation and pharmaceutical industry. In medicine, especially in the therapy of cancer, radiotherapy is commonly used as a component of therapy for a wide range of malignant conditions along with chemotherapy and surgery. It is estimated that half of all cancer patients will receive radiotherapy during the course of their treatment for cancer [1]. During this therapy along with the cancer cells, normal cells also are exposed to irradiation which may lead to serious side effects. So it is important to protect the normal cell from ill effects of radiation by using radioprotective agents which are less harmful. Exposure to radiation during war and industrial accidents is a distinct possibility as exemplified by Fukushima nuclear disaster in 2011.

When ionizing radiation falls on biological system it plays an important role in the generation of free radicals and reactive oxygen species (ROS) like O_2^-, OH^-, H_2O_2, etc [2]. These radiation mediated free radical generation interferes with various physiological activities which results in several pathological conditions like oxidative stress, genetic, haemopoetic abnormalities and extensive tissue injury. The radiation generated free radicals mainly react with key biomolecules like deoxyribonucleic acid (DNA), lipids and proteins inhibiting the proliferation of cells leading to apoptosis [3]. Due to the strong evidence for the generation of free radicals during radiation injury, an antioxidant may provide protection against such injury. In an attempt to discover an ideal radioprotectant drug, several compounds have been synthesized in the recent past. Some of the compounds having thiol group are found to be good free radical scavengers and are effective against radiation induced damage [4], but these compounds exhibited high toxicity at the optimum radioprotective dose [5].

Traditional Indian system of medicine, has described various medicinal plants for the treatment of free radical-mediated ailment; therefore, it is logical to expect such plants may render protective action against radiation induced damage. Hence, the development of less toxic herbal radioprotective agents with extended window of protection has gained attention.

An extensive survey of literature has revealed that a large number of plants have Phytochemical containing thiol moiety. *Drypetes roxburghii* Wall. (Family: Euphorbiaceae) is an indigenous to Western Ghats and locally known as Putranjiva, is a deciduous, evergreen tree and traditionally used in cold, fever, rheumatism and inflammation [6,7]. Seeds contain fatty oil and kernel contains an essential oil with mustard smell, isothiocyanate yielding glycosides, glucoputranjivin, glucocochlearin, glucojiaputin and glucocleomin. The essential oil contains isopropyl and 2-butyl isothiocyanates as the main constituents and 2-methyl-butyl isothiocyanate as a minor component [6]. The studied have confirmed the antimicrobial, anthelmintic, anti-inflammatory, cytotoxic, antioxidant and free radical scavenging *in-vitro* activities of the plant [7-10].

To best of our knowledge, no study has been carried out to assess the radioprotective activity of *Drypetes roxburghii* Wall. Hence we have undertaken the present investigation to evaluate the radioprotective potential of ethanolic extract of seeds of *Drypetes roxburghii* Wall. against electron beam irradiation.

MATERIALS AND METHODS

Chemicals

2-mercaptopropionylglycine (MPG), DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis(3-ethylbenzthiozoline-6-sulphonic acid) were procured from Sigma Aldrich, USA. Thiobarbituric acid, Trichloroacetic acid and all other chemicals were of analytical grade and were procured from Himedia, Mumbai, India.

Plant material

The seeds of *Drypetes roxburghii* Wall. was collected from the surrounding regions of Western Ghats from Deralakatte, Mangalore and Vitla, Dakshina Kannada (Dist), Karnataka. The plant material was authenticated by Dr. Nagalaxmamma, Professor & Head, Dept. of Biotechnology (UG), St. Aloysius College, Mangalore. The herbarium specimen of the plant materials was retained in Department of Pharmacognosy, NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Karnataka, India. The selected plant material was chopped in to small pieces & dried under shade, coarsely powdered and subjected to ethanolic soxhlet extraction. The extract thus obtained was concentrated in flash evaporator to a syrupy consistency and stored in desiccators.

Preliminary phytochemical screening:

The preliminary phytochemical tests of extract were performed by standard methods described in Practical Pharmacognosy by Dr. C.K. Kokate and K.R. Khandelwal [11].

Experimental animals

All the experiments were carried out using wistar rats, 250±20 g and Swiss albino mice, 20±2 g. Animals were kept in the animal house of NGSM Institute of Pharmaceutical Sciences, Mangalore under controlled conditions of temperature (23±2ºC), humidity (50±5%) and 12 hour light-dark cycle. Animals were fed pellet diet (Venkateshwara enterprises, Bangalore) and water *ad libitum*. All the animals were acclimatized for seven days before the study. The experimental protocol was approved by institutional animal ethical committee (Approval No.KSHEMA/AEC/02/2012, Date-14-06-2012)

Acute toxicity test

Acute toxicity of ethanolic extract of *Drypetes roxburghii* Wall. was carried out as per OECD guidelines 425 [12].

Experimental design

***In-vivo* Survival study**

Animals (Swiss albino mice) were pretreated with 200 & 400 mg/kg doses of *Drypetes roxburghii* Wall. (ethanolic extract) and 20 mg/kg dose of standard 2-mercaptopropionylglycine (MPG) for 5 days. After 30 min of last dose, animals were exposed to irradiation (6 Gy of electron beam radiation). The 30 days survival study [which is expressed as % of survival] and determination of Dose Reduction Factor (DRF) of extract was carried out.

The radioprotection was quantified by using biochemical assays after 1, 7 and 30 days of post irradiation. Animals from each groups were sacrificed and the biochemical assays like lipid peroxidation (LPO), glutathione-S-transferase (GST), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and glutathione reductase (GR) and were conducted [13-19].

***In-vitro* studies**

Isolation of mitochondrial fraction from rat liver and exposure for inducing oxidative stress

Mitochondrial fraction from rat liver was prepared by using female Wistar rats (250 g). Rat livers were isolated and homogenized in 0.25 M sucrose containing 1 x 10^{-3} mol dm^{-3} EDTA. Then the homogenate was centrifuged at 3000 x g for 10 min. the mitochondria was sedimented from the above supernatant by centrifugation at 10,000 x g for 10 min. Then the pellet was washed thrice with 5 mM potassium phosphate buffer, pH 7.4, to remove sucrose. Protein was estimated and pellets were suspended in the above buffer at the concentration of 10 mg protein/ml.

Oxidative damage was induced by exposure of mitochondrial fraction to the radiation source at the dose of 450 Gy. The mitochondrial fraction (final concentration 2 mg/ml) were suspended in 5 Mm phosphate buffer (pH 7.4) and exposed to radiation with and without *Drypetes roxburghii* Wall. extract (final concentration of 0.1 %). The unexposed samples to radiation served as normal controls. After exposure of mitochondrial fraction to oxidative stress by irradiating the radiation, the products of lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) [20].

Free-radical scavenging activity [21,22]

DPPH, ABTS, total antioxidant capacity and hydrogen peroxide scavenging assay were carried out with different concentrations of selected plants extract *in-vitro* for free-radical scavenging activities.

DPPH radical scavenging assay

Mix 1 ml of DPPH (0.1 mM in methanol) solution with 3ml of sample solutions in water at different concentrations; incubate at 30°C for 30 min in the dark, measure the absorbance at 517 nm.

Calculate DPPH radical-scavenging activity by the following equation.

% Inhibition = (A0 - A1) / A0 × 100

Where, A0 = absorbance of the control (without extract),

A1 = absorbance in the presence of the extract

ABTS radical scavenging assay

Keep the mixture (1:1, v/v) of ABTS (7.0 mM) and potassium persulfate (4.95 mM) over night at room temperature in dark to form radical cation ($ABTS^{+}$). Dilute the working solution with phosphate buffer solution and adjust the absorbance values between 1.0 and 1.5 at 734 nm. Mix aliquot (0.1 ml) of each sample with the working solution (3.9 ml) and the decrease of absorbance is measured at 734 nm after 10 min at 37°C in the dark against appropriate blank. Calculate the percentage inhibition.

% Inhibition = (A0 - A1) / A0 × 100

Where, A0 = absorbance of the control (without extract),

A1 = absorbance in the presence of the extract

Total Antioxidant Capacity

Dissolve 0.1 ml of the extract (10 mg/ml) in water and mix with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Incubate at 95°C for 90 min. After cooling to room temperature, measure the absorbance of the mixture at 695 nm against appropriate blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.

Hydrogen peroxide scavenging assay

Prepare Hydrogen peroxide solution (2 mM/L) with standard phosphate buffer (pH 7.4). Add different concentration of the aqueous extracts to 0.6 ml of hydrogen peroxide solution. Measure the absorbance at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and calculate the percentage inhibition of different concentrations of the extracts comparing with the standard, ascorbic acid.

Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.) of 6 animals per experimental group. Parametric one way analysis of variance (ANOVA) followed by Tukey's post test. Statistical analyses were performed using Graph pad prism 5.0. The minimal level of significance was identified at $P < 0.05$.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of the ethanolic extract of *Drypetes roxburghii* Wall. reveals the presence of alkaloids, carbohydrates, proteins, flavanoids and tannins. (Table 1)

Table 1 Qualitative preliminary phytochemical test

Sl. No	Tests	Inference
1	**Alkaloids**	
	a) Dragendorff's test	-ve
	b) Hager's test	+ve
	c) Wagner's test	+ve
	d) Mayer's test	+ve
2	**Carbohydrates**	
	a) Molisch's test	+ve
	b) Benedict's test	+ve
	c) Fehling's test	+ve
	d) Selwinoff's test	-ve
3	**Proteins**	
	a)Biuret test	-ve
	b)Million's test	-ve
	c)Xanthoprotein test	-ve
	d)Protein with sulphur	+ve
	e)Ninhydrin	+ve
	f)Cystein	+ve
4	**Flavanoids**	
	a)NaoH	+ve
	b)Lead acetate	+ve
5	**Glycosides**	
	a)Keller Killiani test	-ve
	b)Modified Borntrager's test	-ve
6	**Triterpenoids**	
	a)Liebermann–Burchard test	-ve
7	**Saponins**	-ve
8	**Steroids**	
	a)Liebermann-Burchard test	-ve
	b)Salkowski reaction	-ve
9	**Tannins**	+ve

+ve = Positive/present, -ve = Negative/absent

Acute toxicity study: The ethanolic extract of *Drypetes roxburghii* Wall. was found to be safe upto 2000 mg/kg dose. Screening of the extract was done with two dose levels- 10% of the maximum safe dose (200 mg/kg) and twice that dose (400 mg/kg) in the present study.

Survival study

The data on survival of the animals exposed to the sub lethal dose of 6 Gy whole body electron beam radiation is presented in figure 1. Mortality of the irradiated animals commenced on the 5th day of post radiation in control irradiated group, 200 mg/kg doses of *Drypetes roxburghii* Wall. and 20 mg/kg dose of standard 2-MPG treated groups whereas, mortality was started on 7th day in 400 mg/kg doses of *Drypetes roxburghii* Wall. treated group.

The death of animals continued at a slower rate in *Drypetes roxburghii* Wall. and standard 2-MPG treated groups whereas, complete mortality of the animals was observed on 15th day in the control irradiated group. The 200 mg/kg doses of *Drypetes roxburghii* Wall. treated groups showed 20% survival where as 400 mg/kg doses of *Drypetes roxburghii* Wall. and standard 2-MPG treated groups shows 40% survival at the end of the 30 days survival study.

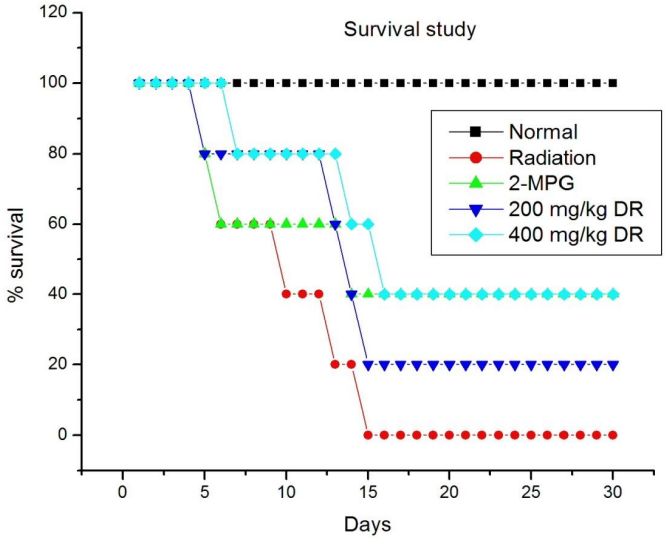

Figure 1 Survival study of *Drypetes roxburghii* Wall.

DR=Drypetes roxburghii Wall. and 2-MPG=2-mercaptopropionylglycine

DRF

The efficacy of a radioprotector is expressed in terms of dose reduction factor (DRF). DRF is evaluated by plotting the percentage survival at the end of 30 days survival studies against different doses of radiation.

$$DRF = \frac{\text{Radiation LD50 in the presence of the protector}}{\text{Radiation LD50 in the absence of protector}}$$

Where, LD_{50} is the lethal dose of radiation causing 50% death in animals.

DRF of *Drypetes roxburghii* Wall. was found to be 1.132.

Biochemical assays

The various biochemical assays results were tabulated in Table 2.

Lipid peroxides (LPO) assay (µmol/g)

There was significant (P<0.01) increase in LPO activity of radiation control group compared to normal control group on day 1 and day 7 of post irradiation. At day 1 of post irradiation the standard 2-MPG and 400 mg/kg dose of *Drypetes roxburghii* Wall. showed significant (P<0.05) decrease in LPO activity compared to radiation control. The standard 2-MPG and 400 mg/kg dose of *Drypetes roxburghii* Wall. groups shows significant (P<0.001) decrease in LPO activity where as the 200 mg/kg dose of *Drypetes roxburghii* Wall. shows significant (P<0.01) decrease compared to radiation control on 7th day of post irradiation. At 30th day of post irradiation (except the radiation control animals which were died) all treated groups shows the LPO activity was restoring towards the normal values.

Glutathione-S-Transferase (GST) assay (µ moles of GSH-CDNB conjugate formed/min/mg protein)

At 1st and 7th day of post irradiation the GST levels were significantly (P<0.001) diminished in radiation control group compared to normal control where as the standard 2-MPG and extract treated groups shows significant (P<0.001) increase in the GST activity. At the 30th day of post irradiation restoration of the GST level towards the normal was observed in all the treated groups.

Reduced glutathione (GSH) assay (µg/ml)

GSH levels were significantly (P<0.001) decreased in irradiated mice when compared to control mice on 1st and 7th day of post irradiation. The treated groups shows significant increase in GSH levels compared to radiation control group on 1st and 7th day of post irradiation and were restoring towards to the normal levels at day 30 of post irradiation.

Superoxide dismutase (SOD) assay (U/mg of protein)

There was significant (P<0.001) decrease in the SOD activity in radiation control group compared to normal control at 1st and 7th day of post irradiation. The all treated groups shows increase in SOD activity at 1st and 7th day of post irradiation and at 30th day of post irradiation the SOD levels of all treated groups were restoring towards to the normal levels.

Table 2 Effect of *Drypetes roxburghii* Wall. on various biochemical assays on mice liver

	Day	Normal control	Radiation control	Std 2-MPG	*Drypetes roxburghii* 200mg/kg	*Drypetes roxburghii* 400mg/kg
LPO activity (µmol/g)	1	0.488±0.134 ---	0.882±0.019 **	0.571±0.011 #	0.684±0.053	0.584±0.048 #
	7	0.436±0.042	4.262±0.207 ***	2.465±0.118 ###	3.145±0.214 ##	2.909±0.110 ###
	30	0.435±0.038 --	---	0.627±0.047 --	0.716±0.025 --	0.692±0.011 --
GST activity (µ moles of GSH-CDNB conjugate formed /min./mg protein)	1	39.79±0.421 ---	31.25±0.603 ***	37.84±0.367 ###	34.55±0.151 ###	35.27±0.091 ###
	7	40.76±0.390 ---	14.75±0.544 ***	32.74±0.840 ###	21.70±0.340 ###	23.40±0.302 ###
	30	39.72±1.024 --	---	34.86±0.393 --	29.68±0.434 --	30.97±0.458 --
GSH activity (µg/ml)	1	240.0±2.887 ---	202.0±2.309 ***	230.7±2.728 ###	217.7±3.283 #	225.0±2.887 ###
	7	222.3±3.712 ---	144.3±3.756 ***	200.3±6.119 ###	164.7±2.906 ---	177.3±4.333 ###
	30	233.7±3.756 ---	---	217.3±1.453 ---	174.7±2.603 ---	179.7±1.764 ---
SOD activity (U/mg of protein)	1	6.50±0.230 ---	3.10±0.173 ***	4.93±0.088 ###	3.90±0.057 ##	4.46±0.068 ###
	7	6.53±0.120 ---	2.10±0.173 ***	4.00±0.251 ###	2.73±0.145 ---	3.46±0.088 ###
	30	6.53±0.120 ---	---	5.70±0.057 ----	4.40±0.208 ----	5.46±0.088 ---
GSHPx activity (U/mg of protein)	1	4.33±0.108 ---	2.30±0.094 ***	3.71±0.084 ###	3.03±0.047 ###	3.26±0.099 ###
	7	4.47±0.173 ---	2.04±0.055 ***	3.04±0.064 ###	2.43±0.059 ---	2.99±0.084 ###
	30	4.47±0.085 ---	---	4.11±0.075 ---	3.23±0.055 ---	3.73±0.052 ---
GR activity (nm of NADPH consumed/min/mg of protein)	1	0.348±0.012 ---	0.140±0.008 ***	0.262±0.006 ###	0.175±0.008 ----	0.215±0.011 ###
	7	0.342±0.015 ---	0.114±0.005 ***	0.232±0.006 ###	0.155±0.008 #	0.199±0.005 ###
	30	0.335±0.006 ---	---	0.278±0.008 ---	0.202±0.005 ---	0.225±0.005 ---

*Each value is expressed as mean ± SEM for 6 animals in each group. One-way ANOVA followed by Tukeys post test. *** P<0.001 w.r.t normal control group. ### P<0.001, ## P<0.01, # P<0.01 w.r .t radiation control group.*

Glutathione peroxidase (GSHPx) assay (U/mg of protein)

There was significant (P<0.001) decrease in the GSHPx activity in radiation control group compared to normal control at 1st and 7th day of post irradiation. The all treated groups shows increase in GSHPx activity in 1st and 7th day of post irradiation and at 30th day of post irradiation the levels of GSHPx of all treated groups were towards the normal levels.

Glutathione reductase (GR) assay (nm of NADPH consumed/min/mg of protein)

There was significant (P<0.001) decrease in the GR activity in radiation control group compared to normal control at 1st and 7th day of post irradiation. The all treated groups shows increase in GR activity in 1st and 7th day of post irradiation when compared to radiation control group. At 30th day of post irradiation the levels of GR of all treated groups were towards the normal levels.

***In-vitro* studies**

Isolation of mitochondrial fraction from rat liver and exposure for inducing oxidative stress

The radiation induced lipid peroxidation products and their inhibition by *Drypetes roxburghii* Wall. (Final concentration 0.1 %) extract were shown in table 3. The mitochondrial fractions were exposed to 450 Gy. The lipid peroxides (LPO) assay showed significant (P<0.001) increase in LPO activity in radiation control group compared to normal control group whereas, the drug treated *Drypetes roxburghii* Wall. and standard 2-MPG groups showed significant (P<0.001) reduction in LPO activity compared to the radiation control group.

Table 3 Effect of *Drypetes roxburghii* Wall. on *in-vitro* LPO activity (µmol/g) in rat liver mitochondria.

Normal control	Radiation control	*Drypetes roxburghii*
0.629±0.035 --	1.746±0.089 ***	0.885±0.112 ###
--	P<0.001	P<0.001

*Each value is expressed as mean ± SEM for 6 samples in each group. One-way ANOVA followed by Tukeys post test. *** P<0.001 w.r.t normal control group. ### P<0.001 w.r.t radiation control group.*

Free-radical scavenging activity

DPPH radical scavenging assay

In DPPH assay, *Drypetes roxburghii* Wall. extract showed 58.51±0.28% of DPPH inhibition whereas ascorbic acid shows 87.23±1.76% inhibition. The IC_{50} of ascorbic acid and *Drypetes roxburghii* Wall. Were found to be 1.97µg/ml and 8.42 µg/ml respectively. [Figure 2]

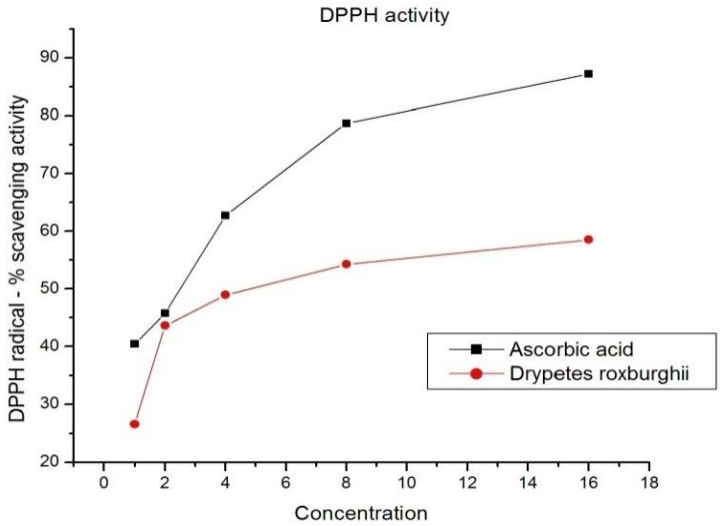

Figure 2 DPPH radical scavenging activity of *Drypetes roxburghii* Wall.

ABTS radical scavenging assay

Ascorbic acid shows 68.33±0.19% inhibition. *Drypetes roxburghii* Wall. extract showed 49.01±0.05% of ABTS inhibition. The IC_{50} of ascorbic acid was 9.07µg/ml whereas *Drypetes roxburghii* Wall. shown 15.82 µg/ml. [Figure 3]

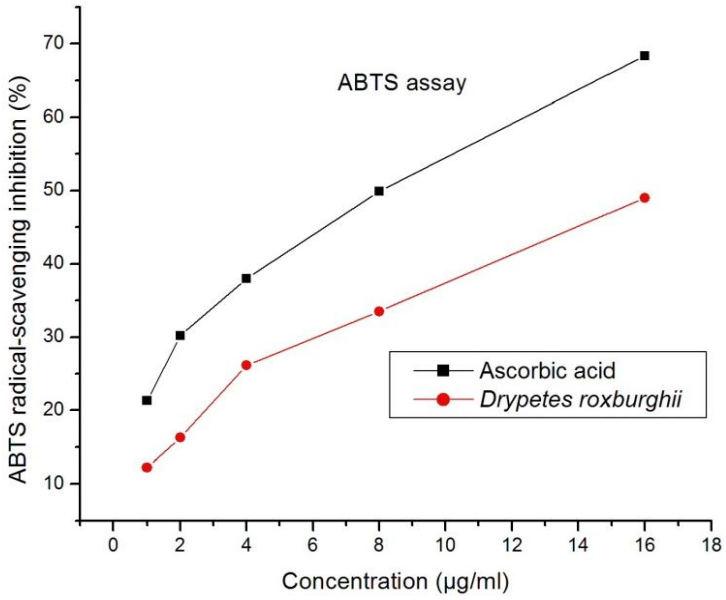

Figure 3 ABTS radical scavenging activity of *Drypetes roxburghii* Wall.

Total Antioxidant Capacity

In total antioxidant capacity assay, it was found that 10 mg of *Drypetes roxburghii* Wall. was equivalent to 60.8 µg of ascorbic acid.

Hydrogen peroxide scavenging assay

Ascorbic acid shows 52.61±0.56% inhibition whereas *Drypetes roxburghii* Wall. extract showed 40.10±0.05% of hydrogen peroxide scavenging inhibition. The IC_{50} of ascorbic acid was 10.60 µg/ml and *Drypetes roxburghii* Wall. shown 19.88 µg/ml. [Figure 4]

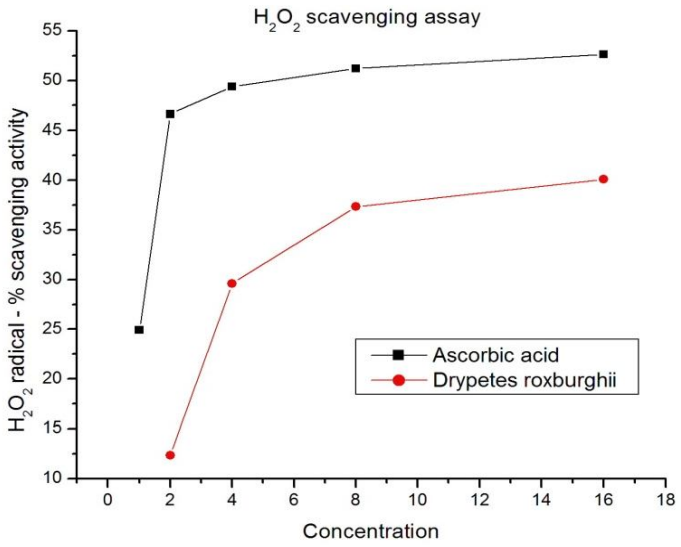

Figure 4 Hydrogen peroxide scavenging activity of *Drypetes roxburghii* Wall.

DISCUSSION

Traditional Indian system of medicine, Ayurveda denotes many medicinal plants which have beneficial therapeutic effects. However, they received very little attention towards radioprotective as well as antioxidant activities. Herbal radioprotectors have been gaining prime importance in radioprotective drug discovery due to lesser side effects which has been reviewed extensively [23]. Ionizing radiation is known to induce oxidative stress through generation of reactive ROS. The mechanisms implicated in the protection of cells by radioprotectors include free radical-scavenging, that protects against ROS generated by ionizing radiation, thus reducing the harmful effects of ionizing radiation [24]. The synthetic compounds having thiol group are found to be good free-radical scavengers and are effective against radiation induced damage. However, these compounds produce serious side effects and were toxic at the doses required for radioprotection [4]. Hence, there is a need for the development of less toxic herbal radioprotective agents.

In the present study we selected potent medicinal plant *Drypetes roxburghii* Wall. (Family: Euphorbiaceae) which is available in Western Ghats containing thiol/sulphur moiety [7-10]. From the 30 days survival study the 400 mg/kg dose of ehanolic extract of *Drypetes roxburghii* Wall. shows 40% survival rate. Hence the pretreatment of the extract documented the survival advantage to the mice exposed to sub lethal dose of 6 Gy of electron beam radiation which was comparable to the standard 2-MPG.

Lipid peroxidation is a good biomarker of damage to cell membrane which primarily occurs due to ionizing radiation through the generation of free-radicals and hence the inhibition of lipid peroxidation is suggestive of radioprotective action [25]. The *Drypetes roxburghii* Wall. treated group shows significant decrease in LPO activity in mice liver which indicates the reduction of liver cell damage. The free-radical scavenging enzymes such as GST, GSH, SOD, GSHPx and GR, which are the first line of defense against free-radicals generated oxidative injuries, were significantly increased with *Drypetes roxburghii* Wall. extract treatment which reflects the potent antioxidant activity *in-vivo*. Hence the radioprotective effect of *Drypetes roxburghii* Wall. extract can be attributed to the antioxidant properties.

The oxidative damage to mitochondria can also lead to membrane permeability transition, cytochrome c release and dysfunction of mitochondria associated with decrease in membrane potential, respiratory control, apoptosis etc [26]. Our results showed that *Drypetes roxburghii* Wall. extract when present during radiation exposure with mitochondrial fraction, prevent the damage by significant decrease TBARS formation, thus it reduces the radiation-induced damage to cellular biomolecules.

Drypetes roxburghii Wall. shows concentration dependent *in-vitro* free-radical scavenging activity in DPPH, ABTS, total antioxidant capacity and hydrogen peroxide scavenging assays, which were attributed due to the presence of thiol/sulphur moiety in the ethanolic extract.

CONCLUSION

From the present study it may be concluded that 400 mg/kg dose of ethanolic extract of *Drypetes roxburghii* Wall. shows a potent radioprotective activity in the irradiated mice. The antioxidant and free-radical scavenging appears to be important mechanisms of radioprotection. Further investigations are essential for the promotion of *Drypetes roxburghii* Wall. as a radioprotector in human applications (Clinical studies).

CONFLICT OF INTEREST: The author(s) declare(s) that they have no conflicts of interest to disclose.

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