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Radioprotective Potential of an Herbal Extract of *Tinospora cordifolia*

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Radioprotection/*Tinospora cordifolia*/Cell proliferation inducer/Micronuclei/Immunostimulator/Herbal radioprotector.

A preparation of *Tinospora cordifolia* (RTc) administered i.p. (200 mg/kg b.w.) to strain "A" male mice 1 h before whole body gamma-irradiation was evaluated for its radioprotective efficacy in terms of whole body survival, spleen colony forming units (CFU), hematological parameters, cell cycle progression, and micronuclei induction. Preirradiation treatment with RTc rendered 76.3% survival (30 days), compared to 100% mortality in irradiated control and prevented radiation induced weight loss. On 10th postirradiation day, the endogenous CFU counts in spleen were decreased with increasing radiation doses 12.0 (5 Gy), 2.16 (7.5 Gy) and 0.33 (10 Gy) but pre-irradiation administration of 200 mg/kg b.w. of RTc increased CFU counts to 31.16, 21.83 and 3.00 respectively. Pre-irradiation RTc treatment could restore total lymphocyte counts (TLC) by the 15th day to normal. It also increased the S-phase cell population that was reduced following 2 Gy irradiation in a time dependent manner. 2 Gy irradiation-induced micronuclei were also decreased by a pre-irradiation administration of RTc from 2.9 to 0.52%. Because the radioprotective manifestation of RTc observed in several systems in experimental animals can be exploited for human applications.

INTRODUCTION

Many synthetic or natural agents have been investigated in the recent past for their efficacy to protect against radiation damage.[1)] Among the natural radioprotective agents compounds, cystine, cysteamine, 5-hydroxytryptophan, 5-hydroxytryptamine, glutathione, and vitamins like A, C, and E[2)] have been extensively studied. Important synthetic molecules include amino-ethyl-isothiouronium bromide hydrobromide (AET), WR-2721. However, the inherent toxicity of these agents at the radioprotective concentration warranted further search of a safer and effective radioprotector. To reduce toxicity, a strategy of combining radioprotective molecules working through different modes of action has also been attempted.[3)] In fact, no radioprotective agent now available, either singularly or in combination, meets all the requisites of an ideal radioprotector.[4)] Recently several isolated plant products and crude extracts that may have a natural combination of several bio-active molecules capable of giving radioprotection through different mechanisms, have been investigated.[5–8)]

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Tinospora cordifolia (Family-Menispermaceae) is a glabrous, succulent, climbing shrub and is a native of India that thrives easily in plain regions. It has been widely used in the Indian system of medicine as *Rasayana* for the treatment of several ailments, such as jaundice, diabetes, rheumatoid arthritis, gout, general weakness, skin diseases, anemia, emaciation, and infections.[9)] It has also been used as a vitalizer, antistress and adaptogenic,[10)] anti-ulcer, and immunomodulatory agent.[11–15)] The aqueous extract of this plant possesses anti-inflammatory and anti-allergic[16)] properties. It has been proved to have hepatoprotective,[17)] hypolipidaemic,[18)] and anti-neoplastic properties.[19)] It improves the phagocytic and bactericidal capacity of polymorphs,[20)] protects against gastric mucosal damage,[21)] and scavenges free radicals.[22,23)]

The whole extract of *T. cordifolia* has been reported to contain several bioactive components, such as glucoside, alkaloids, bitter principle crystalline compounds,[12,24,25)] and nonditerpene furan glucosides cordifoliside A, B, and C.[25)] The bitter principles present in *Tinospora* have been identified as columibin, chasmanthin, and palmarin. The leaves are rich in calcium, phosphorus, protein, and alkaloids, such as protoberberine, tinosporide,[26)] tinosporic acid, and tinosporol. Phytohemical analysis indicated the presence of several diterpenes furan lactones, phenolic lignans, phenyl propane glycosides,[27)] and arbinogalactan.[14)]

For radioprotection, various mechanisms such as free radical scavenging, calcium channel blocking, inhibition of lipid

peroxidation, enhancement of DNA repair, and stimulation of stem cell proliferation are considered important.[28] *Tinospora cordifolia* has several of the above-mentioned properties under different experimental conditions. Therefore it became necessary to investigate its wholesome radioprotective efficacy in experimental animals in terms of whole body survival, genotoxicity, cell proliferation, and hematological parameters.

MATERIALS AND METHODS

Preparation of plant extract

The fresh stems of *Tinospora cordifolia* were collected from Maranda Distt, Kangra, Himachal Pradesh, India, at an altitude of 3,000–3,200 ft. An ethnobotanist identified the samples; the stems were thoroughly washed with water and shade dried. A known quantity of the dried material was extracted with absolute alcohol and triple-distilled water (50:50, v/v; three changes), filtered, lyophilized and stored at 4°C. The extract was tested for endotoxins, using Lal assay kit as described by the manufacturer, and was found to be free from endotoxins. It was assigned a code name RTc. For standardization purposes, the HPLC spectra of RTc extracted from three lots of *Tinospora cordifolia* obtained during different periods were compared (Fig. 1). An HPLC profile of RTc was obtained by using C-18 column (4.6 × 250 mm; Water's HPLC system), using a gradient mobile phase of 2% water in acetonitrile to 85% water in acetonitrile. The flow rate of mobile phase was kept between 0.3 to 1.5 ml/min.

Animals

Swiss albino strain 'A' male mice (10–12 weeks) weighing about 25 ± 2 g were maintained under controlled laboratory conditions (25 ± 2°C; RH 60 ± 5%; 12 h photo-period), fed standard animal food pellets (Amrut Laboratory Animal Feed, India), and tap water *ad libitum*. Animal experiments were conducted according to the guideline of the institutional ethical committee.

Administration of plant extract

The lyophilized preparation was dissolved in triple-distilled water for an administration of desired concentration, and the doses were expressed in mg/kg b. w. referring to the weight of lyophilized extract (RTc). Different doses of RTc were administered to mice through intraperitoneal (i.p.) route in a maximum volume of 0.2 ml. Control animals received 0.2 ml of normal saline.

Maximum tolerated dose (MTD)

An acute toxicity of RTc was studied in terms of percent survival, changes in behavior, alterations in neuromuscular coordination, and respiratory disorder for two days after the administration of singular doses of different concentrations of RTc. The maximum concentration of RTc, which yielded no toxic manifestation, was considered as MTD.

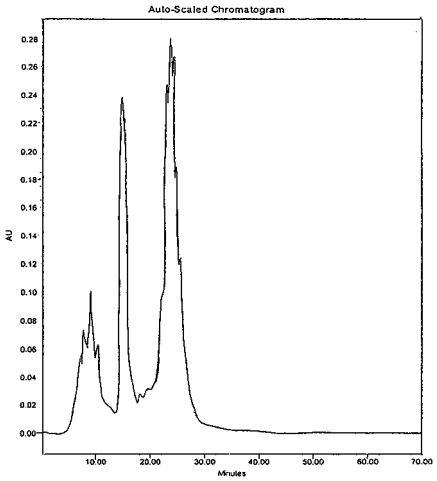

Fig. 1. An HPLC fingerprint of RTc was carried out with a C 18 column (4.6 × 250 mm). The solvent gradient was 2 to 85% water in acetonitrile, and the flow rate was maintained at 0.32 to 1.5 ml/min.

Irradiation of animals

Desired doses of gamma-radiation were delivered to mice by ^{60}Co gamma chamber (Model-220, Atomic Energy of Canada Ltd.) at a dose rate ranging from 0.64 to 0.59 Gy/min during the experimental period. Dosimetry was carried with Baldwin Farmer's secondary dosimeter and Fricke's chemical dosimetry. Each mouse was kept in a perforated plastic container and irradiated individually with a continuous supply of fresh air through a rubber pipe to avoid hypoxia.

Animal survival and body weight

The effects of time interval between administration of different concentrations of RTc and irradiation on survival and body weight of mouse were investigated. Survival was observed daily up to 30th post-irradiation day, and data was expressed as % survival. Mice used for this study were distributed into different treatment groups, including normal control, irradiated control, drug control, and drug plus radiation group. The body weights of the animals were recorded every alternate day. The change in the average weights of the animals at different time intervals due to various treatments was calculated in consideration of the initial body weight of the animal (zero day of experiment) as 100%.

Endogenous spleen colony forming unit (CFU) Assay

The mice were sacrificed by cervical dislocation on the 10th post-irradiation day in all groups. For endogenous CFU assay[29] spleens were dissected out and fixed in Bouin's solution for 24 h. Macroscopic colonies (CFU) visible to naked eyes were scored from each spleen.

Haematological studies

The mice were distributed into 4 groups:

(i) The control animals were treated with 0.2 ml normal

saline

(ii) The irradiation group animals were exposed to 10 Gy

(iii) The drug-alone group animals were administered 200 mg/kg RTc, and

(iv) The drug + irradiation group animals were irradiated (10 Gy), 1 h after an administration of 200 mg/kg RTc.

Haemoglobin and total lymphocyte counts (TLC) were studied in peripheral blood drawn from the heart of mouse on the 7th, 10th, or 15th post-irradiation day.

Micronucleus assay

The effect of 3 doses of RTc (150, 200, and 250 mg/kg b.w.) on 2 Gy induced MN formation in mice bone marrow cells was studied at 24 and 48 h time intervals as described earlier.[30] The epiphyses of femur bones were cut, and bone marrow cells were flushed out with 0.5 ml PBS into 15 ml centrifuge tubes. The cells were centrifuged once at 1,000 rpm and resuspended in a few drops of fetal calf serum. Smears of the cells were drawn on clean glass slides, fixed with methanol for 30 min, and stained with Giemsa stain. At least 2,000 cells were scored from each animal to determine the ratio of polychromatic and normochromatic erythrocytes (PCE and NCE). The number of micronucleated PCEs was expressed in a percentage value to depict the MN frequency.

Cell cycle analysis

The mice were sacrificed at 8, 24, 48, or 72 h time intervals after various treatments, and bone marrow cells were collected as described earlier.[30] Treating the cells with RBC lysis buffer containing ammonium chloride for 2–3 min removed the RBCs, and the remaining nucleated cells were fixed with 70% chilled alcohol. Approximately 1 million cells from each animal were suspended in 1 ml PBS, treated with 200 mg/ml RNase A for 30 min at 37°C, and stained with propidium iodide (50 µg/ml) for another 30 min at 37°C under dark conditions. A minimum of 10,000 cells from each sample was acquired on a FACS Calibure (Beckton, Dikinson) flow cytometer equipped with suitable optics, and the data were analyzed by using Cell-Quest software.

Statistical analysis

The data were subjected to statistical analysis, and significance was determined by use of a Student's *t*-test. The MN frequency was expressed as percentage value. The level of significance was determined by an ANOVA test; $p < 0.05$ was considered significant.

RESULTS

Maximum tolerated dose (MTD)

The single doses of RTc up to 400 mg/kg b.w. were tolerated well by mice with no adverse manifestations, except for the mice being slightly drowsy for 3 to 5 min. However, RTc dose of 500 mg/kg b.w. or above manifested a lack of activity and mortality within 2–3 post-treatment days in a dose-dependent manner. The death rate of mice treated with 500, 550, or 600 mg/kg b.w. RTc was 40, 60, and 100%, respectively. The dose of 400 mg/kg b.w. therefore was considered as MTD.

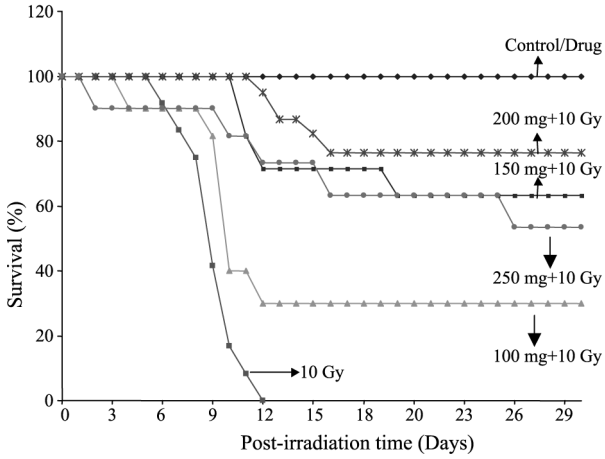

Fig. 2. The radioprotective effect of RTc was studied in terms of a 30 day survival. The mice were administered different doses of RTc 1 h before 10 Gy whole body gamma-irradiation and observed for survival until the 30th post-irradiation day. Data represented mean ± SD of three independent experiments carried out with a minimum of four animals/group each time.

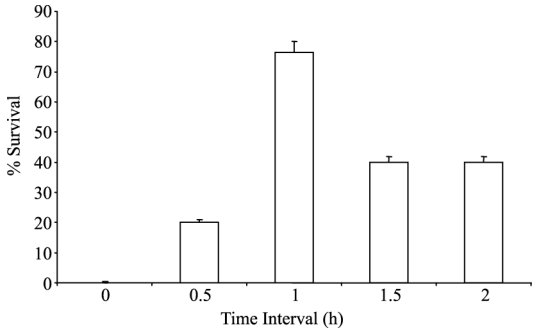

Fig. 3. The effect of time interval between the administration of RTc (200 mg/kg b.w., i.p.) and irradiation on survival in strain "A" mice. The mice were 10 Gy irradiated after various time intervals of RTc treatment and observed for 30 days. The data represented mean ± SD of three independent experiments, each group having a minimum of six animals.

Survival studies and body weight

All irradiated animals without RTc treatment died by the 12th post-irradiation day (Fig. 2). The administration of RTc (200 mg/kg b.w.) 1 h before 10 Gy whole body gamma-irradiation rendered 76.3 ± 3.71% (30 days) survival. Doses of RTc less than or more than 200 mg/kg b.w. were less effective. The effect of time intervals between the administration of RTc and irradiation was also studied in terms of 30 days postirradiation survival. For the dose of 200 mg/kg b.w of RTc, an interval of 1 h between the administration of RTc and

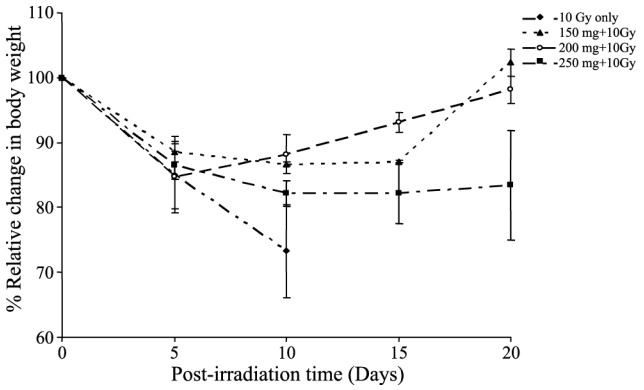

Fig. 4. The effect of RTc on radiation-induced change in the body weight of mice was studied until the 30th post-treatment day. RTc (200 mg/kg b.w.) was administered 1 h before 10 Gy irradiation, and the body weight of mice was recorded every alternate day. Normal control, RTc control, and irradiation controls were kept for comparison. The data from 3 independent experiments having a minimum of 4 animals to a group were pooled for a statistical analysis.

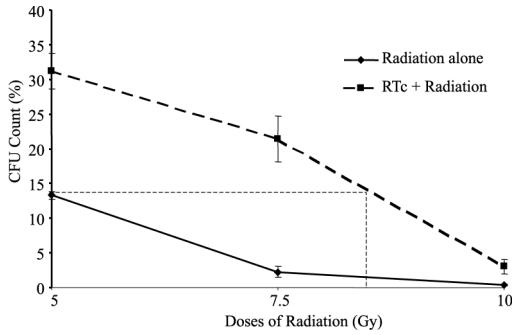

Fig. 5. CFU counts observed on the 10th post-irradiation day in the spleen of animals administered different radiation doses or 200 mg/kg b.w. of RTc, or both. The time interval between drug administration and irradiation was 1 h. Experiments were repeated thrice with a minimum of 4 animals in each group. The data represented the mean ± SD, a Students' *t*-test was applied to calculate statistical significance, and $p < 0.05$ was considered significant.

irradiation was found to be optimal (Fig. 3).

The relative change in the average weight of animals depicted in Fig. 4 demonstrated that RTc administration before irradiation allowed a recovery of the loss in body weight faster than the untreated irradiated mice.

Colony forming unit (CFU) assay

The effect of various doses of radiation on endogenous CFU and its modulation by a pre-irradiation administration of RTc (200 mg/kg b.w., –1 h) are depicted in Fig. 5. CFU counts in spleen decreased with increasing radiation doses (5, 7.5, and 10 Gy). Animals given RTc with no other treatment rendered no significant change in CFU counts. Pre-irradiation treatment with RTc rendered significantly higher ($p < 0.05$) CFU counts in comparison to the corresponding irradiated groups. An estimation of DRF (dose reduction factor) on the basis of CFU counts gave an approximate value of 1.7 (the effect produced by 5 Gy without RTc treatment could be expected with ~8.5 Gy dose in the case of pre-irradiation administration of RTc). However, a more detailed study is required to make an accurate estimation of the DRF.

Hemoglobin (Hb)

Changes in the amount of Hb in different treatment groups have been shown at different post-irradiation intervals (Fig. 6a). RTc alone depressed (statistically non-significant) the Hb level during the first week of treatment, but later it became normal. In the irradiated group Hb, decreased continuously up to the10th post-irradiation day, and the data for later periods could not be collected because all the irradiated animals had died by that time. In RTc + 10 Gy group, the Hb level decreased initially up to the 10th post-treatment day, but recovered steeply thereafter.

Total leukocyte counts (TLC)

The administrations of only a single dose of RTc (200 mg/kg b.w.) increased TLC ($5.9 \times 10^3 \pm 173.74$) up to the 7th post-treatment day, but attained control value gradually by the 10th day (Fig. 6b). Irradiation decreased TLC sharply up to the 10th day, and data for the 15th day could not be obtained because of the death of all animals by that time. Pre-irradiation treatment with RTc did not exhibited change in TLC in comparison to the irradiated group up to the 10th day. However, the recovery became evident thereafter, and the TLC values overshot the control value by the 15th day. The changes in differential leukocyte count (DLC) (lymphocyte, polymorph, and monocyte), in animals of all four groups, were similar to the changes in TLC.

Micronucleus (MN) assay

The effect of irradiation on micronucleus (MN) induction and its modification by treatment with different doses of RTc has been depicted in Table 1. MN frequency in the control group (Group-I) was 0.33 and 0.35% at 24 and 48 h respectively. Exposure to 2 Gy gamma-irradiation (Group-II) resulted in a significant ($p < 0.01$) increase in the MN frequency (2.75 and 2.9% at 24 and 48 h, respectively) in comparison to the control value. Administration of different doses of RTc alone was found to render dose dependent increase in micronuclei frequency (groups 3–6), as compared to untreated controls at all time intervals. Doses of RTc (150, 200, and 250 mg/kg b.w.) rendered 0.4, 0.45, and 0.49% MN at 24 h, respectively. At 48 h the corresponding MN frequencies were 0.43, 0.47, and 0.51%, respectively. Treatment with 150, 200, or 250 mg/kg b.w. of RTc 1 h before irradiation (groups 7–10), however significantly ($p < 0.05$), reduced the frequency of radiation-induced MN, in comparison to the radiation alone group. A pre-irradiation administration of 200 mg/kg b.w. of RTc was most effective, and it reduced the radiation-

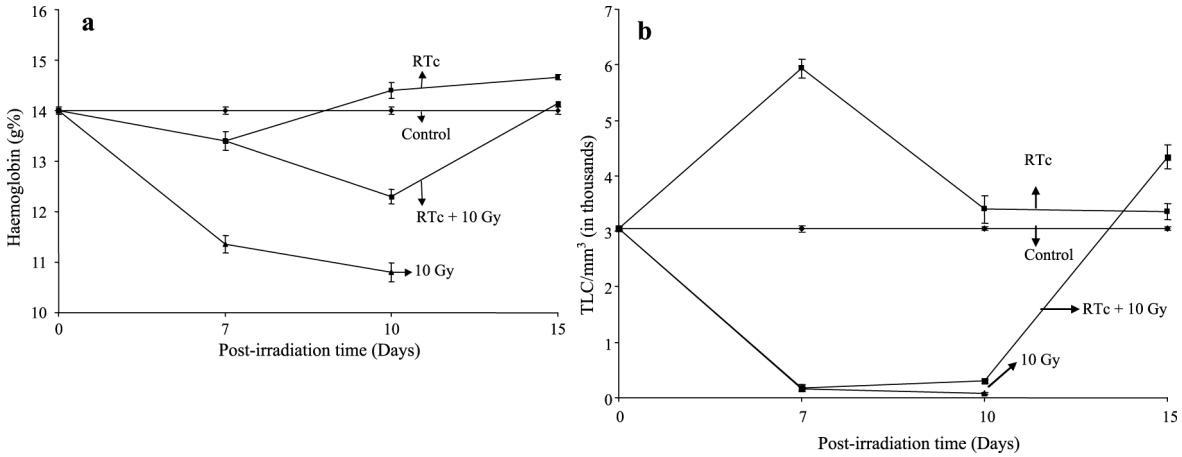

Fig. 6. The effect of RTc pre-irradiation treatment (200 mg/kg b.w) on haematological parameters was studied in strain "A" in mice. Blood was drawn from the heart of mice on the 7th, 10th or 15th post-irradiation days, and data represented the mean values of two independent sets of experiments: (a) Hb content; (b) Total leukocyte counts.

Table 1. The effect of different doses of RTc on 2 Gy-induced MN frequency and bone marrow suppression in mice. Smears of bone marrow cells were fixed with methanol and stained with Giemsa stain. At least 2,000 cells were scored from each animal. The values are mean ± SD of three independent experiments performed with three animals in each group; $p < 0.05$ was considered significant.

Treatment group	Time interval (h)	PCE/NCE ± SD	%MN frequency ± SD
Control	24	0.98 ± 0.1	0.33 ± 0.02
	48	0.98 ± 0.09	0.35 ± 0.02
2 Gy	24	0.65 ± 0.07	2.75 ± 0.08
	48	0.59 ± 0.05	2.91 ± 0.07
RTc alone			
150 mg/kg	24	0.94 ± 0.05	0.4 ± 0.01
	48	0.92 ± 0.07	0.43 ± 0.03
200 mg/kg	24	0.92 ± 0.08	0.45 ± 0.03
	48	0.91 ± 0.05	0.47 ± 0.02
250 mg/kg	24	0.89 ± 0.06	0.49 ± 0.05
	48	0.87 ± 0.9	0.51 ± 0.05
RTc + 2 Gy			
150 mg/kg	24	0.76 ± 0.07	1.1 ± 0.04
	48	0.77 ± 0.06	1.4 ± 0.06
200 mg/kg	24	0.85 ± 0.08	0.52 ± 0.02
	48	0.89 ± 0.06	0.54 ± 0.02
250 mg/kg	24	0.79 ± 0.04	0.96 ± 0.03
	48	0.8 ± 0.06	0.98 ± 0.04

induced MN frequency up to 0.52 and 0.54% at 24 and 48 h time intervals, respectively.

In the control group, the PCE/NCE ratio (Table 1) was 0.98 at both time intervals. Different doses of RTc alone reduced the PCE/NCE ratio in a dose-dependent manner. At 24 h post-treatment time, the values were 0.94, 0.92, and 0.89 for 150, 200 and 250 mg/kg b.w. of RTc, respectively. The corresponding values at 48 h were 0.92, 0.91and 0.87 respectively. Two Gy irradiation rendered significant ($p < 0.05$) bone marrow depression, and the PCE/NCE values were 0.65 and 0.59 at 24 and 48 h, respectively. A pre-irradiation administration of different doses rendered a dose-dependent increase in PCE/NCE values at all time intervals in comparison with the irradiated group. The values were 0.76, 0.85, and 0.79 at 24 h and 0.77, 0.89 and 0.80 at 48 h for 150, 200 and 250 mg/kg b.w. of RTc respectively.

Cell cycle analysis

Control group animals rendered about 12% cells in S-phase (Fig. 7). Two Gy irradiation reduced the S-phase cell population in a time-dependent manner until 24 h and reverted back to normal values by 72%. A maximum reduction in the S-phase cell population was observed at 24 h postirradiation time (8.7%). RTc at a dose of 150 mg/kg b.w. rendered no significant change in the distribution of cells in the different phases of cell cycle at any time intervals. A dose of 200 mg/kg b.w. RTc, however, increased the S-phase cell population to 14.5% at 24 h, whereas a dose of 250 mg/kg b.w. increased the G1/G0 cell population as compared to the control value. Pre-irradiation administration of 200 mg/kg b.w. RTc increased the S-phase cell population in comparison to irradiated animals at all time intervals. In this group, the S-phase fraction increased even above the control group at 72 h, and the value was about 15.5 %.

DISCUSSION

The present study revealed that a pre-irradiation administration of a single dose of RTc (200 mg/kg b.w., –1 h) rendered 76.3% survival in mice exposed to (10 Gy) whole body lethal gamma-irradiation, but irradiated mice without RTc treatment suffered from 100% mortality within 10–15 days. It also protected significantly against radiation-induced loss in body

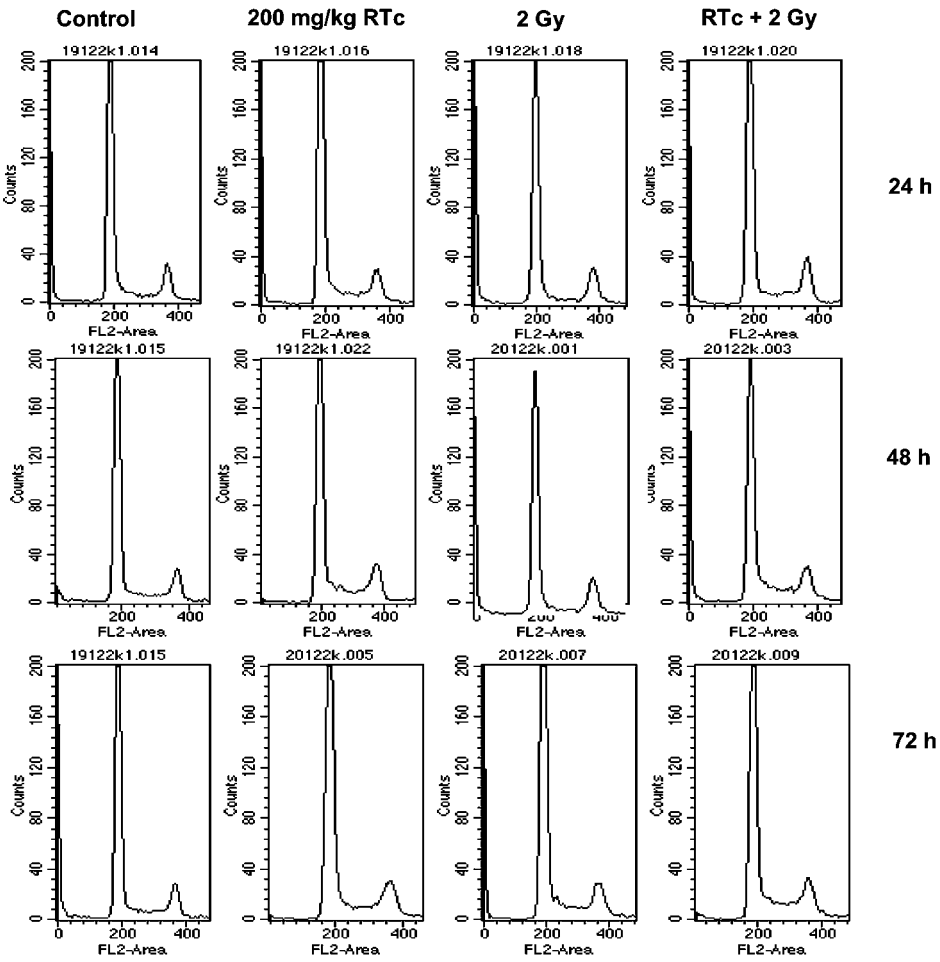

Fig. 7. The effect of RTc on 2 Gy-induced cell cycle perturbations was studied in mouse bone marrow cell flow cytometrically. Alcohol-fixed cells were treated with RNase and stained with propidium iodide to quantify the DNA content. Three animals were taken in each group, and the experiments were repeated thrice. The flowcytograms are representative of all animals in each group.

weight, with a dose of 200 mg/kg b.w. being most effective. Most of the synthetic and herbal radioprotective agents often render maximum radioprotective effect at doses approaching MTD.[7,31–33] However, RTc rendered significant radioprotection at 50% concentration of its MTD. Therefore it might render potential benefits in clinical applications. Most radiation damages arise from and interaction of the radiation-induced free radicals with the biomolecules. Molecules with the ability to scavenge free radicals, therefore, can prevent radiation damage. Since the free radicals are short-lived, it is necessary for such radioprotective molecules to be present in the cellular milieu in sufficient concentrations at the time of irradiation. RTc has already been shown to scavenge free radicals.[12] In this study, a time interval of 1 h between RTc treatment and irradiation was found to be maximally effective. Possibly the major mechanism of the action of RTc in rendering radioprotection is the scavenging of free radicals generated by irradiation, and to achieve the optimal cellular concentration of the free radical scavenging constituents of RTc it takes about 1 h.

The survival against irradiation, in fact, is a result of several factors, such as the prevention of damage through inhibition of free radical generation or efficient scavenging of free radicals, repair of DNA, membrane and other damaged target molecules, and the replenishment of severely damaged or dead cells. The recruitment of cells to substitute the apoptotic and necrotic cells could add to survival. This process significantly contributes toward the recovery of several target systems, such as bone marrow, gastrointestinal tract and skin. Chemicals or other agents, which enhance stem cell proliferation, also can therefore yield an appreciable recovery of the damaged tissue following radiation exposure[34–36] and thereby contribute to the survival. The enhancement of CFU counts in the spleen of RTc treated irradiated mice in comparison to irradiated control (Fig. 5) indicates the role of RTc in protecting the stem cells and/or stimulating the proliferation of the surviving cells. The ability of RTc to enhance cell proliferation is further supported by the cell cycle studies. RTc alone or its preirradiation administration rendered increased cell population in the S-phase (Fig. 7), indicating increased DNA synthesis. However, the cell proliferation enhancing ability of

RTc needs further confirmation. The hemoglobin content was observed to decrease in RTc alone, as well as in RTc treated irradiated animals initially. Irradiation might have caused damage to the RBCs; liver and spleen might have sequestrated the defective RBCs resulting in the initial decrease of Hb. The initial reduction in the Hb content in spite of the preirradiation treatment with RTc may also be explained on this basis. In Ayurvedic literature, *Tinospora* has been reported to be a blood purifier[37] that possibly acts by stimulating liver and spleen, which remove defective and damaged RBCs from peripheral blood circulation. The feedback mechanism, however, stimulated haemopoiesis in the bone marrow, and therefore higher Hb levels were observed on the 10th and 15th post-treatment days (Fig. 6a). RTc pre-irradiation administration rendered a significant increase in TLC that was reduced because of irradiation. The increase in CFU counts in spleen associated with the increase in TLC in RTc treated irradiated animals (Fig. 6b) in comparison to irradiated control animals indicated the immunostimulatory role of RTc and could therefore be attributed to the already known immunomodulatory constituents present in *T. cordifolia*. Since immunosupression following radiation exposure and subsequent opportunistic infections are the major drawbacks of radiation therapy, the use of RTc in radiotherapeutic applications can also be exploited. *T. cordifolia* has already been reported to contain a large number of bio-active molecules, which elicit protection against several stress and pathological conditions by acting through different mechanisms, such as antioxidant,[23] stimulation of cell proliferation,[17,38] immunomodulation1,[3,39] and anti-inflammatory activity.[16] RTc has been demonstrated to manifest antioxidant properties both *in vitro* and *in vivo*.[22,40,41] The present study rendering increased CFU counts, TLC, and survival could also be the result of the above said properties of *T. cordifolia*.

The present study dealing with different doses of RTc (Table 1) in protecting against radiation-induced genotoxicity as observed by MN frequency depicts that 200 mg mg/kg b.w doses were maximally effective. This corroborates the findings of whole body survival experiments. However, RTc alone also induced MN in bone marrow cells in a dose-dependent manner. Therefore RTc possibly renders some toxicity when present with no other stress, but renders protection against subsequent exposure to various stress conditions, a mechanism by which most flavonoids act.[42,43] It is also possible that RTc stimulates the cellular repair machinery and thereby reduces the MN frequency induced by radiation. No direct evidence in support of DNA repair enhancing efficacy by RTc is, however, presently available. Although, radiation-induced bone marrow suppression was protected by RTc, different doses of RTc alone were found to induce bone marrow suppression, indicating that the doses were toxic to the haemopoitic system. These data are corroborated by the Hb content analysis. However, its pre-irradiation administration could protect against radiation-induced bone marrow suppression that could significantly contribute to radioprotection.

The enhancement of cell proliferation, immunomodulation, stimulation of haematopoisis, and protection against radiation-induced genotoxicity together could contribute to the radioprotective efficacy of RTc. The radioprotective manifestations need to be further investigated in other model systems to assess its potential utility for human applications. The identification and characterization of individual constituents of RTc would be also a necessary step in this direction.

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