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Original Article

Ficus racemosa Stem Bark Extract: A Potent Antioxidant and a Probable Natural Radioprotector

V. P. Veerapur[1], K. R. Prabhakar[1], Vipan kumar Parihar[1], Machendar Reddy Kandadi[1], S. Ramakrishana[2], B. Mishra[4], B. S. Satish Rao[2], K. K. Srinivasan[3], K. I. Priyadarsini[4] and M. K. Unnikrishnan[1]

[1]Department of Pharmacology, Manipal College of Pharmaceutical Sciences, [2]Radiobiology, Kasturba Medical College, [3]Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal-576 104, Karnataka, India and [4]Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Mumbai-400085, India

Ethanol extract (FRE) and water extract (FRW) of *Ficus racemosa* (family: Moraceae) were subjected to free radical scavenging both by steady state and time resolved methods such as nanosecond pulse radiolysis and stopped-flow spectrophotometric analyses. FRE exhibited significantly higher steady state antioxidant activity than FRW. FRE exhibited concentration dependent DPPH, $ABTS^{\bullet -}$, hydroxyl radical and superoxide radical scavenging and inhibition of lipid peroxidation with IC_{50} comparable with tested standard compounds. *In vitro* radioprotective potential of FRE was studied using micronucleus assay in irradiated Chinese hamster lung fibroblast cells (V79). Pretreatment with different doses of FRE 1h prior to 2 Gy γ-radiation resulted in a significant ($P < 0.001$) decrease in the percentage of micronucleated binuclear V79 cells. Maximum radioprotection was observed at 20 μg/ml of FRE. The radioprotection was found to be significant ($P < 0.01$) when cells were treated with optimum dose of FRE (20 μg/ml) 1 h prior to 0.5, 1, 2, 3 and 4 Gy γ-irradiation compared to the respective radiation controls. The cytokinesis-block proliferative index indicated that FRE does not alter radiation induced cell cycle delay. Based on all these results we conclude that the ethanol extract of *F. racemosa* acts as a potent antioxidant and a probable radioprotector.

Keywords: Chinese hamster lung fibroblast (V79) cells – *Ficus racemosa* – free radical scavenger – micronucleus assay – radioprotection

Introduction

Many present day diseases are reported to be due to the shift in balance of pro-oxidant and antioxidant homeostasis in the body (1). Reactive oxygen species (ROS), which include superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$) are well documented as cytotoxic intermediates. These ROS differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission (2), modification of polypeptides, lipid peroxidation etc. (3). Ionizing radiation also generates ROS causing non- selective damage to both tumor and normal cells. Therefore development of selective free radical scavengers which could protect normal cells during radiotherapy is an important strategy in radioprotector drug development.

Several members of the genus *Ficus* (family: Moraceae) are being used traditionally in a wide variety of ethnomedical remedies. One among them, *Ficus racemosa*

For reprints and all correspondence: Dr M. K. Unnikrishnan, Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal-576 104, Karnataka, India. Tel: +91 820 2572195; Fax: +91 820 2570061; E-mail: cheruvaloor@yahoo.com

syn. *Ficus glomerata* (Gular; Udumbara) (4), is widely distributed all over India, northern Australia and other parts of Asia. Its stem bark has shown anti-diarrhoeal, antidiuretic, antitussive, anti-pyretic and hypoglycemic activities (5–9). The chemomodulatory effect of *F. racemosa* against ferric nitrilotriaceatate (Fe-NTA) induced renal carcinogenesis and oxidative damage response in rats was reported recently (10). Since detailed *in vitro* antioxidant activity of *F. racemosa* and its radioprotection ability have not yet been explored, we undertook a detailed investigation with the following objectives: (i) To screen antioxidant activity of both ethanol and water extract of *F. racemosa* stem bark. (ii) To determine whether the extract reduces the radiation-induced micronuclei (MN) yields in V79 cell lines *in vitro* (iii) To assess whether this reduction, if present, is concentration dependent.

Materials and Methods

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{2-}$), ascorbic acid (ASC), Deoxy-D-ribose, thiobarbutyric acid (TBA), butylated hydroxytoluene (BHT), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), butylated hydroxyl anisole (BHA) Dulbecco's minimal essential medium (DMEM), L-glutamine, fetal calf serum (FCS), cytochalasin-B were purchased from Sigma Chemical Co, USA acridine orange was procured from Gurr [BHD, UK]. mannitol was procured from SD Fine Chemicals, Mumbai. Trolox and phenazine methosulphate (PMS) were procured from Himedia, Mumbai. All the other chemicals were of analytical grade, solvents for HPLC were of HPLC grade procured from Qualigens Fine chemicals, India. IOLAR grade nitrogen and N_2O were used for degasing the samples. Nanopure water from Millipore Milli-Q system was used to prepare the solutions and all the solutions were prepared fresh.

Irradiation

The cells were exposed to γ-irradiation from a ^{60}Co Theratron teletherapy unit (Siemens, Erlanger, Germany) at Shiridi Saibaba Cancer Research Centre, Kasturba Medical College Hospital, Manipal, at a dose rate of 1.0 Gy/min. The source to surface distance of 87.5 cm was maintained.

Plant Material

The dried bark of *F. racemosa* was collected from Valsad, Gujarath, India in May 2004, identified and authenticated by a qualified botanist. A voucher specimen is deposited in the Herbarium of Manipal College of Pharmaceutical Sciences, Manipal.

Extraction and Fractionation

The shade dried and powdered bark of *F. racemosa* (1 kg) was extracted exhaustively with 95% ethanol in a soxhlet apparatus (55°C; 25–30 cycles), followed by water extraction on a hot water bath (70°C; 3–4 h). The ethanol extract [FRE] (Herb: extract = 5) and water extract [FRW] (Herb: extract = 8) were concentrated to a small volume and then evaporated to afford a free flowing powder. The dried extract was subjected to various chemical tests to detect the different class of phytoconstituents.

HPLC Fingerprint Profile of FRE

The HPLC finger printing (LC-10AD*v*P, Shimadzu Corporation, Japan) was carried out using 700 ppm solutions of FRE on a reverse phase packed column (RP C-18 column; Supleco, USA; $250 \times 4.6\,mm^2$; particle size 5 μm) using gradient elution. Gradient elution was performed using water and acetonitrile at a total flow rate of 1.0 ml/min with a run time of 30 min and elution was monitored by a PDA detector.; gradient composition (min,% acetonitrile): 0, 20; 5, 40; 8, 75; 12, 90; 15, 95; 25, 95; 27, 20; 30, 20. The chromatograms at 270 nm were analyzed and compared.

Free Radical Scavenging Studies

Reaction with DPPH Radical

For steady state measurements, 100 μM DPPH in methanol was added to FRW and FRE (2–200 μg/ml) in methanol/water, mixed well and kept in dark for 20 min. The absorbance at 517 nm was measured with or without the extracts as described earlier (11). Kinetics of DPPH reaction with the FRE was studied using stopped-flow kinetic spectrometer Model SX 18 MV (Applied Photophysics, UK) in single mixing mode using two syringes. In this experiment, syringe I contained 100 μM DPPH in methanol and syringe II contained solution of FRE (27.5–275 μg/ml) (separately). With a time delay of 1.3 s, both the solutions in syringe I and II were mixed and the relative change in the absorbance at 517 nm as a function of time at 25°C was measured. Analysis of the kinetic traces was carried out with an exponential function using the built in software. At least three independent runs were used to determine the observed decay rate constant at different concentrations (12).

ABTS Radical Anion Reaction

For steady state measurements, 100 μM $ABTS^{\bullet-}$ [prepared by the reaction of 2 mM ($ABTS^{2-}$) was mixed with

Table 1. Comparison of the IC_{50} in μg/ml of FRW, FRE against various free radicals and their total antioxidant capacity

Description	DPPH scavenging	$ABTS^{\bullet-}$ scavenging	$^{\bullet}OH$ scavenging	LpX inhibition	$O_2^{\bullet-}$ scavenging	Ascorbic acid equivalents (mg)/mg of extract
FRE	5.99	4.29	59.08	0.61	66.88	4.35
FRW	42.53	34.53	319.28	190.61	–	1.29
ASC	2.78	1.64	–	–	–	–
Mannitol	–	–	2950.0	–	–	–
Trolox	–	–	–	7.99	–	–
BHA	–	–	–	–	1.46	–

Values are expressed as a mean of triplicate analysis.
FRE, ethanol extract of *Ficus racemosa*; FRW, water extract of *Ficus racemosa*; ASC, ascorbic acid; BHA, butylated hydroxyl anisole; –, not done.

0.17 mM potassium persulphate in 20 mM phosphate buffer pH 7.4, kept overnight before use] was mixed with FRW and FRE (2–200 μg/ml) and decrease in absorbance was measured at 734 nm as described earlier (11). Kinetics of $ABTS^{\bullet-}$ reaction with the FRE was studied using stopped-flow kinetic spectrometer Model SX 18 MV (Applied Photophysics, UK) in single mixing mode. Syringe I contained 200 μM $ABTS^{\bullet-}$ in methanol and syringe II contained solution of FRE (9.8–122.5 μg/ml) (separately). Time dependent absorbance changes at 645 nm were measured (13).

Reaction with Hydroxyl Radical

Steady state $^{\bullet}OH$ scavenging activity of FRW and FRE (34–665 μg/ml) was measured by degradation of deoxy-D-ribose method as described (14). Reaction of FRE with $^{\bullet}OH$ was carried out using nanosecond pulse radiolysis technique employing high-energy electron pulses (50 ns, 7 MeV) obtained from a linear electron accelerator and the transients detected by kinetic spectrometry. Radiation dosimetry was done using an aerated aqueous solution of 0.01 M potassium thiocyanate (KSCN). The dose per pulse was 18.5 Gy. Competition kinetics of $^{\bullet}OH$ scavenging by FRE against 250 μM KSCN at pH 6.8 was studied by monitoring $(SCN)_2^{\bullet-}$ absorbance at 500 nm (15).

Lipid Peroxidation Assay

Egg phospatidylcholine (20 mg) in chloroform (2 ml) was dried under vacuum in a rotary evaporator to give a thin homogeneous film, and further dispersed in normal saline (5 ml) with a vortex mixer. The mixture was sonicated to get a homogeneous suspension of liposomes. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid to a mixture containing liposome (0.1 ml), 150 mM potassium chloride, 0.2 mM ferric chloride, FRW and FRE (0.10–300 μg/ml) in a total volume of 0.4 ml. The reaction mixture was incubated for 40 min at 37°C. After incubation, the reaction was terminated by adding 1 ml of ice cold 0.25 M hydrochloric acid containing 20% w/v of trichloroacetic acid, 0.4% w/v of thiobarbituric acid and 0.05% w/v of butylated hydroxytoluene. After heating at 80°C for 20 min, the samples were cooled. The pink chromogen was extracted with a constant amount of butan-1-ol, and the absorbance of the upper organic layer was measured at 532 nm (16).

Reaction with Superoxide Radical Anion

Steady state superoxide radical anion ($O_2^{\bullet-}$) scavenging activity of FRE (23–323 μg/ml) was measured (17). Superoxide radical anion are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT to blue formazan. Briefly, $O_2^{\bullet-}$ were generated by adding 60 μM (0.05 ml) to a mixture containing 156 μM NBT (0.5 ml), 468 μM NADH (0.5 ml) and different concentration of extract/fraction in a total volume of 1.55 ml. All the reagents were prepared in 100 mM phosphate buffer pH 7.4. The reaction mixture was incubated for 5 min at 25°C and the absorbance measured at 560 nm.

Total Antioxidant Capacity

The assay is based on the reduction of molybdate-VI (Mo^{VI}) to molybdate-V (Mo^{V}) by the extracts and subsequent formation of a green phosphate/Mo^{V} complex in acidic pH. FRW and FRE were mixed separately with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate), incubated at 95°C for 90 min, cooled to room temperature and absorbance measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid (ASC) using standard plot (18).

FRE exhibited significantly higher steady state antioxidant activity than FRW (Table 1), therefore FRE was selected to study the kinetics of free radical reactions and *In vitro* radioprotection in V79 cells.

In vitro Cytokinesis Blocks Micronucleus Assay

Chinese hamster lung fibroblast (V79) cells procured from National Centre for Cell Sciences, Pune, India,

maintained in our laboratory were used in the present study. The cells were cultured in DMEM supplemented with 10% FCS, L-glutamine (2 mM), 100 unit/ml of penicillin and 100 μg/ml of streptomycin. Cells were routinely cultured in 25 cm^2 flasks (Nunc, Roskilde, Denmark) with loosened caps and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Influence of FRE on Radiation Induced Cytogenetic Damage

The exponentially growing V79 cells were trypsinized and an appropriate number of cells were seeded into 25 cm^2 T-flasks in triplicate for each treatment group and allowed to grow. Once they attain exponential phase (i.e. 24 h after seeding), the culture flasks were divided into different groups for various treatments. The first triplicate set of the culture flasks served as sham treated control (without any treatment). In the second group, cells were treated with FRE at a dose of 150 μg/ml for 1 h without irradiation. In the third group, cells were treated with increasing doses of FRE (2.0, 5.0, 10, 20, 50, 75, 100, 125 and 150 μg/ml), 1 h before 2 Gy of γ-irradiation. The cells in the fourth and fifth groups were exposed to various doses of γ-irradiation (0.5, 1, 2, 3 and 4 Gy) with or without exposure of the selected optimum dose of FRE (20 μg/ml) for 1 h prior to radiation treatment.

Cells Treated with Various Concentrations of FRE were Exposed to γ-irradiation

The FRE was dissolved in DMEM and sterilized by passing through 0.22 μ syringe filter (Millipore). Various concentrations of extract were prepared freshly just before the treatment. V79 cells were treated with various concentration of FRE (2–150 μg/ml) for 1 h and the drug treatment was terminated by gently washing with culture media followed by addition of 5 ml of fresh medium. The cells were exposed to γ-irradiation at a dose of 1.0 Gy/min. Immediately after irradiation, 4 μg/ml of cytochalsin-B (stock dissolved in DMSO and diluted in media before use) was added to each culture flask in order to block cytokinesis. Cells were incubated further for 16 h, and detached from the flask by trypsin treatment (0.1%) for 2–3 min with subsequent inactivation of trypsin by the addition of 1 ml of DMEM containing serum. The single cell suspensions, thus prepared were centrifuged (1000 rpm for 10 min), washed with PBS and subjected to mild hypotonic (0.75% KCl) treatment for 1 min. The cells were then centrifuged (1000 rpm for 10 min) and fixed in Carnoy's fixative (3: 1 methanol: acetic acid) for 30 min. Finally, the cells were resuspended in small volume of (100–200 μl) of fixative and dropped on to precleaned slides and air-dried. The slides were coded to avoid observer's bias and stained with 0.002% acridine orange in Sorensen's buffer (pH 6.8) for few seconds. Slides were covered with a coverslip and observed under fluorescent microscope (Photomicroscope III, Carl Ziess, Germany) using 40X neofluar objective.

Micronucleated Binuclear Cells Containing One, Two or Many Micronuclei were Scored

A minimum of 1000 binucleated cells with well-preserved cytoplasm were scored along with the incidence of cells containing one, three or many nuclei. The micronucleated binuclear cells containing one, two or many micronuclei were scored according to the criteria of Fenech *et al.* (19) and expressed as percent micronucleated binuclear cells (MNBNC%). Cytokinesis-block proliferation index (CBPI) was also determined among the groups using $CBPI = [(MI + 2MII + 3(MIII + MIV)]/N$ where MI–MIV represent the number of cells with one to four nuclei, respectively, and N is the number of cells scored (20).

Statistical Analysis

All the data were expressed as Mean ± SEM. The dose response curve for MN was fitted on a linear model ($Y = \alpha + \beta X$). The level of statistical significance was determined by one-way ANOVA between the groups followed by Tukey's test and student '*t*' test using Graph PAD Instat, Software, USA.

Results

Phytochemical Screening

Preliminary phytochemical screening of FRE indicated the presence of steroids, triterpenoids, polyphenolics, coumarins, flavonoids and tannins, while alkaloids and saponins were absent. HPLC chromatogram of FRE (700 ppm) was found to contain constituents eluting between 1.02 min to 3.60 min and 12.7 to 18.42 min with major peaks at 1.877 and 16.277 min. This fingerprint served as a standard for comparison in the subsequent preparation of FRE. Bergenin was identified in FRE by HPLC using sample isolated from a different source and comparing its UV spectrum (Fig. 1).

Free Radical Scavenging

DPPH Radical Scavenging Activity

FRE (IC_{50} 5.99 μg/ml) was found to have comparable DPPH (100 μM) scavenging activity with standard ascorbic acid (ASC) under steady state conditions (IC_{50} 2.78 μg/ml) (Table 1). In the absence of FRE, the DPPH (50 μM) signal in stopped-flow spectrophotometer did not show any decay and remained stable. However, in the presence of the extract the absorption due to the DPPH radical decayed completely in 50 s (Fig. 2). This absorption-time plot was fitted to a single exponential function to obtain observed decay rate constant (k_{obs}), which was found to increase linearly with increasing

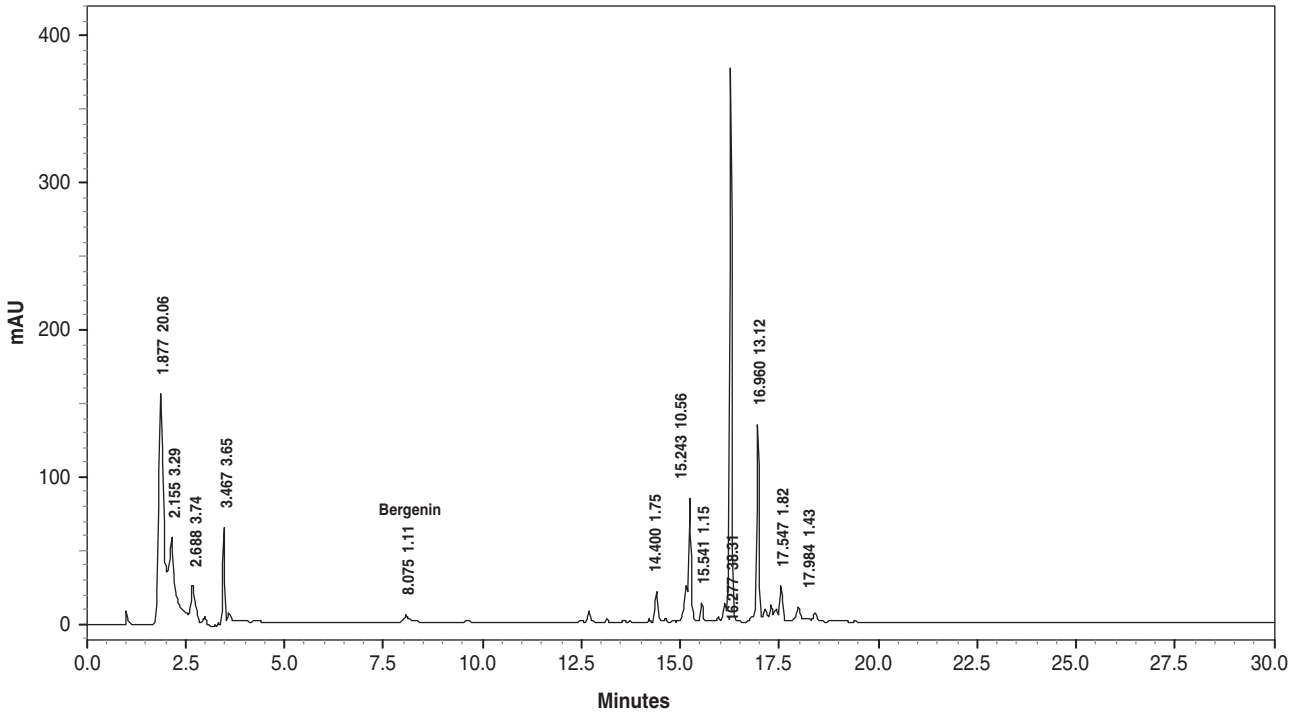

Figure 1. HPLC finger printing: HPLC chromatogram of FRE, separated on a RP-C18 column, Supelcosil, USA ($250 \times 4.6\,mm^2$; particle size 5 μm) using gradient elution—water and acetonitrile at a total flow rate of 1.0 ml/min; gradient composition (min,% acetonitrile): 0, 20; 5, 40; 8, 75; 12, 90; 15, 95; 25, 95; 27, 20; 30, 20. The chromatograms at 270 nm were analyzed and compared.

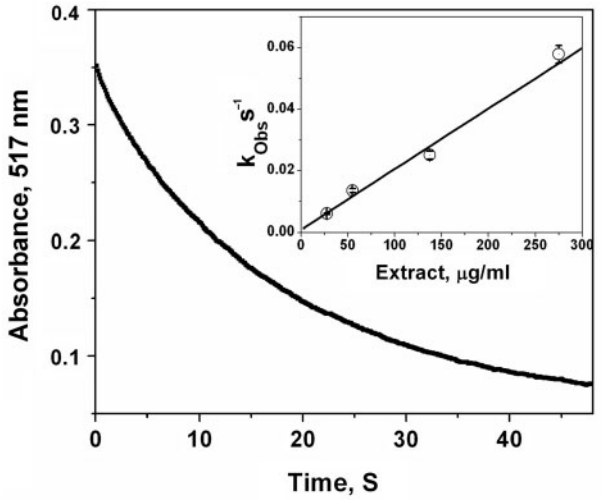

Figure 2. Absorption-time plot showing the decay of 50 μM DPPH at 517 nm in presence of 275 μg/ml of the FRE. Inset shows linear plot for the observed decay rate constant at 517 nm as a function of different concentrations of the FRE.

FRE concentration. The k_{obs} for the decay of DPPH radical in presence of FRE at 125 μg/ml was found to be $0.026\,S^{-1}$ (Inset of Fig. 2).

ABTS Radical Anion Scavenging Activity

FRE (IC_{50} 4.29 μg/ml) exhibited concentration dependent scavenging of $ABTS^{\bullet-}$ (100 μM) under steady state conditions. The activity of FRE is comparable to ASC (IC_{50} 2.78 μg/ml) (Table 1). For kinetic studies, the concentration of $ABTS^{\bullet-}$ was kept at 100 μM. In the absence of the FRE, the $ABTS^{\bullet-}$ signal did not show any decay and remained stable. However, in the presence of FRE, the absorption due to the $ABTS^{\bullet-}$ decayed completely in 20 s (Fig. 3). This absorption time plot was fitted to a single exponential function to get observed decay rate constant, which was found to be increase with increasing concentration of FRE. The k_{obs} for the decay of $ABTS^{\bullet-}$ in presence of FRE at 50 μg/ml was found to be $8.2\,S^{-1}$ (Inset of Fig. 3).

Hydroxyl Radical Scavenging Activity

FRE exhibited better $^{\bullet}OH$ scavenging activity (IC_{50} c59.08 μg/ml) than standard mannitol (IC_{50} of 2.95 mg/ml) under the same experimental conditions (Table 1). The reactivity, in nanosecond time scales towards $^{\bullet}OH$, by FRE was studied using nanosecond pulse radiolysis. A transient spectrum was plotted, which showed prominent peaks at 310 nm (Fig. 4). The spectra do not indicate presence of any detectable standard polyphenolic antioxidants like gallic acid, flavonoids etc., which probably indicates presence of low molecular weight phenolics. Competition kinetics method was used to determine the $^{\bullet}OH$ scavenging ability of FRE

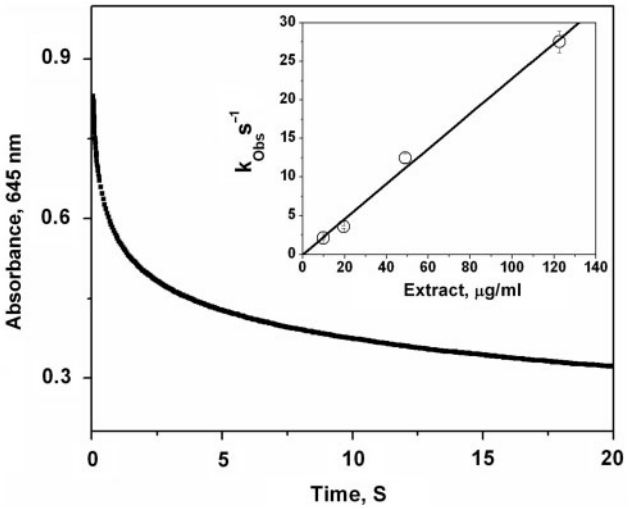

Figure 3. Absorption-time plot showing the decay of 100 µM $ABTS^{\bullet-}$ at 645 nm in presence of 19.6 µg/ml of the FRE. Inset shows linear plot for the observed decay rate constant at 645 nm as a function of different concentrations of the FRE.

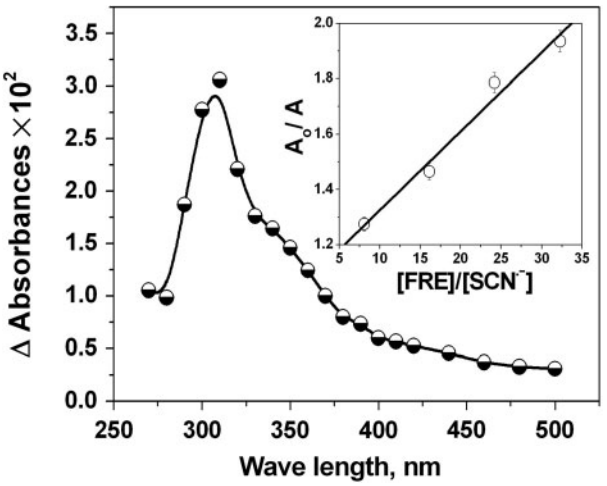

Figure 4. Absorption spectrum of transient species obtained by $^{\bullet}OH$ attack on FRE during pulse radiolysis of aqueous solutions containing 84 µg/ml of FRE at pH 6.8 and saturated with N_2O. Dose per pulse = 18.5 Gy, spectra taken 10 µs after the pulse. Inset shows $^{\bullet}OH$ scavenging ability of FRE as determined by competition kinetics with KSCN, monitoring $(SCN)_2^{\bullet-}$ at 500 nm. Slope of this curve gives the rate constant. A_0, Absorbance of $(SCN)_2^{\bullet-}$ in the absence of FRE; A, Absorbance of $(SCN)_2^{\bullet-}$ in the presence of FRE.

(Inset of Fig. 4). FRE was found to be 2.9% as reactive as $SCN^{\bullet-}$ towards $^{\bullet}OH$.

Inhibition of Lipid Peroxidation

Table 1 shows the effect of FRE on inhibition of Fe^{3+}/ascorbate induced lipid peroxidation in liposomes containing egg phospatidylcholine. FRE (IC_{50}–0.61 µg/ml) was found to show better activity compared to standard Trolox (IC_{50}–7.99 µg/ml).

Superoxide Radical Anion Scavenging Activity

Superoxide radical anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT in this system. Under steady state conditions, FRE exhibited concentration dependent scavenging of $O_2^{\bullet-}$ with IC_{50} of 66.88. The IC_{50} of BHA under the same experimental conditions was found to be 1.46 µg/ml (Table 1).

Total Antioxidant Capacity

FRE was found to possess higher antioxidant capacity in reducing Mo^{VI} to Mo^{V} with ASC equivalents of 4.35 mg per mg of the FRE (Table 1).

In vitro Cytokinesis Blocks Micronuclei Assay

The FRE by itself did not produce any clastogenic effect on V79 cells at the higher dose (150 µg/ml). A radiation-dose response (0.5–4Gy) was analyzed to optimize the best radiation dose to induce micronuclei with less cell killing. Irradiation with 2 Gy was optimal with 21.1% of micronucleated binucleate cells (MNBNC). Treatment of V79 cells with various concentration of FRE (2, 5, 10, 20, 50, 75, 100, 125 and 150 µg/ml) for 1 h before 2 Gy γ-irradiation resulted in a significant ($P < 0.05$ and $P < 0.001$) decrease in percentage of MNBNC as compared to radiation alone group. Further, a linear dose dependent decrease was observed up to a concentration of 20 µg/ml. The maximum reduction (51.42%) was observed at 20 µg/ml. However, further increase in concentration of FRE did not exhibit significant enhancement in the protection (Fig. 5).

The optimum radioprotective dose, namely 20 µg/ml of FRE was selected for radiation dose response experiments. The V79 cells exposed to different doses of radiation induced a dose dependent linear increase in MN count ($R = 0.99918$, Fig. 6). Pretreatment of V79 cells with optimum dose of FRE for 1 h before irradiating with different doses of radiation (0.5, 1, 2, 3 and 4 Gy) produced significant ($P < 0.01$) decrease in percentage MNBNC compared to the respective radiation alone groups ($R = 0.99201$). Optimum dose of FRE was most effective in protecting (41.61%) the cells at a radiation dose of 3 Gy compared to radiation alone group, which is therapeutically relevant in clinical practice (Fig. 6).

The determined CBPI indicated a significant ($P < 0.001$) cell cycle delay under the influence of radiation; FRE treatment did not have any significant influence on cell cycle kinetics. FRE alone (150 µg/ml) also did not show any significant delay compared to sham control (Fig. 7).

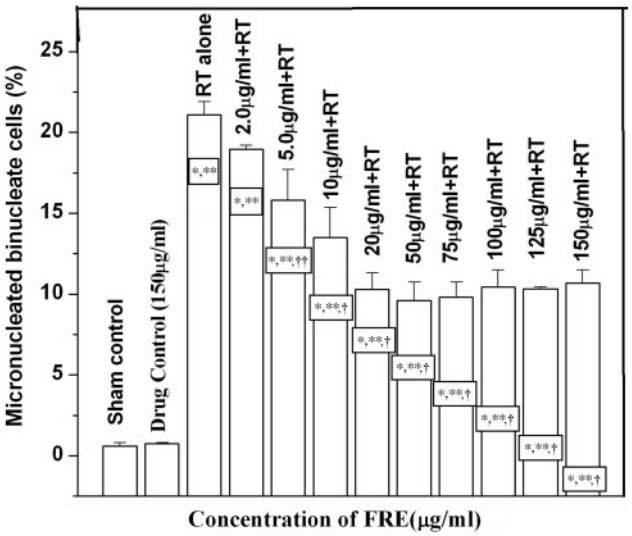

Figure 5. Influence of various concentrations of FRE on micronucleus induction in Chinese hamster lung fibroblast cells (V79) administered 1 h before 2 Gy γ-irradiation. $^{*}P < 0.001$ compared to Untreated; $^{**}P < 0.001$ compared to Drug control (150 μg/ml); $^{\dagger}P < 0.001$ compared to Radiation treated (RT) alone; $^{\dagger\dagger}P < 0.05$ compared to RT alone.

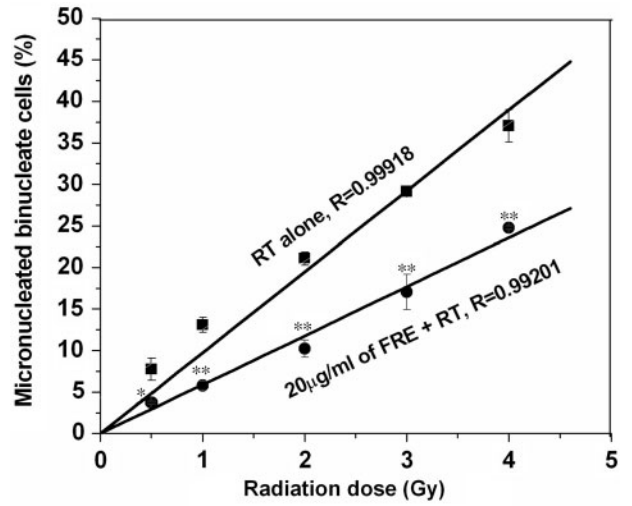

Figure 6. Radiation dose response curve: Influence of FRE (20 μg/ml) on micronucleus induction in Chinese hamster lung fibroblast cells (V79) administered 1 h before increasing doses of γ-irradiation. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to RTalone.

Discussion

Herbal radioprotectors have been gaining prime importance in radioprotective drug discovery due to lesser side effects as reviewed extensively by many authors (21,22). The damage to DNA and membrane lipids are critical factors in radiation induced cellular damage and reproductive cell death. The results of *in vitro* antioxidant data showed a significant free radical scavenging activity of FRE in a dose

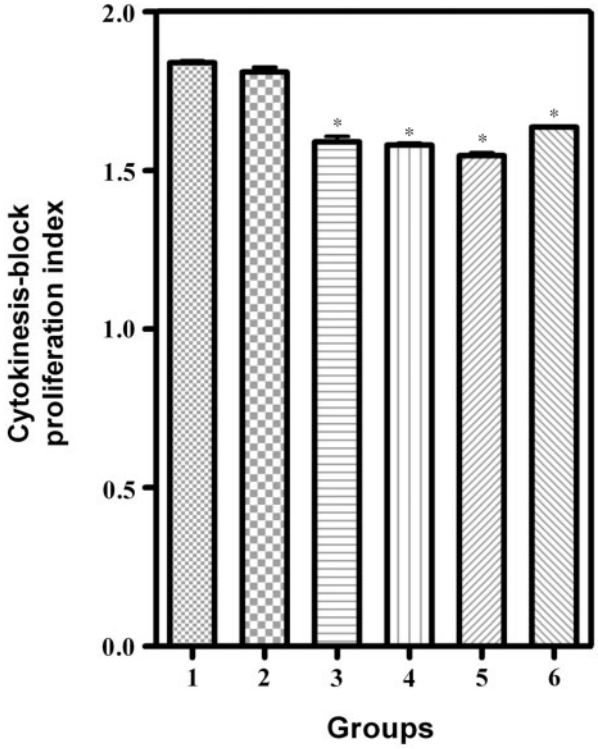

Figure 7. Effect of different concentrations of FRE on cytokinesis-block proliferation index (CBPI) under the influence of 2 Gy γ-radiation in V79 fibroblasts. Group 1: Sham Control; 2: Drug control (150 μg/ml); 3: RTalone; 4: RT + 10 μg/ml FRE; 5: RT + 20 μg/ml FRE; 6: RT + 50 μg/ml FRE; Results are expressed as Mean ± SEM ($n = 3$; $^{*}P < 0.001$ compared to sham control.

dependent manner. Such free radical scavengers exert a key role in radioprotection, because radiation induced cytotoxicity is mediated mainly through generation of free radicals in the biological system (23).

It is well established that ionizing radiation induces different types of lesions in the DNA, including single and double strand breaks, base damage and DNA cross links. Among all these, double strand break has been considered as the critical lesion for radiation induced cell death. There is a correlation between the induction of cell death and frequency of micronuclei induction and chromosome aberrations. Therefore micronucleus assay is a useful parameter to assess the cytogenetic damage. It is extensively used to screen the cytoprotective/radio-modifying potential of synthetic and natural products (24). FRE is found to exhibit potent radioprotection ability in V79 cells. A linear dose response was observed only up to 20 μg/ml indicating probable cell saturation with active principles. Our study also suggests no significant change on cell cycle kinetics by FRE either independently or under the influence of radiation. Collectively, these findings indicate that the antioxidant effects of FRE may make an important contribution to its radioprotective potential.

The earlier reports on chemical constituents of FRE have shown the presence of antioxidant and chemopreventive principles namely, racemosic acid, bergenin, tannins, kaempferol, rutin, bergapten, psoralenes, ficusin, coumarin and phenolic glycosides (25,26). We are currently isolating such antioxidant and anticlastogenic components and exploring their radioprotective potential.

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