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Indian Medicinal Herb Guduchi (*Tinospora cordifolia* Meirs) Exerts its Radiosensitizing Activity by Accelerating Chromosome Damage in HeLa Cells Exposed to Different Doses of γ-Radiation

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ABSTRACT

Radiotherapy is an important treatment modality and screening of phytoceuticals may enhance the clinical outcome of radiotherapy, therefore radiosensitzing activity of various guduchi (*Tinospora cordifolia*) extracts was studied in HeLa cells. Chromosomal aberrations were scored in HeLa cells treated with 10 µg/ml of aqueous, methanol, or methylene chloride guduchi extracts or doxorubicin before exposure to 0, 0.5, 1, 2 or 3 Gy of γ-radiation at 12, 24, 36 or 48 h post-irradiation. Irradiation of HeLa cells caused a dose dependent rise in the chromatid breaks, chromosome breaks, dicentric, centric rings, acentric fragments and total aberrations at all post-irradiation times and the dose response was linear quadratic for all types of aberrations scored. Chromatid breaks increased up to 12 h post-irradiation and declined steadily up to 48 h post-irradiation, whereas chromosome breaks, dicentric, acentric fragments and total aberrations elevated up to 24 h post-irradiation and declined thereafter. However, centric rings continued to rise steadily up to 48 h post-irradiation. Treatment of HeLa cells with aqueous, methanol or methylene chloride guduchi extract or doxorubicin before irradiation significantly enhanced various types of chromosomal aberrations and a maximum rise in the chromosome aberrations was observed in the HeLa cells treated with methylene chloride extract before irradiation when compared to other groups. Various guduchi extracts enhanced the effect of radiation in HeLa cells by increasing the molecular damage to cellular genome and their effect was similar to or even greater than doxorubicin (positive control) pretreatment, depending on the type of guduchi extract used.

Keywords: Acentric fragments, centric rings, chromatid and chromosome breaks, dicentric

INTRODUCTION

New approaches to systemic treatment for unresectable neoplasms are continually being explored. These approaches include the search for new but more effective antitumor agents and the utilization of combination of drugs and/or ionizing radiations. In one approach the radiosensitizer like nitroimidazoles have been used to make hypoxic cells sensitive to radiation (Stratford 1982). The experimental studies have always been promising; however, clinical success was limited owing to their high toxicity (Hirst *et al.* 1991; Oya *et al.* 1995). The alternative approach has been to use chemotherapeutic agents in combination with radiation, where a remarkable success has been achieved in treating difficult neoplasia. Over recent years, many attempts have been made to combine chemotherapy with radiation to improve the therapeutic management of malignant tumors. *Cis*-dichlorodiammine-platinum (II), nucleoside analogues (5-fluorouracil, mitomycin C, gemcitabine), oxazaphosphorines, imidzotetrazines, paclitaxel, docetaxel, topotecan, irinotecan, crytophycins, camptothecin and combretastatin A-4 have been increasingly used in combination with radiation to improve the therapeutic outcome of solid tumors (Kvols 2005; Anderson *et al.* 2008; Page and Yang 2010). Although chemoradiotherapy is successful, the toxic manifestations are greater than either treatment given alone. Therefore, newer approaches are always required to reduce the toxic side effects of combination regimens with optimum therapeutic efficacy and good quality of life after successful treatment (Anderson *et al.* 2008). It could be done by investigating new and novel pharmacological approaches to increase the effect of ionizing radiation to treat cancer and reduce the toxic side effects of combination regimens.

Herbs have attracted the attention of humans for healthcare since the advent of human history. There could be a multitude of approaches to use herbs for cancer treatment. Herbs can be used in the form of crude extracts as has been practiced by various herbal based systems or isolate pure compounds and use them for medication as has been done in the modern allopathy system of medicine (Bremner and Heinrich 2002; Jagetia 2007; Harvey 2008). Various plant extracts including *Scutellaria baicalensis*, *Indigofera trita*, *Oldenlandia diffusan*, *Nigella sativa* and *Acanthus ilicifolius* have been reported to exert their anticancer activity *in vivo* and *in vitro* (Scheck *et al.* 2006; Kumar *et al*. 2007; Islam *et al.*2009; Svejda *et al.* 2010; Khajure and Rathod 2011). HeLa cells have been used as a model cell line to assess the anticancer activity of various, pharmacological agents including plant extracts in vitro by various workers (Jagetia and Baliga 2005; Cuca *et al*. 2011; Almehdar *et al.* 2012; Berrington and Lall 2012; Puoci *et al*. 2012). Guduchi is a Sanskrit name of *Tinospora cordifolia* that means one that protects entire body. Guduchi has been traditionally used as a rasayana drug to treat various ailments in the Ayurvedic system of medicine in India (Nadkarni and Nadkarni 1976). It possesses a wide spectrum of activities including antiinflammatory, antibacterial, antiviral, antimalarial, antileprotic, hypoglycemic and immunomodulatory (Singh *et al.* 2003; Li *et al*. 2004; Singh 2005; Rose *et al.* 2007; Aher and Wahi 2010; Singh and Banyal 2011; More and Pai 2011). Various extracts of guduchi have been reported to protect rats against carbon tetrachloride-induced hepatotoxicity (Kavitha *et al*. 2011). Guduchi has been found to act as an aphrodisiac agent and reduce cisplatin-induced nephrotoxicity and urotoxicity *in vivo* (Hamsa and Kuttan 2010; Khanam *et al*. 2011; Wani *et al*. 2011). It has been reported to be antimutagenic and active against the HIV virus (Sharma *et al*. 2010; Estari *et al*. 2012).

Guduchi has been clinically used to treat throat cancer in man (Chauhan 1995). Reports by CHEMEXCIL (1992)

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indicated its non-toxic nature and our earlier studies have also shown that methylene chloride extract of guduchi was non-toxic up to a dose of 1.2 g/kg in mice (Jagetia *et al.* 2002). Likewise, aqueous and methanol extracts of guduchi have been reported to be non-toxic up to a dose of 3.5 g in mice and rats (Devbhuti *et al.* 2009). Various guduchi extracts have been reported to kill cervical cancer cells in a dose dependent manner earlier (Jagetia *et al.* 1998; Jagetia and Rao 2006). Our earlier studies have also shown that guduchi increased the cell killing effect of radiation *in vivo* and *in vitro* (Jagetia *et al.* 2002; Jagetia 2008). A recent study has shown that dichloromethane extract of guduchi induced molecular damage into DNA as indicated by comet assay (Jagetia and Rao 2011). The crude extract of another species, *Tinospora crispa* has been found to exert cytotoxic effect on HeLa, MCF-7, MDAMB-231 and 3T3 cells *in vitro* (Ibahim *et al*. 2011). The induction of chromosome damage has been reported to be the principal cause of loss of reproductive integrity and clonogenicity of cells (DeMarini *et al.* 1989; Evans 1994). Therefore, it was desired to evaluate the effect of various guduchi extracts on the radiation-induced genomic damage in HeLa cells exposed to different doses of γ-radiation by evaluating chromosome aberrations at different post-treatment times.

MATERIALS AND METHODS

Drugs and chemicals

The stem extracts (aqueous, methanol and methylene chloride) of *Tinospora cordifolia* were provided by Krüger Pharmaceuticals (Mumbai, India) and henceforth, aqueous, methanol and methylene chloride extracts of guduchi will be abbreviated as AQE, MEE, and MCE, respectively. Doxorubicin (DOX) was purchased from Dabur Pharmaceuticals, New Delhi, India. Colchicine, Minimum Essential Medium (MEM), L-glutamine, gentamycin sulfate, and fetal calf serum were procured from Sigma Chemical Co., St. Louis, USA. Dimethylsulfoxide (DMSO) and other routine chemicals including, ammonium oxalate, methanol, glycerol, petroleum ether, methylene chloride and acetic acid were supplied by Ranbaxy fine Chemicals, Mumbai, India. Giemsa stain was procured from BDH, England.

Preparation of drug solutions

The methanol, aqueous extracts or doxorubicin hydrochloride were dissolved in MEM, whereas the methylene chloride extract was dissolved in DMSO in such a way that its concentration never exceeded 0.002%. All solutions were freshly prepared immediately before use and diluted with MEM. Colchicine was dissolved in Milli-Q (Millipore, USA) water at a concentration of 1 mg/ml, stored at -80°C and diluted with MEM immediately before use.

Cell line and culture

HeLa S3 cells procured from National Centre for Cell Science, Pune, India, were used throughout the study. The HeLa S3 cells have a doubling time of 20 ± 2 h and consist of 57-65 chromosomes. The cells were routinely grown in 25 cm^2 culture flasks (Techno Plastic Products, Trasädingen, Switzerland) with loosened caps containing Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% L-glutamine and 50 µg/ml gentamicin sulfate at 37°C in an atmosphere of 5% CO_2 in humidified air in a CO_2 incubator (NuAir, Plymouth, USA).

Experimental design

A fixed number (5×10^5) of exponentially growing cells were plated on to several individual culture flasks (Techno Plastic Products, Trasädingen, Switzerland) and were allowed to grow for 24 h. The non-confluent cell cultures were divided into the following groups (Jagetia *et al.* 2002):

MEM+irradiation group: The cell cultures of this group did not receive any treatment before irradiation.

AQE+irradiation group: The cell cultures of this group were inoculated with 10 µg/ml of aqueous extract before exposure to different doses of γ-radiation.

MEE+irradiation group: This group of cultures was treated with 10 µg/ml of methanol extract before irradiation to different doses of γ-radiation.

MCE+irradiation group: This group of cell cultures was treated with 10 µg/ml of methylene chloride extract.

DOX+irradiation group: The cell cultures of this group were treated with 10 µg/ml of doxorubicin hydrochloride before irradiation to different doses of gamma radiation and served as a positive control.

Irradiation

After two hours of the above treatments (Jagetia *et al.* 2002), the culture flasks containing the cells were exposed to 0 (sham-irradiation), 0.5, 1, 2 or 3 Gy γ-radiation from a telecobalt therapy source (Gammatron, Siemens, Germany) at a dose rate of 1 Gy/min and at a distance (SSD) of 54.5 cm.

Chromosomal analysis

Immediately after irradiation the cells were dislodged by trypsin EDTA treatment. Usually 1×10^6 cells were plated in triplicate for each dose of radiation for each group. The cells were allowed to grow at 37°C in 5% CO_2 in air in a humidified atmosphere. The cells were harvested at 12, 24, 36 and 48 h post-irradiation. The metaphase plates were prepared as described earlier (Jagetia 1994). Briefly, the cells were incubated at 37°C with 1 µg/ml colchicine 1.5 h before each harvesting time. The medium was discarded and the cells were washed twice with phosphate buffered saline. The cells were dislodged by trypsin EDTA treatment and were collected in individual centrifuge tubes, pelleted by centrifugation and the supernatant was discarded. The cell pellet was disturbed and the cells were exposed to hypotonic treatment (0.56% ammonium oxalate) for 10 min at room temperature. The tubes were centrifuged again and the resultant cell pellet was fixed in Carnoy's fixative (3:1 methanol, acetic acid). Finally, the cells were centrifuged, resuspended in a small volume of fixative and dropped on to precleaned, chilled, and coded slides.

Staining and scoring of aberrations

The cells were stained with 4% Giemsa (BDH, England, Gurr Cat. No. 0546750) at 6.8 pH. The slides were dried, cleared in xylene and observed under a transmitted light microscope (Carl Zeiss Photomicroscope III, Oberkochen, Germany) using a 60X planapochromatic objective. A minimum of 125 well spread metaphase plates were scored for each culture and a total of 375 metaphase plates were counted for each dose of radiation. The chromosomal aberrations were identified and scored according to the criteria of Savage (1975). The data were collected for aberrant cells, chromatid and chromosome breaks, acentric fragments, dicentric, and centric ring (rings) chromosomes, and total aberrations.

Statistical analyses

The statistical analysis was carried out using Fisher's exact test. The dose response relationship was evaluated using the equation ($Y = C + \alpha D + \beta D^2$), where C is control chromosomal frequency, D is irradiation dose and α and β are the constants. The Solo 4 statistical package (BMDP Inc., USA and Ireland) was used for statistical analysis. The results were confirmed by repetition of the experiment.

The dose enhancement factor (DEF) was calculated by the following formula (Akashi *et al*. 2008):

DEF = [Guduchi (extracts)+irradiation-Guduchi+sham-irradiation (0 Gy)] / [MEM+irradiation-MEM+sham-irradiation (0 Gy)]

where guduchi = MEE or AQE or MCE guduchi extract.

RESULTS

The results are expressed as percent aberrant cells, chromatid and chromosome breaks, acentric fragments, centric rings, dicentric and ring chromosomes and total aberrations/ cell ± SEM (**Tables 1-4**). Our earlier study (Jagetia *et al.* 2002) showed that 10 µg/ml guduchi extracts showed a maximum cytotoxic effect, and therefore this concentration was selected in the present study.

Aberrant cells

Since HeLa cells are genomically unstable, the frequency of aberrant cells did not change significantly in AQE, MEE, MCE or DOX treated groups than that of MEM treated control irrespective of the post-irradiation scoring time (**Tables 1-4**). Irradiation of HeLa cells resulted in a dose dependent increase in the frequency of aberrant cells in all the groups. However, this increase was statistically non-significant in AQE+irradiation, MEE+irradiation, MCE+irradiation and DOX+irradiation groups when compared to MEM+irradiation group at all the post-irradiation times. The aberrant cells increased with time in all groups up to 24 h post-irradiation, remained unaltered up to 36 h and declined thereafter (**Tables 1-4**).

Chromatid breaks

Treatment of HeLa cells with 10 µg/ml of AQE, MEE, MCE or DOX increased the frequency of chromatid breaks significantly when compared to MEM+sham-irradiation group at all post-irradiation times (**Table 1**). The formation of chromatid breaks declined with time in the AQE, MEE, MCE or DOX+ sham-irradiation group and the lowest frequency of chromatid breaks was observed at 48 h post-irradiation. This decline was approximately 1.7-fold for all the drug-treated groups, except the MCE+sham-irradiation group, where it was 2-fold when compared with the other concurrent groups at 12 h post-irradiation (**Tables 1-4**). Irradiation of HeLa cells to different doses of gamma radiation resulted in a dose-dependent increase in the chromatid breaks in MEM+irradiation and AQE or MEE or MCE or DOX+irradiation groups at all the post-irradiation times (**Fig. 1**). The chromatid breaks accrued by a factor of 2.8 in MCE+irradiation group exposed to 2.0 Gy at 36 h post-irradiation when compared to concurrent MEM+irradiation group, however, this factor was approximately 1.5-fold for AQE and MEE and 1.4 for DOX+irradiation group (**Table 5**). The pattern of elevation in the chromatid breaks was similar to that of 12 h, for the remaining post-irradiation scoring times i.e. 24, 36 and 48 h post-irradiation, except that the frequency of chromatid breaks declined steadily with time reaching a nadir at 48 h post-irradiation, where the frequency of chromatid breaks was 1.3 to 1.7 folds lower than that of 12 h post-irradiation depending on the exposure dose (**Tables 1-4**). The dose response was linear quadratic at all post-irradiation times for all the groups (**Tables 1-4**).

Chromosome breaks

The frequency of chromosome breaks increased significantly in the AQE, MEE, MCE or DOX treated non-irradiated controls at 12 h post-irradiation, which continued to elevate up to 24 h post-irradiation, where the frequency of chromosome breaks reached a peak level. A decline in the frequency of chromosome breaks was observed at 36 h post-irradiation that continued to decline up to 48 h post-irradiation. The increase in the frequency of chromosome breaks was almost similar for the AQE-, MEE-, MCE- or DOX-treated group. The frequency of chromosome breaks increased in a dose dependent manner in MEM+irradiation group at all the post-irradiation times (**Fig. 2**). Similarly, pretreatment of HeLa cells with AQE, MEE, MCE or DOX further increased the frequency of chromosome breaks significantly in AQE+irradiation, MEE+irradiation, MCE+irradiation and DOX+irradiation groups at all the post-irradiation times when compared with the MEM+irradiation group. The dose enhancement factor was approximately between 1.14 to 3.5 depending on radiation dose, type of treatment and scoring time (**Table 5**). The trend of increase in chromosome breaks in all the irradiated groups was similar to that of sham-irradiated groups, where a peak level of chromosome breaks was observed at 24 h post-irradiation, which declined thereafter (**Tables 1-4**). The dose response relationship was linear quadratic for all the groups at various scoring times (**Tables 1-4**).

Acentric fragments

Treatment of HeLa cells with various extracts of guduchi i.e. AQE, MEE, MCE or DOX+ sham-irradiation group resulted in a significant increase in the frequency of acentric fragments when compared to MEM+sham-irradiation controls. The highest rise in acentric fragments was observed at 24 h post-irradiation. The frequency of acentric fragments elevated in a dose dependent manner with the increase in exposure dose in MEM+irradiation, AQE+irradiation, MEE +irradiation, MCE+irradiation or DOX+irradiation groups at all the post-irradiation scoring times (**Fig. 3**). The frequency of acentric fragment was significantly greater in AQE+irradiation, MEE+irradiation, MCE+irradiation and DOX+irradiation groups when compared to MEM+irradiation group (**Tables 1-4**). The dose enhancement factor for acentric fragments was 1.9 to 3 depending on the type of treatment and dose of irradiation at 12 h post-irradiation (**Table 5**). The frequency of acentric fragments increased with time and reached a peak level at 24 h post-irradiation for all the groups and declined thereafter (**Tables 1-4**). The dose response relationship for all the groups was linear quadratic at various post-irradiation times (**Tables 1-4**).

Dicentrics

The frequency of dicentrics in HeLa cells increased significantly in the sham-irradiation group pretreated with AQE, MEE, MCE or DOX at 12 h post-irradiation than that of MEM+sham-irradiation controls. This increase in dicentrics was approximately 3-fold for AQE, MEE and MCE treated groups, except for the DOX treated group, where it was 2.5-fold. Irradiation of HeLa cells caused a dose dependent rise in the frequency of dicentrics in MEM+irradiation group at all the post-irradiation times. The frequency of dicentric increased with time reaching a peak at 24 h post-irradiation and declined steadily thereafter in MEM +irradiation group (**Tables 1-4**). Treatment of HeLa cells with AQE, MEE, MCE or DOX before irradiation resulted in a further elevation in the frequency of dicentrics that was significantly higher than that of MEM+irradiation group (**Tables 1-4**). This elevation in dicentric frequency was irradiation dose dependent in all the AQE+irradiation, MEE+irradiation, MCE+irradiation and DOX+irradiation groups (**Fig. 4**). A peak frequency of dicentric was observed at 24 h post-irradiation that declined thereafter (**Fig. 4**). The dose enhancement factor varied between 1.9 to 3.5 at 24 h after exposure depending on the type of guduchi extract treatment and exposure dose (**Table 5**). The isoeffective dose for 0.5 Gy guduchi+irradiation was equivalent to 3 Gy of MEM+ irradiation group. The data for all the groups fitted well on linear quadratic model at all post-irradiation times (**Tables 1-4**).

Centric rings

Treatment of HeLa cells with 10 µg/ml of AQE, MEE, and MCE or DOX resulted in a steady, but significant increase in the frequency of rings from 12 to 48 h post-treatment in sham-irradiation group and a maximum increase was observed at 48 h post-treatment (**Fig. 5**). The frequency of rings increased in a dose-dependent manner in the MEM

Table 1 Alteration in the radiation-induced chromosomal aberrations by various guduchi extracts in HeLa cells at 12 h post-irradiation.

Dose (Gy)	Treatments	Aberrant cells (%)	Aberrations per cell ± SEM					
			Chromatid breaks	Chromosome breaks	Acentric fragments	Dicentrics	Centric rings	Total aberrations
0.0	MEM+IR	84 ± 0.377	0.21 ± 0.011	0.03 ± 0.002	0.24 ± 0.009	0.01 ± 0.003	0.02 ± 0.003	0.52 ± 0.004
	AQE+IR	87.2 ± 1.131	0.62 ± 0.032^{f}	0.10 ± 0.005^{f}	0.49 ± 0.018^{f}	0.02 ± 0.003	0.05 ± 0.005^{b}	1.28 ± 0.042^{f}
	MEE+IR	87.2 ± 0.377	0.64 ± 0.050^{f}	0.11 ± 0.012^{f}	0.62 ± 0.003^{f}	0.03 ± 0.005^{a}	0.05 ± 0.003^{b}	1.45 ± 0.056^{f}
	MCE+IR	85.6 ± 0.377	0.79 ± 0.050^{f}	0.11 ± 0.005^{c}	0.53 ± 0.014^{f}	0.03 ± 0.003^{a}	0.03 ± 0.003	1.50 ± 0.047^{f}
	DOX+IR	84.8 ± 0.377	0.66 ± 0.021^{f}	0.07 ± 0.004^{d}	0.43 ± 0.017^{f}	0.02 ± 0.00	0.05 ± 0.002^{b}	1.24 ± 0.022^{f}
0.5	MEM+IR	86.67 ± 0.576	0.36 ± 0.018	0.06 ± 0.002	0.48 ± 0.028	0.03 ± 0.004	0.03 ± 0.002	0.96 ± 0.036
	AQE+IR	90.67 ± 0.785	0.78 ± 0.022^{f}	0.17 ± 0.002^{f}	1.04 ± 0.030^{f}	0.06 ± 0.003^{a}	0.07 ± 0.005^{a}	2.13 ± 0.037^{f}
	MEE+IR	90.13 ± 0.576	0.86 ± 0.017^{f}	0.16 ± 0.013^{f}	1.08 ± 0.022^{f}	0.07 ± 0.004^{b}	0.08 ± 0.007^{b}	2.26 ± 0.032^{f}
	MCE+IR	89.6 ± 0.754	0.96 ± 0.016^{f}	0.20 ± 0.011^{f}	1.01 ± 0.005^{f}	0.08 ± 0.013^{b}	0.07 ± 0.007^{a}	2.33 ± 0.032^{f}
	DOX+IR	89.33 ± 0.435	0.81 ± 0.011^{f}	0.15 ± 0.005^{f}	0.92 ± 0.003^{f}	0.06 ± 0.003	0.07 ± 0.003^{a}	2.01 ± 0.034^{f}
1.0	MEM+IR	89.33 ± 0.576	0.48 ± 0.014	0.10 ± 0.007	0.56 ± 0.015	0.04 ± 0.003	0.05 ± 0.007	1.20 ± 0.013
	AQE+IR	91.73 ± 0.576	1.08 ± 0.013^{f}	0.24 ± 0.009^{f}	1.45 ± 0.039^{f}	0.09 ± 0.009^{b}	0.10 ± 0.004^{b}	2.96 ± 0.039^{f}
	MEE+IR	91.47 ± 0.785	1.13 ± 0.018^{f}	0.24 ± 0.012^{f}	1.57 ± 0.036^{f}	0.12 ± 0.011^{d}	0.10 ± 0.003^{b}	3.17 ± 0.047^{f}
	MCE+IR	92.00 ± 0.377	1.11 ± 0.028^{f}	0.27 ± 0.017^{f}	1.31 ± 0.025^{f}	0.13 ± 0.011^{c}	0.11 ± 0.007^{b}	2.94 ± 0.004^{f}
	DOX+IR	90.67 ± 0.576	1.06 ± 0.011^{f}	0.20 ± 0.013^{f}	1.24 ± 0.039^{f}	0.09 ± 0.007^{b}	0.10 ± 0.003^{b}	2.68 ± 0.028^{f}
2.0	MEM+IR	90.67 ± 0.785	0.59 ± 0.007	0.15 ± 0.007	0.71 ± 0.011	0.06 ± 0.003	0.07 ± 0.004	1.59 ± 0.021
	AQE+IR	92.80 ± 0.377	1.26 ± 0.009^{f}	0.30 ± 0.026^{f}	1.69 ± 0.030^{f}	0.14 ± 0.005^{c}	0.13 ± 0.005^{b}	3.53 ± 0.062^{f}
	MEE+IR	92.27 ± 0.217	1.28 ± 0.032^{f}	0.31 ± 0.030^{f}	1.85 ± 0.037^{f}	0.16 ± 0.007^{d}	0.13 ± 0.005^{b}	3.73 ± 0.058^{f}
	MCE+IR	92.80 ± 0.377	1.13 ± 0.030^{f}	0.35 ± 0.017^{f}	1.59 ± 0.035^{f}	0.19 ± 0.009^{f}	0.14 ± 0.003^{b}	3.58 ± 0.057^{f}
	DOX+IR	92.00 ± 0.377	1.16 ± 0.020^{f}	0.29 ± 0.018^{d}	1.55 ± 0.024^{f}	0.12 ± 0.009^{b}	0.13 ± 0.002^{b}	3.26 ± 0.053^{f}
3.0	MEM+IR	90.93 ± 0.785	0.72 ± 0.007	0.18 ± 0.007	0.93 ± 0.029	0.10 ± 0.007	0.09 ± 0.005	2.00 ± 0.029
	AQE+IR	95.47 ± 0.576	1.45 ± 0.041^{f}	0.47 ± 0.011^{f}	1.88 ± 0.045^{f}	0.20 ± 0.009^{c}	0.20 ± 0.009^{b}	4.19 ± 0.068^{f}
	MEE+IR	93.60 ± 0.754	1.52 ± 0.021^{f}	0.48 ± 0.009^{f}	2.00 ± 0.049^{f}	0.22 ± 0.005^{c}	0.16 ± 0.005^{b}	4.40 ± 0.064^{f}
	MCE+IR	93.87 ± 0.217	1.53 ± 0.035^{f}	0.48 ± 0.017^{f}	1.84 ± 0.030^{f}	0.25 ± 0.003^{f}	0.19 ± 0.021^{c}	4.28 ± 0.045^{f}
	DOX+IR	91.47 ± 0.217	1.42 ± 0.022^{f}	0.44 ± 0.013^{f}	1.76 ± 0.050^{f}	0.19 ± 0.009^{b}	0.17 ± 0.005^{b}	3.92 ± 0.084^{f}
r	MEM+IR	0.99	0.99	0.99	0.99	0.99	0.99	0.99
	AQE+IR	0.96	0.99	0.99	0.99	0.99	0.99	0.99
	MEE+IR	0.97	0.99	0.99	0.99	0.99	0.99	0.99
	MCE+IR	0.97	0.97	0.99	0.99	0.99	0.99	0.99
	DOX+IR	0.98	0.98	0.99	0.99	0.98	0.99	0.99

MEM= minimum essential medium; AQE = aqueous extract; MEE= methanol extract; MCE = methylene chloride extract and DOX = doxorubicin
P<a=0.05; b=0.02; c=0.01; d=0.002; e=0.001; f=0.0001 and No symbols = non-significant when MEM+IR compared with the other groups.
N=3; Significance between the treatments was calculated using Fisher's Exact test.

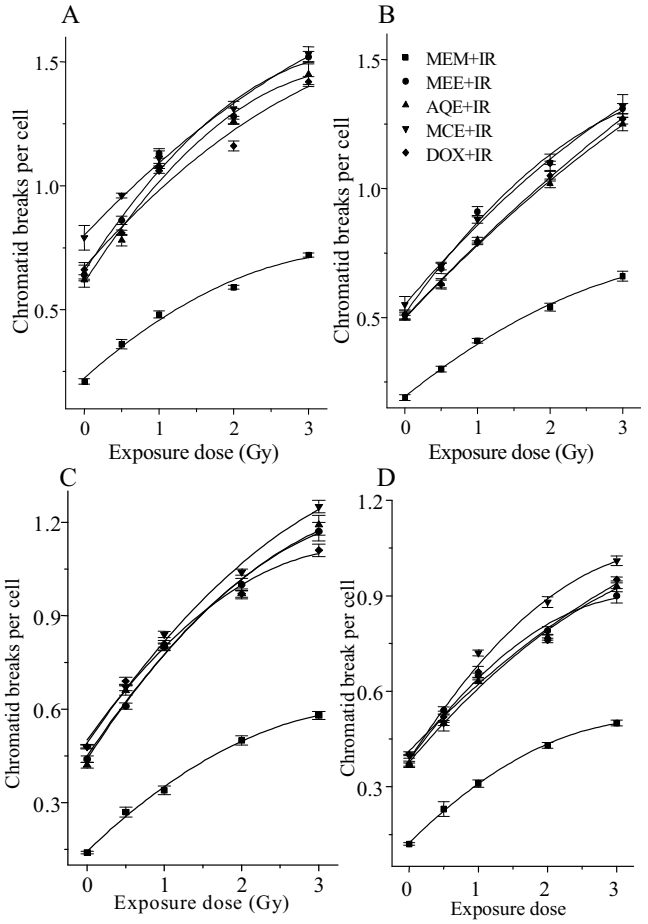

Fig. 1 Altered frequency of radiation-induced chromatid breaks by various guduchi extracts in HeLa cells exposed to different doses of γ-radiation. (**A**) 12 h; (**B**) 24 h; (**C**) 36 h; (**D**) 48 h post-irradiation.

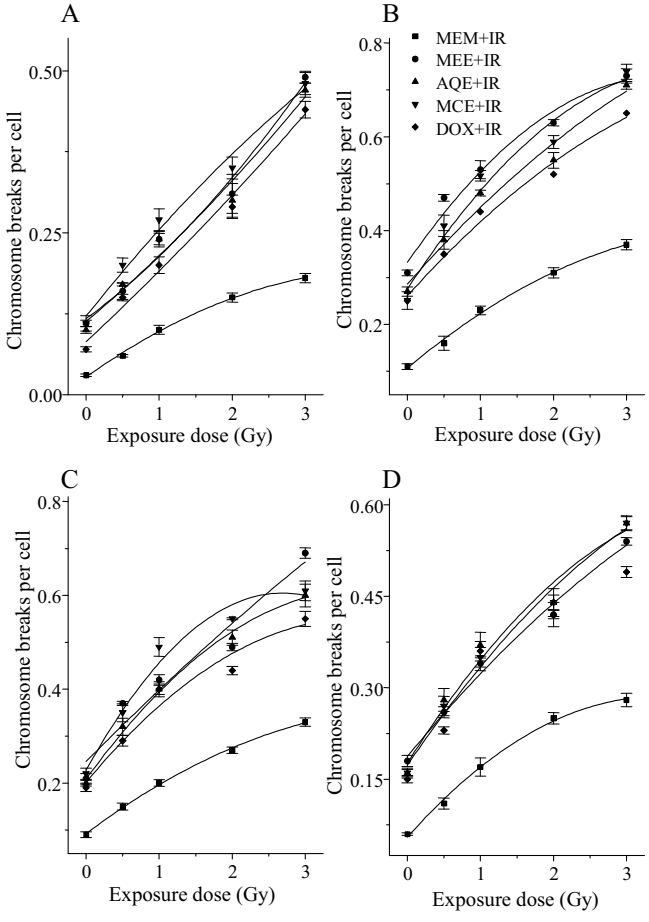

Fig. 2 Altered frequency of radiation-induced chromosome breaks by various guduchi extracts in HeLa cells exposed to different doses of γ-radiation. (**A**) 12 h; (**B**) 24 h; (**C**) 36 h; (**D**) 48 h post-irradiation.

Table 2 Alteration in the radiation-induced chromosomal aberrations by different guduchi extracts in HeLa cells at 24 h post-irradiation.

Dose (Gy)	Treatments	Aberrant cells (%)	Aberrations per cell ± SEM					
			Chromatid breaks	Chromosome breaks	Acentric Fragments	Dicentrics	Rings	Total aberrations
0.0	MEM+IR	91.47 ± 0.949	0.19 ± 0.011	0.11 ± 0.005	0.81 ± 0.017	0.03 ± 0.002	0.03 ± 0.002	1.17 ± 0.027
	AQE+IR	94.67 ± 0.435	0.50 ± 0.009^{f}	0.27 ± 0.014^{f}	1.90 ± 0.024^{f}	0.09 ± 0.005^{c}	0.06 ± 0.005^{a}	2.82 ± 0.026^{f}
	MEE+IR	92.80 ± 0.377	0.512 ± 0.019^{f}	0.31 ± 0.005^{f}	1.82 ± 0.026^{f}	0.10 ± 0.005^{c}	0.06 ± 0.004^{a}	2.80 ± 0.046^{f}
	MCE+IR	93.33 ± 0.785	0.55 ± 0.031^{f}	0.25 ± 0.018^{d}	1.83 ± 0.022^{f}	0.11 ± 0.007^{d}	0.07 ± 0.003^{b}	2.81 ± 0.054^{f}
	DOX+IR	92.53 ± 0.217	0.51 ± 0.015^{f}	0.25 ± 0.014^{f}	1.57 ± 0.023^{f}	0.08 ± 0.002^{b}	0.08 ± 0.009^{b}	2.50 ± 0.045^{f}
0.5	MEM+IR	93.87 ± 0.217	0.30 ± 0.011	0.16 ± 0.015	1.25 ± 0.016	0.07 ± 0.005	0.05 ± 0.005	1.84 ± 0.020
	AQE+IR	94.40 ± 0.377	0.63 ± 0.015^{f}	0.38 ± 0.016^{f}	2.43 ± 0.087^{f}	0.18 ± 0.007^{d}	0.09 ± 0.009^{a}	3.73 ± 0.082^{f}
	MEE+IR	94.67 ± 0.435	0.69 ± 0.024^{f}	0.47 ± 0.007^{f}	2.11 ± 0.044^{f}	0.24 ± 0.026^{f}	0.10 ± 0.007^{b}	3.61 ± 0.053^{f}
	MCE+IR	95.47 ± 0.785	0.70 ± 0.015^{f}	0.42 ± 0.022^{d}	2.20 ± 0.042^{f}	0.22 ± 0.007^{f}	0.10 ± 0.007^{b}	3.51 ± 0.089^{f}
	DOX+IR	93.07 ± 0.217	0.63 ± 0.028^{f}	0.35 ± 0.013^{f}	1.91 ± 0.067^{f}	0.21 ± 0.005^{f}	0.10 ± 0.006^{b}	3.22 ± 0.082^{f}
1.0	MEM+IR	94.40 ± 0.653	0.41 ± 0.009	0.23 ± 0.009	1.48 ± 0.036	0.11 ± 0.005	0.07 ± 0.005	2.31 ± 0.015
	AQE+IR	95.20 ± 0.377	0.80 ± 0.011^{f}	0.48 ± 0.005^{f}	2.55 ± 0.046^{f}	0.24 ± 0.015^{f}	0.14 ± 0.009^{b}	4.21 ± 0.036^{f}
	MEE+IR	94.67 ± 0.217	0.91 ± 0.027^{f}	0.53 ± 0.019^{f}	2.40 ± 0.043^{f}	0.26 ± 0.007^{f}	0.15 ± 0.007^{b}	4.26 ± 0.059^{f}
	MCE+IR	96.00 ± 0.377	0.88 ± 0.015^{f}	0.52 ± 0.011^{f}	2.60 ± 0.035^{f}	0.35 ± 0.019^{f}	0.15 ± 0.002^{b}	4.50 ± 0.054^{f}
	DOX+IR	93.60 ± 0.377	0.79 ± 0.007^{f}	0.44 ± 0.017^{d}	2.38 ± 0.042^{f}	0.30 ± 0.003^{f}	0.14 ± 0.005^{b}	4.04 ± 0.042^{f}
2.0	MEM+IR	94.47 ± 0.217	0.54 ± 0.015	0.31 ± 0.011	1.79 ± 0.046	0.15 ± 0.003	0.10 ± 0.007	2.89 ± 0.049
	AQE+IR	94.67 ± 0.217	1.02 ± 0.017^{f}	0.55 ± 0.017^{f}	2.82 ± 0.066^{f}	0.37 ± 0.007^{f}	0.16 ± 0.004^{a}	4.92 ± 0.063^{f}
	MEE+IR	95.20 ± 0.377	1.10 ± 0.007^{f}	0.63 ± 0.007^{f}	2.67 ± 0.029^{f}	0.39 ± 0.018^{f}	0.17 ± 0.007^{b}	4.96 ± 0.055^{f}
	MCE+IR	95.47 ± 0.217	1.10 ± 0.032^{f}	0.59 ± 0.014^{d}	2.99 ± 0.054^{f}	0.43 ± 0.017^{f}	0.19 ± 0.015^{c}	5.30 ± 0.053^{f}
	DOX+IR	94.67 ± 0.435	1.05 ± 0.028^{f}	0.52 ± 0.009^{c}	2.59 ± 0.033^{e}	0.34 ± 0.008^{f}	0.16 ± 0.008^{b}	4.66 ± 0.049^{f}
3.0	MEM+IR	95.20 ± 0.377	0.66 ± 0.018	0.37 ± 0.011	2.05 ± 0.042	0.19 ± 0.015	0.12 ± 0.005	3.39 ± 0.039
	AQE+IR	96.53 ± 0.217	1.25 ± 0.026^{f}	0.71 ± 0.007^{f}	3.17 ± 0.038^{f}	0.46 ± 0.011^{f}	0.24 ± 0.011^{c}	5.80 ± 0.035^{f}
	MEE+IR	96.53 ± 0.217	1.31 ± 0.021^{f}	0.73 ± 0.015^{f}	3.09 ± 0.044^{f}	0.48 ± 0.025^{f}	0.23 ± 0.003^{c}	5.86 ± 0.019^{f}
	MCE+IR	97.87 ± 0.217	1.32 ± 0.044^{f}	0.74 ± 0.015^{f}	3.25 ± 0.052^{f}	0.59 ± 0.030^{f}	0.26 ± 0.003^{d}	6.16 ± 0.028^{f}
	DOX+IR	95.73 ± 0.217	1.27 ± 0.027^{f}	0.65 ± 0.017^{f}	2.80 ± 0.026^{d}	0.42 ± 0.011^{f}	0.24 ± 0.009^{c}	5.38 ± 0.045^{f}
r	MEM+IR	0.94	0.99	0.99	0.99	0.99	0.99	0.99
	AQE+IR	0.88	0.99	0.99	0.98	0.99	0.98	0.98
	MEE+IR	0.93	0.99	0.99	0.99	0.99	0.97	0.99
	MCE+IR	0.86	0.99	0.98	0.99	0.99	0.99	0.99
	DOX+IR	1.00	0.99	0.99	0.99	0.99	0.98	0.99

MEM= minimum essential medium; AQE = aqueous extract; MEE= methanol extract; MCE = methylene chloride extract and DOX = doxorubicin
P<a=0.05; b=0.02; c=0.01; d=0.002; e=0.001; f=0.0001 and No symbols =non-significant when MEM+IR compared with the other groups.
N=3; Significance between the treatments was calculated using Fisher's Exact test.

Table 3 Alteration in the radiation-induced chromosomal aberrations by various guduchi extracts in HeLa cells at 36 h post-irradiation.

Dose (Gy)	Treatments	Aberrant cells (%)	Aberrations per cell ± SEM					
			Chromatid breaks	Chromosome breaks	Acentric fragments	Dicentrics	Centric rings	Total aberrations
0.0	MEM+IR	90.40±0.754	0.14±0.003	0.09±0.005	0.74±0.042	0.04±0.003	0.04±0.002	1.06±0.035
	AQE+IR	92.53±0.217	0.42±0.009^{f}	0.20±0.005^{f}	1.40±0.031^{f}	0.09±0.009^{b}	0.09±0.005^{b}	2.19±0.038^{f}
	MEE+IR	91.20±0.377	0.44±0.013^{f}	0.21±0.012^{f}	1.44±0.054^{f}	0.10±0.003^{b}	0.08±0.005^{a}	2.28±0.064^{f}
	MCE+IR	91.73±0.435	0.48±0.005^{f}	0.22±0.012^{c}	1.40±0.029^{f}	0.10±0.003^{b}	0.11±0.004^{c}	2.31±0.039^{f}
	DOX+IR	91.47±0.217	0.48±0.007^{f}	0.19±0.007^{d}	1.10±0.028^{d}	0.07±0.005^{a}	0.29±0.005^{b}	1.93±0.015^{f}
0.5	MEM+IR	93.6±0.377	0.27±0.015	0.15±0.007	1.08±0.022	0.07±0.002	0.07±0.007	1.64±0.039
	AQE+IR	94.40±0.377	0.66±0.015^{f}	0.32±0.017^{d}	2.07±0.067^{f}	0.16±0.003^{f}	0.13±0.005^{b}	3.34±0.058^{f}
	MEE+IR	93.60±0.377	0.61±0.011^{f}	0.37±0.003^{f}	1.89±0.045^{f}	0.19±0.011^{f}	0.11±0.003^{a}	3.17±0.045^{f}
	MCE+IR	94.13±0.435	0.67±0.017^{f}	0.35±0.018^{f}	1.97±0.025^{f}	0.20±0.007^{f}	0.13±0.005^{b}	3.33±0.037^{f}
	DOX+IR	93.07±0.217	0.69±0.013^{f}	0.29±0.011^{f}	1.48±0.025^{d}	0.13±0.005^{b}	0.13±0.004^{b}	2.72±0.045^{f}
1.0	MEM+IR	93.33±0.576	0.34 ± 0.014	0.20 ± 0.007	1.33 ± 0.026	0.09 ± 0.005	0.09 ± 0,007	2.05 ± 0.042
	AQE+IR	94.40±0.377	0.80 ± 0.012^{f}	0.40 ± 0.011^{f}	2.24 ± 0.064^{f}	0.21 ± 0.005^{d}	0.16 ± 0.009^{b}	3.82 ± 0.053^{f}
	MEE+IR	94.40±0.653	0.80 ± 0.015^{f}	0.42 ± 0.011^{f}	2.16 ± 0.067^{f}	0.26 ± 0.007^{f}	0.15 ± 0.003^{b}	3.80 ± 0.070^{f}
	MCE+IR	94.40±0.754	0.84 ± 0.015^{f}	0.49 ± 0.018^{d}	2.28 ± 0.046^{f}	0.27 ± 0.013^{f}	0.18 ± 0.005^{c}	4.07 ± 0.055^{f}
	DOX+IR	93.60±0.377	0.81 ± 0.011^{f}	0.40 ± 0.015^{f}	1.75 ± 0.067^{d}	0.18 ± 0.004^{b}	0.16 ± 0.005^{b}	3.30 ± 0.045^{f}
2.0	MEM+IR	94.67 ± 0.785	0.50 ± 0.015	0.27 ± 0.007	1.57 ± 0.028	0.13 ± 0.005	0.12 ± 0.005	2.60 ± 0.036
	AQE+IR	95.20 ± 0.377	0.97 ± 0.015^{f}	0.51 ± 0.016^{d}	2.62 ± 0.062^{f}	0.31 ± 0.005^{f}	0.19 ± 0.005^{b}	4.61 ± 0.060^{f}
	MEE+IR	95.20 ± 0.377	1.00 ± 0.019^{f}	0.49 ± 0.007^{f}	2.39 ± 0.038^{f}	0.32 ± 0.005^{f}	0.22 ± 0.005^{c}	4.45 ± 0.045^{f}
	MCE+IR	94.93 ± 0.576	1.04 ± 0.017^{f}	0.55 ± 0.002^{d}	2.50 ± 0.039^{f}	0.34 ± 0.009^{f}	0.24 ± 0.007^{c}	4.67 ± 0.064^{f}
	DOX+IR	94.40 ± 0.00	0.97 ± 0.011^{f}	0.44 ± 0.009^{c}	2.10 ± 0.043^{d}	0.31 ± 0.005^{f}	0.20 ± 0.011^{b}	4.02 ± 0.042^{f}
3.0	MEM+IR	94.67 ± 0.576	0.58 ± 0.013	0.33 ± 0.009	1.84 ± 0.032	0.16 ± 0.002	0.14 ± 0.005	3.05 ± 0.035
	AQE+IR	96.27 ± 0.217	1.19 ± 0.032^{f}	0.60 ± 0.024^{f}	3.08 ± 0.064^{f}	0.40 ± 0.013^{f}	0.28 ± 0.013^{d}	5.56 ± 0.121^{f}
	MEE+IR	96.27 ± 0.435	1.17 ± 0.026^{f}	0.69 ± 0.011^{f}	2.93 ± 0.038^{f}	0.44 ± 0.020^{f}	0.28 ± 0.012^{d}	5.51 ± 0.041^{f}
	MCE+IR	96.27 ± 0.217	1.25 ± 0.026^{f}	0.61 ± 0.020^{f}	2.88 ± 0.007^{f}	0.46 ± 0.007^{f}	0.31 ± 0.007^{f}	5.50 ± 0.022^{f}
	DOX+IR	95.20 ± 0.377	1.11 ± 0.020^{f}	0.55 ± 0.015^{f}	2.56 ± 0.012^{e}	0.36 ± 0.009^{f}	0.28 ± 0.007^{c}	4.87 ± 0.035^{f}
r	MEM+IR	0.93	0.99	0.99	0.99	0.99	0.99	0.99
	AQE+IR	0.95	0.99	0.97	0.98	0.99	0.98	0.99
	MEE+IR	0.97	0.99	0.99	0.98	0.99	0.99	0.99
	MCE+IR	0.94	0.99	0.99	0.98	0.99	0.99	0.99
	DOX+IR	0.99	0.99	0.98	0.99	0.99	0.99	0.99

MEM= minimum essential medium; AQE = aqueous extract; MEE = methanol extract; MCE = methylene chloride extract and DOX = doxorubicin
P<a=0.05; b=0.02; c=0.01; d=0.002; e=0.001; f=0.0001 and No symbols =non-significant when MEM+IR compared with the other groups.
N=3; Significance between the treatments was calculated using Fisher's Exact test.

Table 4 Alteration in the radiation-induced chromosomal aberrations by different guduchi extracts in HeLa cells at 48 h post-irradiation.

Dose (Gy)	Treatments	Aberrant cells (%)	Aberrations per cell ± SEM					
			Chromatid breaks	Chromosome breaks	Acentric fragments	Dicentrics	Centric rings	Total aberrations
0.0	MEM+IR	85.60±0.377	0.12 ± 0.004	0.06 ± 0.002	0.49 ± 0.041	0.03 ± 0.003	0.06 ± 0.003	0.76 ± 0.039
	AQE+IR	88.53±1.57	0.37 ± 0.005^{f}	0.16 ± 0.005^{c}	1.10 ± 0.039^{f}	0.07 ± 0.003^{b}	0.12 ± 0.003^{b}	1.82 ± 0.033^{b}
	MEE+IR	87.73±0.949	0.37 ± 0.015^{f}	0.18 ± 0.009^{f}	1.02 ± 0.047^{f}	0.07 ± 0.005^{b}	0.13 ± 0.006^{b}	1.77 ± 0.062^{b}
	MCE+IR	87.73±0.576	0.40 ± 0.015^{f}	0.16 ± 0.007^{a}	1.08 ± 0.029^{f}	0.08 ± 0.007^{c}	0.13 ± 0.007^{b}	1.85 ± 0.039^{b}
	DOX+IR	87.47±0.785	0.40 ± 0.002^{f}	0.15 ± 0.005^{c}	1.01 ± 0.022^{f}	0.05 ± 0.007	0.10 ± 0.007^{a}	1.71 ± 0.017^{a}
0.5	MEM+IR	88.80 ± 0.377	0.23 ± 0.023	0.11 ± 0.009	0.74 ± 0.019	0.07 ± 0.005	0.09 ± 0.005	1.24 ± 0.033
	AQE+IR	91.20 ± 0.377	0.54 ± 0.023^{f}	0.28 ± 0.018^{c}	1.53 ± 0.047^{f}	0.15 ± 0.004^{c}	0.15 ± 0.005^{b}	2.61 ± 0.095^{b}
	MEE+IR	92.27 ± 0.949	0.54 ± 0.011^{f}	0.26 ± 0.009^{f}	1.48 ± 0.051^{f}	0.16 ± 0.005^{c}	0.16 ± 0.005^{b}	2.60 ± 0.044^{b}
	MCE+IR	93.07 ± 0.576	0.57 ± 0.008^{f}	0.27 ± 0.016^{c}	1.60 ± 0.011^{f}	0.17 ± 0.007^{d}	0.19 ± 0.005^{c}	2.80 ± 0.017^{c}
	DOX+IR	92.27 ± 0.576	0.52 ± 0.018^{f}	0.23 ± 0.005^{c}	1.39 ± 0.047^{f}	0.13 ± 0.005^{c}	0.15 ± 0.009^{b}	2.43 ± 0.038^{b}
1.0	MEM+IR	90.67 ± 0.217	0.31 ± 0.011	0.17 ± 0.015	0.99 ± 0.020	0.10 ± 0.005	0.12 ± 0.003	1.69 ± 0.034
	AQE+IR	92.80 ± 0.377	0.63 ± 0.005^{f}	0.37 ± 0.020^{f}	1.94 ± 0.066^{f}	0.17 ± 0.015^{b}	0.22 ± 0.007^{b}	3.32 ± 0.079^{b}
	MEE+IR	93.60 ± 0.377	0.65 ± 0.007^{f}	0.34 ± 0.012^{d}	1.80 ± 0.036^{f}	0.20 ± 0.005^{c}	0.22 ± 0.007^{c}	3.22 ± 0.022^{c}
	MCE+IR	94.40 ± 0.377	0.72 ± 0.009^{f}	0.35 ± 0.015^{c}	1.87 ± 0.047^{f}	0.22 ± 0.003^{d}	0.24 ± 0.007^{c}	3.41 ± 0.073^{c}
	DOX+IR	92.53 ± 0.217	0.66 ± 0.018^{f}	0.36 ± 0.015^{c}	1.70 ± 0.046^{f}	0.17 ± 0.011^{b}	0.21 ± 0.007^{b}	3.11 ± 0.013^{b}
2.0	MEM+IR	92.53 ± 0.576	0.44 ± 0.009	0.25 ± 0.009	1.15 ± 0.029	0.12 ± 0.005	0.15 ± 0.003	2.11 ± 0.042
	AQE+IR	94.13 ± 0.576	0.77 ± 0.009^{f}	0.42 ± 0.012^{c}	2.12 ± 0.047^{f}	0.25 ± 0.007^{d}	0.26 ± 0.009^{b}	3.83 ± 0.046^{b}
	MEE+IR	94.93 ± 0.217	0.79 ± 0.013^{f}	0.42 ± 0.020^{c}	2.10 ± 0.046^{f}	0.31 ± 0.020^{f}	0.28 ± 0.005^{c}	3.90 ± 0.081^{c}
	MCE+IR	94.93 ± 0.576	0.88 ± 0.017^{f}	0.44 ± 0.022^{d}	2.17 ± 0.039^{f}	0.27 ± 0.007^{f}	0.34 ± 0.007^{f}	4.82 ± 0.064^{f}
	DOX+IR	93.60 ± 0.377	0.76 ± 0.007^{f}	0.42 ± 0.007^{c}	1.79 ± 0.024^{f}	0.24 ± 0.003^{c}	0.25 ± 0.003^{b}	3.46 ± 0.032^{b}
3.0	MEM+IR	93.60 ± 0.377	0.51 ± 0.009	0.28 ± 0.011	1.40 ± 0.062	0.16 ± 0.005	0.18 ± 0.002	2.54 ± 0.057
	AQE+IR	95.73 ± 0.576	0.93 ± 0.017^{f}	0.57 ± 0.011^{d}	2.40 ± 0.041^{f}	0.32 ± 0.017^{d}	0.32 ± 0.009^{b}	4.54 ± 0.023^{b}
	MEE+IR	96.00 ± 0.377	0.90 ± 0.023^{f}	0.54 ± 0.005^{d}	2.32 ± 0.041^{f}	0.37 ± 0.026^{f}	0.36 ± 0.015^{d}	4.49 ± 0.091^{d}
	MCE+IR	96.27 ± 0.435	1.01 ± 0.015^{f}	0.57 ± 0.015^{f}	2.36 ± 0.042^{f}	0.35 ± 0.020^{f}	0.41 ± 0.017^{f}	4.76 ± 0.060^{f}
	DOX+IR	94.40 ± 0.00	0.95 ± 0.009^{f}	0.49 ± 0.009^{d}	1.95 ± 0.041^{d}	0.30 ± 0.020^{d}	0.31 ± 0.009^{c}	4.00 ± 0.064^{c}
r	MEM+IR	0.99	0.99	0.99	0.99	0.99	0.99	0.99
	AQE+IR	0.99	0.99	0.99	0.99	0.99	0.99	0.99
	MEE+IR	0.96	0.99	0.99	0.99	0.99	0.99	0.99
	MCE+IR	0.94	0.99	0.99	0.99	0.99	0.99	0.99
	DOX+IR	0.93	0.99	0.99	0.97	0.99	0.99	0.99

MEM= minimum essential medium; AQE = aqueous extract; MEE = methanol extract; MCE = methylene chloride extract and DOX = doxorubicin
P<a=0.05; b=0.02; c=0.01; d=0.002; e=0.001; f=0.0001 and No symbols =non-significant when MEM+IR compared with the other groups.
N=3; Significance between the treatments was calculated using Fisher's Exact test.

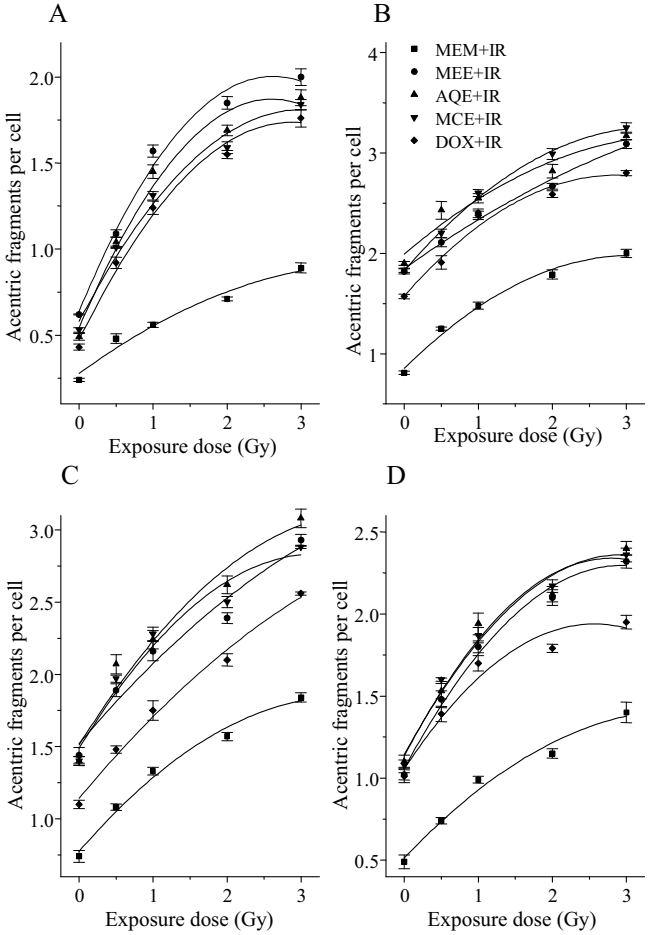

Fig. 3 Altered frequency of radiation-induced acentric fragments by various guduchi extracts in HeLa cells exposed to different doses of γ-radiation. (**A**) 12 h; (**B**) 24 h; (**C**) 36 h; (**D**) 48 h post-irradiation.

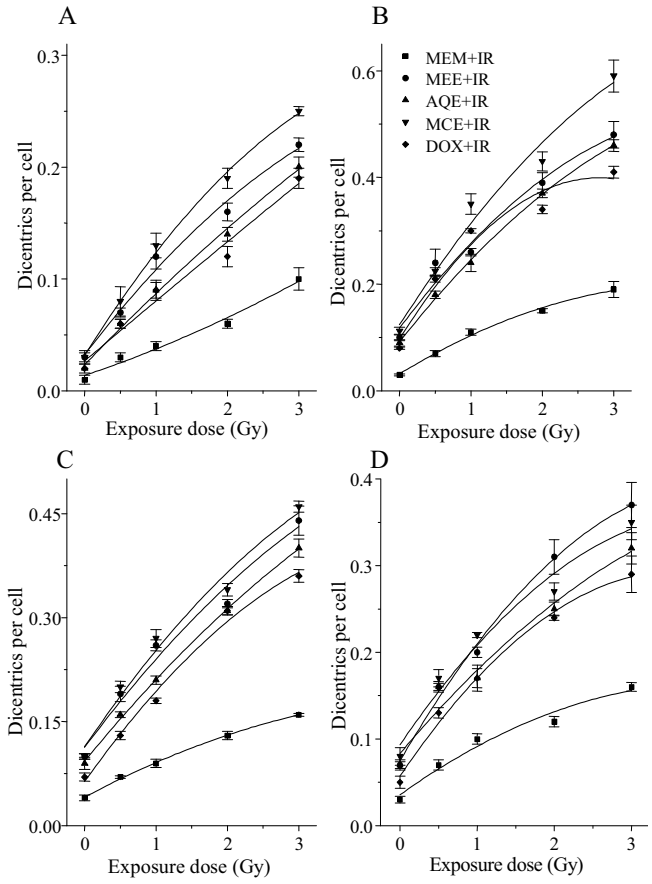

Fig. 4 Altered frequency of radiation-induced dicentric chromosomes by various guduchi extracts in HeLa cells exposed to different doses of γ-radiation. (**A**) 12 h; (**B**) 24 h; (**C**) 36 h; (**D**) 48 h post-irradiation.

Table 5 Dose enhancement factor for various guduchi extracts at different post-irradiation times.

Post-irradiation time (h)	Exposure Dose (Gy)	Treatments	Dose enhancement factor (DEF)					
			Chromatid breaks	Chromosome breaks	Acentric fragments	Dicentrics	Centric rings	Total aberrations
12	0.5	AQE+IR	1.07	2.33	2.29	2.00	2.00	1.93
		MEE+IR	1.47	1.67	1.92	2.00	3.00	1.84
		MCE+IR	1.13	3.00	2.00	2.50	4.00	1.89
		DOX+IR	1.00	2.67	2.04	2.00	2.00	1.75
	1	AQE+IR	1.70	2.00	3.00	2.33	1.67	2.47
		MEE+IR	1.81	1.86	2.97	3.00	1.67	2.53
		MCE+IR	1.18	2.28	2.44	3.33	2.67	2.12
		DOX+IR	1.48	1.86	2.53	2.33	1.67	2.12
	2.0	AQE+IR	1.68	1.67	2.55	2.40	1.60	2.10
		MEE+IR	1.68	1.67	2.62	2.60	1.60	2.13
		MCE+IR	0.89	1.14	2.25	3.20	2.20	1.94
		DOX+IR	1.31	1.83	2.38	2.00	1.60	1.89
	3.0	AQE+IR	1.63	2.47	2.01	2.00	2.14	1.97
		MEE+IR	1.72	2.47	2.00	2.11	1.57	1.99
		MCE+IR	1.45	2.47	1.90	2.44	2.28	1.89
		DOX+IR	1.49	2.47	1.93	1.89	1.71	1.81
24	0.5	AQE+IR	1.18	2.20	1.20	2.25	1.50	1.36
		MEE+IR	1.64	3.50	0.66	3.50	2.00	1.21
		MCE+IR	1.36	3.40	0.84	2.75	1.50	1.04
		DOX+IR	1.09	2.00	1.50	3.25	1.00	1.07
	1.0	AQE+IR	1.36	1.75	0.97	1.87	2.00	1.21
		MEE+IR	1.82	1.83	0.86	2.00	2.25	1.28
		MCE+IR	1.50	2.25	1.15	3.00	2.00	1.48
		DOX+IR	1.27	1.58	1.20	2.75	1.50	1.35
	2.0	AQE+IR	1.68	1.67	2.55	2.40	1.60	2.10
		MEE+IR	1.68	1.67	2.62	2.60	1.60	2.13
		MCE+IR	0.89	1.14	2.25	3.20	2.20	1.94
		DOX+IR	1.31	1.83	2.38	2.00	1.60	1.89
	3.0	AQE+IR	1.59	1.69	1.02	2.31	2.00	1.34
		MEE+IR	1.70	1.61	1.02	2.37	1.89	1.38
		MCE+IR	1.64	1.88	1.14	3.00	2.11	1.51
		DOX+IR	1.62	1.64	0.99	2.12	1.78	1.30
36	0.5	AQE+IR	1.85	2.00	1.97	2.33	1.33	1.98
		MEE+IR	1.30	2.67	1.32	3.00	1.00	1.53
		MCE+IR	1.46	2.17	1.68	3.33	0.67	1.76
		DOX+IR	1.61	1.67	1.12	2.00	1.33	1.36
	1.0	AQE+IR	1.90	1.82	1.42	2.20	1.40	1.65
		MEE+IR	1.80	1.91	1.22	3.20	1.40	1.53
		MCE+IR	1.80	2.45	1.49	3.40	1.40	1.78
		DOX+IR	1.65	1.91	1.10	2.20	1.40	1.38
	2.0	AQE+IR	1.53	1.72	1.47	2.44	1.25	1.57
		MEE+IR	1.55	1.55	1.14	2.44	1.75	1.41
		MCE+IR	2.80	1.83	1.32	2.67	1.62	1.53
		DOX+IR	1.36	1.39	1.20	2.67	1.37	1.36
	3.0	AQE+IR	1.75	1.67	1.53	2.58	1.90	1.69
		MEE+IR	1.66	2.00	1.35	2.83	2.00	1.62
		MCE+IR	2.75	1.62	1.34	3.00	2.00	1.60
		DOX+IR	1.43	1.50	1.33	2.42	1.90	1.48
48	0.5	AQE+IR	1.54	2.40	1.72	2.00	1.00	1.64
		MEE+IR	1.54	1.60	1.84	2.25	1.00	1.73
		MCE+IR	1.54	2.20	2.08	2.25	2.00	1.98
		DOX+IR	1.09	1.60	1.52	2.00	1.67	1.50
	1.0	AQE+IR	1.37	1.91	1.68	1.43	1.67	1.61
		MEE+IR	1.47	1.45	1.56	1.86	1.50	1.56
		MCE+IR	1.68	1.73	1.58	2.00	1.83	1.68
		DOX+IR	1.37	1.91	1.38	1.71	1.83	1.50
	2.0	AQE+IR	1.25	1.37	1.54	2.00	1.55	1.49
		MEE+IR	1.31	1.26	1.64	2.67	1.67	1.58
		MCE+IR	1.50	1.47	1.65	2.11	2.33	1.68
		DOX+IR	1.12	1.42	1.18	2.11	1.67	1.30
	3.0	AQE+IR	1.43	1.86	1.43	1.92	1.67	1.53
		MEE+IR	1.36	1.64	1.43	2.31	1.92	1.53
		MCE+IR	1.56	1.86	1.41	2.08	2.33	1.63
		DOX+IR	1.41	1.54	1.03	1.92	1.75	1.29

+irradiation, AQE+irradiation, MEE+irradiation, MCE +irradiation, and DOX+irradiation groups at all the post-irradiation times (**Fig. 5**). The elevation in the frequency of rings was significantly higher in AQE+irradiation, MEE +irradiation, MCE+irradiation, and DOX+irradiation groups when compared to MEM+irradiation group. The frequency of rings increased steadily with time in all the groups and a maximum number of rings was observed at 48 h post-irradiation (**Tables 1-4**). The dose enhancement factor for ring formation varied between 1.7 to 2.3 at 48 h depending

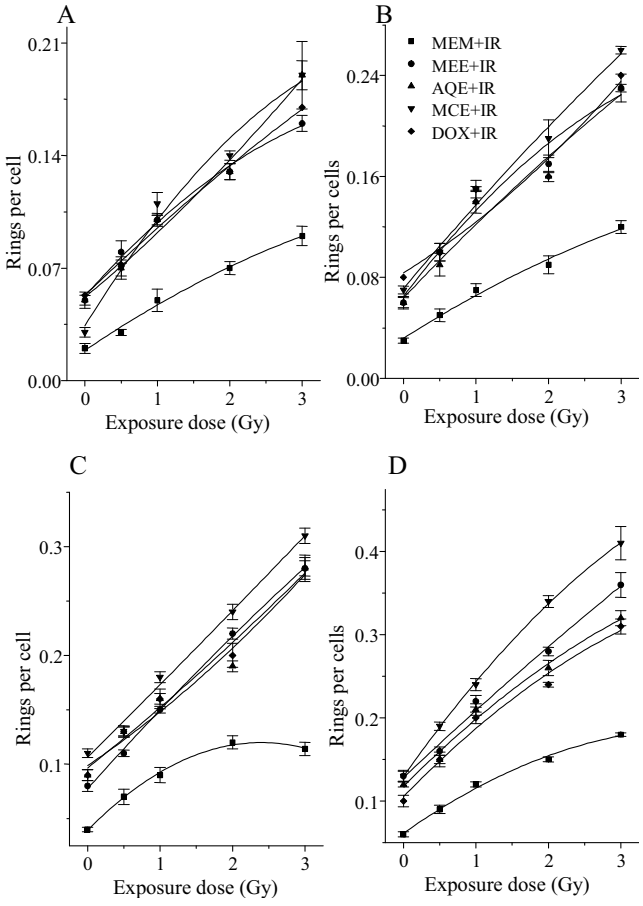

Fig. 5 Altered frequency of radiation-induced ring chromosomes by various guduchi extract in HeLa cells exposed to different doses of g-radiation. (**A**) 12 h; (**B**) 24 h; (**C**) 36 h; (**D**) 48 h post-irradiation.

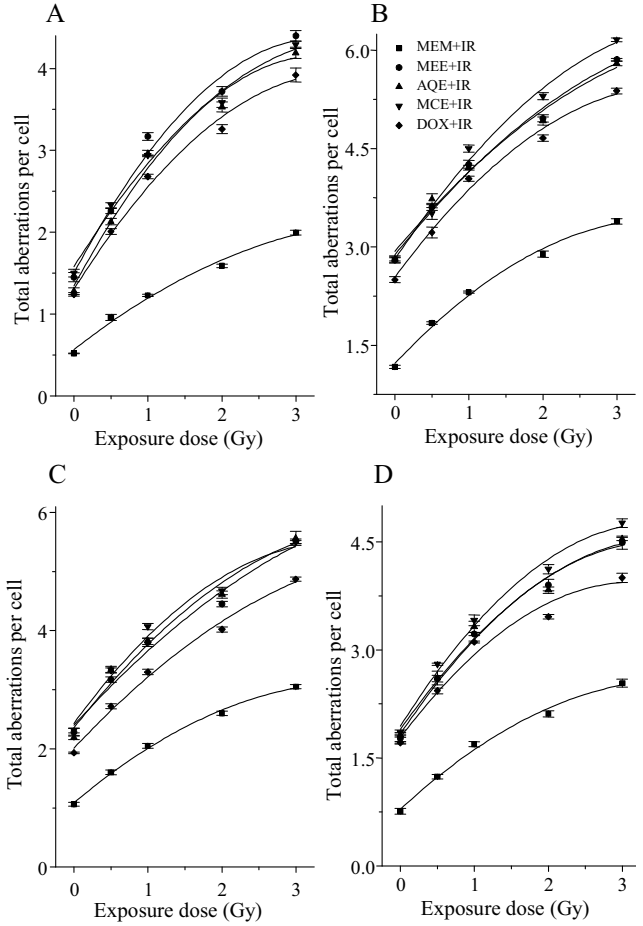

Fig. 6 Altered frequency of radiation-induced total aberrations by various guduchi extracts in HeLa cells exposed to different doses of g-radiation. (**A**) 12 h; (**B**) 24 h; (**C**) 36 h; (**D**) 48 h post-irradiation.

on the type of guduchi extract treatment and exposure dose (**Table 5**). The dose-response relationship for all the groups was linear quadratic at different post-irradiation times (**Tables 1-4**).

Total aberrations

The total aberrations per cell increased significantly in AQE, MEE, MCE or DOX+sham-irradiation groups when compared to MEM+sham-irradiation. The total aberrations per cell increased with the increase in post-irradiation scoring time, reached a peak level at 24 h post-treatment and declined thereafter (**Fig. 6**). Irradiation of HeLa cells to different doses of γ-rays resulted in a dose related but significant rise in the total aberrations per cell when compared to MEM+sham-irradiation group. Treatment of HeLa cells with 10 µg/ml of AQE or MEE or MCE or DOX before irradiation caused a significant rise in the total aberrations at all the post-irradiation scoring times when compared to MEM+irradiation group (**Tables 1-4**). The total aberrations increased with time and the highest frequency was scored at 24 h post-irradiation that declined thereafter without restoration to MEM+sham-irradiation level at 48 h post-irradiation. The dose enhancement factor was between 1.2 to 1.5 depending on the type of treatment and dose of irradiation at 24 h post-irradiation (**Table 5**). The dose response relationship was linear quadratic for all the groups at various post-irradiation times (**Tables 1-4**).

DISCUSSION

Cell culture provides a rapid, efficient and economic system for cytotoxicity screening, allowing elucidation of the mode of action of a drug in a controlled, and systematic manner with a high degree of resolution. Chromosomal aberrations are often considered the chief or even the only cause of reproductive cell death following irradiation. The reproductive cell death is mainly because of the loss or mis-assortment of genetic material during cytokinesis (Evans 1967; Savage 1975; Jagetia 1994). It has usually been considered that damage caused by irradiation is converted into aberrations within a few hours, with none arising later, after which the aberrations are stable unless the cells undergo cytokinesis, when mechanical problems like breakage or chromosome bridges or loss of acentric fragments can occur (Bedford and Cornforth 1987; Jagetia 1994; Jagetia and Venkatesha 2006). Our earlier study has shown a maximum cytotoxic and radiosensitizing effects of various guduchi extracts at a concentration of 10 µg/ml therefore, radiosensitizing action of 10 µg/ml of aqueous, methanol and methylene chloride extracts of guduchi was evaluated in cultured HeLa cells exposed to different doses of γ-radiation at various post-irradiation times by screening asymmetrical chromosome aberrations.

Despite the fact that the HeLa cells are genomically aberrant, irradiation of HeLa cells resulted in a dose dependent rise in the frequency of aberrant cells in both MEM +irradiation and AQE, MEE, MCE or DOX +irradiation groups. A dose dependent increase in the aberrant cells has been reported in cultured human peripheral blood lymphocytes exposed to different doses of radiation and combination with other drugs earlier (Swanson *et al.* 1967; Lloyd *et al.* 1975; Littlefield *et al.* 1987). Exposure of SW1116 cells to ^{125}I monoclonal antibody has been reported to increase the chromosome breaks in a dose dependent manner (Woo *et al*. 1989). Similarly, an increased yield of acentric fragments, dicentrics and simple exchanges have been observed in the cultured lymphocytes of prostate cancer patients undergoing radiotherapy (Hille *et al*. 2010). The chromosome aberrations like chromatid breaks, chromosome

breaks, acentric fragments and total aberrations increased significantly in a dose dependent manner in the MEM +irradiation group. These findings are in conformation with the earlier findings, where a dose related increase in chromatid breaks has been reported in cultured human lymphocytes (Chu *et al.* 1961; Norman *et al.* 1988; Antoccia *et al.* 1992; Terzoudi *et al*. 2011) and mouse bone marrow cells (Bender and Gooch 1963; Kligerman 1988; Jagetia 1994; Jagetia *et al.* 2003; Jagetia and Venkatesha 2006). The asymmetrical exchanges like dicentric and centric rings increased in a dose dependent manner in MEM+irradiation group and the dose response was linear quadratic. Similarly, dicentric and rings have been reported to increase with increasing dose of radiation in vivo and in vitro with a linear quadratic dose response relationship (Chu *et al.* 1961; Bender and Gooch 1963; Fabry 1986; Jagetia 1994; Jagetia *et al.* 2003; Jagetia and Venkatesha 2006; Terzoudi *et al*. 2011). The cells irradiated in different phases of cell cycle produce different types of chromosomal aberrations. The irradiation of cells in G_0/G_1 or S-phase of the cell cycle induces chromosome-type or chromatid-type aberrations and chromatid exchanges, respectively, whereas irradiation of cells in G_2-phase induces mainly chromatid breaks in mammalian cells. However, if the cells are irradiated during M-phase, both chromatid and chromosome type of aberrations are produced (Natarajan and Meyers 1979; Terzoudi *et al*. 2011). Since asynchronous HeLa cells were irradiated, all types of chromosome aberrations could be scored in the present study. The induction of chromosome aberration in an irradiated cell is a good indicator of radiosensitization, cell killing and induction of cancer (Natarajan 2002; Terzoudi *et al*. 2011). Treatment of HeLa cells with AQE, MEE, MCE or DOX before exposure to various doses of gamma radiation enhanced the formation of asymmetrical aberrations by a factor of 2 or more at all the post-irradiation scoring times depending on the irradiation dose, irrespective of the treatment when compared to MEM+irradiation group. The dose enhancement factor varied between 1.7 to 3.5 depending on the type of guduchi extract, radiation dose and scoring time. A potentiation of ≥ 1.4 or > 1.4 indicates a good radiosensitizing effect. At lower doses highest potentiation was observed at 12 h, whereas at higher doses the maximum enhancement in the effect of radiation was found at 36 h. This may be due to prolongation of delay in the cell division by pre-treatment with various guduchi extracts. A similar effect has been observed in CHO cells treated with turmeric extract before exposure to different doses of γ-radiation (Araújo *et al*. 1999). Ionizing radiation and cytostatic drugs have been reported to enhance the chromosome aberrations in human bone marrow cells earlier (Preston *et al.* 1972; Awa 1974; Lucas *et al.* 1989). Chlorpromazine has been reported to increase the radiation-induced dicentric, rings and deletions in human lymphocytes (Goetz *et al.* 1975). Similarly, MMC has also been reported to increase the chromosome aberrations in combination with radiation (Goetz *et al.* 1976). Treatment of lymphocytes in combination with neutron or X-irradiation and caffeine resulted in the increase in chromosome aberrations (Natarajan *et al*. 1980). A similar effect was observed for hydroxyurea, caffeine, novobiocin and mitomycin C (Kihlman *et al*. 1982; Takahashi *et al.* 1986; Ijima *et al.* 1991; Karmakar *et al.* 1994). The chromosome aberrations persisted in the cells until 48 h and did not reach to normal level in both MEM +irradiation and guduchi pretreated irradiated groups, which may be the reason for its cell killing and radiosensitizing effects in our earlier study. A similar effect has been observed earlier in irradiated human lymphocytes and TK6 lymphoblastoid cells *in vitro* and mice bone marrow cells *in vivo* (Jagetia and Ganapathi 1989; George 2001; Andreev and Eidelman 2011).

The mechanism of enhanced radiosensitivity by various guduchi extracts is not well understood. However, the radiosensitizing effect of guduchi may be attributed to several putative mechanisms. Ionizing radiation interacts with cellular genome by induction of free radicals (Becker and Sevilla 1993) and guduchi extracts may have further escalated the induction of free radicals aggravating the radiation-induced chromosome damage. Guduchi may also have reduced the antioxidant status of cells by generating reactive oxygen species (ROS) thus increasing damage to cellular genome. Guduchi has been reported to increase superoxides, hydrogen peroxide and TNFα (More and Pai 2011). Ionizing radiations have been reported to cause single and double strand breaks, sugar and base damages as well as protein crosslinks in DNA (Goodhead 1994), which are subsequently expressed as various types of asymmetrical aberrations (Bryant *et al*. 2010). The presence of guduchi before irradiation may have increased the molecular DNA damage leading to a further rise in the asymmetrical chromosome aberrations. This contention is supported by our recent study, where an increase in the radiation-induced molecular DNA damage by methylene chloride extract of guduchi in HeLa cells has been reported by comet assay (Jagetia and Rao 2011). Phytochemical analysis of guduchi has shown the presence of berberine alkaloid, which has been reported to induce internucleosomal DNA fragmentation resulting in the formation of a complex with DNA and inhibit topoisomerase II enzyme *in vitro* (Hande 1998). A similar action cannot be ruled out in the present study, where inhibition of topoisomerase II enzyme by guduchi may have increased the radiation-induced chromosome damage as compared to irradiation alone. Although no attempt has been made to investigate the molecular mechanisms of action in the present study, there is no reason to believe that guduchi may have not employed this pathway to exert its action on HeLa cells. Irradiation has been reported to increase the transactivation of NF-κB in HeLa cells (Bradbury *et al.* 2001) and inhibition of transactivation of NF-κB by guduchi may have resulted in the increased molecular damage to DNA, and accrued radiosensitization. The inhibition of NF-κB and DNA double strand break repair has been reported to increase the effect of radiation (Estabrook *et al*. 2011). Recently, berberine, an isoquinoline alkaloid and other phytochemicals present in the extracts of guduchi have been reported to suppress the transactivation of NF-κB (Pandey *et al.* 2008). Similarly, octacosanol present in the stem of guduchi has also been reported to suppress the transactivation of NF-κB (Thippeswamy *et al.* 2008). Guduchi may have also reduced the radiation-induced activation of COX-II and LOX-5 mRNA and thus might have increased the effect of radiation in HeLa cells. Alcoholic extract of guduchi has been reported to inhibit their activities *in vitro* (Li *et al.* 2004).

It is clear from our study that cytotoxic effects of guduchi is due to its ability to enhance the radiation-induced DNA damage as evidenced by increased chromosomal aberrations. This may be due the presence of various phytochemicals including disaccharides like cordifolioside acetates and alkaloids like tinosporin, tinosporic acid, berberine and tinosporol, octacosanol, N-formyl-asimilobine, 2-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (tinoscorside A, 1), *N*-acetylasimilobine 2-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (tinoscorside B, 2) and β sitosterol (Gangan *et al.* 1995, 1996; Sarma *et al.* 2009; Thippeswamy *et al.* 2009; Phan *et al.* 2010; Sharma *et al*. 2010).

CONCLUSIONS

From the present study it is clear that all guduchi extracts were able to enhance the effect of radiation by bringing out changes in the fidelity of DNA resulting in various types of chromosomal aberrations which may be responsible for greater cell kill as reported earlier (Jagetia *et al.* 2002). Out of all the extracts tested the MCE was most potent followed by MEE in enhancing the radiation-induced DNA damage in HeLa cells, an observation identical to our earlier study (Jagetia *et al.* 2002). The exact mechanism of action of guduchi is not known but it is certain that it has increased the DNA damage, which is expressed as increased chromosome aberrations. This increase in asymmetrical chromo-

some aberrations may be due to increase in radiation-induced oxidative stress, and inhibition of topoisomerase II by guduchi extracts. Guduchi may have also blocked the transactivation of radiation-induced NF-κB, COX-II and LOX-5 and increased the effect of radiation.

This increased radiosensitization by guduchi may be due to the presence of various phytochemicals including various disaccharides and alkaloids. The comparison of the effects of guduchi treatment with doxorubicin revealed that guduchi extracts were as effective as doxorubicin in enhancing the radiation-induced chromosome aberrations and MCE enhanced the effect of radiation even greater than doxorubicin treatment before irradiation. Therefore, methylene chloride extract which has been reported to be more cytotoxic than doxorubicin (Jagetia *et al.* 2002) deserves consideration as a drug that can enhance the effect of radiation.

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