cess against diseases of a wide variety of economically important crops including Fusarial rots and wilts[28]. Thus, they have the potential to suppress the fish pathogenic *Fusaria*. Intensive research is needed to exploit their full potential against *Fusarium* spp. pathogenic to fish. Moreover, studies on the integrated use of several micro-organisms will be needed to control the diseases caused by different fungi.

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Radioprotective effect of *Phyllanthus niruri* on mouse chromosomes

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Micronuclei (MN) induction in the mouse bone marrow polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) was studied 24 h after whole body exposure to 4 Gy of gamma radiation with or without a single intraperitoneal injection of 25 mg/kg to 150 mg/kg of 50% methanolic extract of *Phyllanthus niruri* (whole aerial part). The extract significantly reduced the radiation-induced MN induction in both PCE and NCE and increased the ratio of PCE to NCE. The effect increased linearly with extract dose from 25 to 125 mg/kg.

PHYLLANTHUS niruri (syn. *P. fraternus* Webster, f. Euphorbiaceae) is a winter weed, growing in the tropical parts of India, and the whole plant, fresh leaves and roots are used as medicine[1]. It is found to be effective in treating infective hepatitis, and is used as an adjuvant with other herbal medicines. Sane *et al.*[2] reported that *P. amarus* (syn. *P. niruri*[3]) protected rats against carbon tetrachloride induced liver toxicity. Some drugs used in liver treatment like Liv. 52 and Thiola (2-mercaptopropionylglycine, MPG) have shown good radioprotective effect against radiation-induced chromosome damage in mouse[4–6]. But there is no study on the radioprotective effect of *Phyllanthus*. Therefore, an experiment was conducted to see if the plant possesses any protective effect against radiation damage *in vivo*.

Fresh *Phyllanthus niruri* plants were collected from the fields near Mangalore, during the months of February to April and were identified. Complete aerial parts were removed, washed, shade dried and powdered. Extract (PNE) was prepared by refluxing with methanol 50% at 60°C and concentrated under vacuum. Extract is not soluble in water. A fine suspension was prepared using 0.5% carboxy methyl cellulose (CMC) in phosphate-buffered saline, freshly before use.

Six- to eight-week-old Swiss albino mice weighing 25 ± 3 g were selected from an inbred colony maintained in our laboratory, under controlled conditions of temperature (23 ± 2°C) and humidity (50 ± 5%). The animals were administered intraperitoneally (i.p.) with 0.2 ml of 0.5% CMC or PNE, at 25 to 150 mg/kg body weight one hour before whole body irradiation with 4 Gy of gamma radiation. Animals were restrained in well-ventilated polypropylene boxes and whole body exposed to cobalt-60 gamma rays from a Gammatron

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Table 1. Effect of *Phyllanthus niruri* extract on micronucleus induction by radiation in mouse bone marrow

Treatment	MPCE/1000	MNCE/1000	P/N ratio
Control	00.00	00.00	1.02 ± 0.01
RT (4 Gy)	123.45 ± 0.12	21.30 ± 0.08	0.58 ± 0.02[a]
PNE, 25 mg/kg + RT	109.31 ± 0.10[b]	18.21 ± 0.10[c]	0.62 ± 0.01[ac]
PNE, 50 mg/kg + RT	84.13 ± 0.09[b1]	16.13 ± 0.09[c1]	0.76 ± 0.02[ac1]
PNE, 75 mg/kg + RT	67.17 ± 0.10[b1]	12.11 ± 0.09[c1]	0.88 ± 0.01[ac1]
PNE, 100 mg/kg + RT	53.21 ± 0.09[b1]	8.41 ± 0.15[c1]	0.95 ± 0.03[c]
PNE, 125 mg/kg + RT	34.12 ± 0.12[b1]	5.10 ± 0.10[c1]	0.99 ± 0.01[c]
PNE, 150 mg/kg + RT	33.61 ± 0.11[b]	5.09 ± 0.14[c]	1.01 ± 0.02[c]

a: $P < 0.001$ compared to control, b: $P < 0.0001$, c: $P < 0.001$ compared to RT alone, 1: $P < 0.001$ compared to immediate lower dose of extract.

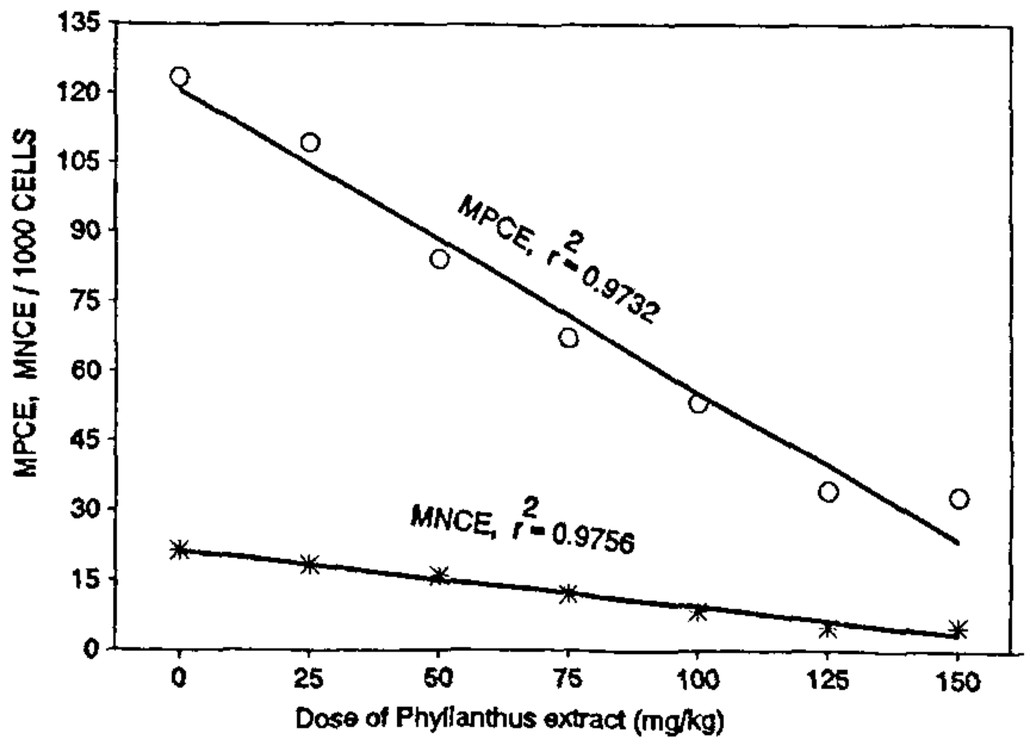

Figure 1. Dose response curves for MPCE and MNCE in the bone marrow of mice treated with different doses of *Phyllanthus* extract before whole body irradiation (4 Gy). Data from Table 1.

teletherapy unit (Siemens, Germany) at a dose rate of 1 Gy/min. One group of animals was sham irradiated to serve as control. Five animals were used in each group. Twenty four hours after irradiation, the animals were killed by cervical dislocation and micronuclei were prepared according to the method of Schmid[7]. Briefly, the femur of each animal was dissected out and the bone marrow was flushed out into Dulbeco's modified Eagle's medium and centrifuged. Supernatant was discarded and the pellet was dispersed in a few drops of fetal calf serum (FCS). Smears were prepared and stained with 0.125% acridine orange (BDH, England) in Sorensen's buffer and observed under a fluorescent microscope (Carl Zeiss Photomicroscope III, Germany). A minimum of 2000 polychromatic (PCE) and normochromatic erythrocytes (NCE) were observed from each animal and the cells containing micronucleus (MPCE and MNCE) were recorded. The data are expressed as the number of MPCE or MNCE per 1000 cells. The ratio of polychromatic to normochromatic erythrocytes (P/N ratio) was also calculated. The statistical significance between the groups was determined by one-way analysis of variance (ANOVA) with application of post-hoc Bonferroni's test.

Whole body irradiation resulted in a significant increase in the MPCE (123/1000 cells) and MNCE (21/1000 cells) and a significant reduction in the P/N ratio (0.5). Pretreatment with PNE, at all doses, significantly reduced the micronucleus induction in both PCE and NCE and increased the P/N ratio (Table 1). This effect increased linearly ($r^2 = 0.9732$ for MPCE, 0.9756 for MNCE and 0.9446 for P/N ratio) with the extract dose (Figure 1). The effect increased significantly with each dose increment from 25 mg/kg to 125 mg/kg, but increase in dose to 150 mg/kg did not give any further decrease in the radiation-induced chromosome damage (Table 1).

Even though hepatic protection against chemical toxicity by this plant was reported earlier[2] and is made use of in the treatment of liver diseases in the traditional medical systems[1,3], this is the first time that a radioprotective effect of this plant is reported. Our results clearly demonstrate that the 50% methanolic extract of the aerial parts of the plant protects mouse chromosomes against radiation damage and this effect increases with the dose administered. But the maximum protection was seen at a dose of 125 mg/kg, and no further protection could be obtained by increasing the extract dose to 150 mg/kg. Thus, there seems to be an optimum dose for protection for this extract, which is much below the toxic dose. We did not find any toxic effects even at a dose of 2 g/kg of the extract; higher doses were not tried. This is similar to the earlier findings on the anti-

oxidant radioprotectors MPG[8] and *Ocimum* leaf extract[9]. Thus, *Phyllanthus niruri* could be a promising candidate for nontoxic radiation protection and, therefore, needs to be studied in more detail.

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Application of FISH-MN technique to probe micronuclei formation in normal, transformed and malignant cells using alpha satellite pan centromeric DNA

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A micronucleus assay employing biotinylated alpha pan centromeric DNA probe was used in the present study. The study sample consisted of 4 normal, 2 transformed and 9 malignant cell lines of human origin. The frequency of centromere positive micronuclei (c + MN) was found to decrease from normal (67%) to transformed (64.9%) to cancer cells (59%). The c + MN/c–MN ratio from normal to malignant cells was found to vary significantly ($P < 0.01$), while the signal ratio showed absence of any correlation ($P > 0.05$). These results demonstrate the usefulness of combining FISH technique and micronuclei assay to trace the mechanism of micronucleus formation in cells of diverse histopathological origin.

THE micronucleus test provides a quick and easy method of evaluating chromosomal damage. Formation of micronuclei (MN) can occur either from lagging chromosomes or due to their loss from the cell during division. The MN assay has been used on a variety of tissues including blood lymphocytes[1,2], fibroblasts[3], mouse splenocytes[4], bone marrow[5] and tumour cells[6,7]. Although the routine MN assay is used for determining the extent of genomic damage, it suffers from one serious drawback, i.e. it does not uncover the nature of damage. The problem becomes more relevant in the light of findings that indicate preferential chromosome integration in micronuclei[8,9]. To overcome these limitations, fluorescence *in situ* hybridization technique (FISH) has been successfully used in the past. FISH assay has been employed for visualizing the whole chromosomes[8,10], kinetochores[3,5,11], centromeres[12–17] and telomeres[18] in the micronuclei. By using this technique it is also possible to distinguish between clastogenic and aneugenic activity of chemicals or physical agents[4,19,20]. It provides a convenient way of analysing chromosome segregation in interphase cells or detecting amplified genes in tumour cells[6]. Thus, problems which flaw the routine staining techniques are overcome using this method. Recently, flow cytometry has been used in combination with FISH technique to make it more useful[10,21,22].

Although many FISH-MN studies have been conducted in the past, most of the work has been carried out in lymphocytes and cell lines. To our knowledge, no report exists on the FISH-MN status in cell lines exhibiting different levels of malignancy. To address this question, the present study was carried out on normal, transformed and malignant human cell lines.

Established human cell lines representing normal fibroblasts (HMB, FRS, TUB, SV350 and SV371), transformed fibroblasts (WI-38 VA 13, GM) and cancer cells, i.e. melanoma (MeWo, Be11), head and neck cancer (4197, 4451), glioblastoma (U87-155), cancer of rectum (RKO) and colon (SW 480) were considered. Cells were grown in RPMI 1640 medium enriched with 10% (v/v) foetal calf serum (pH 7.2) at 37°C in an atmosphere of 5% CO_2 and 95% air mixture. Before harvesting, cells were trypsinized at 37°C under sterile conditions and subcultured for 24 h at 1×10^6 cells/ml concentration. At the termination of culture, cells were fixed in methanol/acetic acid (3:1) and slides were prepared. The FISH staining was carried out as per previously described procedures[23]. Briefly, cells were first treated with RNase, followed by pepsin treatment and post fixation in a solution of phosphate buffered saline, magnesium chloride and formaldehyde for 10 min.

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