Prophylactic effect of *Phyllathus amarus* plant extract on mouse bone marrow cells irradiated with X-rays

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Abstract

The herbal preparations of *Phyllathus amarus* has been used extensively in indigenous system of medicine for treating many infectious diseases. A great deal of interest has been generated on the protective effects of whole plant extracts of *Phyllanthus* against mutagens. The present study was designed to evaluate the modifying effect of *Phyllanthus* extract against X-ray induced chromosomal damage in bone-marrow cells of mice. The animals were irradiated at a single dose and durations and were treated with *Phyllanthus* extract to evaluate the modifying mechanism in the exposed cells.

Keywords: Antimutagen; Clastogenic; Genotoxic; Radioisotopes.

Introduction

Radioisotopes have assumed great importance in medical field for diagnostic as well as therapeutic purposes. These applications inevitably expose normal tissues to ionizing radiations that induce lesions in DNA in living cells, which contribute to great genetic burden. The lesions are expressed as structural aberrations of chromosomes. These hazards of radiation emphasize the need to seek certain prophylactic measures to counter the radiation induced genetic damage.

Interest in dietary anti-mutagens of plant origin was reviewed after observation that crude plant extracts reduced the activity of a number of known environmental mutagens (Abraham et al. 1986; Ito & Sugiyama, 1985; Iain et al. 1978). Herbal preparations of *Phyllanthus amarus* and its related species have generated a great deal of attention in recent years for a wide variety of protective effects against various mutagens (Alldrick et al. 1986; Debisari Sankar, 1981; Dhir et al. 1991; Gowrishankar & Vivekanandan, 1994). These extracts were used extensively in indigenous systems of medicine for treating ailments such as leucorrhoea (Rao et al. 1985, scurvy Chopra et al. 1956), and ulcerations (Gupta, 1908). Besides, they have been recorded to have antibacterial (Vinayagamoorthy, 1982), antiviral (Saigopal et al. 1986), antipyretic and laxative properties (Rao & Siddique, 1964). Since, these preparations are extensively consumed in varying quantities, their effects in organisms exposed to physical and chemical pollutants in our environment need to be assessed. Hence, this preliminary study involved the evaluation of modifying effects of the crude extract of *Phyllathus amarus* against X-irradiation, induced chromosomal damage in mouse bone marrow cells.

Materials and methods

A total Eight-week-old Swiss Albino mice weighing 25-30 g were procured from The King Institute, Guindy, Chennai, India and were maintained in pathogen-free condition. Pellet diets (Lipton India Ltd.) and water were provided ad libitum.

The crude extract of *Phyllathus amarus* was freshly prepared by homogenizing 0.5g of leaves in 10 ml distilled water and further diluting the suspension to obtain 0.5% decoction.

Irradiation was carried out at MERADO Lab, CSIR Complex, Chennai, India. Animals were held in a well-ventilated poly-propylene holder and exposed to 3 Gy of X-rays. The dose rate as determined by Fricke’s ferrous sulphate dosimetry.

The experiments were carried out on three sets of animals, each consisting of six animals. The first set was administered with a single doses of intra-peritoneal injection of *Phyllanthus* extract at a dose rate of 200mg/kg body weight, while the second set of animals were exposed to 3 Gy of X-rays at a dose rate 0.5 Gy/min. These animals were killed by cervical dislocation after 48, 72 and 96 hr to obtain the bone marrow samples. The third set of animals were given acute dose of Phyllanthus decoction (200 mg/kg b.w) after exposure to X-rays at 0.5 Gy/min dose rate and were sacrificed after 96 hr. Six animals were also maintained as control. Colchicine (Labo Chemicals/Sigma Chemicals) of 0.005% was given (0.5 ml) intraperitoneally to each treated and exposed animals for 2 hr before sacrifice. The animals were killed by cervical dislocation. The bone marrow samples were removed from both femur bones and flushed in 0.075 M potassium chloride, incubated at 37°C for 20 minutes, and thereafter centrifuged at 800 rpm for 5 mins and the cells were fixed in cold 1:3 glacial acetic acid and ethanol after discarding the supernatant. Slides were prepared by flame-drying and stained in Giemsa (Preston et al. 1981).

Received: March 2014
Accepted: April 2014

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Research Article
THE SCITECH JOURNAL ISSN 2347-7318 ISSN 2348-2311 Online

Mitotic indices were recorded from the treated and the control animals by examining 800-1500 cells per animal. For the determination of chromosomal aberrations, a minimum of 100 metaphase cells were scored under oil-immersion form each animal. The types of structural aberrations were classified according to Buckton and Evans (Buckton & Evans, 1973).

Statistical significance of the difference between the control and treated groups were evaluated using Student's t-test (Zar, 1974).

Results
Mice exposed to X-rays at 3 Gy and sacrificed 48, 72 and 96 hr after irradiation showed a significant decrease in mitotic index when compared to the control set of animals (P<0.005) (Table 1). Thus, the significant reduction in mitotic index reveals the mitotoxic property of X-rays. Phyllanthus crude extract also has an effect similar to that of X-rays in lowering the frequency of mitosis (P< 0.005) which would indicate that Phyllanthus is also mitotoxic when administered alone (Table 1).

A significant increase in the chromosomal aberrations was found in response to exposure to 3 Gy of X-rays (P<0.005). The response was high in the set of animals sacrificed after 96 hr. The chromosomal anomalies such as chromatid breaks, chromosome fragments, rings and pulverisation of chromosomes were recorded in the irradiated samples (Fig.1).

Phyllanthus extract, however, induce mitotic anomalies only in the set of animals sacrificed immediately after the chemical treatment. Mitotic anomalies, were at a minimum level in the animals sacrificed at 72 and 96 hr after treatment, which would indicate that Phyllanthus extract is neither clastogenic nor genotoxic. However, the toxicity is observed to be less when compared to the irradiated cells.

The X-irradiated bone marrow cells post-treated with Phyllanthus extract and sacrificed after 96 hr., showed a significant decrease in the frequency of aberrant cells (P<0.005) (Table 1). The results clearly establish the presence of some anti-genotoxic agent (presumably Phyllanthin) in Phyllanthus extract, is capable of counteracting the radiation-induced genetic toxicity. However, no significant alteration in mitotic index was observed in the combined treatments (Table 1).

Discussion
Radiotherapy is inevitable in the treatment of cancer. Their continuous application in medical practice remains debatable as they are harmful to some healthy cells as well. Survey of literature, showed that various chemicals such as sulphahydryl compounds, glycols, alcohol, etc., reduce the efficacy of radiation per unit dose. Several micronutrients in the diet were also shown to modify the free-radical reactions and afford protection against radiation (Micozzi, 1989).

Various plant alkaloids are being increasingly recognized as desmutagens and anti-mutagens as several of them shown to exhibit anti-mutagenic and anticanicogenic properties (Yoshikawa et al. 1981; Vinayagamoorthy, 1982). A large number of vegetable juices were also found to reduce chromosomal aberrations in rat bone marrow cells induced by dimethy benz(a)anthracene (Ito et al. 1985). Abraham et al., 1986 also showed that carrot and spinach juices suppressed the incidence of micronuclei in mice induced by cyclophosphamide.

Various plant extracts have also been reported to afford protection against radiation. Fruit extracts of brassica, papaya, bitter gourd and mint have been shown to inhibit radiation-induced lipid peroxidation, significantly in a concentration-dependent manner (Sandhya & Kale, 1993). Root extracts of Plumbago rosea were shown to have tumor-inhibitory effect in sarcoma 180 cell (Solomon, 1993). Ganasoundary and Uma Devi (Ganasoundary & Umadevi, 1993) reported that leaf extracts of Ocinum sanctum afforded significant protection against gamma ray induced mortality in mice.

Plant extracts of a variety of Phyllanthus species have been reported to afford protection against various chemical and physical mutagens (Debrisari Sankar, 1991; Dhir et al. 1991; Gowsrishankar & Vivekanandan, 1994; Gowrishankar et al. 1993). In the present analysis, Phyllanthus afforded protection against 3 Gy of X-irradiation by reducing significantly the frequency of aberrant cells in bone marrow cells of mice (Table 1). Therefore, the radiation-induced damage, due to free-radicals in DNA is either prevented or repaired, thus indicating the presence of some antioxidants (presumably Phyllanthin) in the extracts of Phyllanthus which provide protection by its ability to scavenge the oxidizing free radicals. Similar situations exist wherein, a significant decrease in the frequency of micronuclei and aberrant metaphases was observed following pre- and post treatments with vitamin C and E in y-irradiated mouse bone marrow cells (Sarma & Kesavan, 1993). Post-treatment with vitamin E enhanced the 30-day survival of mice treated with 8 Gy of y-radiation has been reported by Malick et al., 1978. The present observation on the occurrence of decreased mitotic index during longer exposure and critical chromosomal aberrations were similar to the reports of conger (Conger, 1956); in the samples treated with gamma rays. Farooqi and Kesavan, 1992 reported that caffeine post-treatments afforded significant radioprotection to bone marrow cells of whole-body-irradiated mice.

The higher protection afforded by the plant extract observed in the study may be due to Phyllanthin, an active principle of Phyllanthus amarus, or to the combined action of all the ingredients. Hence, the finding, that Phyllanthin or all the ingredients of Phyllanthus collectively reduce the X-radiation-induced chromosomal damage when administered immediately after radiation raises hopes regarding its applicability in radiotherapy of tumours, in view of protecting the healthy cells from damage. However, further investigations are necessitated in this direction in order to elucidate the probable mechanisms of radioprotection, before recommending this drug for clinical application.

Acknowledgements
The authors wish to thank Dr. Ishari K. Ganesh and the management members of VeI's Educational Trust for the encouragement and support.
Table 1. Modifying effect of phyllanthus on bone marrow cells of mice after exposure to x-rays

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>Dose</th>
<th>Sampling time (hr)</th>
<th>No. Total Secured</th>
<th>Mitotic index x ± SE</th>
<th>Aberrant Cells [%] ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>15000</td>
<td>4.65 ± 0.40</td>
<td>8.4 ± 0.57</td>
</tr>
<tr>
<td>X-rays</td>
<td>3Gy</td>
<td>48</td>
<td>8999</td>
<td>2.12 ± 0.12*</td>
<td>58.44 ± 3.05*</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>5004</td>
<td>132</td>
<td>0.63 ± 0.04*</td>
<td>56.46 ± 5.17*</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>7672</td>
<td>75</td>
<td>0.97 ± 0.01*</td>
<td>64.09 ± 5.17*</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phyllanthus (0.05%)</td>
<td>200 mg/kg</td>
<td>48</td>
<td>10976</td>
<td>0.69 ± 0.01*</td>
<td>18.09 ± 1.34*</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>8959</td>
<td>77</td>
<td>0.35 ± 0.10*</td>
<td>6.11 ± 3.88</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>10102</td>
<td>32</td>
<td>0.16 ± 0.01*</td>
<td>4.16 ± 4.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-rays + Phyllanthus extract</td>
<td>3 Gy + 200 mg /kg</td>
<td>96</td>
<td>8949</td>
<td>0.76 ± 0.02***</td>
<td>25.88 ± 1.45</td>
</tr>
</tbody>
</table>

*P<0.005 Statistically significant from control samples  **P<0.01 Statistically significant from treated samples

**References**


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