

RESEARCH ARTICLE

Protective Efficacy of *Phyllanthus amarus* L against Radiation – Induced Chromosomal Damages in Onion Root Meristems

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ABSTRACT:

The protective effect of *Phyllanthus amarus* L in the irradiated roots of onion was evaluated. The results were indicated that the *Phyllanthus* extract showed a radio modifier effect by reducing the frequency of aberrant cells in the meristematic cells exposed to gamma rays.

KEYWORDS: Gamma rays, aberrant cells, chromosomes, gyres, antimutagens.

INTRODUCTION:

With the increasing application of diagnostic radiotherapy in medical practice and the possible expanded use of nuclear fuel as a substitute for our dwindling fossil reserves, there is growing concern over the impact of radiation exposure on human health especially with respect to genetic hazards. Deleterious reactions of these oxyradicals with biomolecules result in the induction of a variety of structural aberrations of chromosomes including DNA strand breaks, protein oxidation and lipid peroxidation^{11,1}. Unrepaired and misrepaired damages lead to drastic biological effects like cell death, induction of mutations, cell transformation and carcinogenesis¹. Hence, it has become absolutely essential to seek a measure to minimize the radiation – induced genetic damage. A variety of pre and post- treatments have been studied with an attempt to reveal, the protective effects of vitamin C and E against gamma radiation³. The role of various plant extracts as desmutagens and antimutagens are being increasingly recognized, as several of them are shown to have anti-mutagenic and anti-carcinogenic properties²¹.

A large number of vegetable juices were also found to reduce chromosomal irregularities in rat bone marrow cells induced by dimethyl benz(a) anthracene¹². Abraham et al., (1986), also showed the suppression of micronuclei induced by cyclophosphamide in mice feed with carrot and spinach juice². Herbal preparations of *Phyllanthus amarus* L and its related species have generated a great deal of interest in recent years for a wide range of protective effects against various chemical mutagens^{16,7}. The herbal extracts are used extensively in indigenous systems of medicine for treating ailments such as leucorrhoea¹⁷, scurvy⁵. Besides, they have been recorded to have antibacterial²⁰ and antiviral¹⁸ properties. Since these preparations are extensively consumed in various quantities, their effects in organisms exposed to physical and chemical pollutant in our environment need to be assessed⁸. Hence, this study involved the evaluation of modifying effects of the crude extract of *Phyllanthus amarus* L against gamma radiation – induced chromosomal changes in *Allium cepa* L root meristems.

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MATERIALS AND METHODS:

The experiments were carried out using the root meristems of onion as the test system ($2n=16$). Irradiation was carried out in a Gamma camera having the source of Co^{60} , and potency of 7 curie, at MERADO laboratory, CSIR complex, Taramani, Chennai, India. Aqueous extracts were prepared from the leaves of *Phyllanthus amarus* by grinding them in distilled water and further diluting to make 0.5 and 1% decoction. The onion root meristems were exposed to gamma rays at 1.5 and 2.00 Gy for 1h. Some of the irradiated cells were fixed in 1:3 acetic acid and ethanol while the other exposed root tip cells were thoroughly washed and post-treated with the crude extract of *Phyllanthus* extract. Parallel distilled water controls were maintained. The treated and control samples were squashed following the Heamatoxylin staining technique¹⁴. Mitotic indices were recorded from the treated and control root samples by examining 2000 to 5000 cells involving 6 root tips from 3 bulbs¹⁰. The types of structural changes in chromosomes were classified⁴. The magnitude of protection afforded by *Phyllanthus* during post-treatments was calculated¹⁹. Statistical significance of the difference between the control and treated groups were evaluated using students t-test. Further analysis was also carried out to compare the significance of differences, if any, between the irradiated and post-irradiated samples²².

RESULTS AND DISCUSSION:

Table 1, shows the effect of Co^{60} and aberrations compared to control samples ($P<0.005$) Mitotic inhibition was also observed in the meristems treated with the plant extracts of *Phyllanthus* and distilled water ($P<0.005$) (Table 1). The irradiated root meristems were post-treated with *Phyllanthus* extract for 1 hour to study mitogenic potential. However, these cells did not attain the normal frequency of cell division. And the extent of radiation induced mitotic inhibition was further increased following treatment with *Phyllanthus* ($P<0.005$). Hence, the results clearly demonstrate that *Phyllanthus* does not exert discernible influence on the mitotic frequency and that therefore it is mitotoxic. The gamma exposure of meristems resulted in a substantial increase ($P<0.005$) in the induction of mitotic irregularities, and structural aberrations. The irradiated root meristems showed the occurrence of many anaphasic irregularities such as anaphasic bridge, multiple

fragments and lagging chromosomes. The frequency of radiation-induced alterations were progressively decreased when the exposed roots were left for recovery in distilled water. The optimal dose of *Phyllanthus* for eliciting maximum radioprotection appears to be 0.5%. Further, the protection afforded was greater if the irradiated cells were exposed to *Phyllanthus* extract for 24h (81.99%). Continuous application of radiation in medical practice remains debatable as they are harmful to some healthy cells as well. Plant extracts of a variety of *Phyllanthus* species have been reported to afford protection against various chemical mutagens^{6,15,9}.

In the present analysis, *Phyllanthus* extract significantly reduces the frequency of damaged cells at 0.05% during longer exposure following 1.5 Gy of gamma radiation (Table 1). This decrease in frequency of aberrations following treatment with *Phyllanthus* indicated its interference with the formation of radiation induced aberrations. A reduction in the yield of X-rays induced damage by treatment with *Phyllanthus amarus* has been shown in mouse bone marrow cells¹³. A similar decrease in the frequency of micronuclei and aberrant metaphases were observed following pre and post-treatments with vitamin C and vitamin E in the gamma irradiated mouse bone marrow cells¹⁹. The results reveal that the *Phyllanthus* extract exerts radioprotection when administered immediately after irradiation. A similar situation, where, in post-treatment with vitamin E enhanced the 30 day survival of mice treated with 8 Gy of gamma radiation has been reported¹³. Farooqi and Kesavan (1992), also reported that caffeine post treatments afforded significant radio-protection to bone marrow cells of whole body irradiated mice. The possible mechanism of *P.amarus* for exerting radioprotection is due to the suppression of the formation of reactive oxygen species generated as a result of radiation¹⁸. 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 compounds of *Phyllanthus*. Hence, the findings indicate that *Phyllanthin* or all ingredient of *Phyllanthus amarus* collectively reduce the radiation induced chromosomal damage when administered immediately after the radiation raises hopes regarding its suitability as therapeutic agent in emergencies.

Table: I Protective efficacy of *Phyllanthus* plant extract in the onion root tip cells exposed to gamma rays.

Mode of Treatment	Radiation dose gy	Plant extract Concentration	Duration of treatment Hr	No. of cells in division	Mitotic index X+SE	Percentage of Aberration X+SE	Magnitude of protection %
Control DW	-	-	1	1179	7.85±0.33	1.48±0.39	-
Gamma Radiation	1.5	-	-	149	2.86±0.44	64.20±5.12	-
-do-	2	-	-	143	2.88±2.03	73.31±6.95	-
Plant extract	-	0.5	1	111	2.20±0.41	12.80±3.23	-
-do-	-	1	1	120	2.31±0.26	51.30±5.87	-
Irradiated roots post treatment with D.W	1.5	-	-	33	1.36±0.21	35.07±8.93	45.42
Irradiated roots post treatment with plant extract	2	0.5	24	37	0.77±0.12	11.57±5.93	81.99

X- Mean; SE- Standard error; P<0.05; P<0.01; P<0.005.; Gy; gyres; Magnitude of protection = (Irradiated-Treated sample) X 100/ Control

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