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Short Communication

Effect of *Phyllostachys parvifolia* leaf extract on ionizing radiation-induced genetic damage: A preliminary *in vitro* cytogenetic study

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ABSTRACT

The ionizing radiation is a known carcinogen as well as cancer therapeutic agent however, the side effect on normal tissue is a limiting factor and inadequate doses necessitates search for an ideal radioprotective agent. Bamboo species are rich source of antioxidants hence have therapeutic value in many free radical mediated diseases. This is the first report regarding in vitro protective effect of bamboo leaf extract against radiation induced genetic damage in human peripheral blood lymphocytes by cytokinesis blocked micronuclei (CBMN) assay. Fresh whole blood was exposed to 5Gy of cobalt-6o gamma radiation with or without 30 min pre-treatment with 3 μl and 5 μl of hydro alcoholic leaf extract of *Phyllostachys parvifolia*. In addition to whole extract the effect of potential active compound orientin was also assessed. The frequency of radiation induced micronuclei decreased significantly in a dose dependent manner following treatment with whole extract as well as orientin. The extent of reduction in micronuclei frequency was higher with whole bamboo leaf extract as compared to orientin alone.

1. Introduction

Plants have been used since time immemorial for the treatment of free radical-mediated diseases in humans such as rheumatoid arthritis, atherosclerosis, cancer, Alzheimer's disease, Parkinson's disease and several other conditions including inflammatory diseases.

Medicinal plants were explored for the radio-protective effect against cellular damage induced by ionizing radiation [1,2]. Number of herbal preparations, both in wholesome form of complete extract or their components, have been shown to render protective activity against radiation in both in vivo and in vitro models [3]. The bamboo plant is native of Asian Countries and India is second largest reserve of bamboo in world after China. Bamboo leaves have been used in traditional Chinese medicine for treating fever and detoxification for over 1000 years [4]. Bamboo leaves are a folk remedy for plethora of diseases like leprosy, hematemesis, haemoptysis, fever, cough, dysmenorrhea, amenorrhea, osteoarthritis, osteoporosis, diarrheoa, dyspepsia, flatulence and worm problems [5]. The bamboo manna-a siliceous matter (tabasheer, vanshalochana) and other herbal formulations viz., Sitopaladi Churna, Talisadi Churna, Chyavanaprash Avaleha, and Tincture of Bambusa, etc. of Ayurveda include bamboo leaves as one of its constituents [6]. The anti-diabetic, anti-inflammatory, antioxidant, anti-microbial, arthritis, anti-hyperglycemic, anti-tumor activity of various bamboo species is also reported [7,8]. Till date no reports are available on protective effect of bamboo leaves on ionizing radiation induced damage. *Phyllostachys parvifolia* is from one of the medicinally important genera *Phyllostachys* [9]. It is growing in south Gujarat region of India. Reported phytoconstituents in bamboo leaves are flavones C-Glucosides like orientin, homoorientin, vitamins and isovitexin [7,8]. Along with that Orientin is known to show radioprotective activity against ionizing radiation [10]. Therefore present study is aim to evaluate protective effect of leaf extract against the ionizing radiation induced genetic damage by preliminary in vitro CBMN Assay. Orientin was taken as a reference compound to compare the protective effect.

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2. Materials, reagents and equipment

HikaryoxI™ RPMI-1640 Culture Media, Cytochalasin-B (Cyt-B, Sigma Aldrich, St. Louis, USA), Potassium Chloride (Merck, Mumbai, India), Carnoy's Fixative Agent, Aluminum Trichloride, Giemsa Staining Solution (Himedia, Mumbai, India) DPX mounting medium (S.D. Fine-Chemicals, Mumbai, India), Microscope (Labomed LX 300), Clinical Centrifuge (Remi Pvt Ltd., Mumbai, India), Gamma Radiation 5000 Chamber (Cobalt 60 Radiation Source), Orientin (≥97% HPLC, Sigma Aldrich, St. Louis, USA).

Fresh Leaves of *P. parvifolia* were collected, dried under the shade, and coarse powder was prepared.

3. Experimental work

3.1. Preparation of sterile leaf extract of P. parvifolia

Dried Leaf powder of *P. parvifolia* (10 gm) was mixed with 100 ml of 30% ethanol. It was refluxed for 1 hr on heating water bath and filtered through Whattman filter paper [0.45 μm]. This procedure was repeated till flavonoid detection showed negative result. This extract was reduced by evaporation, it was then filtered using 0.45 μm syringe filter and diluted up to 10 ml. The diluted sample was further filtered through 0.22 μm syringe filter and tested for sterility.

3.2. Estimation of flavonoid content by $AlCl_3$ method

One ml of the above prepared extract was diluted up to 10 ml with ethanol. 3 ml of this diluted ethanolic extract was added to 3 ml of ethanolic $AlCl_3$. Absorbance reading was taken at 430 nm after 10 min. Results were expressed in g/100 g of dry matter using rutin as a standard.

3.3. Preparation of orientin standard solution

Stock solution of 100 μg/ml of Orientin was prepared in methanol and further diluted as per the requirement in same solvent.

3.4. Sampling and blood collection

Venous Blood of a non-smoking healthy volunteer (23/M) was collected (following signing of consent form by him as per the ICMR guideline) from median cubital vein in aseptic conditions in sterile heparinized vacutainer.

3.5. Irradiation of blood cultures

One ml of whole blood was added with 3 μl and 5 μl hydro alcoholic leaf extract respectively in aseptic conditions. After 30 min, blood sample was exposed to 5Gy γ-radiation and used for short term culture using standard protocol as reported earlier [11,12]. The short term cultures were set-up including a negative control i.e. blood without radiation; a positive control i.e. blood with Radiation (5Gy), along with treatment groups i.e. bamboo leaf extract 3 μl and 5 μl, orientin 3 μl and 5 μl; with and without radiation. All the cultures were setup in duplicates and coded.

3.6. CBMN assay and scoring

CBMN assay was done according to the method of Fenech and Morley [11,12]. After 46 h of incubation 90 μl of 6 μg/ml of Cyt-B was added to all cultures to block cytokinesis. After 24 h, cells were harvested by treating with 5 ml of 0.56% KCl for 1–2 min to ensure controlled swelling of cells and better visibility of nucleus inside the well preserved cytoplasm. Cells were fixed in Carnoy's Fixative, washed thrice with the fixative, and re-suspended in a small quantity of fixative. Fixed cells were dropped gently on clean, pre-chilled microscope slides, air-dried and stained with 4% Giemsa for 5 min. These slides were observed under a Microscope (Labomed LX 300) using 40× objective. Selection of Binucleated cells (BNC) and identification of Micronuclei (MN) were according to the criteria given by Fenech [12]. Total of 100 BNC cells from each culture's slides were scored and the frequency of cells with one (MN1), two (MN2) and three (MN3) micronuclei were recorded. The mononucleate and multinucleate cells were counted separately and the Nuclear Division Index (NDI) was calculated as the ratio of dividing cells to that of total number of cells scored [13]. The data are graphically represented as the frequency of MN in binucleated

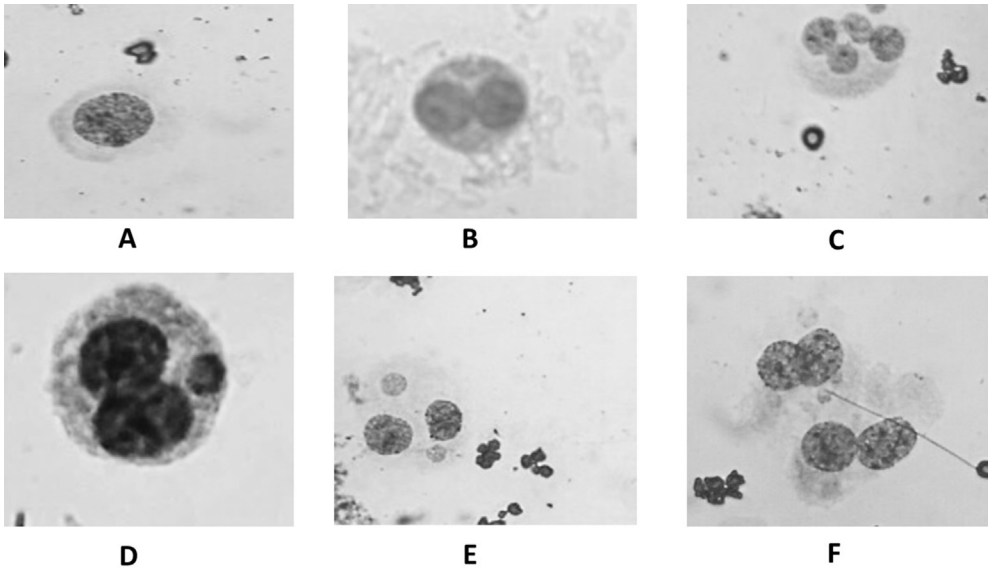

Fig. 1. Photomicrographs showing genetic damage after exposing to 5Gy radiation (digitally magnified images of 1000×; **A**: a mononucleated cell, **B**: a binucleated cell, **C**: a multinucleated cell, **D**: a binucleated cell with one micro nuclei, **E**: a binucleated cell with two micronuclei, **F**: a binucleated cell with three micronuclei).

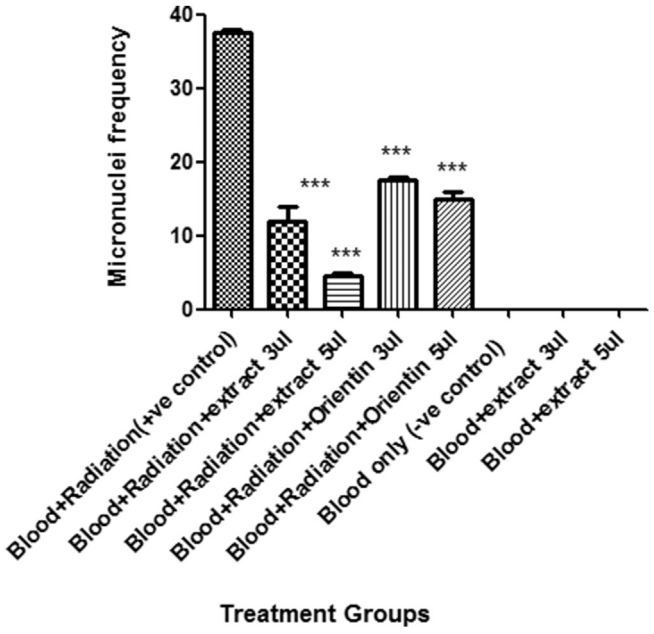

Fig. 2. Graphical representation of Micro nuclei scoring.

cell treated with two concentration of extract (5 μl and 3 μl) irradiated with 5Gy, along with positive and negative controls.

3.7. Statistical analysis

The analysis was performed using Graph Pad Prism 5 software. To compare the data resulting from different set-up, One Way Analysis of Variance (ANOVA) was used. P-value $\leq$0.005 was taken as the basis of assigning significance.

4. Results

Bamboo leaves are rich in flavonoid content in form of c-glycosides like orientin, homo orientin, iso orientin and isovitexin. Therefore flavonoid rich extract was prepared using 30% ethanol. Total flavonoid content was estimated to be 1.578%w/w against rutin as a standard. Flavonoid content was 47.3 μg and 78.9 μg in 3 μl and 5 μl respectively. Sterility of the prepared extract was checked following incubation on nutrient agar and potato dextrose agar slants for 72hrs. No bacterial and fungal growth was observed indicating sterility of the extract. In vitro CBMN assay was performed to examine the radioprotective effect of the prepared leaf extract against the radiation induced genetic damage. The extent of genetic damage is shown by micronuclei formation in binucleated cell which is shown in Fig. 1.

On exposure of whole blood to 5Gy gamma radiation, NDI values of control and treated culture indicated that the concentration of extract and radiation dose, used in the study, didn't affect the cell cycle progression. Numbers of diving cells were in comparable amount and frequency of formation of micronuclei was very high. When whole blood was mixed with bamboo leaf extract, frequency of formation of micronuclei was reduced by 90%; which was 43% when only orientin was used (Fig. 2). Thus, frequency of micronuclei was reduced by two folds in presence of bamboo leaf extract as compared to only orientin. This finding indicates that the leaf extract contains other active phytoconstituents which are collectively giving more protective effect to cell against radiation.

5. Discussion

The preliminary results of present study can be further validated using other in vitro cytogenetic endpoints of genetic damage like COMET assay and chromosome aberration assay. The radio protective effect of bamboo leaf extract is more compare to isolated orientin; hence isolation and characterization of other phyto constituents are also proposed.

6. Conclusions

This is the first report regarding protective effect of *P. parvifolia* leaf extract on genetic damage of human peripheral blood lymphocytes induced by radiation. The levels of genetic damage significantly decreased when cell culture was treated with bamboo leaf extract as well as orientin ($P < 0.001$). It was a significant observation that compare to isolated orientin, whole bamboo leaf extract exhibited more protective effect against radiation.

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