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Protective effect of *Bixa orellana* L. against radiation induced chromosomal aberration in Swiss albino mice

M.S. Karchuli[1], Ganesh N[2]*

***Corresponding author:**

Ganesh N

[1]VNS Institute of Pharmacy, Bhopal, M.P. India

[2]*Dept. of Research, Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah Hills, Bhopal

Email- nganesh_research2@yahoo.co.in

Abstract

Radioprotective effect of hydroalcoholic extract of seeds of *Bixa orellana* have been studied by examining chromosome aberration in cells of bone marrow in irradiated mice. Healthy adult Swiss mice were injected intraperitoneally (ip) with 500 mg kg^{-1} body weight, 1000 mg kg^{-1} of or double distilled water (DDW). They were exposed to whole body irradiation of 2.0 Gy gamma radiation 30 min later. After 24 h, chromosomal aberrations were studied in the bone marrow of the femur by routine metaphase preparation after colchicine treatment. Radiation (4.0 Gy) increased the number of aberrant cells from less than 1% in controls to almost 20%. Pre-treatment with the extract compounds resulted in a significant reduction in the percentage of aberrant metaphases as well as in the different types of aberration scored. The extract was not toxic at 1500 mg kg^{-1} body weight. Being non toxic and easily available natural source *Bixa orellana* extract may be use for as radioprotective for human beings.

Key Words – radioprotective, *Bixa orellana*

Introduction

They were Pat and his coworker who introduced that pretreatment of rats with cysteine protected them against the radiation-induced lethality.[1] There are many investigations which proved the potential of plant extract as radioprotective. Some of the plant's extract having potential radioprotective are *Gingko biloba*,[2] *Centella asiatica*,[3] *Hippophae rhamnoides*,[4] *Osimum sanctum*,[5] *Panax ginseng*,[6] *Podophyllum hexandrum*,[7] *Tinospora cordifolia*,[8] *Emblica officinalis*,[9] *Phyllanthus amarus*,[10] *Amaranthus paniculatus*,[11] *Piper longum*,[12] *Syzigium cumini*,[13] *Mentha arvensis*,[14] *Mentha piperita*,[15] *Zingiber officinale*,[16] *Ageratum conyzoides*,[17] *Aegle* marmelos [18] and *Aphanamixis polystachya*.[19] *Bixa oreallana* is a shrub used as an ornamental plant in India and is best known as the source of the natural pigment annatto, produced from the fruit. Parts of the plant has been used to make medicinal remedies for such conditions as microbial infections, sunstroke, tonsilitis, burns,

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leprosy, pleurisy, apnoea, rectal discomfort, and headaches. The protective effect of *Bixa orellana* against against the response of Escherichia coli cells to DNA damage induced by UV radiation, hydrogen peroxide and superoxide anions [20,21] promoted us to go assessing it's radioprotective potential at chromosomal level.

Materials and methods

Animal

Swiss albino mice (*Mus musculus*) of either sex, 6–8 weeks old with body weight of 24 ± 2 g, were used from animal house of department of research, Cancer Hospital and Research Center, Bhopal, India, as per norms laid down by CPCSEA. Mice were given standard mouse feeding pellets and water *ad libitum*.

Irradiation

Mice were irradiated by ^{60}Co source in the cobalt teletherapy unit (ATC-C9) at Radiation Oncology Department, Jawahalal Nehru Cancer Hospital and Research Center, Bhopal, India. Mice were placed in ventilated Plexiglas cages and irradiated in a group of 6 mice. The source to skin distance was 80 cm with irradiation time 2'.99" min. The mice were irradiated with 4.0 Gy γ-rays.

Preparation of extract

Seeds were collected from medicinal garden of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, India. The *Bixa orellana* extract (BE) was prepared from dried seed powder by macerating with 50 % ethanol. The dried extract was stored at 4 ° C.

Cytogenetics of Bone Marrow

The extract was dissolved in DDW. Extract (BE) was given as 100, 200, 500, 1000 and 1500 mg kg^{-1} body weight of mouse per day in DDW orally to Swiss albino mice for 15 consecutive days. The extract was non-toxic and no mortality was observed till day 30. An optimum dose of 500 mg kg^{-1} body weight and 1000 mg kg^{-1} of BE was selected 500 mg kg^{-1} body weight and 1000 mg kg^{-1} body weight dose was taken for the study. Groups of four mice were injected intraperitoneally (ip) with 500 mg kg^{-1} or 1000 mg kg^{-1} of extract 30 min before whole body exposure to 4.0 Gy gamma radiation. One group of six animal was injected with DDW and exposed to 2 Gy gamma radiation (Rt) and another four animals were sham-exposed (control). 24 h after irradiation/sham-irradiation the bone marrow chromosomes were prepared for analysis.[22] The animals were injected ip with 0.025% colchicine (Sigma, USA) and 2 h later they were killed by cervical dislocation. Bone marrow from femur was flushed out into normal saline, treated with 1% sodium citrate and fixed in methanol-acetic acid (351). The cells were spread on clean slides and stained by 3% Giemsa (Sigma, USA); metaphase plates were observed and chromosomal aberrations were scored using oil immersion (with 100x object lens) under a light microscope. 400 metaphases were scored per animal. The number of aberrant cells as well as different types of aberration, such as chromosome and chromatid breaks (total breaks), fragments and rings.

Statistical Analysis

Student's *t*-test was employed to analyze the results. *P*-values <0.05 were considered significant. Regression analysis was done to obtain $LD_{50/30}$ values and to determine DRF.

Result and discussion

The results are presented in Table 1. The sham-treated control group had 0.56% aberrant cells which consisted of breaks and fragments. No complex aberrations such as dicentrics or rings were noted. Radiation significantly increased the percentage of aberrant cells, along with all types of aberration (breaks, fragments, dicentrics and rings). Pre-treatment with all the BE significantly reduced the percentage of aberrant cells, breaks and fragments compared with Radiation treated.

High levels of gamma irradiation can induce mortality in mammals. With respect to radiation damage to humans, it is important to protect biological systems from radiation-induced

genotoxicity or lethality. The main radioprotective class is thiol synthetic compounds such as amifostine. Amifostine is a powerful radioprotective agent compared with other agents, but this drug is limited in the use in

Table .1 Chromosomal aberrations in mice after 4 Gy administration

Aberration	Group[a]		
	Control	Radiation only	Drug (1000 Mg Kg^{-1} Body Weight)[B] + Radiation
Total Aberration	0.56±0.5	70.25±1.26[d]	34.4±0.58[c]
Break	0.04±0.02	14.5±1.3	10.75±0.5
Fragment	0.42±0.5	50.5±2.08	23.82
Ring	0	6±0.82	3±0.82
Dicentric	0	4.25±1.26	2.25±0.5
Polyploidy	0	6.25±0.96	2.25±0.5
Pulverized	0	5.75±0.5	2±0.82
Severely damaged	0	4±0.82	0
Double minutes	0	2.25±0.5	0

a- Each group consist of six animals
b- Drug was administered regularly 3 days before exposure to 4 Gy radiation.
c- Significant protection against radiation at $P < 0.05$
d- Significant level of induction , $P < 0.05$

clinical practice due to side effects and toxicity. [23-25]. The search for less-toxic radiation protectors has spurred interest in the development of natural products.

Bixa orellana extract is non toxic to the rat at 2000 mg kg^{-1} body weight [26] and No-Observed-Adverse-Effect-Level (NOAEL) for human beings was judged to be at dietary level of 0.1% (69 mg/kg body weight/day for males, 76 mg/kg body weight/day for females) of annatto extract (norbixin). The present finding that *Bixa orellana* extract are good radioprotectors of bone marrow at non-toxic dose suggests that it may be promising agents for human radiation. [27]

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