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Study of protective potential of *Adhatoda vasica* Extract against radiation induced genotoxicity: An *in-vivo* analysis

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Abstract

In the present paper, we provide an insight of the radioprotective potential of Adhatoda vasica leaf extract. Mice bone marrow cells were used as the test system. Animals were fed with 500mg/kg body wt. and 1000mg/kg body wt. of ethanolic extract of the plant prior to 9 Gray γ-radiation exposure and chromosomal aberration assay and micronucleus assay were performed. The frequency of chromosomal aberrations and micronuclei among different treatment groups and the controls was compared. The results revealed a significant decrease in the frequency of chromosomal aberrations as well as the micronucleus possessing cells in the groups pre-treated with A. vasica extract as compared to the untreated group. Better radioprotection was observed in 1000mg/kg-body wt. dosage group as compared to 500mg/kg-body-wt. dosage group. These findings were suggestive of radioprotective potential of A. vasica leaf extract.

Key Words: Radioprotection, Adhatoda vasica, Chromosomal aberrations, Micronucleus.

Introduction

Radiotherapy is one of the most common modalities followed for treating human cancers [1]. Together with other modalities such as surgery and chemotherapy, it plays an important role in the treatment of 40% of those patients who are cured of their cancer [2]. Radiotherapy is also a highly effective treatment option for palliation and symptom control in cases of advanced or recurrent cancers. To obtain optimum results, a judicious balance between the total dose of radiotherapy delivered and the threshold limit of the surrounding normal critical tissues is required. In order to obtain better tumor control with a higher dose, the normal tissues need to be protected against radiation injury. Aqueous radiolysis caused by ionizing radiation generates free radicals which may interact directly or indirectly with the genetic material causing damage and subsequent cell death. [3] Thus, the role of radioprotective compounds is very important in clinical radiotherapy. Evidence suggests that various compounds and formulations are being used as adjuvants to radiotherapy in order to minimize its harmful effects on the normal tissues. In addition, researchers have explored numerous plant materials as potential radioprotectors. The radioprotective potential of several herbal plants is well established and the noteworthy studies include those on *Gingko biloba* [4], *Ocimum sanctum* [5], Panax ginseng [6], *Tinospora cordifolia* [7], *Emblica officinalis* [8], *Piper longum* [9], *Mentha piperita* [10], and *Zingiber officinale* [11].

Adhatoda vasica, an evergreen, gregarious, stiff and perennial shrub of family Acanthaceae has been used as herbal medicine in treating a wide variety of diseases in India. Leaves of the plant are the main source of drug preparation. A number of different principles including, alkaloids: vasicine, vasicinone, vasinol, essential oil: betane, vitamins: vitamin C, b-carotene, a non-crystalline steroid: vasakin and a mixture of fatty acids have been identified as contributing to the observed medicinal effects of the plant [12]. The shrub is the source of the drug 'vasaka', well known in the indigenous system of medicine for its beneficial effects, particularly in bronchitis. Furthermore, A. vasica has also been accredited to afford protection against allergen-induced bronchial obstruction in guinea pigs [13]. The leaves as well as flowers, fruits and roots are extensively used for treating cold, whooping cough, asthma and as antihelminthic. The leaf juice is stated to cure diarrhoea, dysentry and glandular tumor. In vivo investigations have revealed that 90% ethanolic extract of A. vasica possesses promising teratologic effects in rats [14]. Bromohexine and ambroxol, semisynthetic derivatives of vasicine from A. vasica have growth inhibitory effect on Mycobacterium tuberculosis, thereby proving useful in the therapy of tuberculosis [15]. In the context of the aforementioned literature, the present study is aimed at investigating the radioprotective potential of A. vasica leaf extract using Swiss albino mice.

Material and Methods

Collection and Identification of plant

A. vasica plants were collected from areas in and around Bhopal, Madhya Pradesh (India). Samples were identified at Department of Botany, MLB College, Bhopal.

Extraction

A. vasica leaves were washed thoroughly, shade-dried and finely powdered in a mixer. Extraction, using 80% ethanol solvent system [16], was performed in order to obtain the leaf extract, which was then lyophilized, weighed and stored for further usage.

Animals

Female Swiss albino mice, 6-8 weeks old with an average body weight of 30 gm, from an inbred colony were obtained from CPCSEA approved animal breeding centre of Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah Hills, Bhopal. These were maintained under controlled temperature and light conditions in an animal house and provided with standard diet and acidified water ad libitum.

Irradiation

Cobalt-60 gamma radiation unit of Radiotherapy Department, Jawaharlal Nehru Cancer Hospital & Research Centre, Idgah Hills, Bhopal, was used for whole body irradiation of mice. Single fraction dose of 9 Gy gamma radiation was used.

Table 1 shows the animal groups and the respective treatments provided.

Groups	Number of Mice	Treatment (6 days duration)	Irradiation (Post treatment)
Normal Control (NC)	4	No drug	No radiation
Vehicle Control (VC)	4	2% gum acacia	No radiation
Radiation Control (RC)	4	No drug	9Gy gamma radiation
Drug Dose 1 (D1)	4	500 mg extract/ kg-body wt.	No radiation
Drug Dose 2 (D2)	4	1000 mg extract/ kg-body wt.	No radiation
Radiation + Drug1 (RD1)	4	500 mg extract/ kg-body wt.	9Gy gamma radiation
Radiation + Drug2 (RD2)	4	1000 mg extract/ kg-body wt.	9Gy gamma radiation

Bone Marrow Chromosomal Aberration Assay [17]

All the animals were sacrificed by cervical dislocation after 2 hours of intraperitoneal colchicine (0.025%) injection. The femoral bone was excised and the bone marrow was aspirated by flushing it with normal saline. The cells were centrifuged and treated with 0.056M KCl solution at 37°C for 30 minutes. The cell pellet was fixed with freshly prepared acetic acid-methanol solution. The pellet was washed and finally resuspended in 2ml of the fixative. The slides were prepared by standard air drop method and stained with 4% Giemsa stain [18]. The slides were observed under 100X magnification of BX60 Olympus microscope. The chromosomal aberrations were observed and recorded in standard format.

Bone Marrow Micronucleus Assay

For micronucleus assay the bone marrow cells were washed with normal saline and fixed with freshly prepared acetic acid-methanol solution. The cells were centrifuged and repeatedly washed with the fixative prior to slide preparation. The slides were prepared by smearing and stained with 4% Giemsa stain. The dried slides were observed under 100X magnification of BX60 Olympus microscope and the micronuclei were scored.

Statistical Analysis

The data was analyzed by performing One-way ANOVA by using GraphPad Prism version 4 software.

Results

Chromosomal Aberration Assay

Chromosomal aberrations (Figure 1) were found to be significantly increased in RC group as compared to the NC group ($p<0.001$). The mean aberrant metaphases in the RD1 and RD2 groups were observed to be statistically increased as compared to NC group. However, the incidence of mean aberrant metaphases in RD2 group to be significantly decreased as compared to the RC group. The mean aberrant metaphases in the VC, D1 and D2 groups were observed to be statistically non-significant ($p>0.05$) as compared to the NC group. The mean incidence of chromosomal aberrations observed in various groups is given in Table 2.

Table 2. Frequency of chromosomal aberrations in mice bone marrow cells of treatment and control groups.

Groups	NM (Mean ± S.E.)	AM (Mean ± S.E.)	Types of Aberrations (Mean ± S.E.)							
			MIN	R	ACA	DC	F	ICD	PCD	PP
NC	26.75±0.48	3.25±0.48	0.75±0.25	0.0±0.0	1.5±0.29	0.0±0.0	1.0±0.41	0.0±0.0	0.0±0.0	0.0±0.0
VC	26.25±0.48	3.75±0.48	1±0.41	0.0±0.0	1.5±0.29	0.0±0.0	1.25±0.48	0.0±0.0	0.0±0.0	0.0±0.0
RC	3.75±0.63	26.25±0.63*	12.75±2.56	7.0±1.29	8.25±1.31	14.0±2.16	13.25±0.95	0.25±0.63	2.75±0.48	1.75±0.25
D1	23.75±0.63	6.25±0.63	2.75±0.48	0.50±0.29	4.50±0.65	2.2±0.25	1.75±0.48	2.0±0.41	0.0±0.0	0.0±0.0
D2	24.0±0.71	6.0±0.71	1.5±0.29	0.5±0.29	3.5±0.29	1.25±0.65	1.5±0.65	1.0±0.41	0.0±0.0	0.0±0.0
RD1	7.75±0.85	22.25±0.85*	11.0±1.08	6.75±0.63	7.75±0.75	7.25±0.25	4.5±0.65	1.0±0.65	0.50±0.29	0.0±0.0
RD2	19.5±1.11	10.25±1.11*Ψ	8.75±0.48	3.75±0.48	2.25±0.65	3.25±0.63	0.5±0.29	0.25±0.25	0.0±0.0	0.25±0.25

* Significant when compared to normal control group (P<0.001)

Ψ Significant when compared to radiation alone group

Table 3. Frequency of micronucleus possessing cells in mice bone marrow cells of treatment and control groups.

Group	Number of Cells scored	Normal Cells (Mean ± S.E.)	Micronuclei Cells (Mean ± S.E.)
NC	100	99.00 ± 0.41	1.0 ± 0.41
VC	100	99.00 ± 0.41	1.0 ± 0.41
RC	100	44.25 ± 1.89	56.0 ± 1.73*
D1	100	95.0 ± 0.71	5.0 ± 0.71
D2	100	97.25 ± 0.25	2.75 ± 0.25
RD1	100	78.75 ± 1.03	21.25 ± 1.03*Ψ
RD2	100	89.25 ± 0.63	10.75 ± 0.63*Ψ

* Significant when compared to normal control group (P<0.001).

Ψ Significant when compared to radiation alone group.

Micronucleus Assay

The mean number of micronucleus possessing cells (Figure 2) in RC, RD1 and RD2 groups was recorded to be significantly increased (p< 0.001) as compared to NC group. However, that of VC, D1 and D2 groups was found to be statistically non-significant as compared to the NC group. A marked decrease (p<0.001) in the number of micronuclei of RD1 and RD2 groups was seen when compared to that of RC group micronuclei. Table 3 represents the mean incidence of micronucleus possessing cells found in different groups.

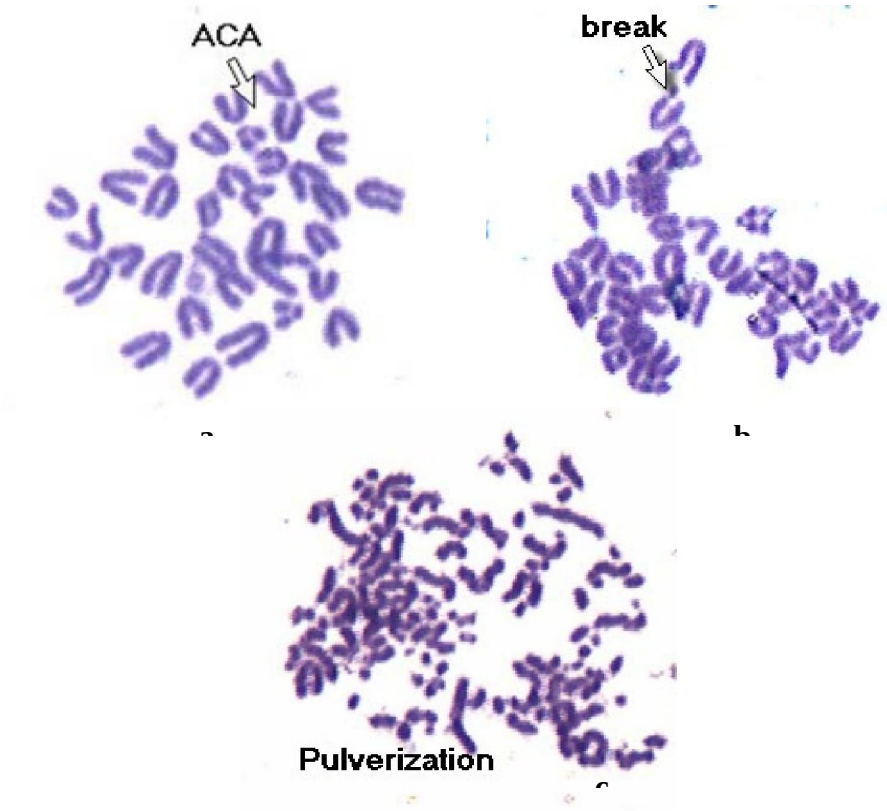

Figure 1: Chromosomal aberrations in the radiation exposed groups.

a- metaphase showing acrocentric association, b- metaphase with a chromatid break, c-severely damaged metaphase (pulverization)

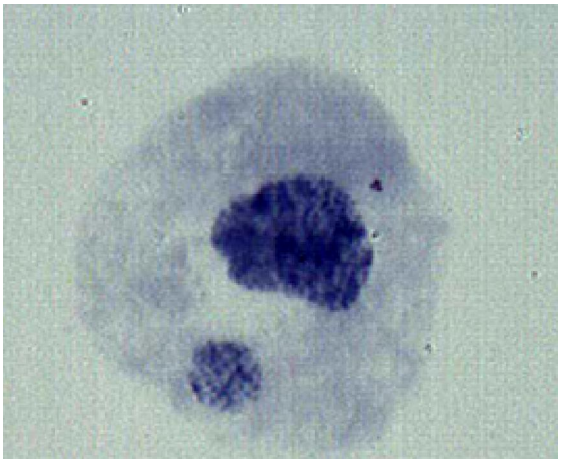

Figure 2 : Mice bone marrow cell with a micronucleus

Discussion and Conclusion

Ionising radiation is a known genotoxic agent [3]. The results of the present study revealed significantly increased chromosomal aberrations in the RC group as compared to NC group. The various aberrations observed include single and double minutes, rings acrocentric associations, fragments, interchromatid breaks, premature centromeric division and polyploidy. However, marked decrease in the mean aberrant metaphases was recorded in the groups pre-treated with A. vasica extract. Moreover, the extract could provide better radioprotection at 1000mg/kg-body wt. as compared to 500mg/kg-body-wt. dosage. The findings of chromosomal aberration assay were analogous to that of Meenal et al (2007), who concluded from their study that A. vasica is an effective radioprotector at the dosage of 800 mg/kg-body wt., administered for 15 days prior to 8Gy whole body radiation exposure [16]. However, in our study, a similar effect was observed after 6 day pre-treatment with 1000 mg/kg-body wt. dose of A. vasica extract in 9 Gy whole body radiation exposed group.

In another study, Kumar et al (2005) investigated the modulatory influence of A. vasica leaf extract in γ-irradiated Swiss albino mice by performing hematological and biochemical analyses including GSH assay, lipid peroxidation assay and serum phosphatases activity [19]. They concluded that a significant radioprotection is achieved at 800 mg/kg body-wt/day for 7 consecutive days prior to 8 Gy γ-radiation. Ample evidence suggests that ionizing radiation results into the generation of free radicals due to radiolysis of water in a biological system (Hall, 1978) [20]. GSH being an endogenous free radical scavenging enzyme offers protection against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation (Biaglow et al, 1987) [21]. However, marked reduction in blood GSH following radiation exposure has been revealed by Kumar et al (2005). The GSH reduction is minimized by pretreatment with A. vasica leaf extract. The decrease in the chromosomal damage exhibited by the A. vasica pretreatment group of our study may thus be attributed to the restoration of glutathione and the subsequent scavenging of free radicals generated on exposure to γ-radiation. To conclude, the findings of the present study unveil the fact that A. vasica has a potential radioprotective property. However, the role of the individual phyto-constituents needs to be explored for further validation, so that an alternative radioprotective therapeutic agent may be developed.

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