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Research Article

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Radiation Protective Potentiality of *Adhatoda vasica*

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Abstract

The present study forecasts the extensive damage caused by radiotherapy and chemotherapy treatment for cancer in the genetic setup of an individual. To counteract this damage, radioprotective potentiality of 50% Methanolic extract of Adhatoda vasica was evaluated through Cyto-Geno analysis.

50% Methanolic extract of Adhatoda vasica leaves was used as a drug whose radioprotective potentiality was to be investigated. Human peripheral lymphocytes were cultured using RPMI-1640 media, harvested and observed under microscope for chromosomal aberration assay.

Total 200 metaphases were counted for each group. All the metaphases were found to be normal in Control group. The frequency of normal metaphases greatly increased after pretreatment with both low and high drug dose (50mg/Kg body weight and 100mg/Kg body weight) concentration. The average normal metaphase in Radiation Control was 118 out of 200 metaphases but after pretreatment with 50% Methanolic extract of *Adhatoda vasica* the number of normal metaphase increased to 179 at 50mg/Kg body weight and 184 at 100mg/Kg body weight. It was concluded that 50% Methanolic Extract of *Adhatoda vasica* with both low and high drug doses have shown its potentiality as a radio protector against the therapeutically induced mutations which can prove to be a contributor in cancer management in future. Such indigenous Indian, herbal, cost effective, poor man friendly drug will definitely be a potential adjuvant to cancer treatments like radiotherapy and chemotherapy since Amifostine a well known radioprotector given to the patients at the time of cancer therapy is expensive and has its own side effects.

Keywords: Radiation Protective, *Adhatoda vasica,* metaphases

Introduction

Herbs have been used for time uncounted for healing the sick and infirm [1]. No doubt Indian herbs are the most sought after herbs in the world due to their organic quality, purity and lowest cost of cultivation. Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century [2].

At the moment, most successful cancer therapy is done by radiation (radiotherapy) in spite of the fact that radiation is hazardous to health. DNA is considered to be the prime target of radiation action in the cells [3]. To increase the therapeutic index of radiation therapy, various modes of radioprotection have been developed that selectively reduce cytotoxic effects to normal tissues. Radioprotection is the protection of

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normal cells from hazardous effects of ionizing radiation and the agents used for this purpose are known as radioprotectors. Chemical radioprotectors may involve sulfhydryl radioprotecting compounds like Amifostine, [4-9] MEA (Mercaptoethylene amine), AET (Aminoethyl isothiourea)] free radical scavengers and antioxidants like ethanol, ethylene glycol, glycerol, vitamin A, C and E[10-11] etc. are found to be good radioprotectors. Chromosomal aberration study in animals is recognized as one of the most important sensitive parameters in assessing radiation damage and toxic effects as very small dose can produce detectable chromosome changes. Chromosomal aberrations may be numerical or structural. Structural aberrations may involve breaks, intrachromosomal exchange, deletion, interstitial deletion, rings, interchromosomal exchange, dicentrics, isochromosome, insertion, duplication, double minutes, and Micronuclei etc. [12-13]

Adhatoda vasica a small evergreen sub-herbaceous bush belonging to family Acanthaceae has been selected for our study to investigate the radioprotective potentiality. It's a small evergreen sub herbaceous bush having many medicinal properties of importance like anti-diabetic, anti-asthmatic, anti-bacterial, diuretic, anti-inflammatory etc.

Materials and Methods

50% Methanolic extract of Adhatoda vasica leaves was prepared and used as a drug whose radioprotective potentiality was to be investigated. Human peripheral lymphocytes were cultured using RPMI-1640 media and harvested. Slides were prepared by Air drop technique, stained with Giemsa and observed under microscope for chromosomal aberration assay. To evaluate the radioprotective potentiality, four groups were taken viz. Control, Radiation Control, Drug Control and Drug+Radiation. Control group consisted of only blood without any radiation and without any drug. Radiation Control group consisted of blood plus radiation where peripheral lymphocytes were irradiated by Cobalt 60- γ radiation source with radiation doses ranging from 1Gray (RG1), 2 Gray (RG2), 4 Gray (RG4), 6 Gray (RG6) and 9 Gray (RG9) respectively. Drug control group consisted of blood plus drug. Drug dose was calculated according to 50mg/Kg body weight (D1) and 100mg/Kg body weight (D2) respectively. Drug + Radiation group where peripheral lymphocytes were pretreated with the drug before irradiation consisted of following sub groups: DRA1 (1Gray + 50mg), DRA2 (1Gray + 100mg), DRB1 (2Gray + 50mg), DRB2 (2Gray + 100mg), DRC1 (4 Gray + 50mg), DRC2 (4Gray + 100mg), DRD1 (6Gray + 50mg), DRD2 (6Gray + 100mg), DRE1 (9Gray + 50mg) and DRE2 (9Gray + 100mg).

Observation and Result

Total 200 metaphases were counted for each group. All the metaphases were found to be normal in Control group. In Radiation Control group the percentage of aberrant metaphases was found to be as following: RG1-8%, RG2- 28%, RG4 -44%, RG6-60% and RG9-65% respectively. In Drug Control group for both D1 and D2 sub groups percentage of aberrant metaphases was found to be similar i.e. 6%. In Drug + Radiation group the aberrant metaphases were found to be in following percentage: Sub group DRA1- 1%, DRA2- 5%, DRB1- 3%, DRB2- 7%, DRC1-7%, DRC2- 1%, DRD1-10%, DRD2 -7%, DRE1- 13% and DRE2 20% respectively. In our study the induction of Micronuclei in the experimental group was also observed as it is an indirect evidence of strand breaks, fragments and deletions in the chromosomes.

Discussion and Conclusion

In the present study a dose dependent radioprotection was obtained in all the 5 doses of radiation (1Gray, 2Gray, 4Gray, 6Gray and 9Gray). When compared to the Radiation control group (radiation alone) the frequency of normal metaphases greatly increased after pretreatment with low drug dose (50mg/Kg body weight) concentration. Good metaphase index was shown by normal metaphases at high drug dose (100mg/Kg body weight), however in 1Gray,

Table 1: Total count of aberrant, normal metaphase and different types of aberrations in each group

GROUPS		TM	NM	AM	TYPES OF ABERRATIONS								
					DC	MIN	PCD	FBD	PP	BB	RG	ER	OT
CT	C	200	200	0	0	0	0	0	0	0	0	0	0
RC	RG1	200	184	16	4	2	2	1	1	0	1	0	0
	RG2	200	144	56	30	10	16	4	2	4	6	1	2
	RG4	200	112	88	40	21	26	13	4	10	6	0	1
	RG6	200	80	120	70	28	46	13	16	1	14	2	10
	RG9	200	70	130	80	28	54	16	26	0	32	6	10
DC	D1	200	188	12	8	0	1	1	1	1	0	1	0
	D2	200	188	12	6	0	1	1	1	1	0	1	0
DR	DRA1	200	198	2	0	0	1	0	0	0	1	0	0
	DRA2	200	190	8	2	0	2	0	0	0	6	0	0
	DRB1	200	194	6	2	2	0	0	2	0	0	0	0
	DRB2	200	186	14	2	0	6	4	0	0	2	2	4
	DRC1	200	186	14	8	2	0	0	2	0	6	0	2
	DRC2	200	198	2	0	0	0	0	0	0	0	2	0
	DRD1	200	180	20	2	4	6	4	4	0	6	2	2
	DRD2	200	186	14	2	0	0	0	4	0	12	0	0
	DRE1	200	174	26	4	2	2	4	4	0	12	0	0
	DRE2	200	160	40	6	22	6	4	4	0	22	0	4

TM-Total metaphase, NM-Normal metaphase, AM-Aberrated metaphase, DC-Dicentric, MIN-Minute, PCD-Premature Centromeric Division, FBD-Fragment Break Deletion, PP-Polyploidy, BB-Bubbling, RG-Ring, ER-Endoreduplication, OT-Other

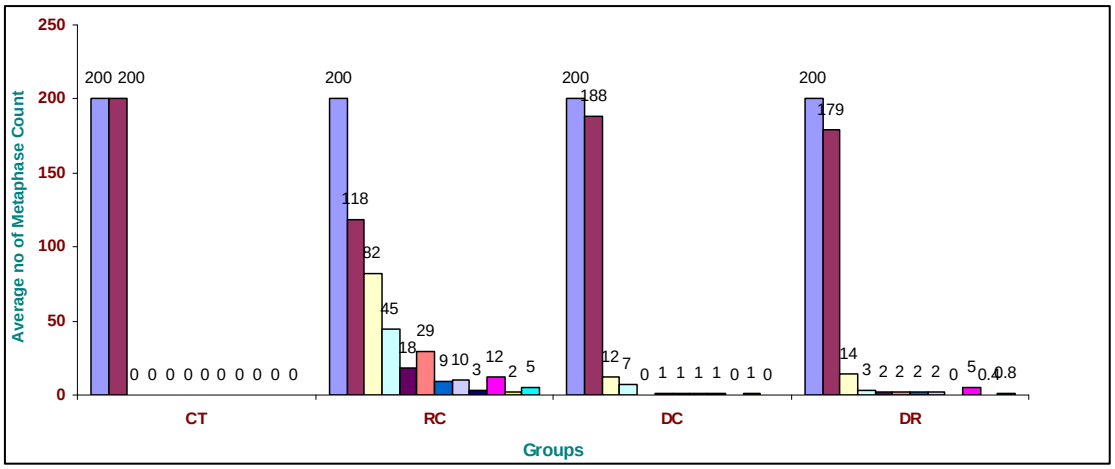

Fig 1: Comparision of Average of control, Radiation, Drug & Drug+ Radiation

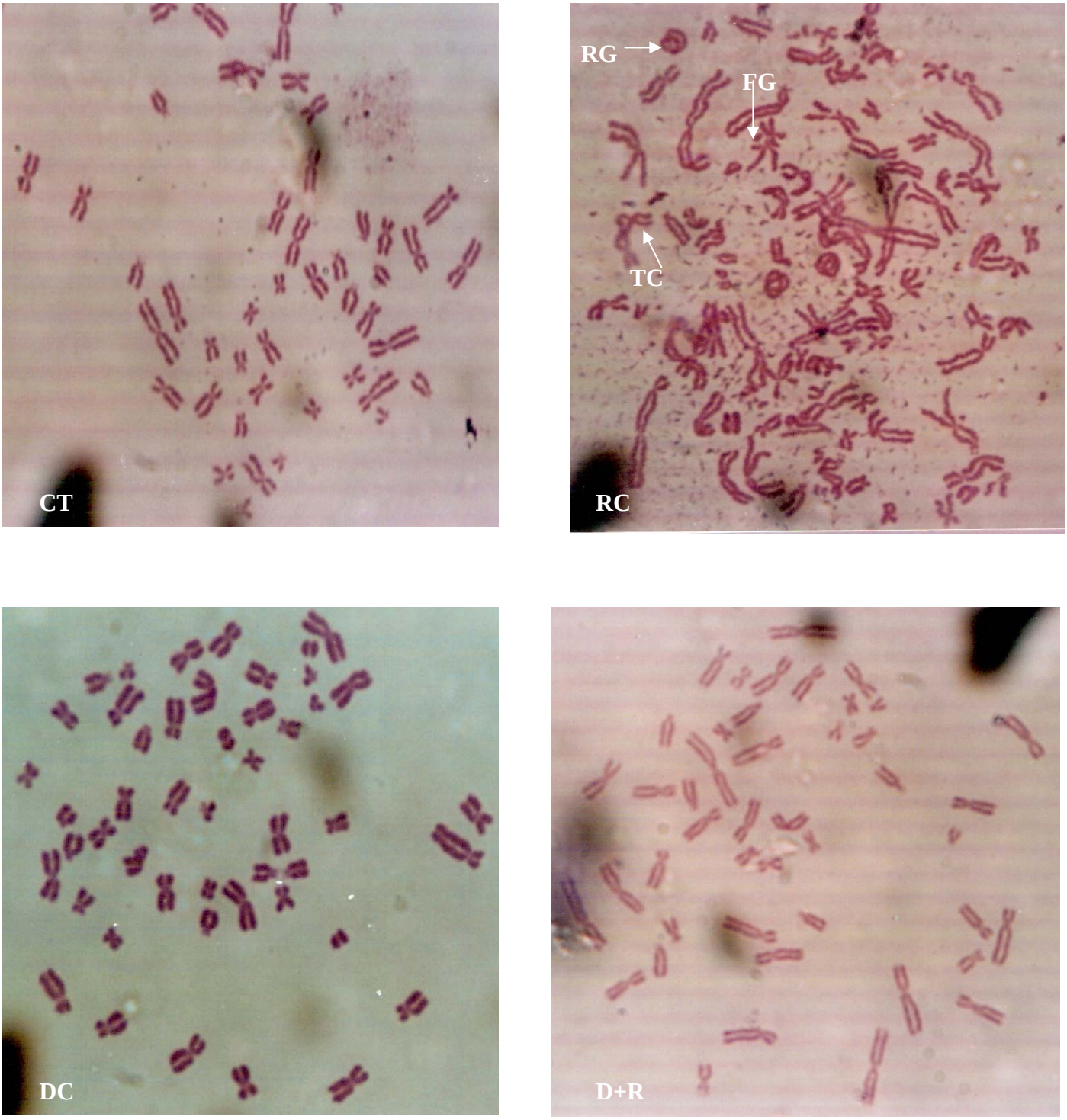

Fig 2: Plates showing metaphase, CT- Control Group Showing Normal metaphase, RC- Radiation Control plate showing Tricentric (TC), Ring (RG) and fragment (FG) in radiated lymphocytes, DC- Drug control plate showing normal metaphase, D+R-Drug+Radiation plate showing normal metaphase

2Gray and 9Gray there was a slight deviation in normal metaphase count as compared to the 50mg/Kg body weight concentration with mean and standard error values as 47.5±1.19 for 1Gray, 46.5±1.25 for 2Gray, 49.5±0.28 for 4Gray, 46.5±1.5 for 6Gray and 40±3.46 for 9Gray. The survival percentage in the Drug control group was similar in both the drug doses (50 and 100mg/Kg body weight). The Dicentrics and similar other aberrations were reduced to a minimal percentage after pretreatment with drug before irradiation in comparison to Radiation control group.

Populations of cancer patients undergoing cancer treatment by radiotherapy and chemotherapy are still in the high risk factor group for therapy induced mutations. Therefore complementary and alternative medicine is proving to be a better cure to minimize the side effects. Thus it is concluded that 50% Methanolic Extract of *Adhatoda vasica* with both low and high drug doses have shown its potentiality as a radio protector against the therapeutically induced mutations which can prove to be a contributor in cancer management in future. Such indigenous Indian, herbal, cost effective, poor man friendly drug will definitely be a potential adjuvant to cancer treatments like radiotherapy and chemotherapy since Amifostine a well known radioprotector given to the patients at the time of cancer therapy is expensive and has its own side effects.

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