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PROTECTIVE EFFECTS OF CIRCUMA LONGA ON INDUCED GENOTOXIC EFFECTS OF DOXORUBICIN ON BONE MARROWCELLS OF MICE

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ABSTRACT

The anticancer drug Doxorubicin tested for its genotoxic effects in the bone marrow cells of Swiss mice along with Circumin . The end points investigated were chromosomal aberrations in bone marrow cells of male mice. The doses tested were 40mg/kg body weight of mice. Significant percentages of chromosomal aberrations (CA) recorded from bone marrow of each of the Doxorubicin and Circumin treated groups of mice. The results of this present study indicated the decrease the values when compared to the earlier results mice treated only with only Doxorubicin, which proves that circumin reduces the effects induced by the combined drug dosage.

Key words: Doxorubicin(DXR) ,Circumin ,Chromosomal Aberrations

No: of Tables 1 **No: of References 9**

INTRODUCTION

More than 50 cytotoxic drugs are commercially available for treating cancer patients. Clinical and laboratory studies have proved many of them to be mutagens, carcinogens, or teratogens in humans and animals, while patients that received therapeutic dosages of these drugs have exhibited a long list of acute and chronic adverse effects, including second cancers (Baker ES, Connor TH, 1996).

Most antineoplastic drugs are soluble in body fluids and can thus be delivered systemically. Although therapeutic efforts are directed toward cancer tissue, the DNA of non-cancer cells is also subjected to damage during chemotherapy. There is an increasing interest in the assessment of biological markers that detect the damage produced after cancer chemotherapy. As the target cells in determination of the correlation between exposure of cancer patients to antineoplastic drugs and / or radiation and alterations of DNA repair efficiency, peripheral blood lymphocytes are frequently used (Rigaud O, Guedeney G etal., 1990). After intravenous administration of various antineoplastic drugs in the peripheral blood leukocytes of all cancer patients studied showed a significantly increased level of DNA damage compared to the pre-treatment values. Administration of antineoplastic drugs in standard protocols is accompanied by significant DNA damage in peripheral blood leukocytes (Baker ES, Connor TH, 1996).

In early studies (Arinaga S, Akiyoshi T etal., 1985) of the lymphocyte activation induced by allogeneic cells in a mixed lymphocyte culture, it was found that peripheral blood lymphocytes obtained from cancer patients 7 days after treatment with doxorubicin showed an about twice as high cytotoxicity with respect to the pretreatment control values.

Doxorubicin has a wide variety of toxic side-effects, including cardiotoxicity, cytotoxicity and the induction of chromosomal aberrations (Singal et al., 2000; Jung and Reszka , 2001).

The chromosome aberration assay is a powerful classical cytogenetic tool for genotoxicity testing and can be used as a validation test for Comet assay(technique for evaluation of DNA damage/repair, genotoxicity testing and biomonitoring) results (Hartmann et al., 2003).

Genotoxicity studies have frequently been conducted on mammalian systems to evaluate the mutagenic potential associated with acute or chronic exposure to chemical agents. Recently, particular attention has been devoted to the Comet assay in order to identify substances with genotoxic activity. This test allows the detection of DNA damage such as single and double-strand breaks and alkali labile lesions in individual cells after acute and/or chronic exposure to a genotoxic agent (Tice et al., 2000).

Doxorubicin induced a significant increase ($p < 0.05$) in DNA damage score and the frequency of chromosome abnormalities, these results being consistent with those reported by other authors (Anderson et al., 1998). The anticancer drug doxorubicin (DOX) is toxic to target cells, but also causes endothelial dysfunction and edema, secondary to oxidative stress in the vascular wall. Thus, the mechanism of action of this drug may involve chemotoxicity to both cancer cells and to the endothelium (Matthew B, Wolf and John W, Baynes, 2006).

The effect of various concentrations of Aegle marmelos (AME) on the doxorubicin (DOX)-induced genotoxic effects in mice bone marrow was studied. Treatment of mice with different concentrations of DOX resulted in a dose-dependent elevation in the frequency of micronucleated polychromatic (MPCE) as well as normochromatic (MNCE) erythrocytes in mouse bone marrow. The frequencies of MPCE and MNCE increased with scoring time, and the greatest elevation for MPCE was observed at 48 hours post-DOX treatment, whereas a maximum increase in MNCE was observed at 72 hours post-DOX treatment. This increase in MPCE and MNCE was accompanied by a decline in the polychromatic erythrocytes–normochromatic erythrocytes (PCE/NCE) ratio, which showed a DOX-dose-dependent decline. Treatment of mice with 200, 250, 300, 350, and 400 mg/kg body weight of AME, orally once daily for 5 consecutive days before DOX treatment, significantly reduced the frequency of DOX-induced micronuclei accompanied by a significant elevation in the PCE/NCE ratio at all scoring times. The greatest protection against DOX-induced genotoxicity was observed at 350 mg/kg AME. The protection against DOX-induced genotoxicity by AME may be due to inhibition of free radicals and increased antioxidant status (Ponemone Venkatesh, Bellary Shantala etal., 2007).

MATERIALS & METHODS

In the present study on dose effect relationship the animals were injected intraperitonially with various doses of 40mg/kg body weight. For each concentration of Drug, 3 mice were maintained which are to be studied at different time intervals i.e., 24hrs, 48hrs & 72hrs..For Control group, 3 Mice were also maintained which received equal volume of distilled water. All the control and treated 2.5mg, 5mg & 7.5 mg of circumin for body weight ,animals were sacrificed at 24h, 48h and 72h by cervical dislocation after the administration of the test compound. ,colchicine was added to all the incubated mice 2hrs before scarifying to inhibit the spindle formation.. Animals were dissected

out for femur bones and flushed out bone marrow into a Petridish containing 0.75m KCl (hypotonic) solution to get a homogenous suspension. The cell suspension was collected in clean centrifuge tubes and incubated at 370c for 45minutes. After incubation the tubes were centrifuged for 10minutes at 1000rpm. The supernatant was discarded and to the pellet 5ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) was added and allowed to stay at room temperature for 10minutes.

This step was repeated 4 to 5 times. Finally the cells were fixed in fresh fixative.

Air-dried slides were prepared by dropping one or two drops of the final suspension on the grease-free, pre-chilled slides with the Pasteur pipette. The slides were dried immediately by air-drying method, coded and stained in 2% Geimsa (2mL of Geimsa + 2mL of Sorenson's buffer + 46 mL of distilled water) for 10 minutes.

RESULT & DISCUSSION

The mutagenic potential of any test compound is evaluated by scoring and analyzing the frequency of structural aberrations and numerical aberrations (Datta et al., 1970). Although chromosomal damage in somatic cells is not transmitted to the offspring (Evans, 1976), there is every possibility of transmission of these aberrations to further filial generation as stable abnormalities (Hirschhorn And Collins, 1969).

Among the structural abnormalities, gaps are termed as achromatic lesions and remain unstained by the feulgen technique (Reiger et al., 1976) reported gaps in metaphase chromosomes to be the result of insufficient folding of chromosome fibres.

TREATMENT	DOSES µl/culture	NUMBER OF METAPHASES SCORED	CHROMATID ABERRATIONS.			ISOCHROMATID ABERRATIONS.		TOTAL NO: OF ABERRATIONS	TOTAL NO: OF POLYPLOID CELLS.
			GAPS	BREAKS	ACENTRIC FRAGMENTS	GAPS	BREAKS		
CONTROL	-	250	1 (0.40)	2 (0.80)	0 (0.00)	1 (0.40)	0 (0.00)	2 (0.80)	0 (0.00)
DOXORUBICIN	40	250	3 (1.20)	4 (1.60)	3 (1.20)	3 (1.20)	2 (0.80)	9 (3.60)	2 (0.80)
DOXORUBICIN + CIRCUMIN	2.5	250	3 (1.20)	5 (2.00)	2 (0.80)	2 (0.80)	1 (0.40)	8 (3.20)	3 (1.20)
	5	250	2 (0.80)	3 (1.20)	1 (0.40)	2 (0.80)	2 (0.80)	6 (2.40)	2 (0.80)
	7.5	250	1 (0.40)	2 (0.80)	1 (0.40)	2 (0.80)	1 (0.40)	4 (1.60)	1 (0.40)

NOTE: - 250 metaphases were scored for each dose. Gaps and polyploids are not included in total aberrations. Values in paranthesis are percentages. $P<0.05$

Table 1: Chromosomal Aberrations of Showing Induced genotoxic effects

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