

MODULATORY EFFECT OF CHENOPODIUM ALBUM EXTRACT AGAINST CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY DAMAGE IN CULTURED MAMMALIAN CELLS

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ABSTRACT

The chemoprotective effect of *Chenopodium album* extract was tested against the genotoxicity induced due to Cyclophosphamide (CPA) in human lymphocytes using chromosomal aberrations (CA) as a parameter. About 100 µg/ml of CPA was treated with *Chenopodium album* extract at dosages of 3,6,9 mg/ml of culture medium. A dose dependent decrease in genotoxic damage of CPA was observed. So the result clearly suggests the modulatory potential of *Chenopodium album* against the genotoxicity of CPA *in-vitro*.

Keywords: CPA, CA, Genotoxicity, Modulatory effect

INTRODUCTION

Chenopodium album commonly known as "bathua" is a well known food as well as a medicinal herb. In traditional system of medicine, it is used as an anthelmintic, antimicrobial, antirheumatic, contraceptive, laxative, cardiogenic, antiscorbutic, bloodpurifier & also in treatment of hepatic disorder, spleen enlargement, intestinal ulcers, digestive, carminative, seminal weakness, pharyngopathy, splenopathy, hemorrhoids, cardiac disorder^{1,2,3}. Some studies showed cytotoxic activity of this plant against various tumor cell lines *in-vitro*⁴. Recent studies also highlighted the potential of this plant to counteract malignancy developed in breast cancer⁵.

CPA is a commonly used as an anti-cancerous drug against a broad range of cancers. With increasing use of these neoplastic drugs, there occurs long term side effects. Several reports indicate the carcinogenic & mutagenic effects of CPA in humans as well as animals^{6,7}.

In this present study we have analysed the modulatory effect of *Chenopodium album* plant extract (both aq. & methanol) against CPA induced genotoxicity by using CA method in cultured human lymphocytes. The chromosome aberration assay *in vitro* is a useful and sensitive test for detection of genotoxins⁸. So CA assay method is widely used for testing modulatory effect of various natural compounds against chemical induced genotoxicity.

MATERIALS & METHOD

Plant collection & extract preparation

Fresh plant materials of *Chenopodium album* were collected. The plant material was allowed to air-dry at ambient temperature and then milled. Fifty grams of the sample was extracted with 250 ml each of methanol and water. Extraction was done exhaustively by

taking 250 ml of methanol & double distilled water as the solvent respectively at a temperature of 70°C for 24 hrs. The filtrate was collected using Whatman No. 41 filter paper. Solvents were removed by evaporation using a rotary evaporator at a temperature of 50°C and the dried crude extracts were stored at 4°C. The extracts were subsequently diluted to working concentrations by dissolving in PBS.

Lymphocyte Culture

Heparinized blood samples were obtained from healthy donors. Then lymphocyte isolation was done by using Ficoll paque plus⁹. Then the blood sample (0.5 ml) was placed in a sterile culture vial containing 5ml of RPMI 1640 medium supplemented with 1 ml of fetal calf serum & 0.1ml of phytohaemagglutinin & incubated at 37°C for 24 hr in a CO₂ incubator.

Chromosomal aberration analysis

For this after 24 hr, CPA & plant extract were added in conc. of 3,6,9 mg/ml individually as well as in combination & incubated for another 48 hr. Colchicine (0.2 ml) was added to each culture vial an hour before harvesting in order to arrest metaphase. Cells centrifuged at 1000 rpm for 10 min, pre-warmed 0.075M KCl solution was added after removal of supernatant. Cells were resuspended & incubated at 37°C for 20 min. After hypotonic treatment culture was centrifuged and supernatant was removed. Then cells were fixed by adding chilled fixative (Methanol:Acetic acid; 3:1). The slides were prepared by air drying method and stained with giemsa stain for 20 min. Then the slides were screened for CA¹⁰. Two hundred metaphases were examined for the occurrence of different types of abnormality.

Statistical analysis

Student *t*-test was used for analysis of CAs

Table 1: Chromosomal aberrations after *Chenopodium album* (aqueous) treatment

Treatments	Abnormal metaphases without gaps		Chromosomal aberrations		
	Number	Mean% ± SE	Gaps	Chromatid	Chromosome
CPA(100µg/ml)	18	9.0 ± 2.02	Break7	10	Break 8
Plant extract					
3mg/ml	4	2.0 ± 1.8	3	3	1
6mg/ml	2	1.0 ± 0.42	1	2	-
9mg/ml	2	1.0 ± 0.42	1	2	-
CPA + Plant extract (Aq.)					
CPA + 3	10	5.0 ± 1.45	5	6	4
CPA + 6	9	4.5 ± 1.36	4	6	3
CPA + 9	8	4.0 ± 1.27	4	5	3

Table 2: Chromosomal aberrations after *Chenopodium album* (Methanol)treatment

Treatments	Abnormal metaphases without gaps		Chromosomal aberrations		
	Number	Mean% ± SE	Gaps	Chromatid Break	Chromosome Break
CPA(100µg/ml)	18	9.0 ± 2.02	7	10	8
Plant extract					
3mg/ml	3	1.5 ± 0.63	2	2	
6mg/ml	1	0.5 ± 0.1	1	1	1 -
9mg/ml	1	0.5 ± 0.1	1	1	-
CPA + Plant extract (Methanol)					
CPA +3	9	4.5 ± 1.36	4	6	3
CPA +6	7	3.5 ± 1.17	3	5	2
CPA + 9	6	3.0 ± 1.06	3	5	1

S.E.: Standard error; CPA: Cyclophosphamide;

RESULTS & DISCUSSION

The results show dose dependent decrease in induction of CA by CPA when treated with plant extract. Statistically significant maximum decrease was found at higher dose of methanol extract in comparison to low doses & with aq. extract also. When CPA treated alone the total number of aberrations scored was 18.00 which decreased to 6.0 when treated with both CPA & 9mg/ml of plant extract (methanol) & decrease to 7.0 when treated with aqueous extract. Such decrease was analysed statistically using student's t-test & found to be significant. The effects on CA after treatment with CPA & various doses of *Chenopodium album* extract (aqueous, methanol) individually as well as in combination are shown in Table -1 & Table-2 respectively.

As many reports have suggested the mutagenicity & carcinogenicity effect of CPA^{6,7}. So in order to minimize the side effects various kinds of natural components showing modulatory effect are well established¹¹⁻¹³. The protective effect of this plant extract against carcinogen bioactivation, DNA damage induced by other compounds & anti-oxidant, free radical scavenging activity have been reported earlier^{14,15}.

The present results clearly indicate modulatory activity of this plant extract (both aq. & methanol) against CPA induced genotoxicity in human lymphocytes *in-vitro*. We expect that this finding will give idea for design & development of a new protective drug having modulatory activity over existing chemotherapies.

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