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Chromosome Aberrations Induced by Eumaitenine, a Sesquiterpene Isolated from *Maytenus boaria* Mol. in Cultured CHO Cells

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A screening program of Chilean plants for antitumor and/or antineoplastic activity has been going on for the last decade. A sesquiterpene Eumaitenine (EUM) has been isolated from seeds of *Maitenus boaria* Mol. Celastraceae (Becerra et al. 1987).

Antineoplastic activity has been proved in KB cells from nasopharyngeal human carcinoma. It also shows inhibitory activity in lymphocytic leukemia P388. The LD_{50} was determined to be 7.3×10^{-5} mg/ml. These determinations were carried out by the National Cancer Institute, USA.

Since chemotherapy for human malignancies is being widely used, it is necessary to know the undesirable side effects, which often include genetic damage. Through these experiments we wish to determine the genotoxic characteristics of this sesquiterpene.

It is for this purpose that a chromosomal aberration test in CHO cells has been used. This bioassay has been established as a good indicator of genotoxicity (Tsuda and Kato, 1977; Derrudi and Natarajan,1985). The CHO line (Chinese hamster ovary cells) was first established in 1958 by Puck. They have been routinely employed to evaluate genotoxicity using Chromosome Aberrations (CA) as a parameter.

MATERIAL AND METHODS

Chinese hamster ovary cells were cultured in McCoy's 5a

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medium with 10% fetal calf serum (Gibco), 100 units penicillin, 100 ug/ml streptomycin (Sigma Chemical Co).

The cells were plated into 6 cm petri dishes at a concentration of 5 X 10^4 cells/dish in a humidified, 5% CO_2 atmosphere at 37°C. EUM was obtained from the Phytochemical Laboratory, University of Concepcion.

Cultured were set up in duplicate and three different doses of the chemical were tested. The chemical was dissolved in dimethyl sulfoxide (DMSO) and tested at concentrations of $4.6x10^{-5}$ mg/ml, $7.3x10^{-5}$ mg/ml, $14.6x10^{-5}$ mg/ml . Negative control cultures were treated with 20 ul of DMSO (Sigma Chemical Co) and the positive control with 20 ul of ethyl methanesulfonate (EMS) at a final concentration of 350 ug/ml. Cells were harvested in the first division after treatment (12h). They were exposed to colcemid (Gibco) 0.1 ug/ml for 2 h and collected by mitotic shake-off and incubated in 0.075 M KCl hypotonic solution for 15 min at 37°C. The cells were fixed in Carnoy's following the standard procedures. Slides were stained with 2% Giemsa buffered solution (pH 6.8) for 15 min.

The number of cells with CA was recorded on 100 well-spread methaphases at the magnification of 1000X. Types of aberrations were classified into seven groups: cells with chromosome and chromatid breaks, cells with rings, fragments, dicentric, triradial and quadriradial figures.

The X^2 test was used to determine whether any value deviated significantly from the negative control. (Tayama et al. 1989, Xue-jun et al. 1991).

RESULTS AND DISCUSSION

Table 1 shows the occurrence of chromosomal aberrations in CHO cells treated with EUM. Table 2 shows the types of CA induced by EUM. The three doses tested were 3.6 x 10^{-5} mg/ml ($1/2LD_{50}$), 7.3 x 10^{-5} mg/ml (LD_{50}), and 14.3 x 10^{-5} mg/ml ($2LD_{50}$).

The analysis of the data show that there is a clear dose related increase in the frequency of CA. The type of aberrations induced by EUM was similar for all three doses, and mainly consisted of

single and double breaks and fragments . Complex interchanges were rarely detected.
Gaps were scored, but not included in the statistical analysis because under the light microscope it is difficult to distinguish chromosome breaks from the achromatic regions. (Derrudi and Natarajan, 1989).

Statistical analysis shows that the higher doses of EUM have a highly significant genotoxic activity as compared to the negative control. The lower dose tested has a slightly less significant genotoxic activity.

Table 1. Effects of Eumaitenine on the frequency of chromosome aberrations in cultured CHO cells.

Treatment	Doses	Cells scored	% of cells aberrant	% of Chromosome Aberrations
EUM	mg/ml $X10^{-5}$			
	3.6	100	5	8 *
	7.3	100	10	10 **
	14.6	100	13	16 ***
EMS	350 ug/ml	50	34	50 ***
DMSO	0.5 %v/v	100	2	2

Values marked with asterisks are significantly different from negative control by X^2 test. (* $P< 0.05$; ** $P< 0.01$; *** $P< 0.001$).

Table 2. Percentage of the different types of chromosomal aberrations in cultured CHO cells treated with different doses of Eumaitenine.

Treatment	Doses	Types of Chromosome Aberrations						
		ctb	csb	ace	dic	r	tr	qr
EUM	mg/ml $X10^{-5}$							
	3.6	1	2	5	0	0	0	0
	7.3	6	1	3	0	0	0	0
	14.6	7	5	3	0	1	0	0
EMS	350 ug/ml	20	16	6	1	3	1	3
DMSO	0.5 % v/v	2	0	0	0	0	0	0

ctb: chromatid break; csb: chromosome break; ace: acentric fragment; dic: dicentric chromosome; r: ring; tr: figure triradial; qr: figure quadriradial.

The analysis of the results of the LD_{50} dose ($7.3x10^{-5}$ mg/ml) showed a significantly higher frequency of CA than the negative control. Considering that the type of aberrations are mainly

breaks and fragments, it can be concluded that the cytostatic activity showed by EUM in KB cells could be due to its clastogenic potential.

As it has been previously proven (Cea et al.1991, Alarcón et al. 1992) these sesquiterpenic molecules have an evident genotoxic activity. The high genotoxicity of the dose with cytostatic effect does not allow the authors to recommend it as an antitumor drug.

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