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EFFECTS OF TURMERIC AND ITS ACTIVE PRINCIPLE, CURCUMIN, ON BLEOMYCIN-INDUCED CHROMOSOME ABERRATIONS IN CHINESE HAMSTER OVARY CELLS

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ABSTRACT

Naturally occurring antioxidants have been extensively studied for their capacity to protect organisms and cells from oxidative damage. Many plant constituents including turmeric and curcumin appear to be potent antimutagens and antioxidants. The effects of turmeric and curcumin on chromosomal aberration frequencies induced by the radiomimetic agent bleomycin (BLM) were investigated in Chinese hamster ovary (CHO) cells. Three concentrations of each drug, turmeric (100, 250 and 500 μg/ml) and curcumin (2.5, 5 and 10 μg/ml), were combined with BLM (10 μg/ml) in CHO cells treated during the G_1/S, S or G_2/S phases of the cell cycle. Neither turmeric nor curcumin prevented BLM-induced chromosomal damage in any phases of the cell cycle. Conversely, a potentiation of the clastogenicity of BLM by curcumin was clearly observed in cells treated during the S and G_2/S phases. Curcumin was also clastogenic by itself at 10 μg/ml in two protocols used. However, the exact mechanism by which curcumin produced clastogenic and potentiating effects remains unknown.

INTRODUCTION

Recently a variety of compounds that possess antimutagenic properties has been detected in vegetables and spices, and evidence is accumulating that their dietary intake decreases the risk of cancer and other malignant diseases in human (Kada *et al.*, 1986). Antioxidant compounds, especially phenols such as gallic acid, tannins, curcumin, ellagic acid and eugenol, are of considerable interest from the viewpoint of dietary antioxidant supplementation (Halliwell, 1994).

Turmeric, a spice obtained from the rhizome of *Curcuma longa* Linn (Zingiberaceae), has been regularly used for its coloring, flavoring and medicinal properties (Ammon and Wahl, 1991). Studies suggest that turmeric is a potent antimutagenic *in vivo* against carcinogens such as benzo[a]pyrene and methylcholanthrene (Polasa *et al.*, 1991), and it is effective in inhibiting the formation and excretion of urinary mutagens in smokers (Polasa *et al.*, 1992).

Curcumin, the active principle of turmeric, is commonly used as a coloring agent in foods, drugs and cosmetics, and has a wide range of effects. Curcumin is known to act as an antioxidant, antimutagen and anticarcinogen (Sharma, 1976; Kunchandy and Rao, 1990; Nagabhushan and Bhide, 1992; Rao *et al.*, 1995; Anto *et al.*, 1996). Curcumin also has therapeutic properties for some human diseases (Srimal and Dhawan, 1973; Srivastava *et al.*, 1985).

Bleomycin (BLM) is an antibiotic and radiomimetic glycopeptide that is routinely used in cancer chemotherapy as an antineoplastic agent. BLM is mutagenic in diverse genetic assays (Povirk and Austin, 1991) and is thought to exert its genotoxic effects through free radical production and the induction of oxidative damage to DNA (Lown and Sim, 1977; Cunningham *et al.*, 1984; Sikic, 1986).

The aim of the present study was to investigate the action of the antioxidants turmeric and curcumin on chromosomal damage induced by the oxidative agent BLM, by determining their effect on the frequency of BLM-induced chromosomal aberrations in Chinese hamster ovary (CHO) cells.

MATERIAL AND METHODS

Chemicals

Turmeric was donated by Quest International Company (Brazil), curcumin (diferuloylmethane; C.I. 75300) was purchased from Sigma Chemical Co. (USA), and bleomycin sulfate was obtained from Hospital das Clínicas, USP, Brazil (Blenoxane®; Bristol-Myers Squibb, Brazil). Turmeric and BLM were dissolved in distilled water and curcumin was dissolved in dimethyl sulfoxide (DMSO; Sigma), just before each treatment. The final concentration of DMSO in the medium was 0.5%. Concentrations of BLM (10 μg/ml), turmeric (100, 250 and 500 μg/ml) and curcumin (2.5, 5 and 10 μg/ml) were chosen based on results obtained in pilot experiments (data not shown).

Cell culture and treatments

Chinese hamster ovary cells (CHO-9 cell line) were kindly supplied by Prof. A.T. Natarajan (University of Leiden, The Netherlands). Cells were maintained as monolayers growing at 37°C in 25-cm^2 flasks (Corning) containing HAM-F10 (Sigma) plus DMEM (Dulbecco's modi-

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fied Eagle's medium; Sigma), supplemented with 10% newborn bovine serum (Cultilab), antibiotics (0.1 mg/ml streptomycin and 0.06 mg/ml penicillin), and 2.38 mg/ml HEPES (Sigma). Cells were subcultured two or three times a week. The experiments were performed with cells at six to 12 passages.

Cells were seeded at a density of 10^6 cells/flask and then treated with either turmeric or curcumin, that remained in the culture until cell harvesting, 1 h before BLM addition. BLM treatment lasted 30 min. After this, cells were washed twice with PBS and refed complete medium plus either turmeric or curcumin. Exponentially growing CHO cells have a doubling time of 12-14 h (Preston *et al.* 1987; Cozzi *et al.*, 1993). In this work, total culture time was 14 h. Cells were harvested three, eight and 12 h after initiation of BLM treatment. According to Preston *et al.* (1981), cells fixed three, eight and 12 h after the beginning of treatment are, respectively, in the late G_2/S, middle S and early G_1/S phases of the cell cycle at the moment they are treated. Untreated controls were handled identically, with the same changes in medium. Colcemide (100 µl at a concentration of 5 µg/ml) was added to the culture medium 2 h before harvesting. Each experiment was repeated three times.

Cells were harvested by the method of Moorhead *et al.* (1960), with modifications (1% sodium citrate hypotonic solution, methanol/acetic acid 3:1 fixative). The air-dried chromosome preparations were stained with 3% Giemsa, diluted in phosphate buffer. Only well-spread metaphases presenting 21 ± 1 chromosomes were analyzed. Three hundred metaphases (100 in each experiment) were analyzed per treatment in order to determine the frequencies of chromosomal aberrations (blind test). The mitotic index was obtained by counting the number of mitotic cells in 3,000 cells per treatment (1,000 in each experiment).

Statistical analysis

Data concerning number of abnormal metaphases and total number of chromosomal aberrations, including gaps, and mitotic index were statistically analyzed by analysis of variance, with calculations of the F-statistic and respective P values. In all cases in which $P < 0.05$ the mean values of each treatment were compared by the Tukey test, with calculation of the minimum significant difference for $P = 0.05$.

RESULTS

Frequencies of abnormal metaphases, chromosomal aberrations and mitotic indices in CHO cells treated with turmeric combined or not with BLM during G_1/S, S or G_2/S phases of the cell cycle were determined (Tables I, II and III). Mitotic indices observed in cultures treated with turmeric were not significantly different from those found in untreated controls, except when 500 µg/ml turmeric was associated with BLM at the G_1/S phase (Table I). Turmeric was not clastogenic in any protocol tested. Although a statistically significant difference was not detected when compared with BLM alone, there was a tendency for the highest concentration of turmeric combined with BLM to increase the frequency of abnormal metaphases and chromosomal aberrations in cells treated at G_1/S phase. On the other hand, turmeric did not show any significant effect on bleomycin-induced aberrations.

A 30-min pulse treatment with BLM at 10 µg/ml to CHO cells produced a marked induction of chromosome aberrations. BLM at this concentration was also used as a clastogenic agent by other authors (Salvadori *et al.*, 1994; Anderson *et al.*, 1994). In this paper, the main aberration types after BLM exposure at G_1/S were isochromatid breaks and dicentrics, and in the case of treatment

Table I - Distribution of the different types of chromosomal aberrations in 300 cells analyzed per group of treatment and mitotic index[ψ] (MI) observed in CHO cells after treatment with turmeric (13 h) and/or bleomycin (BLM, 10 µg/ml) during G_1/S phase.

Treatment (µg/ml)		MI (%)	AM	Aberrations						Total
				Chromatid type			Chromosome type			
				gaps	breaks	exch	breaks	dic	rings	
Untreated control		12.6	8	3	3	0	3	0	0	9
Turmeric	100	11.5	5	3	0	0	5	0	0	8
	250	11.4	14	14	2	0	3	1	1	21
	500	10.2	9	4	2	2	1	0	0	9
BLM		10.3	63*	9	20	21	61	28	5	144*
Turmeric	100 + BLM	10.4	77*	9	13	14	74	39	10	159*
	250 + BLM	9.9	99*	12	22	45	67	37	11	194*
	500 + BLM	4.5*	87*	24	26	36	59	51	14	210*

[ψ]3000 cells were counted per treatment. AM = Abnormal metaphases; dic = dicentrics; exch = exchanges (including: triradials, quadriradials, complex exchanges and sister union). *Statistically different when compared with untreated control.

Table II - Distribution of the different types of chromosomal aberrations in 300 cells analyzed per group of treatment and mitotic index[ψ] (MI) observed in CHO cells after treatment with turmeric (9 h) and/or bleomycin (BLM, 10 µg/ml) during S phase.

Treatment (µg/ml)		MI (%)	AM	Aberrations						Total
				Chromatid type			Chromosome type			
				gaps	breaks	exch	breaks	dic	rings	
Untreated control		16.8	15	4	4	3	0	4	0	15
Turmeric	100	11.9	14	4	5	2	1	3	0	15
	250	11.5	16	4	7	2	1	4	0	18
	500	17.3	12	3	4	2	2	2	0	13
BLM		10.6	68*	9	23	52	15	7	2	108*
Turmeric	100 + BLM	9.6	64*	7	23	48	16	7	5	106*
	250 + BLM	11.3	64*	13	33	46	10	6	4	112*
	500 + BLM	7.2	71*	10	40	49	8	9	2	118*

For symbols and abbreviations see Table I.

Table III - Distribution of the different types of chromosomal aberrations in 300 cells analyzed per group of treatment and mitotic index[ψ] (MI) observed in CHO cells after treatment with turmeric (4 h) and/or bleomycin (BLM, 10 µg/ml) during G_2/S phase.

Treatment (µg/ml)		MI (%)	AM	Aberrations						Total
				Chromatid type			Chromosome type			
				gaps	breaks	exch	breaks	dic	rings	
Untreated control		13.8	4	1	1	0	1	2	0	5
Turmeric	100	11.1	13	1	6	0	3	3	1	14
	250	13.1	11	5	2	0	1	4	0	12
	500	14.8	7	2	2	0	0	2	1	7
BLM		5.3	68*	25	55	16	7	3	1	107*
Turmeric	100 + BLM	7.3	68*	37	56	16	7	5	0	121*
	250 + BLM	9.2	57*	22	56	8	7	3	2	98*
	500 + BLM	6.2	60*	32	40	13	4	4	1	94*

For symbols and abbreviations see Table I.

at S and G_2/S phases, chromatid breaks, gaps and exchanges were the most frequent.

The effects of curcumin on the frequencies of abnormal metaphases, chromosomal aberrations and mitotic indices induced by BLM in cells treated during G_1/S, S or G_2/S phases were determined (Tables IV, V and VI). Curcumin at 10 µg/ml was clastogenic but not cytotoxic. The chromosomal damage induced by 10 µg/ml curcumin was statistically significant when compared with the untreated control, and chromatid breaks and gaps were the most frequent alterations observed (Tables IV and V). A cytotoxic effect was observed when curcumin at 2.5 and 10 µg/ml was combined with BLM at S phase (Table V) and curcumin at 10 µg/ml combined with BLM at G_2/S phase (Table VI). When compared with BLM alone plus curcumin control, curcumin at 5 and 10 µg/ml potentiated the clastogenicity of BLM at S (Table V) and in all three concentrations tested in G_2/S (Table VI). These effects were observed for both abnormal metaphases and chromosome aberrations. The number of abnormal metaphases increased 109 and 83% in cultures treated at S phase by BLM plus curcumin at 5 or 10 µg/ml, respectively, and 41, 60 and 115% in cultures treated at G_2/S phase by BLM plus curcumin at 2.5, 5 or 10 µg/ml, respectively. The total number of chromosome aberrations increased 239 and 229% in cultures treated at S phase by BLM plus curcumin at 5 or 10 µg/ml, respectively, and 153 and 359% in cultures treated at G_2/S by BLM plus curcumin at 5 or 10 µg/ml, respectively. These values were calculated by comparing those observed for the association BLM + curcumin with the expected values (sum of the effects caused by each agent alone). The main aberration types observed in cells treated with curcumin combined with BLM, in which a synergistic effect had occurred, were chromatid breaks and gaps.

DISCUSSION

The CHO cell system has been used to evaluate mutagenesis and antimutagenesis activities of different

Table IV - Distribution of the different types of chromosomal aberrations in 300 cells analyzed per group of treatment and mitotic index$^{\Psi}$ (MI) observed in CHO cells after treatment with curcumin (13 h) and/or bleomycin (BLM, 10 µg/ml) during G_1/S phase.

Treatment (µg/ml)	MI (%)	AM	Aberrations						Total
			Chromatid type			Chromosome type			
			gaps	breaks	exch	breaks	dic	rings	
Untreated control	14.1	5	0	1	0	1	3	0	5
DMSO	13.0	14	6	2	0	3	4	0	15
Curcumin 2.5	18.0	17	11	2	1	3	1	0	18
5	20.3	17	10	6	2	0	0	0	18
10	9.9	78*	24	42	13	1	4	1	85*
BLM	11.5	76*	5	15	24	91	52	12	199*
Curcumin 2.5 + BLM	10.2	85*	7	16	17	109	65	22	236*
5 + BLM	10.9	91*	8	29	25	115	58	14	249*
10 + BLM	8.2	125*	5	34	126	60	53	14	292*

For symbols and abbreviations see Table I.

Table V - Distribution of the different types of chromosomal aberrations in 300 cells analyzed per group of treatment and mitotic index$^{\Psi}$ (MI) observed in CHO cells after treatment with curcumin (9 h) and/or bleomycin (BLM, 10 µg/ml) during S phase.

Treatment (µg/ml)	MI (%)	AM	Aberrations						Total
			Chromatid type			Chromosome type			
			gaps	breaks	exch	breaks	dic	rings	
Untreated control	13.9	8	4	4	0	0	0	0	8
DMSO	11.6	8	3	2	1	1	1	0	8
Curcumin 2.5	12.4	9	1	4	1	1	2	0	9
5	9.2	15	2	8	3	1	1	0	15
10	7.2	37*	10	22	2	2	2	0	38*
BLM	11.9	48*	6	30	20	12	7	0	75*
Curcumin 2.5 + BLM	5.7*	57*	8	26	57	8	6	2	107*
5 + BLM	7.5	132**	26	113	142	14	8	2	305**
10 + BLM	2.9*	156**	25	225	97	15	6	4	372**

For symbols and abbreviations see Table I. *Statistically different when compared with untreated control. **Statistically different when compared with BLM alone plus curcumin control.

Table VI - Distribution of the different types of chromosomal aberrations in 300 cells analyzed per group of treatment and mitotic index$^{\Psi}$ (MI) observed in CHO cells after treatment with curcumin (4 h) and/or bleomycin (BLM, 10 µg/ml) during G_2/S phase.

Treatment (µg/ml)	MI (%)	AM	Aberrations						Total
			Chromatid type			Chromosome type			
			gaps	breaks	exch	breaks	dic	rings	
Untreated control	12.7	6	1	3	0	0	2	0	6
DMSO	13.9	4	0	1	1	0	3	0	5
Curcumin 2.5	13.4	7	2	4	1	0	0	0	7
5	14.0	9	3	1	1	1	3	0	9
10	10.6	12	1	2	2	1	4	1	11
BLM	5.0	46	11	31	17	4	2	1	66
Curcumin 2.5 + BLM	5.9	75**	43	48	15	5	4	0	115*
5 + BLM	5.8	88**	68	92	23	2	4	1	190**
10 + BLM	2.9*	125**	78	223	28	18	6	1	354**

For symbols and abbreviations see Table I. *Statistically different when compared with untreated control. **Statistically different when compared with BLM alone plus curcumin control.

compounds (Cozzi *et al.*, 1991; Musk *et al.*, 1997). Several natural compounds and antioxidant agents, such as vanillin (Sasaki *et al.*, 1987a,b, 1990), garlic acid (Knasmüller *et al.*, 1989), humic acid (Cozzi *et al.*, 1993), fatty acids (Sasaki *et al.*, 1994), squalene (Fan *et al.*, 1996) and Tochu tea (Nakamura *et al.*, 1997), have shown antimutagenic properties against chromosomal damage in CHO cells.

As oxidative damage in biological systems is considered to cause aging, degenerative diseases and cancer, particular attention has been focused on the possibility of modulating these effects through the use of free-radical scavengers to minimize cellular injury. Curcumin is a natural antioxidant derived from turmeric, which has therapeutic properties and anticancer effects. These beneficial effects of turmeric have been postulated to be due to curcumin (Reddy and Lokesh, 1994).

In the present work, turmeric was not clastogenic by itself whereas curcumin increased both the number of abnormal metaphases and the frequency of chromosomal aberrations at the highest concentration in cells treated for 13 and 9 h. As the cell cultures were treated with curcumin during 13, 9 and 4 h, respectively for cells treated by BLM in G_1/S, S and G_2/S phases, the curcumin-induced damage was higher in the 13-h treatment compared with the 9-h treatment, and was not statistically significant in the 4-h treatment compared with untreated controls (Tables IV, V and VI).

Turmeric was found to be non-mutagenic in mice and rats (Vijayalaxmi, 1980) and in the Ames test (Nagabhushan *et al.*, 1987). However, it induced chromosomal damage in plant systems (Abraham *et al.*, 1976) and was weakly clastogenic in acutely treated mice (Mukhopadhyay *et al.*, 1998). Turmeric can also antagonize the mutagenicity of other substances (Shalini and Srinivas, 1990; Srinivas and Shalini, 1991; Mukundan *et al.*, 1993). According to our results turmeric did not reduce the BLM-induced chromosomal aberrations; instead, it showed a slight tendency to increase the chromosomal damage induced by BLM at G_1/S phase (Table I).

Some authors have noticed that curcumin is non-mutagenic in CHO cells (Au and Hsu, 1979), Ames test (Nagabhushan *et al.*, 1987) and mice (Abraham *et al.*, 1993). Nevertheless, our data clearly indicate that curcumin at 10 μg/ml is clastogenic when present in the culture for more than 9 h. Clastogenic effects of curcumin were also observed by others. Mukhopadhyay *et al.* (1998) reported a slight increase in the number of chromosomal aberrations in acutely treated mice by curcumin. Giri *et al.* (1990) observed a significant increase in SCE and a weak increase in the frequency of chromosomal aberrations in mice and rats treated acute and chronically with curcumin. Ishidate Jr. *et al.* (1984), using a Chinese hamster fibroblast cell line, noticed that curcumin was clastogenic at 30 μg/ml. We have observed that curcumin is cytotoxic at concentrations higher than 20 μg/ml (data not shown).

Curcumin significantly reduced the frequencies of micronucleated polychromatic erythrocytes in mice exposed to γ-radiation (Abraham *et al.*, 1993), and it was also indicated as an antimutagen against environmental mutagens *in vitro* and an antitumor drug *in vivo* (Nagabhushan *et al.*, 1987; Nagabhushan and Bhide, 1992). However, curcumin and turmeric could not inhibit cyclophosphamide- or mitomycin-C-induced chromosomal aberrations in mice (Mukhopadhyay *et al.*, 1998). According to our data, curcumin increased significantly the frequencies of both abnormal metaphases and chromosomal aberrations induced by BLM at S and G_2/S phases when compared with BLM plus curcumin controls. The potentiating influence of curcumin on the clastogenicity of BLM was reproducible in three independent experiments. A potentiation effect with curcumin was also observed by Sahu and Washington (1992). They demonstrated the pro-oxidant properties of ascorbic acid and curcumin on quercetin-induced nuclear damage in presence of iron and copper. Therefore, according to these authors ascorbic acid and curcumin may have a dual role in carcinogenesis.

A potentiation effect of known antioxidant compounds combined with clastogenic compounds has also been reported by others. Chromosome aberrations induced by alkylating agents in cultured Chinese hamster cells were enhanced in the presence of σ-vanillin, an isomer of vanillin (Matsumura *et al.*, 1993). Aruoma (1993) found a prooxidant action of vitamin E in an assay for DNA damage with BLM and iron. Anderson *et al.* (1994) showed that ascorbic acid produced exacerbating effects using Comet assay with human lymphocytes when it was combined with BLM. Cozzi *et al.* (1997) observed that ascorbic acid increased BLM-induced aberrations in CHO cells, whereas β-carotene had no effect on chromosomal aberrations induced by BLM. Conversely, Salvadori *et al.* (1994) observed that β-carotene enhanced the clastogenicity induced by BLM in CHO cells.

Since BLM is a radiomimetic compound, the effects of some radioprotectors on its genetic activity have been evaluated. Radioprotectors, such as DMSO, 2-[(aminopropyl)amino] ethanethiol (WR-1065) (Hoffman *et al.*, 1993) and cysteamine (Hoffman and Littlefield, 1995), potentiated the clastogenic activity of BLM in G_o human lymphocytes. In contrast, WR-1065 has been reported to protect against the effects of BLM in cultured Chinese hamster cells (Nagy and Grdina, 1986).

BLM acts by generating oxygen radical species and it can be destroyed by the reactive oxygen species produced by itself (Scheulen *et al.*, 1981; Lopez-Larraza *et al.*, 1990). Salvadori *et al.* (1994) suggested that the presence of a scavenger, such as ascorbic acid and β-carotene, could cancel or reduce this detoxification. As curcumin is also a free-radical scavenger (Kunchandy and Rao, 1990), a similar mechanism may explain the potentiating effect observed. However, we have observed that curcumin also enhances γ-radiation-induced chromosomal aberrations in

CHO cells (Araújo *et al.*, 1999), and since curcumin was clastogenic by itself, another mechanism could be involved.

Chromosomal aberrations are believed to result from unrepaired and misrepaired DNA lesions, and inhibitors of DNA repair can induce an enhancement of the DNA damage caused by many kinds of compounds (Kihlman and Natarajan, 1984; Noviello *et al.*, 1994). Curcumin increased the BLM-induced chromosomal damage at S and G_2/S phases, but not in G_1/S. These data suggest that curcumin could inhibit a repair mechanism operating during S and G_2/S phases in CHO cells.

Kuo *et al.* (1996) have noticed that curcumin induces apoptotic cell death in promyelocytic leukemia HL-60 cells through free-radical generation. Although the exact mechanism by which curcumin induces reactive oxygen species remains to be elucidated, Kunchandy and Rao (1990) reported the ability of curcumin at low concentrations to generate hydroxyl radical through the Fenton reaction by reducing Fe^{3+} to Fe^{2+}, as well as possessing scavenging properties at higher levels. On the other hand, it has been postulated that BLM interacts with molecular oxygen and Fe^{2+} to produce superoxide anion and other oxygen metabolites (Sausville *et al.*, 1978).

Potentiation of BLM by curcumin could also depend on its pro-oxidant activity. According to Sahu and Washington (1992), the antioxidant curcumin, like ascorbic acid, can become a pro-oxidant agent depending on the redox state of the biological environment. Therefore, the clastogenic and potentiating effects of curcumin found in the present work could be explained by the fact that curcumin would act as a pro-oxidant agent at the highest concentrations tested under the conditions of the present report. Possibly, in other conditions curcumin acts as an antioxidant agent.

On the basis of these data, we may conclude that turmeric and curcumin did not protect against BLM-induced chromosomal aberrations; conversely, curcumin exhibited a clastogenic and potentiating effect. However, the mechanism by which curcumin acts remains to be investigated, and further studies are necessary to clarify this point.

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RESUMO

Antioxidantes de ocorrência natural têm sido exaustivamente estudados quanto a sua capacidade de proteger organimos e células contra danos oxidativos. Muitos constituintes das plantas, incluindo cúrcuma e curcumina, parecem ser potentes antimutágenos e antioxidantes. Os efeitos de cúrcuma e curcumina na freqüência de aberrações cromossômicas induzidas pelo agente radiomimético bleomicina (BLM) foram investigados em células do ovário de hamster chinês (CHO). Três concentrações de cada droga, cúrcuma (100, 250 e 500 μg/ml) e curcumina (2,5, 5,0 e 10 μg/ml), foram combinadas com BLM (10 μg/ml) em células CHO tratadas durante as fases G_1/S, S ou G_2/S do ciclo celular. Nem cúrcuma nem curcumina evitaram o dano cromossômico induzido pela BLM em fase alguma do ciclo celular. Ao contrário, a potenciação da clastogenicidade da BLM pelo curcumina foi nitidamente observada em células tratadas durante as fases S e G_2/S. A curcumina também se mostrou clastogênica na dose de 10 μg/ml nos protocolos de tratamento de 9 e 13 h. Contudo, o mecanismo exato pelo qual a curcumina produziu efeitos potenciadores e clastogênicos permanece desconhecido.

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