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Original Research Paper

Modulating Effects of Curcumin on Chromium Induced Chromosomal Aberrations in Somatic Cells of Mice

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ABSTRACT

The protective effects of curcumin in chromium induced cytotoxicity was evaluated in *in vivo* animal model using analysis of chromosomal aberrations in somatic cells of mice. Three doses of curcumin were selected for modulation and given to animals after priming with chromium. The animals ware sacrificed 48hr after the treatment and slides were prepared. A significant decrease was observed in the percentage of chromosomal aberrations when animals were primed with curcumin. The present results clearly indicate the protective nature of curcumin against heavy metal genotoxicity.

INTRODUCTION

Human population is not only exposed to a variety of environmental mutagens but also inhale a large number of other chemicals. Many of these have shown genotoxicity in mammalian systems. The commonly used spice, *Curcuma longa* yields turmeric, a gold coloured spice used in foods, drugs and cosmetics. The curcumin, present in turmeric, is known for its medicinal properties, considered to have anti inflammatory, anticarcinogenic, antimutagenic, antioxidant, antiseptic, anti-invasive, antiangionic and therapeutic properties for some diseases (Srimal & Dhawan 1973, Srivastava et al. 1985, Kunchandy & Rao 1990, Goel et al. 2000, Aggarwal et al. 2003). The available data on curcumin mutagenicity are controversial (Vijayalaxmi 1980, Abraham et al. 1993, Sandhya Rani & Rudrama Devi 2001, 2002, Anuradha et al. 2007, Giri et al. 1990, Mukhopadhyay et al. 1998).

Chromium has been extensively investigated metal. A number of reviews on this subject are available in the literature. Positive mutagenic effects of chromium are reported using experimental animal models (Sunderman 1986, Szyba et al. 1992, Banukiran et al. 1999, Kunal Das 2009). Hence, in the present investigation studies were carried out to study the protective role of curcumin in chromium induced cytogenetic damage in bone marrow cells of mice.

MATERIALS AND METHODS

Eight week old healthy laboratory bred Swiss Albino mice (*Mus musculus*) weighing 25±3g were maintained under standard laboratory conditions at temperature 22°C. Commercial pellet diet (Hindustan Lever, India) and deionized water were provided to mice by ad labium.

In the present studies, two experiments were conducted. The animals were fed orally with chromium and mitomycin-C. The animals were categorized into the following groups.

Group I	Control
Group II	Mitomycin-C (70 mg/kg)
Group III	Chromium (60 mg/kg)
Group IV	Chromium primed with Curcumin (60 mg/kg + 5 mg/kg)
Group VI	Chromium primed with Curcumin (60 mg/kg + 7.5 mg/kg)
Group VII	Chromium primed with Curcumin (60 mg/kg + 10 mg/kg)

The animals were fed orally with 60 mg/kg of chromium and Curcumin was given by gavage needle for 7 consecutive days. The animals were sacrificed at 48h. The treatment for 48h was kept to allow bone marrow cells to complete two cell cycles. The control and treatment group of animals were sacrificed 6 hr after the last treatment by cervical dislocation. The bone marrow was flushed out in clean glass Petri dishes with hypotonic solution of KCl (0.75 M) to get a fine homogenous cell suspension. It was then collected in centrifuge tubes incubated for 30 min at 37°C. The slides were prepared, stained and screened for 100 well spread metaphases per animal and for the presence of various types of chromosomal aberrations such as gaps, breaks, chromatid separation and polyploids in control and treated groups of animals. The differences in the percentage of chromosomal aberrations in somatic cells of control and treated groups of animals was analysed using chi-square test.

RESULTS AND DISCUSSION

The results on the frequency of various types of chromosomal aberrations in somatic cells of chromium + curcumin treated animals are presented in Tables 1 and 2.

At 48 h exposure the frequency (%) of gaps in control animals was 0.60, which increased to 3.60 in 75 mg/kg treatment, but decreased to 1.60, 1.60, and 1.60 in curcumin primed animals i.e., 60 + 5, 60 + 7.5, and 60 + 10 mg/kg respectively. Similarly, the frequency (%) of breaks in control animals was 1.00, but increased to 4.80 in 60 mg/kg and decreased to 4.20, 3.20, and 2.80 in curcumin primed animals i.e., 60 + 5, 60 + 7.5 and 60 + 10 mg/kg. The frequency (%) of fragments in control animals was 0.00. It was increased to 1.20 in 60 mg/kg and decreased to 1.00, 0.80 and 0.80 in curcumin primed animals i.e., 60 + 5, 60 + 7.5 and 60 + 10 mg/kg. Similarly, the frequency (%) of exchanges in control animals was 0.00 which increased to 0.60 in 60 mg/kg and decreased to 0.40, 0.40 and 0.40 in curcumin primed animals i.e., 60 + 5, 60 + 7.5 and 60 + 10 mg/kg. The frequency (%) of polyploids in control animals was 0.20 which increased to 0.40 in 60 mg/kg and decreased to 0.40, 0.20 and 0.20 in curcumin primed animals i.e., 60 + 5, 60 + 7.5 and 60 + 10 mg/kg. The frequency (%) of chromatid separations in control animals was 0.60 which increased to 1.40 in 60 mg/kg and decreased to 2.40, 2.00, and 1.00 in curcumin primed animals i.e., 60 + 5, 60 + 7.5, and 60 + 10 mg/kg.

The frequency of various types of chromosomal aberrations recorded in mitomycin-C treated groups of control animals for 48h was as follows. The frequency of gaps and breaks was 1.80 and 5.40, whereas the frequency (%) of fragments and exchanges was 1.00 and 0.60. The frequency of polyploids and chromatid separations was 2.60 and 3.00 % respectively. The total frequency (%) of aberrations in mitomycin-C treated control groups was 12.40.

Thus, as a result of various types of chromosomal aberrations the percentages of total chromosomal aberrations at 48h exposure to chromium + curcumin were 2.40 in control and 12.00 in 60 mg/kg of chromium treated animals to 10.00, 8.20 and 6.80 of chromium + curcumin treated animals respectively (Tables 1 and 2).

The actively proliferating cells from the bone marrow provide maximum information on the effect of any test compound (Preston et al. 1987). Chromosomal aberrations observed in the present

Table 1: Frequency of chromosomal aberrations recorded in somatic cells of mice with chromium primed with curcumin for 24, 48 and 72 h time intervals.

Dose (mg/kg) duration of treatment	Non-primed		Primed with curcumin					
			5mg/kg		7.5mg/kg		10mg/kg	
	Normal metaphases scored (%)	Abnormal metaphases scored (%)	Normal metaphases scored (%)	Abnormal metaphases scored (%)	Normal metaphases scored (%)	Abnormal metaphases scored (%)	Normal metaphases scored (%)	Abnormal metaphases scored (%)
48h								
Control II	488 (97.60)	12 (2.40)						
Mitomycin-C 60mg/kg	438 (87.60) 440 (88.00)	62 (12.40) 60 (12.00)*	450 (90.00)	50 (10.00)	459 (91.80)	41 (8.20)	466 (93.20)	34 (6.80)

The values in parentheses are percentages; *P < 0.01

Table 2: Classification of various types of chromosomal aberrations in somatic cells of mice analysed after 48 h chromium treated animals primed with various doses of curcumin. Gaps and polyploids are not included in total aberrations.

Dose (mg/kg) and duration	Structural aberrations				Numerical aberrations		Total no. of aberrations(%)
of treatment	Gaps	breaks	fragments	exchanges	polyploidy	Chromatid seperations	
48h							
Control II	3 (0.60)	5 (1.00)	0 (0.00)	0 (0.00)	1 (0.20)	3 (0.60)	8 (1.60)
Mytomycin-C	9 (1.80)	27 (5.40)	5 (1.00)	3 (0.60)	13 (2.60)	15 (3.00)	50 (10.00)
60 mg/kg	18 (3.60)	24 (5.80)	6 (1.20)	3 (0.60)	2 (0.40)	7 (1.40)	40 (8.00)
60+5 mg/kg	8 (1.60)	21 (4.20)	5 (1.00)	2 (0.40)	2 (0.40)	12 (2.40)	40 (8.00)
60+7.5 mg/kg	8 (1.60)	16 (3.20)	4 (0.80)	2 (0.40)	1 (0.20)	10 (2.00)	32 (6.40)
60+10 mg/kg	8 (1.60)	14 (2.80)	4 (0.80)	2 (0.40)	1 (0.20)	5 (1.00)	25 (5.00)

The values in parentheses are the percentages.

analysis were classified into structural and numerical and other abnormalities. These end points serve as indicators for evaluating the mutagenic potential of test substances. Since, these are considered as stable anomalies which contribute to next generation further such variations in somatic tissues may lead to malignancy.

Analysis of chromosomal aberrations in somatic cell of mice is being carried in this laboratory from last one decade with heavy metals like lead, chromium and cadmium, etc. (Kalyan Swamy et al. 1993, Rajitha & Rudrama Devi 1996, Banukiran et al. 1999).

In biological systems, oxidative damage is considered to cause ageing, 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 cell injury. Curcumin is a natural antioxidant derived from turmeric which has therapeutic properties and anticancer effects. These beneficial effects are due to curcumin (Reddy & Lokesh 1994). Curcumin is non-mutagenic in Chinese Hamster Ovarian cells (Au & Hsu 1979) Ames test (Nagabhushan et al. 1987) and mice (Abraham et al. 1993, Sandhya Rani & Rudrama Devi 1999, Vijayalaxmi 1980).

In the present study curcumin inhibits the percentage of chromosomal aberrations induced by chromium in bone marrow cells of mice. The results are comparable with that Abraham et al. (1993) who showed that curcumin significantly reduces the frequencies of micronucleated polychromatic

erythrocytes in mice exposed to γ-irradiation. Further, curcumin was also indicated as antimutagen against environmental mutagens in *in vitro* and in tumour drug *in vivo* (Nagabushan et al. 1987). Significantly, curcumin reduced the clastogenic activity of cisplatin in somatic cells of mice. However, curcumin and turmeric could not inhibit cyclophosphamide or mitomycin-C induced chromosomal aberrations in mice (Mukhopadhyay et al. 1998). A potentiating effect with curcumin was also observed by Sahu & Washington (1992). They demonstrated that pre-oxidant properties of ascorbic acid and curcumin on quercetin induced nuclear damage in presence of iron and copper. Therefore, according to these studies ascorbic acid and curcumin may have a dual role in carcinogenesis.

The various studies conducted at National Institute of Nutrition, Hyderabad on turmeric and its active principle curcumin suggest that it can impact all stages of carcinogenesis. It prevents activation of carcinogens and attack of electrophiles on DNA, acts as antioxidant and antipromotor, retards the conversion of preneoplasia in addition to repairing the damage to DNA. The antimutagenic nature of turmeric was evaluated in human smokers at administered dose of 1.5g/day for 30 days. When turmeric was given by oral route, the liver and kidney functions were not altered even within 15 days (Polasa 1991). Further, a clinical trial was also carried out in groups of reverse smokers who are at high risk of palatal cancers in a specific area of Andhra Pradesh. A dose of 1g/day was given for a period of 9 months, and the results suggested that it had a significant suppression of precancerous lesions, decreases micronuleii and DNA adduct in oral epithelial cells (Annual report of NIN, Hyderabad 1994-95).

Epidemiological studies have indicated significant differences in the incidence of cancers aiming ethnic groups who have different life styles and have been exposed to different environmental factors. It has been estimated that some human cancers could be prevented by modification of life style including dietary modification (Surh 2002). The use of natural chemicals allowing suppression, retardation or inversion of carcinogenic process is a promising approach especially for the prevention of tumours.

According to large number of epidemiological studies, a high consumption of fruits and vegetables is consistently associated with low incidence of most human cancers (Dorai & Aggarwal 2004). The dietary components as chemopreventive agents have received much attention in the public and medical community. The ability of curcumin to support apoptosis in cancer cells without cytotoxic effects on healthy cells is also important and was described in different cell lines (Tourkina et al. 2004). Hence, the protection afforded by curcumin in chromium induced genetic damage is of great importance as it can be used as preventive medicine in occupationally exposed population as the people do work for may years in same place. A battery of test protocol using curcumin against heavy metal genotoxicity are under progress.

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