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PROTECTIVE EFFECTS OF TURMERIC AGAINST CISPLATIN INDUCED GENOTOXICITY ON BONE MARROW CELLS OF MICE

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ABSTRACT

The inorganic platinum compound cisplatin (CP), has been used as a cancer therapy for decades and exhibits strong anti-tumor activity. However, the wide application of Cisplatin is largely limited due to its side effects. Natural therapies, such as the use of plant-derived products in cancer treatment, may reduce adverse side effects. In the present investigation studied the possible anti genotoxicity effects of Turmeric ethanolic extract on chromosomal aberrations induced by Cisplatin in the bone marrow cells of mice. The results indicate that Turmeric extract decreased significantly the percentage of chromosomal aberrations induced by Cisplatin in a dose dependent manner indicates the anti genotoxic effects of Turmeric. Hence Turmeric can be used as chemopreventive regimen.

KEYWORDS: Cisplatin, Turmeric, Antigenotoxicity, Chromosomal Abberations, Bone Marrow.

1. INTRODUCTION

The exposure of living organisms including humans to toxicants which ultimately cause DNA damage (Bont and Larebeke) and ultimately result in the transformation of normal cells to cancer cells. Cisplatin is one of the most potent chemotherapeutic drugs which is being used effectively against various solid tumors including testicular, ovarian, breast, lung, bladder, head, neck, *etc*.(Dasari and Tchounwou, 2014). The antitumor action of CP is atributted to its action on DNA synthesis (Crul *et al.*, 1997). The high mutagenic potency of CP raises the concern that its use in cancer chemotherapy may be responsible for secondary malignancies

which have been observed in animals and some cured patients treated with CP (Pillaire *et al.*, 1994). The therapeutic efficacy of cisplatin is limited due to the development of acquired drug resistance by the cancer cells (Kartalou and Essigmann, 2001) and various side effects in the host (Khynriam and Prasad, 2001). In fact, cisplatin has important side effects including reduction of antioxidant plasma levels and generation of free radicals in normal cells (Baliga *et al.*, 1998; Weijl *et al*, 1998; Wozniak *et al*, 2004). However, the wide application of Cisplatin is largely limited due to its side effects.

The use of dietary antioxidants to prevent antitumor agent-induced chromosomal damage in nontumor cells is currently eliciting considerable interest. The antioxidant compounds are capable of neutralizing free radicals and may play a major role in the prevention of certain diseases such as cancer (Clayton, 2000). Hence recent research have directed toward phytochemicals for cancer therapy (Lau *et al.*, 2008; Blanchard *et al.*, 2008; Nandakumar *et al.*, 2008). The research indicates that Turmeric have unique antioxidant, antimutagenic, antitumorigenic and anticarcinogenic properties (Majeed *et al.*, 1995; Miquel *et al.*, 2002). Turmeric is the dried rhizome of *Curcuma longa* Linn. a perennial herb of the family Zingiberaceae has several medicinal uses. The root of the plant is normally ground to yield a yellow colored powder commonly known as Turmeric, which is used for various applications like flavoring agent, spice, food preservative, coloring agent or for decoration, textile dye and in drugs and cosmetics (Rao *et al.*, 1995; Anto *et al.*, 1996). Ethanolic extract of Turmeric possesses anti-tumor activity (Rithaporn *et al.*, 2003). Hence the present study is designed to evaluate the protective effects of crude Turmeric ethanolic extract on Cisplatin induced genotoxicity in the bone marrow cells of mice.

2. MATERIALS AND METHODS

2.1 Analysis of chromosomal aberrations in somatic cells of mice

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound (Preston *et al* 1987). Chromosomal analysis in actively proliferating bone marrow cells is recommended as an ideal protocol to assess the chromosome mutational property of environmental agents (Bishun *et al*., 1973; Matter and Tsuchimoto *et al*., 1980; Preston *et al*., 1987). They have cell cycles (Frindel *et al*., 1967; Cole *et al*., 1981; Preston *et al*., 1987) and evaluation of first metaphases following exposure to the test compounds yields the best estimate of chromosome aberrations. Chromosome aberrations observed in the present analysis were classified into structural, numerical and

other abnormalities. These end points serve as indicators for evaluating the mutagenic potentials of test substances. Since they are considered as stable anomalies and continue to next generation. In the present study the air drying technique of Preston *et al* (1987) was employed with slight modifications to study the effect of test compounds on somatic cells of mice. Sampling times were ranged from 24h to 72h to cover short and long term effects on cells at different stages of cell cycle at the time of exposure to the test compound.

2.2 Treatment

2.2.1 For genotoxic studies

Animals were divided into ten groups: Group 1: mice served as a control group and treated daily with saline for 7 consecutive days. Group 2: mice were injected with a single dose of 2.5 mg/ kg Cisplatin. Group 3: mice were injected with a single dose of 5 mg/ kg Cisplatin. Group 4: mice were treated with 10 mg/ kg Cisplatin. Group 5: mice were treated with 10mg/kg body weight of Turmeric Group 6: mice were treated with 20mg/kg body weight of Turmeric. Group 7: mice were treated with 40mg/kg body weight of Turmeric.

2.2.2 For modulating studies

Among the various dose groups tested to study the toxic effects of Cisplatin, the percentage of chromosomal aberrations was high at highest dose of Cisplatin. Hence the highest dose of Cisplatin 10mg/kg selected for modulation of its toxic effects with antioxidants enriched, Turmeric (10, 20, 40 mg/kg) were selected to study in *in vivo* test systems.

Turmeric (10, 20,40mg/kg i.e group 7,8 and 10) extraction was given in split doses orally for 7 consecutive days (to cover the seven cell divisions of erythropoiesis (Adler, 1984) and 10mg/ kg of Cisplatin was administrated on day 7, one hour after regular exposure to antimutagen as a single intraperitonial dose. The animals were sacrificed on 8^{th}, 9^{th}, 10^{th} day which corresponds to 24, 48, 72hours exposure to mutagen respectively. Control group of animals were also maintained simultaneously.

The animals were sacrificed at appropriate time intervals of 24, 48 and 72 h. 2hours prior to sacrificing, 0.2ml of 0.05% colchicine was injected to all the animals to inhibit spindle formation in order to get well spread metaphases. All the animals were killed by cervical dislocation and hind limbs were dissected out for femur bones and freed from connective tissue and muscles with the help of gauge and immediately suspended in hypotonic solution (0.56% KCl).

2.2.3 METHODOLOGY

The bone marrow was flushed out into clean glass Petri dishes with a hypodermic syringe fitted with a 22-guage needle and dispersed well in hypotonic solution (0.56% KCl i.e. 0.75M KCl) to get a homogeneous cell suspension. The suspension was collected in clean centrifuge tubes and incubated at 37^0 C for 45 minutes. After the incubation the tubes were centrifuged for 10 minutes at 1000rpm. The supernatant was removed carefully with the help of Pasteur pipette leaving a small volume over the pellet. To the pellet 5ml of pre chilled fresh fixative (3:1 absolute methanol: glacial acetic acid; prepared freshly before use and preserved in refrigerator for chilled condition) was added drop wise from the sides of the centrifuge tubes and immediately dispersed the cell suspension by aspirating several times with a Pasteur pipette. The tubes were left undisturbed for 10 minutes at room temperature. After 10 minutes the suspension was centrifuged again and the supernatant was removed carefully leaving a small volume of the supernatant over the pellet and 5ml of chilled fresh fixative was added carefully and kept for 10minutes undisturbed. This process was repeated for 4 to 5 times to ensure proper fixation. In the final change the cells were resuspended in 0.5ml of fresh fixative.

2.2.4 Preparation of the slides

Two to three drops of cell suspension were dropped on clean grease free, prechilled slides. The slide was blown once across and allowed to dry on a slide warmer. Two slides from each animal were prepared by air drying technique from control and treated animals. The slides were coded and stored in dust free chambers. The staining was done within 24hours after the slide preparation. The slides were stained with 2% Giemsa (2ml of Giemsa in 46ml of double distilled water plus 2ml of phosphate buffer* pH 6.8) for 7-8 minutes and later they were rinsed in double distilled water and allowed to dry. Finally the slides were soaked in Xylene for overnight and mounted in DPX mountant.

2.2.5 Scoring

For each mice 100 well spread metaphases were examined randomly using Leica CW 4000 Image analyzer.

2.2.6 Statistical analysis

The data on CAs were analyzed statistically using 2x2 contingency

Chi – Square test.

3. RESULTS AND DISCUSSION

The development of Chromosomal abnormalities (or aberrations) are considered as sensitive biological indicators in the mutagenic bioassays (Catherine *et al*., 1998; Tucker and Preston, 1996). The CAs is one of the widely used parameters for testing the protective effects of natural compounds on the drug and chemical induced toxicity. The results on the incidence of CAs in *in vivo* bone marrow after treatment with CP and various doses of Turmeric extract individually and CP primed with Turmeric are shown in the tables 1, 2 and 3.

Various types of chromosomal aberrations in somatic cells of control and treated animals were screened as per the standard protocol indicated by earlier workers (Adler, 1984; Evans and O'Riodan, 1975; Gebhart, 1970; Preston *et al* 1987; Reiger *et al* 1976).

Mice treated with CP (10mg/ kg) had a higher frequency of CAs than the controls and dose dependent decrease was observed when treated with Turmeric extract. Maximum decrease in CAs was found at the higher dose of Turmeric extract rather than with the lower doses. At 24hr for the CP alone treatment the percentage of aberrations scored 13.60 which decreased to 9.80, 6,20, 3.80 in10, 20, 40mg/kg of Turmeric extract primed groups. At 48hrs14.80 CP alone treated animals which decreased to10.20, 7.80, 4.60 in10, 20, 40mg/kg of Turmeric extract primed groups. At 72 hrs for the CP alone treatment the percentage of aberrations scored 16.60 which decreased to 11.20, 8.60, 5.20. The decrease in CAs were statistically significant ($p<0.05$) for 10, 20, 40mg/kg of Turmeric extract primed groups in comparison to CP alone treated animals. For the Turmeric extract alone treated group, the total no of aberrations did not show any significant level of changes. This decrease in CAs were statistically significant.

The frequencies of chromosomal aberrations in unprimed Mitomycin-C and 10mg/kg of Cisplatin treated animals increased when compare with control animals. But they were gradually decreased when Cisplatin treated mice were primed with Turmeric with three different doses. The differences in the frequencies of chromosomal aberrations between control and nonprimed groups at various time intervals was subjected to statistical analysis and found to be significant, when the data of Cisplatin treated and Turmeric primed groups were subjected to statistical analysis using $\chi 2$ and found to be significant decrease in CAs at all dose groups ($P < 0.05$).

In our study In our study we observed the mutagenecity of cisplatin after cisplatin treatment which supports earlier findings of its genotoxic properties in cultured mammalian cells, mouse germ cells and peripheral blood lymphocytes (Choudhury *et al*, 2000; Nersesyan et al 2003; Jin and Ikushima,2004; Misra et al 2006; Anuradha and Rudrama Devi, 2009). Antitumor agents are known to interact with specific biological molecules leads to generation of free radicals in nontumor cells both *in vivo* and *in vitro* (Weijl et al., 1997).

In the present observation Turmeric decreased the cisplatin toxicity. The cytotoxic inhibition effects of the Turmeric crude extract was highly significant (P< 0.05) at 10,20,40 mg/kg. The protection was high at higher doses. These results are exactly in accordance with Hastak et al. 1997 who observed that alcoholic extract of turmeric offered protection against BaP-induced increase in micronuclei in circulating lymphocytes of healthy individuls. In another study Mohammad *et al* (2016) observed that decrease in the oxidative stress in Adriamycin-induced hepatotoxic rats when treated with hydroalcoholic extract *Curcuma longa*. Similar results were observed by Ramsewak *et al*., 2000; Akram *et al*., 2010 that C. longa inhibits the growth of various types of cancer cells and decreases liver injury in experimental models of animals. The protective effects of Curcuma longa may be due to its antioxidant properties (Mohebbati et al., 2016).

Table 1: Frequency of chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of Cisplatin for 24, 48, 72hrs interval.

Dose(mg/kg) and duration of treatment (hr)	24h		48h		72h	
	Normal metaphases scored(%)	Abnormal metaphases scored(%)	Normal metaphases scored(%)	Abnormal metaphases scored(%)	Normal metaphases scored(%)	Abnormal metaphases scored(%)
Control	485 (97.00)	15 (3.00)	483 (96.60)	17 (3.40)	483 (96.60)	17 (3.40)
2.5 mg/kg	468 (93.60)	32* (6.40)	460 (92.00)	40** (8.00)	455 (91.00)	45** (9.00)
5 mg/kg	446 (89.20)	54** (10.80)	432 (86.40)	68** (13.60)	427 (85.40)	73** (14.60)
10 mg/kg	432 (86.40)	68** (13.60)	422 (84.40)	78** (15.60)	415 (83.00)	85** (17.00)

The values in parenthesis are percentages

*P < 0.05 **P < 0.01

Table 2: Frequency of chromosomal aberrations recorded in somatic cells of mice after treatment with various doses of Turmeric extract for 24, 48, 72hrs interval.

Dose (mg/kg) and duration of treatment (hr)	24h		48h		72h	
	Normal metaphases scored(%)	Abnormal metaphases scored(%)	Normal metaphases scored(%)	Abnormal metaphases scored(%)	Normal metaphases scored(%)	Abnormal metaphases scored(%)
Control	485(97.00)	15(3.00)	485(97.00)	15(3.00)	484(96.80)	16(3.20)
10mg/kg	482(96.40)	18*(3.60)	481(96.20)	19*(3.80)	480(96.00)	20*(4.00)
20 mg/kg	480(96.00)	20*(4.00)	478(95.60)	22*(4.40)	477(95.40)	23*(4.60)
40 mg/kg	477(95.40)	23*(4.60)	475(95.00)	25*(5.00)	474(94.80)	26*(5.20)

The values in parenthesis are percentages

* P > 0.05

Table 3: Frequency of chromosomal aberrations recorded in somatic cells of mice between control and Cisplatin treated animals primed with different doses of Turmeric after 24, 48, 72hrs interval.

Dose (mg/kg)	Non-primed		Primed with Turmeric					
			10 mg/kg		20 mg/kg		40 mg/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(%)
24h								
Control I	484 (96.80)	16 (3.20)	-	-	-	-	-	-
Mitomycin C	453 (90.60)	47 (9.40)	-	-	-	-	-	-
10 mg/kg cisplatin	432 (86.40)	68** (13.60)	451 (90.20)	49* (9.80)	469 (93.80)	31** (6.20)	481 (96.20)	19** (3.80)
48h								
Control II	483 (96.60)	17 (3.40)	-	-	-	-	-	-
Mitomycin C	439 (87.80)	61 (12.20)	-	-	-	-	-	-
10 mg/kg cisplatin	426 (85.20)	74** (14.80)	449 (89.80)	51* (10.20)	461 (92.20)	39** (7.80)	477 (95.40)	23** (4.60)
72h								
Control III	483 (96.60)	17 (3.40)	-	-	-	-	-	-
Mitomycin C	431 (86.20)	69 (13.80)	-	-	-	-	-	-
10 mg/kg cisplatin	417 (83.40)	83** (16.60)	444 (88.80)	56* (11.20)	457 (91.40)	43** (8.60)	474 (94.80)	26** (5.20)

The values in parenthesis are percentages

*P < 0.05 **P < 0.01

4. CONCLUSION

It has been suggested that the common use of antimutagens and anticarcinogens in every days life will be most effective procedure for preventing human cancer and genetic diseases. The

results of present investigation conclude a dose dependent antigenotoxic potential of Turmeric against cisplatin induced genotoxicity in *in vivo* bone marrow cells of mice. However, the mechanism by which Turmeric acts remains to be investigated and further studies are necessary to clarify this point. The present investigation highlight the importance of commonly consumed dietary agents which protect the cells from toxic free radicals.

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