Cytogenetical and histochemical studies on curcumin in male rats

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Abstract

Curcumin is a major component of the curcuma species, commonly used as a yellow coloring and flavoring agent in foods. Curcumin has been demonstrated to have potent antioxidant, anti-inflammatory activity and has shown anticancer properties in many rodent models. There is the perception that since compounds are natural, they are devoid of toxicity and safe to use. Some of the active compounds in supplements have inherent toxicity. In this study, five doses (0.5, 5, 10, 25 and 50 mg/kg/daily) of curcumin spice were orally administered to male rats daily for four weeks. The effect of curcumin was studied genetically by evaluation of chromosomal aberrations and micronucleus formation in bone marrow cells of male rats. Histopathological and histochemical investigations were studied in different tissues (liver, kidney) of males. The cytogenetical results showed that curcumin caused a statistically significant dose-dependent increase in the number of micronucleated polychromatic erythrocytes (MNPCEs) and in the frequencies of total chromosomal aberrations over the control. Also, results showed that there were significant differences between positive control and all curcumin-tested doses, except the dose of 50 mg/kg showed no significant difference. Histopathological results showed different degrees of alterations, as manifested by vacuolar degeneration in hepatocytes, tubular degeneration in renal tissues. We conclude that over use of curcumin spice may cause genotoxical and histopathological effects.
Keywords: curcumin spice, ADI, bone marrow, chromosome aberrations, micronucleus polychromatic erythrocytes (MNPCEs), liver, kidney.

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1 Introduction

Tumeric is a spice that comes from the root Curcuma longa, a member of the ginger family, Zingaberaceae. Curcuminoids are components of tumeric, which include mainly curcumin (diferuloyl methane), demethoxycurcumin and bisdemethoxycurcmin [1]. Curcumin, bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione, is a yellow-orange dye derived from the rhizome of the plant Curcuma longa. Curcumin is widely used as a spice and coloring agent in several foods, such as curry, mustard, bean cake, cassava paste and potato chips, as well as in cosmetics and drugs [2]. It exhibits chemopreventive and growth inhibitory activity of the initiation and promotion of many cancers [3, 4]. The dietary photochemical curcumin possesses anti-inflammatory, antioxidant, and cytostatic properties. Curcumin acts as a potent anticarcinogenic compound. Curcumin induces apoptotic cell death by DNA-damage and preventing cancerous cell growth [5, 6]. In spite of curcumin is a natural antioxidant known to possess therapeutic properties and has been reported to scavenge free radicals and to inhibit clastogenesis in mammalian cells [7]. Curcumin has been reported to induce a significant increase in the frequency of chromosomal aberrations in Chinese hamster ovary (CHO) cells [8]. So it was necessary to evaluate the genotoxic effects and histochemical changes induced in male rat cells as a result to oral administration of different doses of curcumin for four weeks.

2 Materials and methods

An experiment was carried out on male Wistar rats (Rattus norvegicus) weighing 150-200gm, receiving standard food and water ad libitum. Each experimental group consisted of 10 animals. The first group (negative control) received distilled water orally by gastric intubation. The second group (positive control) injected IP with single dose of 25mg/kg cyclophosphamide. Other five groups received curcumin spice (0.5, 5, 10, 25 and 50 mg/kg body weight). Curcumin was dissolved in distilled water and 1ml/animal of suspension given orally to male rat daily for four weeks. The dose 0.5 is the curcumin acceptance daily intake (ADI) and the other doses were chosen according to [9]. Twenty-four hours after the last administration, Animals were injected IP with colchicine solution Two hours later animals were killed by cervical dislocation. The femur bones were quickly separated one femur bone was used for preparation of micronucleus polychromatic erythrocytes according to method of [10]. While the other one was used for chromosomal preparation according to [11]. For histopathological and histochemical studies, paraffin sections of liver and kidneys were stained with Hx and E, Feulgen stain for DNA and periodic acid Schiff technique for mucopolysaccharide. DNA and PAS were evaluated as optical density values of their specific color using computer- assisted image analyzer. The analysis of variance test was used for the chromosomal aberrations data analysis. While the *t*- test was used to analyse the data of MNPCEs and histochemical studies.

Table 1: The frequencies of MNPCEs in male albino rate bone marrow cells of all experimental groups.

	Total Counted PCEs/ animal	MNPCEs		Mean ± SD
		No.	%	
Negative control	2000	94	1.57	5.67 ± 2.16
Positive control	2000	604	10.07	100.67 ± 6.77 **
0.5 mg/kg bw curcumin	2000	91	1.52	NS 5.17 ± 1.47 ♦
5mg/kg bw curcumin	2000	142	2 .37	* 23.67 ± 4.59 ♦♦
10 mg/kg bw curcumin	2000	222	3 .70	** 37.00 ± 4.52 ♦♦
25 mg/kg bw curcumin	2000	381	6.35	** 63.50 ± 6.83 ♦♦
50 mg/kg bw curcumin	2000	587	9.78	** 97.83 ± 3.31 NS

*significant comparison to –ve control at ($p< 0.05$) **significant compared to –ve control at (0.01)
♦significant comparison to +ve control at ($p< 0.05$) ♦♦significant compared to +ve control at (0.01)

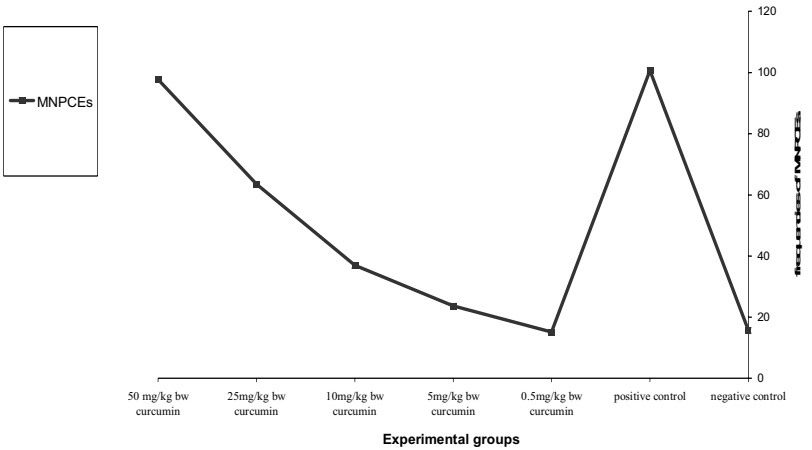

Figure 1: Frequencies of MNPCUs in male albino rat bone marrow cells of all experimental groups.

3 Results

3.1 Cytogenetical results

Results of micronucleus test in bone marrow cells of male rats of all experimental groups are summarized in table 1 and presented in figure 1. Data indicated that curcumin exhibited a dose dependent increase in the frequency of MNPCEs when compared with negative control. Since the dose of 5mg/kg bw induced significant increase at ($p< 0.05$), whereas, the 10, 25 and 50 mg/kg bw doses of curcumin induced significantly increase in the frequencies of MNPCEs at ($p< 0.01$) than control. At the comparison to positive control, results showed that there were statistically significant differences between the frequencies of MNPCEs of curcumin treated animals with doses (0.5, 5 and 25 mg/kg bw) and positive control. In contrast, no statistically significant difference between the dose of 50mg/kg bw and the positive control was observed.

Table 2: Frequency of different chromosomal aberration male rat bone marrow cells of different experimental groups.

	Experimental groups						
	Negative control	Positive control	0.5mg/kg curcumin	5mg/kg curcumin	10mg/kg curcumin	25mg/kg curcumin	50mg/kg curcumin
Hypo-ploidy	0.17c ± 0.41	1.50b ± 1.05	0.50c ± 0.55	0.83c ± 0.75	1.50b ± 0.55	2.50a ± 0.55	3.00a ± 0.00
Hyper-ploidy	0.00d ± 0.00	0.67bc ± 0.88	0.00d ± 0.00	0.00d ± 0,00	0.50cd ± 0.55	1.17ab ± 0.41	1.33a ± 0.52
Polyploidy	0.00b ± 0.00	1.83a ± 0.75	0.00b ± 0.00	0.00b ± 0.00	0.00b ± 0.00	0.17b ± 0.41	1.67a ± 0.75
Chromatid gap	0.67d ± 0.82	4.83a ± 1.47	1.00d ± 0.63	2.17c ± 0.41	2.67bc ± 0.52	3.33b ± 0.82	5.17a ± 0.52
Chromatid break	0.33b ± 0.52	4.83a ± 1.03	0.17b ± 0.41	0.83b ± 0.41	1.50b ± 0.55	2.00a ± 0.89	2.67a ± 0.52
Chromosome break	0.00c ± 0.00	1.00a ± 0.89	0.00e ± 0.00	0.0de ± 0.00	0.17d ± 0.41	0.83bc ± 0.41	1.33b ± 0.75
Fragment	0.00d ± 0.00	3.00a ± 1.16	0.33cd ± 0.52	0.83bcd ± 0.75	1.17cd ± 0.41	1.67b ± 1.05	2.83a ± 0.75
Deletions	0.00b ± 0.00	0.50a ± 0.55	0.00b ± 0.00	0.00b ± 0.00	0.00b ± 0.00	0.33ab ± 0.52	0.67a ± 0.52
Centromeric attenuation	3.33bc ± 1.03	5.17a ± 1.47	2.33c ± 0.82	4.67ab ± 1.37	5.83a ± 1.49	5.17a ± 1.17	5.17a ± 1.17
Total chromosomal aberrations	4.50e ± 1.38	22.83a ± 2.71	4.33e ± 0.82	9.17d ± 0.75	13.33c ± 1.03	17.17b ± 0.75	23.83a ± 1.60

(Means with different letters within each column are significant at 5% level.)

Table 2 and figure 2 illustrate the mean values of structural and numerical chromosomal aberrations in bone marrow cells of all experimental groups. Results showed that curcumin accepted daily intake (ADI) dose not induced significant difference as compared to negative control. In bone marrow cells of animals administered with the other four doses, a dose dependant increase in the frequencies of all individuals and total chromosomal aberrations were observed. At the comparison between the frequencies of all individuals and total chromosomal aberrations induced in positive control and those of different curcumin doses treated animals, results showed that there were significant differences ($p<0.01$) between positive control and groups of 0.5, 5, 10, and 25 mg/kg curcumin. Whereas, there was no significant difference between positive control and the group of 50 mg/kg curcumin treated animals. These results indicated that the high dose of curcumin (50 mg/kg) is the more effective to induce significant increase in the frequencies of MNPCEs and chromosomal aberrations in rat bone marrow cells.

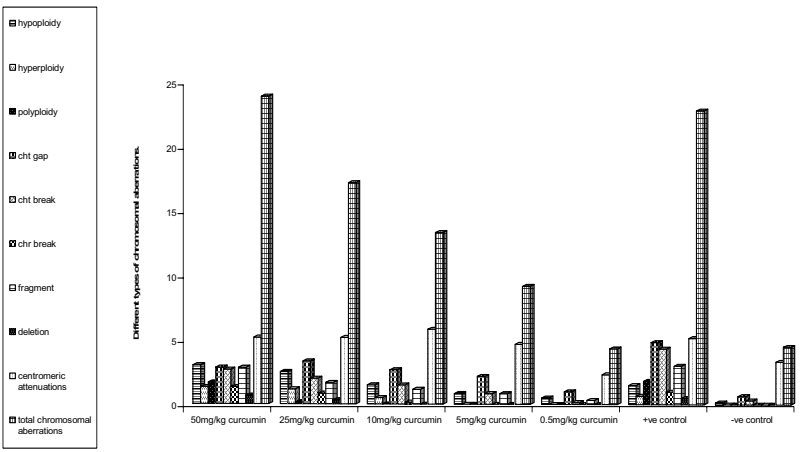

Figure 2: Frequencies of different chromosomal aberrations induced in all experimental groups.

3.2 Histological and histochemical results

The microscopic observation in control and 0.5 mg/kg curcumin treated rats showed normal structure of hepatic lobules in which the hepatocytes arranged in cords radiating from the central vein. Liver sections of curcumin treated rats revealed cytoplasmic degeneration, necrosis, cytoplasmic vacuolar damage and dilated and congested with few inflammatory cells in the portal tract. These observations were highly pronounced in rats treated with 5,10 and 25 mg/kg curcumin and were prominent in rat treated with 50mg/kg curcumin (fig 3). As regard to kidney tissues, histological observation of sections of animals receiving 0.5, 5, and 10 mg/kg of curcumin showed that almost all the renal tubules were normal without histopathological changes. While, the kidney tissues of animals

received the dose of 25 mg/kg of curcumin showed some picture of damage in tubular epithelial cells, thickening in blood vessels, mild cellular infiltration and interstitial hemorrhage. While, the renal tubules of animals received the dose of 50 mg/kg of curcumin revealed advanced tubular degeneration, increased in cellular infiltration, interstitial hemorrhage and some glomeruli were damage (fig 4). Histochemical evaluation to DNA and PAS were measured as mean value per nucleus by image analyzer. DNA was demonstrated by using Feulgen reaction technique. The Results showed that curcumin different doses induced significant decrease ($p<0.01$) in the mean value of DNA content of liver tissues when compared with control (table 3 and fig 5). In kidney cells**,** also results showed that the curcumin induced significant decrease ($p<0.01$) in the mean value of DNA content than in control (table 4 and fig 6). As regard to PAS + ve material, curcumin caused significant decrease ($p<0.01$) in the level of PAS in both liver and kidney tissues of all groups as compared to control as shown in (tables 5, 6 and fig 7, 8). while in renal tubule, all treated groups revealed moderate reaction in the basement membrane and brush border.

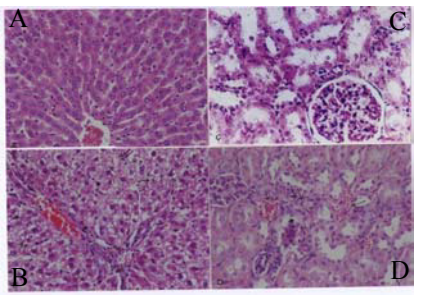

Figure 3: Section of control and treated liver (A and B) and kidneys (C and D) H and E X300.

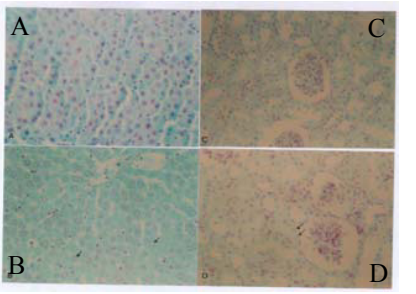

Figure 4: Section of control and treated liver (A and B) and kidneys (C and D) Feulgen reaction X300.

Table 3: Mean values of DNA content in liver tissues of all treated groups.

Groups	Control	0.5mg/kg	5mg/kg	10mg/kg	25mg/kg	50mg/kg
DNA Content	0.35 ± 2.31E02	0.25 ± **3.01E-02**	0.22 ± 2.20E-02	0.20 ± 2.58E-02	0.18 ± **0.19E-02**	0.18 ± 1.49E-02

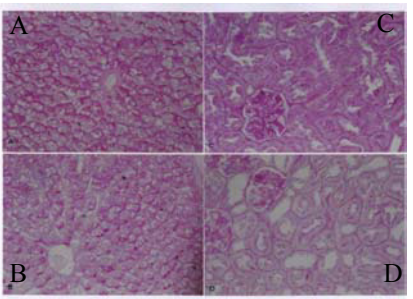

Figure 5: Section of control and treated liver (A and B) and kidneys (C and D) PAS X300.

Table 4: Mean values of DNA content in kidney tissues of all treated groups.

Groups	Control	0.5mg/kg	5mg/kg	10mg/kg	25mg/kg	50mg/kg
DNA Content	0.30 ± 2.87E-02	0.25 ± 3.71E-02	0.25 ± 3.25E-02	0.24 ± 2.75E-02	0.22 ± 1.87E-02	0.22 ± 2.46E-02

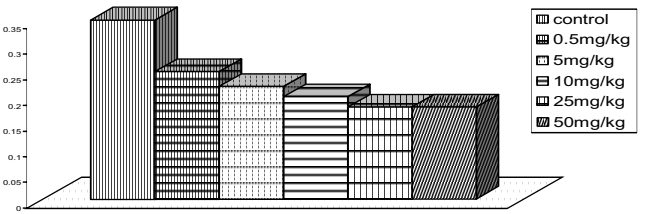

Figure 6: Mean values of DNA content in hepotocytes of all treated groups.

Table 5: Mean values of PAS content in hepatic cells of all treated groups.

Groups	Control	0.5mg/kg	5mg/kg	10mg/kg	25mg/kg	50mg/kg
DNA Content	0.42 ± 4.89E-02	0.18 ± 2.11E-02	0.13 ± 5.18E-02	0.27 ± 6.54E-02	0.13 ± 6.79E-02	0.28 ± 1.61E-02

Table 6: Mean values of PAS content in renal cells of all treated groups.

Groups	Control	0.5mg/kg	5mg/kg	10mg/kg	25mg/kg	50mg/kg
DNA Content	0.37 ± 2.20E-02	0.31 ± 1.99E-02	0.25 ± 1.74E-02	0.28 ± 1.75E-02	0.28 ± 1.56E-02	0.28 ± 1.66E-02

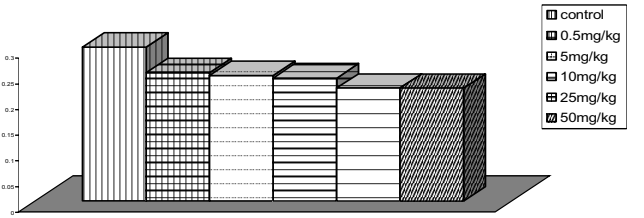

Figure 7: Mean values of DNA content in kidney cells of all treated groups.

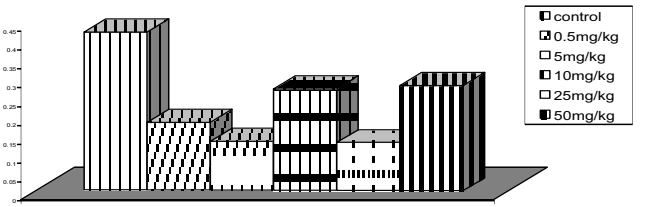

Figure 8: Mean values of PAS content in hepatic cells of all groups.

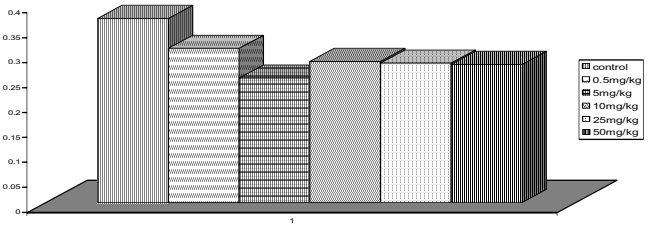

Figure 9: Mean values of DNA control in hepotocytes of all treated groups.

4 Discussion

Curcumin has been used as a herbal medicine. Curcumin shows a variety of physiological and pharmacological effects. Several studies indicate curcumin to be anticarcinogenic [12] and anti-inflammatory [13] Curcumin further shows antioxidant properties, it acts as a superoxide radical scavenger [7, 14] and as a singlet oxygen guencher [15]. Contrary to the antioxidant nature of curcuminoids, much evidence for cytotoxic properties of curcumin was reported, and its cytotoxicity is suggested to be due to production of reactive oxygen species and causes oxidative DNA damage [16]. The cytogenetic results of the present study demonstrate that the curcumin administration induced a dose dependent significant increase in the frequencies of MNPCEs and total chromosomal aberrations in bone marrow cells of male rats. Histochemical studies confirmed these results. Since, the histochemical investigation of the present study showed that curcumin caused significant decrease in DNA content

in both liver and kidney tissues of treated animals. Many studies have found that the antitumor- promoting effects of curcumin, could be contributed to its apoptosis-inducing activity. Since, curcumin induces a growth arrest in the G2/M–phase of the cell cycle, it exerts profound effects on mitotic spindle organization and leads to formation of monopolar spindles that are unable to segregate chromosomes normally. Cells with monopolar spindles arrest in M-phase for extended periods, and eventually leave mitosis and enter interphase with grossly aberrant nuclei consisting of numerous large micronuclei.

The production of cells with extensive micronucleation are due to its ability to disrupt normal mitosis, and raises the possibility that curcumin may promote genetic instability under some circumstances [17]. Histochemical examination of PAS +ve material indicated that curcumin caused significant decrease of PAS content in both liver and kidney tissues of all groups. The decrease in PAS +ve material observed in this study was interpreted due to the most probably consequent to the degenerative changes [18]. Previous literatures have demonstrated that antioxidants can act as prooxidants under some circumstances [19]. It is found that b-carotene [20], vitamin E [21], quercetin [22], N-acetylcysteine [23] , and caffeic acid [24] can act as potent DNA damaging agents. Also, a potential clastogenic effect of known antioxidant compounds has been reported by others. S-vanillin were enhanced the chromosome aberrations induced by alkylating agents in cultured Chinese hamster cells [25]. Bcarotene and ascorbic acid were enhanced the clastogenicity induced by BLM in CHO cells [26and27].Curcumin, like ascorbic acid, can become a pro-oxidant agent depending on the redox state of the biological environment [28]. Therefore, the mutagenic 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 study. Clastogenic effects of curcumin were also observed by others. Curcumin showed a slight increase in the number of chromosomal aberrations in treated mice [29]. Curcumin acute and chronically treated induced a significant increase in SCE and a weak increase in the frequency of chromosomal aberrations in mice and rats [30]. The histological examination of the present study also showed that curcumin induced hepatotoxicity. Liver tissues of curcumin treated animals revealed highly pronounced cytoplasmic degeneration, necrosis and cytoplasmic vacuolar damage. In addition, the portal tract was dilated and congested with few inflammatory cells were present in animals treated with curcumin high dose. Other studies confirmed this result. It was reported that the administration of high curcumin dose for longer duration showed hepatotoxicity represented in focal necrosis or focal necrosis with regeneration both in mice and rats [31, 32]. Thus putative chemo-preventive antioxidants may have carcinogenic effect. Therefore, much consideration to safety should be required when curcumin is used for nutrition supplement.

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