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Evaluation of the Genotoxicity and Antigenotoxicity of Curcumin by Chromosomal Aberrations and Biochemical Studies in the Albino Rats Exposed to Methotrexate

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Abstract: The present work evaluated the genotoxicity and antigenotoxicity of curcumin, a yellow colouring agent, contained in turmeric (*Curcuma longa* L., Zingiberaceae) on albino rats. Curcumin has been extensively investigated for its cancer chemopreventive potential. Curcumin also possesses anti-inflammatory and antioxidant properties chromosomal aberrations assays were performed to study the genotoxic and antimutagenic activity of the curcumin. Also, biochemical studies of the curcumin was tested by measuring; total protein, cholesterol, glucose testosterone, GOT and GPT. Results showed that dose of 0.5 mg/kg b.w. of the curcumin did not induce any genotoxic effects, but in combined treatment with methotrexate, it significantly reduced methotrexate induced chromosomal aberrations. Moreover, continuous treatments 10 and 20 mg/kg b.w. of curcumin induced chromosomal aberrations alone and in combined treatments with methotrexate. Biochemical analysis showed that the low levels of curcumin non significant alone or combined with methotrexate and high doses of curcumin treatments, show significant increases in the activity of plasma protein liver GOT and GPT, cholesterol and testosterone, singly or combined. From this study we conclude that curcumin shows both genotoxicity and antigenotoxicity depending on its concentration, these results stimulate the interest in styding possible new uses of curcumin.

Key words: Curcumin · Genotoxicity · Antimutagenicity · Albino rats

INTRODUCTION

A wide variety of phenolic compounds derived from edible plants have been reported to retain marked anti-oxidant and anti-inflammatory activities, which contribute to their chemopreventive potential [1,2]. Among these compounds, curcumin, it is a yellow odorless pigment isolated from the rhizome of tumeric (*Curcuma Longa* L., zingiberaceae). Pharmacological studies have demonstrated its anti-tumor, anti-inflammatory and anti-oxidant activity with very low toxicity [3-5]. Also, anti-oxidant and anti-inflammatory effects of this compound have been assessed in various *in vitro* and in experimental animal systems, human studies and on other living organisms [6-8].

The practice of disease prevention is the most cost effective means for improving human health. The prolonged use of the anticancer drugs may result in various types of secondary tumors in normal cells. Anticancer drug e.g. mitomycin C is a potent DNA cross-linker [9]. Methotrexate is an antineoplastis agent used to fight a number of different cancers. Substantial evidence supports the concept that methotrexate was mutagenic and carcinogenic in animals [10]. The genotoxic effect of anticancerous drugs may be reduced if supplemented with natural antioxidants plant products. With this view, the effect of curcumin was studied against the genotoxic dose of methotrexate in rate.

MATERIALS AND METHODS

Chemicals: Methotrexate was obtained from sigma U.S.A.

Animals: Eighty male albino rats (100-120g), purchased from the animal house colony, the National Research Center Giza, Egypt). Rats were maintained on standard laboratory diet (protein, 16.04%, fat, 3.63%, fiber, 4.1% and metabolic energy, 0.012 MJ) and water.

After an acclimation period of a week, animals were divided into eight groups (10 rat/ group) and the groups housed individually in filter-top polycarbonate cages housed in a temperature - controlled (23 ± 1°C) and artificially illuminated (12 h dark/ light cycle) room free from any source of chemical contamination. All animals received human care in compliance with the guidelines of the animal care and use committee of the N.R.C.

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Experimental Design: Animals within different treatments groups were treated (daily at a 24-h interval) intragastrically for 37 days as follows:

Group 1 was kept untreated as the control group.
Group 2 was treated with 0.5 mg/kg b.w. of curcumin.
Group 3 was treated with 10 mg/kg of b.w. of curcumin.
Group 4 wastreated with 20 mg/kg b.w of curcumin.
Groups 5 , 6 and 7 were treated with curcumin similar to Groups 2,3 and 4 respectively, plus intra peritoneally single dose of 10mg/kg b.w. of methotrexate on the last day of curcumin treatment.
Group 8 was treated with single dose of 10 mg/kg b.w. of methotrexate at the 37th day of curcumin treatments on another groups.

At the end of the experimental period, all animals were sacrificed and dissected on day 38. Bone marrow and blood samples were collected from all animals for cytogenetic and biochemical analysis, respectively.

Glucose was determine as described by Trinder [11]. The method of Kaplan [12] was adopted to determine total protein. Also, GOT and GPT activities were determined as described by Young [13]. Tesosterone was measured by the method of Hall [14] and cholesterol was determined as indicated by Richmond [15].

Statistical Analysis: Statistical program SPSS version H.O for windows was performed; $P < 0.05$ was regarded as statistically significant.

RESULTS

Cytogentical Results: The data of Table (1) illustrate the mean values of structural and numerical chromosomal aberrations in bone marrow cells of all experimental groups. Results showed that curcumin 0.5 mg/kg b.w. dose not induced significant difference as compared to negative control. In bone marrow cells of animals administered the other two doses, a dose dependant increase in the frequencies of all individuals and total chromosomal aberrations were observed. Comparison between the frequencies of total chromosomal aberrations induced in positive control (methotrexate) and those of different curcumin doses treated animals, results showed that there were significant differences between positive control and groups of 0.5, 10, 20 mg/kg curcumin. Also, there is significant difference between combined treatment of 0.5 mg/kg b.w curcumin with methotrexate and methotrexate alone. While there was no significant difference between combined treatments of 10, 20 mg/kg curcumin with methotrexate and positive control.

Table 1: Frequency of different chromosomal aberration male rat bone marrow cells of different experimental groups

Treatments	GAP	Break	Fragment	Deletion	CA	Total aberration %	- ve %	+ ve %	Poly %
Control –ve	0.00±0.00^{a}	1.60±0.40^{c}	0.00±0.00^{a}	0.80±0.49^{a}	1.60±0.74^{a}	4.00±1.41^{a}	0.00±0.00	0.00±0.00	0.00±0.00
Methotrexate (±ve) 10mg	5.20±0.80^{a}	6.00±0.633^{a}	2.80±0.49^{a}	4.80±0.49^{a}	7.20±0.49^{bc}	26.0±0.633^{a}	0.67±0.66	1.33±0.66^{ab}	0.00±0.00
Curcumin 0.5mg	0.00±0.00^{a}	0.20±0.44^{d}	0.40±0.54^{a}	0.20±0.44^{a}	0.40±0.54^{a}	1.20±0.43^{f}	0.00±0.00	0.00±0.00^{c}	0.67±0.66
Curcumin 10mg	2.40±0.40^{ad}	2.80±0.49^{bc}	1.20±0.49^{b}	2.40±0.40^{d}	3.20±0.49^{de}	12.00±0.63^{de}	1.33±0.66	0.00±0.00^{c}	0.00±0.00
Curcumin 20mg	1.60±0.40^{d}	3.20±0.49^{b}	1.40±0.40^{b}	3.20±0.49^{bcd}	4.40±0.40^{d}	14.80±1.35^{d}	0.67±0.66	2.00±0.00^{a}	0.00±0.00
Curcumin 0.5 mg + methotrexate	1.00±0.63^{d}	1.80±0.49^{d}	0.80±0.49^{a}	1.60±0.40^{d}	6.40±0.40^{c}	21.60±1.16^{e}	0.00±0.00	0.00±0.00^{c}	0.67±0.66
Curcumin 10 mg + methotrexate	3.20±0.49^{bc}	4.80±0.49^{e}	2.40±0.40^{a}	4.40±0.40^{ab}	8.40±0.98^{ab}	23.20±0.80^{bc}	1.33±0.66	0.67±0.66^{bc}	0.00±0.00
Curcumin 20 mg + methotrexate	4.00±0.63^{ab}	5.60±0.74^{a}	3.20±0.49^{a}	4.00±0.63^{abc}	10.00±0.63^{a}	26.80±1.02^{a}	0.67±0.66	1.33±0.66^{ab}	0.00±0.00
L.S.D at <0.05	1.578	1.469	1.152	1.382	1.728	3.142	N.S.	1.224	N.S.

Means with different treatment within each column are significant at control ($P < 0.05$)

Table 2: Influences of curcumin and methotrexate on blood glucose total protein, cholesterol, testosterone, GOT and GPT of male albino rats

Treatment	GOT M±S.E	GPT M±S.E	Glucose M±S.E	Cholesterol M±S.E	Total protein M±S.E	TestosteroneM±S.E
Control -ve	32.00±0.12^{a}	13.00±0.39^{a}	90.00±0.53^{a}	49.80±0.56^{a}	14.00±0.18	7.60±0.81^{a}
Methotrexate (±ve) 10mg	73.00±0.72^{g}	63.80±0.56^{g}	298.00±0.58^{g}	256.00±0.51^{g}	64.00±0.28^{g}	74.00±0.18^{g}
Curcumin 0.5mg	38.00±0.70^{a}	16.00±0.18^{a}	99.00±0.94^{a}	47.00±0.48^{a}	15.40±0.60^{a}	8.20±0.28^{a}
Curcumin 10mg	97.40±0.82^{b}	75.40±0.18^{b}	103.80±0.56^{b}	62.00±0.73^{b}	22.40±0.51^{b}	16.00±0.48^{b}
Curcumin 20mg	115.20±0.45^{c}	90.00±9.90^{c}	114.60±0.56^{c}	92.20±0.58^{c}	30.40±0.64^{c}	39.80±0.56^{c}
Curcumin 0.5mg + methotrexate	39.80±0.45^{a}	19.40±0.64^{a}	93.00±0.82^{a}	53.80±0.87^{a}	35.00±0.70^{d}	19.00±0.48^{b}
Curcumin 10mg + methotrexate	247.20±0.65^{f}	106.2±0.96^{f}	197.60±0.80^{f}	167.40±0.98^{f}	46.00±0.88^{f}	51.25±0.50^{f}
Curcumin 20mg + methotrexate	213.40±0.67^{e}	103.60±0.65^{e}	143.00±0.93^{d}	129.00±0.18^{e}	39.00±0.18^{e}	42.00±0.73^{e}

The different letters (a, b, c, d, e, f, g) in the same colum are significantly different at level ($P < 0.05$). all values were expressed as Mean + S.E

These results indicated that the low dose of curcumin alone or combined with methotrexate is not effective to induce significant increase in chromosomal aberrations in rat bon marrow cells, but also significantly reduced methotrexate induced chromosomal aberrations.

Biochemical Results: The present results involving blood glucose, cholesterol, total protein, testosterone, GOT and GPT were illustrated in Table 2. As shown in the table, blood glucose levels were markedly increased by curcumin concentrations 10, 20 mg/kg b.w. alone or combined with methotrexate, respectively. The evaluation of blood glucose levels with combined treatments also increased more than the curcumin alone, but the curcumin 0.5 mg/kg singly or combined was not significant. Also an increase was noticed in cholesterol, total protein, testosterone, GOT and GPT under the same condition. While, curcumin 0.5 mg/kg singly or combined with methotrexate did not induce significant increased or decreased only slightly increased in testosterone and total protein.

DISCUSSION

Curcumin is a yellow pigment present in the rhizome of turmeric (*Curcuma Longa L.*, ZIngiberaccac). It possesses anti-inflammatory and antioxidant properties [2, 16]. Curcumin, also acts as a single oxygen quencher [17].

Contrary to the antioxidant nature of curcuminoids, much evidence for cytotoxic properties of curcumin was reported and is suggested to be due to production of reactive oxygen species and causes oxidative DNA damage [18].

The cytogenetic results of present study demonstrate that the curcumin administration induced a dose dependent significant increase in the frequencies of total chromosomal aberrations in bone marrow cells of male rats.

Biochemical studies confirmed these abovementioned results, since the biochemical investigation of the present study showed that the high doses of curcumin caused significant increase in the serum glucose, cholesterol, total protein, liver GOT and GPT and testosterone. Previous literature have demonstrated that antioxidants can act as pro-oxidants under some circumstances [19]. It is found that caffeic acid can act as potent DNA damaging agents [20].

Deletion and break are the main type of chromosomal aberrations caused by high doses of curcumin single or combined with methotrexate. Curcumin has been reported to induce a significant increase in the frequency of chromosomal aberrations in Chinese hamster ovary (CHO) cells [21]. 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 [22]. Curcumin can be metabolized to tetrahydrocurcumin, which may have greater antioxidant, antimutagenic or antitumor effects than curcumin [23]. Also, curcumin exerts a global *in vivo* antioxidant effect, but *in vitro* it increased the genomic damage [19, 24-27]. Moreover, *in vivo* curcumin exerted have a protective effect on copper ion genotoxicity which was evaluated by the comet and micronucleus assays [28]. The same phenomenon seems to occur with curcumin as potential herbal agent for mitigation of nickel and chromium induced micronuclei in human blood cultures [29]. In this work, both methods were able to detect genotoxicity due to high curcumin concentrations. Both methods evidence protection by low dose of curcumin against methotrexate and this effect was better supported by the biochemical assays. The chromosomal aberrations assay provided evidence of genotoxicity and antigenotoxicity of curcumin. This is in agreement with other studies [9, 30, 31].

These abovementioned features highlight interest in making further investigations on the mechanism of action and the composition of curcumin extract with a view to possible more extensive applications in genetical practice.

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