

Protective Effect of Curcumin on Mitronidazole Induced Genotoxicity in Bone Marrow Chromosomes of Swiss Albino Mice

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Abstract

Curcumin the yellow colored polyphenolic super antioxidant derived from powdered rhizome of *Curcuma longa* is useful in various debilitating conditions. It is also recommended in cancer prevention and as a treatment adjunct in various diseases. Hence, the aim of the present experimental design was to assess the protective effect of curcumin (CUR) on mitronidazole (MTZ) induced genotoxicity in bone marrow chromosomes of Swiss albino mice. In the present experiment four batches of Swiss-albino mice were maintained. The experimental group was administered five consecutive oral dose of mitronidazole (50mg/kg/animal/day). The subsequent experimental group was administered curcumin (4.5mg/animal/day) for 9 days and on the 10th day Mitronidazole (50mg/kg/animal/day) was administered for five consecutive days. For each experimental group a parallel control group was maintained, one group administered 0.5ml of Dimethylsulphaoxide (DMSO) for 14 days the seconded group administered 50 mg/kg.b.wt. of MTZ for 9 days . Twenty two hours after the administration of the last dose, mice were sacrificed and air-dried metaphase preparations were made and subsequently assessed for chromosomal damage. In animals treated with only mitronidazole there was a significant decline in mitotic index. The aneugenic property of mitronidazole was distinctly discernible, besides other chromosomal aberrations such as polyploidy terminal deletions and breaks were also observed. In comparison an elevation in mitotic index equivalent to normal group was discerned in animals pre- treated with curcumin and subsequently treated with mitronidazole and an overall decline in the mutation rate was also discernible clearly indicating the protective effect of curcumin.

Key words: Mitronidazole; Curcumin; Chromosome aberration; Mitotic index.

Introduction

There is an increasing perception that human social and cultural system is growing at a pace, which is culminating in detrimental changes that are serving as threats to the mankind and also to other biological systems on earth. However, certain measures undertaken by humans, viz. a good diet taken in a routine manner renders protection against numerous toxicants even without anyone being aware of it. Certain compounds are such toxicants and if these are not flushed out from biological systems, the result is manifestation of some debilitating condition. During the course of evolution of human beings, about 4500 years back, chemicals were first fashioned and used as various aids and tools to enhance the mode of living. Since then mining of chemicals and their unlimited and indiscriminate use has culminated in polluting our environment [1].

Mitronidazole (MT) is an anti-parasitic drug widely used against protozoan infection in developed as well as developing countries. It has been approved for the treatment of trichomoniasis, giardiasis and amoebiasis by FDA [2]. MT has been reported as mutagenic in a strain sensitive to oxidative damage, *Salmonella typhimurium* tester strain TA 100 both with and without metabolic activation system, and negative in TA 98 [3,4]. It has also been reported to induce micronuclei in mouse bone marrow cells *in vivo* [5], chromosomal aberrations and sister chromatid exchanges [6,7,8] and DNA single strand breaks in peripheral blood lymphocytes [9]. Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions useful for human kind [10]. In the present study the effect of curcumin was studied on the genotoxic doses of MT

using chromosomal aberration and mitotic index as a parameters.

Various modes and methodologies are being devised to combat drugs-induced toxicity with a focus on herbal formulations [11]. Curcumin, a polyphenolic substance derived from *Curcuma longa*, is one of such formulations. In India since times immemorial the powered form of dried *Curcuma* has been used in various rituals and ceremonies. It is a mandatory spice present in every Indian kitchen. It is usually used in most of the cooked preparations and is generally believed to ward off numerous ailments. Curcumin, a derivative of *Curcuma*, has been given the designation of super antioxidant and in some experiments it has been shown to be 300 times more potent than vitamin E. It has been shown to have a wide spectrum of biological actions, these includes anti-inflammatory, anti carcinogenic, anti mutagenic and antibacterial activities [11,12,13]. However, till date no concrete evidence is available to indicate the protective effect of curcumin on chemicals toxicity viz. Mitronidazole induced genotoxicity based on assessment of chromosomal profile. Hence, the aim of the present experiment was to fulfill the existing lacunae by assessing the protective effect of curcumin on mitronidazole induced genotoxicity.

Materials and methods

Chemicals

The following chemical reagents were used: Mitronidazole was procured from Glaxo company (India), with following features: Molecular weight = 201.32, Minimum assay (ex C₁) 95%, Curcumin crystalline: C₂₁H₂₀O₆ was procured from Loba Chem. Pvt. Ltd. The following features were observed:

Molecular weight = 368.39, Minimum assay (acidimetric) 99%, Melting point= 170-180°C.

Animal treatment

The study was conducted on twenty four adult male Swiss albino mice 32-50 days old and weighing around to 30-40g were maintained in plastic cages under controlled lighting conditions (12:12 light: dark cycle) relative humidity ($50 \pm 5\%$) and temperature ($37 \pm 2^\circ\text{C}$), fed with mice feed and were given ad libitum access to water. A group of 6 mice per experiment were taken and treated with mitronidazole and Curcumin. The doses were prepared daily in Dimethylsulphaoxide (DMSO) and distilled water and were administered by gastric gavage method. The dose protocol was as follows: Group-I Control group (a) were treated with only vehicle (DMSO) for 14 day Group-II Control groups (b) were treated with only curcumin 4.5 mg/animal/day for 14 days. Group-III Experimental batch were administered MT 50 mg/kg/animal for five consecutive days. Group-IV Experimental batch were pre-treated with curcumin 4.5mg/animal/day for 9 days and on the 10th day five consecutive oral doses of MT 50mg/kg/day/animal was administered.

Detection of chromosomal anomalies

Twenty-two hours after administration of last dose, animals were sacrificed and bone marrow from femur was excised and air-dried metaphase preparations were made according to the technique of Tjio and Wang [15]. The slides were subsequently stained in Giemsa and 50 metaphase/ animal were

assessed. Hence for each group a minimum of 300 metaphases were assessed for various chromosomal aberrations such as aneuploidy, breaks, gaps, formation of ring chromosomes. **Calculation of mitotic index**

Mitotic division stages in a minimum of 1000 cells were counted for each animal. Mitotic index was calculated according to the formula:-

$$\text{Mitotic index} = A / A+B$$

Where A= No. of dividing cells (metaphases) B= No. of no dividing cells . To determine the validity and significance of the experiment Z – Test was conducted using the formula $Z = \frac{P_1 - P_2}{\sqrt{pq(1/n_1 + 1/n_2)}}$ Where $P_1 = x_1/n_1$, $P_2 = x_2/n_2$; n_1, n_2 = total number of observations x_1, x_2 = number of variables in sample $p = x_1 + x_2 / n_1 + n_2$; $q = 1 - p$

Results

In the present experiment, an effort has been made to assess the genotoxicity induced by MT as well as to monitor the protection accorded by curcumin against MT rendered toxicity. It was observed that in animals administered MT there was a significant increase in the number of chromosomal aberrations and a decline in mitotic index as compared to the control group. In animals pretreated with curcumin, and subsequently administered MT the number of chromosomal aberrations were less and the mitotic index was equivalent to the control group (Table 1, 2 and Figure 1, 2).

Table 1. Protective effect of curcumin against Mitronidazol induced decline in mitotic index.

S. No.	Dose	No. of dividing cells (b)	No. of non dividing cells (b)	Total cell population	Mitotic index (MI)	Z Test values $p < 0.05$
1	Control group a (DMSO) administered for 14 days)	294 (x_1)	12378	12672 (n_1)	0.023	
2	MT50mg/kg/animal (Five consecutive doses)	360 (x_2)	32460	32820 (n_2)	0.010	9.88
3	Control group b (Curcumin 4.5mg/day/animal administered for 9 days)	362 (x_1)	14188	14550 (n_1)	0.024	
4	Curcumin 4.5mg/day/animal for 9 days + MT 50mg/Kg/animal for 5 consecutive days	288 (x_2)	13404	13692 (n_2)	0.021	2.12

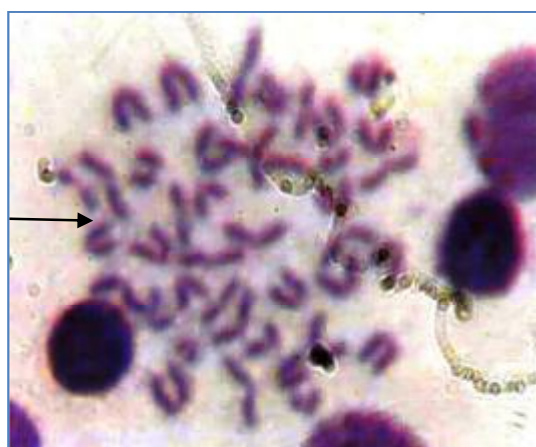
Table 2. Protective effects of curcumin on mitronidazol induced genotoxicity as manifested by chromosomal abnormalities.

S. No.	Dose	Chromosomal abnormalities						Total aberrations	Total population	Z Test values $p < 0.05$
		C.F.	Frag.	Del.	Gaps	Aneuploidy	Euploidy			
1	Control group a (DMSO) administered for 14 days)	3	0	0	0	5	1	9 (x_1)	12672 (n_1)	
2	MT50mg/kg/animal (Five consecutive doses)	22	10	38	4	24	6	104 (x_2)	32820 (n_2)	4.66
3	Control group b (Curcumin 4.5mg/day/animal administered for 9 days)	8	5	12	0	19	2	46 (x_1)	13692 (n_1)	
4	Curcumin 4.5mg/day/animal for 9 days+ MT 0mg/Kg/animal for 5 consecutive days	1	0	0	0	3	2	6 (x_2)	14550 (n_2)	5.76

C. Centric fusion, Frag. Fragment, Del. Deletion.



a



b



c

Fig. 1 Metaphase chromosomes of mice treated with mitronidazol. a. N= 38 and a. deletion b. break c. centric fusion . (Arrows). Geimsa. 1000X.



Fig. 2 Metaphase chromosomes of mice treated with curcumin and mitronidazol. have only deletion (arrow). N=40 Geimsa. 100X.

Discussion

During the last 20 years, nitroimidazole genotoxic agent metronidazole has been studied by several other authors [16,17] and has been associated with differential effects due to the metabolism of this compound and with the genotype of the exposed organisms, or both. When we analyze the potential metronidazole genotoxicity, we found differences in chromosome aberrations frequencies between the control groups and the experimental groups which had received metronidazole. These findings agree with previous reports in the literature [15,16] and widely studied in other models and experimental designs [7,17,18,19].

We are frequently exposed to numerous hazardous agents and protection is rendered to us by numerous means, where innate mechanism of our body flushes out undesired element. In certain countries like India since an individual begins to eat as an infant and till death *Curcuma longa* is a mandatory food additive and an individual in his diet intakes 1-5g/day of the powdered form of *Curcuma* (Turmeric), which is thought to be one such cleansing agent that helps to render protection against various toxicants.

Turmeric has been used historically, as a component of Indian Ayurvedic medicine since 1900 B.C. to treat variety of ailments and research in the later half of the 20th century has identified curcumin, a derivative of *Curcuma longa* to be responsible for most of the biological activities of the turmeric till present, these effects have not been confirmed in humans. However, in 2008, numerous clinical trials in humans were underway, studying the effect of curcumin on numerous diseases including multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer's disease [20].

Hence, in our study, we aimed to assess the protective effect of curcumin against metronidazole induced genotoxicity. Chromosomal aberrations and decline in mitotic index are the most sensitive indicators of bone marrow damage [21,22]. An effort has been made in the current experimental design to observe

whether such toxic effects induced by metronidazole, are neutralized or counterbalanced by administration of curcumin, a polyphenolic compound, derived from *Curcuma longa*. It is the main ingredient in Indian curries and is useful in various inflammatory conditions. It is recommended in cancer prevention and as a treatment adjunct in various diseases [13,23,24]. Antimutagenic and immunomodulatory activities of curcumin on mutagenicity and immunosuppression induced by various mutagens have been reported [24,25,27]. However, The genoprotective effect of curcumin has not been evaluated against metronidazole induced genotoxicity in Iraq. Hence, it was of interest to assess the genotoxicity induced by metronidazole and protection rendered by curcumin against such genetic damage. Curcumin has been shown to exhibit strong antioxidant activity, comparable to vitamins C and E and is eight times more powerful than vitamin E. Several studies have demonstrated curcumin's ability

to reduce oxidative stress [27, 28,29]. These observations are in accord with our results where curcumin has been shown to render protection against metronidazole induced toxicity (Table 1). When mice were pretreated with curcumin and subsequently treated with metronidazole it was observed that mitotic index was equivalent to the mitotic index of control group, however, the chromosomal damage was not significantly repaired by curcumin, as decline in the number of chromosomal aberrations in animals pretreated with curcumin and subsequently treated with metronidazole did not show significant decline in chromosomal aberrations compared to the control groups. Consequently, we can postulate that administration of curcumin for longer period of time then perhaps toxic agents such as metronidazole might not be effective in induction of chromosomal abnormalities. Thus, the current experiments confirm the protective role of curcumin in mice bone marrow cells.

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التأثير الوقائي للكرم في السمية الوراثية للعقار ميترونيدازول في كروموسومات نقي

العظم للفئران السويسرية البهقاء

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الملخص :

يستخلص الكرم (متعدد فينولي أصفر اللون) من مسحوق الريحون المجففة لنبات *Curcuma longa* ويعد مضاداً للتأكسد عالي الكفاءة. وهو مفيد في حالات الضعف المتباينة. ويوصف بالكرم للوقاية من السرطان وعلاج مساعد لأمراض مختلفة. لذا كان الهدف من التصميم التجريبي الحالي تقدير التأثير الوقائي للكرم في السمية الوراثية التي يحثها العقار ميترونيدازول في كروموسومات نقي عظم الفئران السويسرية البهقاء. تم استخدام أربعة مجموعات من الفئران السويسرية البهقاء في التجربة الحالية. وتم إعطاء المجموعة التجريبية خمسة جرعات يومية منفصلة من العقار ميترونيدازول بالتجريب المعدي بواقع 50 ملغم ١ كغم. وزن جسم. وتم إعطاء المجموعة التالية 4.5 ملغم ١ كغم. وزن جسم. ١ يوم من الكرم لتسعة أيام متوالية، وفي اليوم العاشر أعطيت 50 ملغم ١ كغم. وزن جسم من العقار ميترونيدازول ١ يوم ولمدة خمسة أيام متوالية. وتم الاحتفاظ بمجموعة سيطرة مقابل كل مجموعة تجريبية. أعطيت إحداها المادة المذيبة دايثيل سولفواوكسيد لمدة أربعة عشر يوماً بينما أعطيت المجموعة الثانية جرعة يومية 4,5 ملغم ١ كغم. وزن جسم من الكرم ولمدة أربع عشر يوماً متوالية. بعد 22 ساعة من المعاملة الأخيرة في كل مجموعة تم قتل الفئران وتحضير شرائح كروموسومات الطور الاستوائي بطريقة التجفيف بالهواء ثم جرى لاحقاً تقدير التلف الكروموسومي. وقد ظهر انخفاضاً معنوياً في دالة الانقسام الميتوزي للمجموعة المعاملة بالعقار ميترونيدازول وحده. وكان الفعل الانبجيني للميترونيدازول جلياً بشكل خاص، فضلاً عن ملاحظة أنواع أخرى من الشذوذ الكروموسومي مثل التعدد المجموعي، الحذوفات الطرفية، والكسور. وبالمقابل كان هناك ارتفاعاً واضحاً في دالة الانقسام الميتوزي في الحيوانات المعاملة مسبقاً بالكرم وبعدها بالعقار ميترونيدازول. وبشكل عام كان هناك انخفاضاً جلياً في معدل الطفرة مما يثبت الأثر الوقائي للكرم.