

Evaluation of The Genoprotective Effect of Curcumin Against Methotrexate in Bone Marrow and Spleen Cells in Mice

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Abstract

Curcumin is a yellow pigment produced from the rhizomes of the *Curcuma longa* plant and a primary chemo preventive component of turmeric is used as a spice and food coloring ingredient. Curcumin has a large number of pharmacological activities, such as anticancer, anti-diabetic, antioxidant, anti-infectious, and anti-inflammatory properties. Investigation of the geno-protective effect of curcumin on methotrexate induces chromosomal aberrations of spleen and bone marrow cells. In this study, 32 mice were used and divided into four groups (eight mice at each group) as follows: Group1 (negative control): Dimethyl sulfoxide was given intraperitoneally to mice every day for ten days. Group2 (positive control): Mice were received a single dose (20mg/kg) of methotrexate intraperitoneally. Group3: Mice were received (200mg/kg) of curcumin solution intraperitoneally for ten successive days. Group4: Mice were received (200mg/kg) of curcumin solution intraperitoneally for ten successive days and on the day 11, a single dose of methotrexate (20 mg/kg) was injected intraperitoneally. In animals treated with methotrexate, significant elevations of the chromosomal aberrations were observed along with a decline in the mitotic index. Meanwhile, there was a considerable elevation of the mitotic index and no detectable chromosomal changes in the curcumin-supplemented mice. The number of abnormal cells was reduced significantly in the curcumin-treated group in comparison with the methotrexate-treated group. The ability of curcumin to inhibit methotrexate's cytotoxic effects was shown by the compound's ability to raise the mitotic index. According to this finding, curcumin approved to be a protective agent against genotoxic effects of methotrexate.

Keywords: Curcumin, Chromosomal aberrations, Methotrexate, Mitotic index, Mice

تقييم الحماية الجينية للكرمين ضد الميثوتريكسات في خلايا نخاع العظام و الطحال في الفئران طيف محمد مريوش^{*1}، علي فارس حسن²، ساجدة حسين اسماعيل³، احمد حسين صيهود⁴ قاسم سلمان حمود¹ و ندى ناجي الشاوي²

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الخلاصة

الكرمين هو صبغة صفراء تنتج من جذور نبات الكركم ومكون أساسي وقائي كيميائي، يستخدم كتوابل وملون غذائي. يحتوي الكركمين على عدد كبير من الفعاليات الدوائية، مثل خصائص مضادة للسرطان ومضادة للسكري ومضادات الأكسدة ومضادة للعدوى ومضادة للالتهابات. التحقيق في التأثير الوقائي الجيني للكرمين على تأثير الميثوتريكسات الذي يسبب انحرافات صبغية في خلايا الطحال ونخاع العظام. في هذه الدراسة تم استخدام ٣٢ فأراً، تم تقسيمها إلى أربع مجاميع، مكونة من ثمانية فئران لكل منها على النحو التالي: المجموعة ١ (التحكم السلبي): تم إعطاء ثنائي ميثيل سلفوكسيد داخل الصفاق للفئران كل يوم لمدة عشرة أيام. المجموعة ٢ (التحكم الإيجابي): تم تلقي الفئران جرعة واحدة (٢٠ ملجم / كجم) من الميثوتريكسات داخل الصفاق. المجموعة ٣: تم إعطاء الفئران (٢٠٠ ملجم / كجم) من محلول الكركمين داخل الصفاق لمدة عشرة أيام متتالية. المجموعة ٤: تم إعطاء الفئران (٢٠٠ ملجم / كجم) من محلول الكركمين داخل الصفاق لمدة عشرة أيام متتالية وفي اليوم الحادي عشر، تم حقن جرعة واحدة من الميثوتريكسات (٢٠ ملجم / كجم) داخل الصفاق. الاستنتاج: في الحيوانات المعالجة بالميثوتريكسات، لوحظ زيادة كبيرة في كروموسومات الانحراف مع انخفاض في مؤشر الانقسام. مع ذلك، كانت هناك زيادة كبيرة ملحوظة في مؤشر الانقسام الخيطي ولا توجد تغييرات كروموسومية ملحوظة. بينما في الفئران المعالجة بالكرمين. انخفض عدد الخلايا غير الطبيعية في المجموعة مقارنة بالمجموعة المعالجة بالميثوتريكسات. مما أثبت قدرة الكركمين على تثبيط التأثيرات السامة للميثوتريكسات على الخلايا من خلال قدرته على رفع مؤشر الانقسام وتقليل التأثيرات الجينية السامة لعقار الميثوتريكسات، والذي سبب طفرات في الكروموسومات.

الكلمات المفتاحية: الكركمين؛ الانحرافات الصبغية، ميثوتريكسات، مؤشر الانقسام، الفئران

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Introduction

Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadien-3,5-dione) is a potent poly phenolic substance separated from the roots of turmeric (*Curcuma longa*). It was used in the treatment of the common cold, skin diseases, wound healing, and inflammation from the time of ancient Asian medicine ⁽¹⁾.

Curcumin has several pharmacological properties, including anti-cancer, anti-inflammatory, antioxidant, anti-infectious, and anti-diabetic properties⁽²⁾. Curcumin is a flavoring agent with multiple therapeutic properties, most notably anti-cancer effects without adverse effects on the normal cell. Its anti-cancer effects include tumor growth inhibition and induction of apoptosis by modifying signaling pathways *in vivo* and *in vitro*⁽³⁾, a number of studies show that it prevents tumor growth⁽⁴⁾. It has also been documented turmeric has a chemo preventive effect on benzo (a)pyren induced fore stomach and 7,12-dimethylbenz(a) anthracene (DMBA), induced skin tumors in mice ⁽⁵⁾. It has been found that curcumin acts as an anti-inflammatory and anti-cancer agent during the progression /promotion stage of colon tumor^(6,7).

In *Salmonella typhimurium*, curcumin also has an anti-mutagenic action against 4-nitrophenylene-diamine (NPD) and diaminofluorine ⁽⁸⁾. In addition to this, it has been shown that curcumin may protect against clastogenesis that is caused by cisplatin by functioning as a free-radical scavenger ⁽⁹⁾. Curcumin has been shown to prevent DNA damage caused by radiation in rat lymphocytes⁽¹⁰⁾. In a somatic mutation recombination test in *Drosophila*, turmeric showed antimutagenic activity against urethane⁽¹¹⁾. Chromosomal aberrations / abnormalities or chromosomal mutations, are changes to the structure or number of chromosomes which can typically-affect more than one gene; and these abnormalities in the chromosomal -structure or -number may cause birth abnormalities, mental impairment, and a higher risk of infertility⁽¹²⁾. Methotrexate (MTX), is one of the widely used antineoplastic drug and a well-known immunosuppressant introduced for therapeutic use since 1950s. It is used against a broad range of neoplastic disorders including acute lymphoblastic leukemia, non-Hodgkin's lymphoma, breast cancer and testicular tumors ⁽¹³⁾.

Pellizzer et al. ⁽¹⁴⁾ examined the developmentally toxic effects of MTX in a variety of other vertebrate model organisms such as mice, rats and rabbits; where, El -Alfy NZI ⁽¹⁵⁾ at 2016 reported that MTX induced genetic damage on the chromosomes and DNA content of male albino mice after single treatment with low doses.

Aim of study

The current research was designed to examine the protective effect of curcumin against

Methotrexate-induced chromosomal abnormalities in cells of spleen and bone marrow.

Materials and Methods

Materials

Preparation of curcumin extract rhizome (*Curcuma longa*), was obtained from the local Iraqi market and made a coarse powder with mortar and pestle, after that curcumin powder was dissolved in 60-70 ml Dimethyl sulfoxide (DMSO) by swirling or shaking then make to volume 100 ml with the same solvent ⁽¹⁶⁾.

Methotrexate tablets Ebewe (Austria) were bought from a pharmacy, and a methotrexate stock solution was made by dissolving one tablet of methotrexate (2.5 mg) in 2.5 ml of distilled water and mixing it with a vortex apparatus.

Methods

Animals and treatment procedures

In the present study, 32 albino Swiss mice, ranging in weight from 23 to 27 g were used. This was done in compliance with the recommendations made by the Biochemical and Research Ethical Committee at the College of Pharmacy University of Baghdad (Canadian Council on Animal Care guidelines). From the College of Pharmacy at the University of Baghdad, the animals were purchased. Under typical conditions (well ventilated, temperature 22±2°C, relative humidity 50%-60%, and 12-hour day/night cycle), they were kept for two days. After receiving approval from the Institutional Animal Ethical Committee, all animal experiments were carried out.

Experimental design

In this study, thirty two mice were utilized, which were separated into four groups (eight mice of each) as follow: Group1 (negative control): Mice had been injected intraperitoneally with Dimethyl sulfoxide daily for ten successive days. Group2: Mice had been injected with a single dose (20mg/kg) of methotrexate ⁽¹⁷⁾.

Group3: Mice had been injected (I.P) with (200mg/kg) of curcumin solution for ten successive days.

Group4: Mice had been injected (I.P) with (200mg/kg) of curcumin ⁽¹⁸⁾ solution for ten successive days and on day eleven single dose of methotrexate (20 mg/kg) was administered intraperitoneally. At the end of the study, each rat received an injection of 1 mg/kg of colchicine (Sigma, USA) intraperitoneally, and then, two hours later, their cervical dislocation was used to scarify them. Samples of bone marrow were extracted from the femur bones, then processed in an aseptic technique and Spleen cells samples for evaluation of mitotic index.

Evaluation of mitotic index

According to the following formula, the cells number in the division stage was determined as a

percentage of the total number of cells. The mitotic index is equal to 100 (the ratio of the number of mitotic cells to the total number of cells).

Chromosomal aberration assay

Bone marrow chromosome preparations were carried out according to the method of Preston *et al*⁽¹⁹⁾

Statistical analysis

The Statistical Package for Social Sciences (SPSS), version 24 was used for data analysis. The data were calculated as mean \pm standard error of means (SEM). An one-way Analysis of Variance (One way-ANOVA) was used to compare

among groups. When $P < 0.05$, statistically significant differences were accepted.

Results of the Study

Table 1 demonstrated that group 2 had a significantly lower mitotic index of bone marrow cells and spleen cells than the group 1, meanwhile, the group 3 demonstrated a significant elevation ($p < 0.05$) of the same parameter when compared to the negative control group.

In the same table, the mitotic index of group 4 in both spleen cells and bone marrow cells showed a significant elevation, when compared to the negative control group ($p < 0.05$).

Table 1. The incidence of the mitotic index of the spleen and bone marrow cells of albino mice.

Treatment Groups	Mitotic Index	
	Cell of Bone Marrow	Cell of Spleen
Group I (DMSO) (Negative control)	5.36 \pm 0.07	4.34 \pm 0.09 [#]
Group II Methotrexate (MTX) (positive control) 20mg/kg	2.18 \pm 0.06*	1.1 \pm 0.1*
Group III Curcumin extract	6.96 \pm 0.21* [#]	6.17 \pm 0.24* [#]
Group IV (Curcumin+MTX)	4.31 \pm 0.33 [#]	3.8 \pm .37 [#]

The data are expressed as mean \pm SEM (n=8);

* indicates a significant difference from the control.

indicates a significant difference from the MTX group.

Table 2. Incidence of Total chromosomal aberration of the bone marrow and spleen cells of albino mice.

Treatment Groups	Total Chromosomal Aberration	
	Bone Marrow Cells	Spleen Cells
Group I (DMSO) (Negative control)	0.12 \pm 0.004	0.103 \pm 0.003 [#]
Group II Methotrexate (MTX) (positive control) 20mg/kg	0.32 \pm 0.006*	0.27 \pm 0.003*
Group III (Curcumin)	0.102 \pm 0.004 #	0.09 \pm 0.002 #
Group IV (Curcumin+ MTX)	0.168 \pm 0.03 [#]	0.14 \pm 0.014 [#]

The data are expressed as mean \pm SEM (n=8);

* indicates a significant difference from the control.

indicates a significant difference from the MTX group.

Table 2 showed, total chromosomal aberration in bone marrow and spleen cells of group 2 showed a significant elevation, when compared with group 1 ($p < 0.05$), meanwhile groups 3 and 4 showed a no significant differences in the same parameter when compared to the negative control group ($p > 0.05$). In the same table, a total chromosomal aberration for group 4 in both bone marrow cells and spleen cells showed a significant reduction, when compared to a positive control group ($p < 0.05$).

Discussion

Many anticancer drugs used in chemotherapy lead to a variety of symptoms of

direct adverse effects⁽²⁰⁾. In addition, in experimental studies, some anticancer agents have been shown to be carcinogenic, mutagenic and teratogenic⁽²¹⁾.

MTX administration, for example, has been shown to increase the accumulation of oxidative DNA injuries, which have been shown to induce DNA damage.⁽²²⁾

Hence, protecting normal cells from conditions that may cause malignancy is an important way to prevent long-term impairments or damage due to the administration of chemotherapeutic agents⁽²³⁾. The most sensitive indicators of bone marrow and spleen

damage are chromosomal aberrations and a decrease in mitotic index⁽²⁴⁾.

The present results of MTX administration resulted in a significant reduction in the mitotic index and that the incidence of the mean of various types of chromosomal aberrations were significantly increased ($P < 0.05$). These results are in agreement with studies of others^(25, 26).

It has been shown that curcumin has both preventative and therapeutic benefits against a wide variety of malignancies⁽²⁶⁾

The results of several investigations indicate that the curcumin compound may either stop the growth and spread of tumors or minimize their size.⁽²⁷⁾

It has been shown that curcumin has anti-angiogenic properties, induces apoptosis, and interferes with the cell cycle, all of which may prevent the growth of cancer and the spread of malignant cells⁽²⁸⁾. In addition, curcumin has been shown to have a wide range of biological actions, they include its anti-mutagenic, anti-carcinogenic, anti-genotoxic, anti-inflammatory, and antioxidant capabilities⁽²⁹⁾.

When mice were pretreated with curcumin and subsequently treated with MTX it was observed that a significant decrease in total chromosomal aberration when compare group 4 with group 2 in both spleen cells and bone marrow cells, this finding is completely agree with a previous studies that reach to conclusion about a protective effect of curcumin against chromosomal aberrations^(30,31,32). Additionally, when mice were pretreated with curcumin and subsequently treated with MTX, it was observed that a significant elevation of mitotic index, in comparison of group 4 with group 2 of both spleen and bone marrow cells, these results are in line with the work of other study⁽³³⁾. Besides, there is a relationship between chromosomal aberration and cell division, previous study have been showed an increase in the aberration of chromosomes, caused a decrease in the cell division⁽³⁴⁾, this finding completely synonyms with the finding of present study in which the increase in chromosomal aberration associated with a decrease in mitotic index and vice versa The exact mechanism(s) of chromosomal protection of curcumin is not investigated in this study, but previously they found that methotrexate which is used as an inducer for aberration cause chromosomal damage (aberration) by different mechanisms⁽³⁵⁾ according with present finding curcumin counteract that effect and provide a protection to the genetic materials.

Conclusion

In conclusion, curcumin pretreatment resulted in a considerable reduction of the genotoxic and oxidative stress of MTX in vivo. Additionally, pretreatment with curcumin improved the mitotic activity in the cells of bone marrow and spleen.

Conflicts of Interest

The authors declare no conflict of interest.

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Ethics Statements

This study was approved by the scientific and ethical committees of the Department of Pharmacy, Kut University College.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: **Sajida H. Ismael, Taif M. Maryoosh**; data collection: analysis and interpretation of results: **Ali Faris Hassan, Kasim S. Hmood, Nada N Al-Shawi**; draft manuscript preparation: **Taif M. Maryoosh**. All authors reviewed the results and approved the final version of the manuscript.

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