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Open Access Research Article

Evaluation of the Protective effect of Omega-7 Against Methotrexate Genotoxicity in Bone Marrow Cells of Mice

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Abstract:

Background: A substance that can affect DNA or chromosomes is defined as a genotoxin. DNA damage in a somatic cell may result in a somatic mutation (cancer). In contrast, damage to a germ cell (germline mutation) may result in a heritable changed characteristic. Omega-7 is a non-essential monounsaturated free fatty acid with anti-inflammatory, anti-obesity, and antidiabetic effects. **Objectives:** Evaluation of the possible protective effects of omega seven against methotrexate genotoxicity.

Method: Two major equal groups were obtained from seventy mice, and five subgroups (each of seven) were created from these groups as follows: **Group I** received liquid paraffin orally for seven successive days. **Group II**: received liquid paraffin orally for seven successive days, followed by a single intraperitoneal dose of methotrexate (20 mg/kg) on the eighth day. **Group III**: received omega-7 (50mg/kg) orally for seven successive days, followed by a single intraperitoneal dose of methotrexate (20 mg/kg) on the eighth day. **Group IV**: received omega-7 (100mg/kg) orally for seven successive days, followed by a single intraperitoneal dose of methotrexate (20 mg/kg) on the eighth day. **Group V**: received omega-7 (100mg/kg) orally for seven successive days. The first major group was intraperitoneally injected with 1mg/kg colchicine, and then after two hours, all mice were killed by spinal dislocation. Bone marrow cells from the first major group were used to measure the mitotic index and chromosomal aberrations, and bone marrow cell of the second group was used to measure the appearance of the micronucleus. Statistical Package for Social Sciences (SPSS) and ANOVA test were used to compare groups.

Results: Treatment of mice with omega-7 led to a significant decline in chromosomal aberration and micronucleus aberrance with a significant elevation of the mitotic index.

Conclusion: Omega-7 has been shown to have a protective role against methotrexate genotoxicity.

Keywords: Chromosomal aberration; Methotrexate; Micronucleus; Mitotic index; Omega-7.

Introduction

Omega-7 with the chemical formula C16:1n-7 is a monounsaturated fatty acid (1), and has been found in plants and also in marine food products (2,3). It is mostly present in the oils of sea buckthorn and macadamia nut, where it makes 17 % - 29 % of the fatty acids, respectively (4). It is commonly used to prevent thrombosis and strokes (5).

Also, it is used in skin cleansers and may be a good skin lightener due to its ability to prevent melanogenesis (6). In rodents,a study about omega-7 found that it accelerates wound healing, which may be related to its anti-inflammatory properties (3,7).

Genotoxicity refers to a drug's or chemical's ability to cause harm to or modify the genetic material DNA or RNA (8,9). Chromosomal aberrations (CAs) are a type of chromosomal abnormality; that refer to a change in the structure and/or number of chromosomes (10).

Deletions, ring chromosomes, acentric chromosomes, and dicentric chromosomes are instances of structural anomalies (11,12)The mitotic index (MI) is a proliferative state indicator for a cell population that is calculated as the proportion of cells in mitosis to all cells (13).

Micronuclei (MN) are tiny acentric chromatin fragments created when a chromosome or chromosomal fragment is not integrated into one of the daughter cells (14). Breast cancer, acute lymphoblastic leukemia and rheumatoid arthritis have seen widespread usage of the anti-metabolite methotrexate (MTX) (15,16), it prevents the formation of tetrahydrofolic acid, which is necessary for DNA synthesis and cell replication, by competing with the enzyme dihydrofolate reductase's (DHFR) folate binding site. MTX inhibits DNA, RNA, and protein synthesis in cells by preventing the synthesis of thymidylate and purine nucleotides via the inhibition of dihydrofolate reductase and, to a lesser degree, thymidylate synthetase (17). The aim of the present study was to evaluate the possible genoprotective effects of omega-7 with two different

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doses (50mg/kg and 100mg/kg) on bone marrow cells in methotrexate-treated mice

Materials and Methods

Animals and Treatment Protocols Experiments were conducted using 70 albino Swiss mice (Mus musculus). They were taken from the College of Pharmacy/University of Baghdad animal house. At a weight between 23 and 27 grams each, they were separated into two major groups each with five independent groups, the first major group was used to measure MI and CAs, and the second major group was used to measure Micronuclei (MN), each of which was housed in a separate plastic cage. Temperatures ranged from 23 to 25°C, and the animals had access to ad libitum water and free excess food. Five groups of seven mice each were formed from each major group, as follows:

Group I: Liquid paraffin was administered to seven mice. The dosage was administered orally for seven consecutive days. Liquid paraffin was used as an inert vehicle (as omega-7 is an unsaturated fatty acid) and represented the negative control group, as the control group is the vehicle that dissolves the curative material (18).

Group II: Mice were fed liquid paraffin for seven consecutive days, and methotrexate (20mg/kg) was administered intraperitoneally (IP) on day eight. This group represents a positive control (19).

Group III: 50mg/kg of omega-7 was given orally to mice for seven days, and an MTX (20mg/kg) IP injection was administered on day eight (20).

Group IV: 100mg/kg of omega-7 was given orally to mice for seven days and an MTX (20mg/kg) IP injection was administered on day eight(20).

Group V: 100mg/kg of omega-7 was given orally to mice for seven days(20).

The first major group was IP injected with 1mg/kg colchicine 24 hours after therapy finished, as colchicine is a well-established spindle poison that arrests mitotic cell division at the metaphase stage by destabilizing microtubules (21), and then killed by spinal dislocation after two hours. Cells of bone marrow (BM) were collected, and genotoxic assays were performed on the cells to measure MI and Cas (22), The second major group was sacrificed, and BM cells were used to evaluate MN appearance (23).

Statistical Analysis

The statistical package for Social Sciences (SPSS) version 25 was used for data entry and analysis, and the ANOVA test was used to compare test groups. Differences between test groups with *P* values of less than 0.05 were deemed significantly different for all study results (24).

Results:

As shown in Table 1, when contrasted with the liquid paraffin group, MTX resulted in a statistically significant reduction in MI ($P<0.05$). MI was not substantially different in group V mice in comparison to the liquid paraffin group ($P>0.05$); Group III showed a non significant difference as compared to the MTX group in MI. Group IV exhibited substantial increases ($P<0.05$) in MI compared to the MTX group. The MI ($P>0.05$) did not differ significantly across groups III, and IV, in BM.

Table (1). Bone marrow MI occurrence in experimental mice groups as a result of different treatments

Treatment Groups (N=7)	Mitotic Index Bone Marrow Cells
(Group I)Liquid paraffin (Negative control)	8.68±0.44
(Group II)methotrexate (Positive control) 20mg/kg	5.68±0.89*
(Group III) Omega7 at a dose 50 mg/kg plus methotrexate 20 mg/kg	6.08±0.68
(Group IV) Omega7 at a dose 100 mg/kg plus methotrexate 20 mg/kg	7.28±1.19#
(Group V) Omega7 100mg/kg	8.28±0.35

Data are represented as mean±SD.
* showed a significant difference in comparison to the liquid paraffin ($P<0.05$).
# showed a significant difference in comparison to the MTX ($P<0.05$).

As shown in the table 2, when compared with liquid paraffin group, MTX resulted in a statistically significant higher ($P<0.05$) MN. While Group V showed a non-significant difference in the manifestation of MN in comparison to the untreated liquid paraffin group ($P>0.05$). The MN was considerably decreased in both Group III and Group IV ($P<0.05$) in comparison with the MTX group. BM cells from Group III, and Group IV mice showed significant differences in the presence of MN when compared to each other ($P<0.05$).

Table (2): Effects of omega-7 on micronucleus appearance in BM cells.

Treatment Groups (N=7)	Micronucleus Appearance in BM Cells
(Group I)Liquid paraffin (Negative control)	6.67±0.42
(Group II)methotrexate (Positive control) 20mg/kg	9.33±0.51*
(Group III) Omega7 at a dose 50 mg/kg plus methotrexate 20 mg/kg	8.5±0.30#B
(Group IV) Omega7 at a dose 100 mg/kg plus methotrexate 20 mg/kg	7.83±0.59#B
(Group V) Omega7 100mg//kg	7±0.34

Data are represented as mean±SD.
* showed a significant difference in comparison to the liquid paraffin ($P<0.05$).
# showed a significant difference in comparison to the MTX ($P<0.05$).
* B showed a significant difference in comparison between combination group III to combination group IV.

In Table (3), group IV showed a non-significant difference in total CAs as compared to liquid paraffin ($P>0.05$), while MTX at a dose (20mg/kg) showed a significant increase in total CAs as compared to liquid paraffin ($P<0.05$).On the other hand, both combination groups III and IV showed a significant decrease ($P<0.05$) in total CAs in comparison with the MTX group. There was a significant difference when comparing combination group III to

combination group IV ($P<0.05$), in which combination group IV was more effective than combination group III in lowering total chromosomal aberration.

Table (3): Effects of omega7 on the total CAs in BM

Treatment Groups (N=7)	Total chromosomal aberration
(Group I)Liquid paraffin (Negative control)	0.12±0.01
(Group II)methotrexate (Positive control) 20mg/kg	0.20±0.03*
(Group III) Omega7 at a dose 50 mg/kg plus methotrexate 20 mg/kg	0.16±0.02 # B
(Group IV) Omega7 at a dose 100 mg/kg plus methotrexate 20 mg/kg	0.13±0.02#B
(Group V) Omega7 100mg//kg	0.13±0.01

Data are represented as mean±SD.
* showed a significant difference in comparison to the liquid paraffin ($P<0.05$).
# showed a significant difference in comparison to the MTX ($P<0.05$).
* B showed a significant difference in comparison between combination group III to combination group IV.

Discussion

Because of the advances in both success rates and patient survival, chemotherapy is increasingly being employed as a treatment for cancer (25). MTX is one of the most extensively researched and effective chemotherapeutic agents used. During cancer treatment with MTX, quickly dividing normal cells are damaged, resulting in a variety of undesirable toxicities occurring in the bone marrow and spleen (26). There is no previous study focusing on the effect of omega-7 as a geno-protective factor but many other studies showed that unsaturated fatty acids may have a geno-protective effect (27, 28). Treatment group V showed a non-significant effect on the MI, CAs, and MN formation compared with the negative control, indicating that omega-7 did not have any genotoxic effect on BM cells. Table (1) represents the MI test results, there were significant decreases when comparing positive control (Group II) to negative control and these differences were due to the toxic effect of MTX. Previous studies showed strong evidence supporting the role of reactive oxygen species (ROS) in the pathogenesis of MTX damages (29, 30). reduction of MI for mice that were treated with only one dose (20mg/kg) was consistent with the previous results that showed that following treatment with MTX the mitotic index decreased, this was as a result of its capacity to overlap with DNA through lack of dihydrofolate reductase enzyme, a key enzyme in the process of growth and cell division (31). The considerable reduction in MI caused by MTX is related to the suppression of the pathway leading to the production of thymidine, which is a nucleotide important in DNA synthesis. One process that may be implicated in CAs is the depletion of this substrate (19). the combination group IV showed a significant increase in MI as compared with the positive control in the BM, demonstrating that a dose of 100mg/kg of omega-7 was more effective than a dose of 50mg/kg in increasing the MI.

The MN assay is a cytogenetic approach used for toxicological screening to evaluate the probable genotoxic effects of hazardous substances for their possible genotoxic effects. The significant increase in MN in the positive group (II) was due to the fact that MTX is an S-dependent substance that causes DNA lesions that are irreparable before DNA synthesis (32), which results in replication stress (creating reactive oxygen or reactive nitrogen species that damage the phosphodiester backbone of DNA), which can cause chromatid-type or chromosome-type aberrations that stall the DNA replication fork and result in transient or permanent DNA breaks from which acentric chromosome fragments and MN are produced (33). Combination group IV showed a significant decrease in MN in comparison with combination group III ($P<0.05$) indicating that high dose of omega-7 was more effective in lowering the MN. The results of CAs of positive control showed a significant increase compared with negative control agreeing with studies that showed that MTX can induce chromosomal abnormalities (34). All treatment groups for CAs showed a significant decrease when compared with positive controls ($P<0.05$) and when compared with each other. The exact way in which omega-7 produces a protective effect on MTX genotoxicity is not known. However, in this study, antioxidant effects can be taken into consideration as a possible mechanism. It is generally known that oxygen-free radicals are powerful mutagens. Because MTX is widely recognized for inducing oxidative stress, it was employed as a source of free radicals (29,35). Omega-7 demonstrated antioxidant action with antioxidant substance (28,36). Another previous study also showed that omega-7 exhibits antioxidant effect in cardiomyocytes (37).

Conclusions:

Omega-7 is a genoprotective agent, that showed significant protective effects on mouse bone marrow stem cells by increasing mitotic index and decreasing micronucleus appearance, and total chromosomal aberrations caused by MTX in a dose-dependent manner.

Authors' declaration

Conflicts of Interest: None

We hereby confirm that all the tables in the manuscript are ours. Besides, the figures and images, which are not ours, have been given permission for re-publication and attached to the manuscript. Authors sign on ethical consideration's approval- Ethical Clearance: The project was approved by the local ethical committee in the College of Pharmacy, University of Baghdad according to the code number (513 .24. 1.2022).

Authors' Contributions:

Study conception & design: (Ahmed F. Hassan). Literature search: (Zahraa H. Hassani). Data acquisition: (Zahraa H. Hassani). Data analysis &

interpretation: (Ahmed F. Hassan). Manuscript preparation: (Zahraa H. Hassan). Manuscript editing & review: (Zahraa H. Hassani & Ahmed F. Hassan).

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تقييم التأثير الوقائي لأوميغا 7 ضد السمية الوراثية للميثوتريكسات في خلايا نخاع العظام لدى الفئران

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خلاصة
خلفية البحث: تُعرَّف المادة التي يمكن أن تؤثر على الحمض النووي أو الكروموسومات على أنها سم جيني. قد يؤدي تلف الحمض النووي في الخلية الجسدية إلى حدوث طفرة جسدية (سرطان). في المقابل ، قد يؤدي تلف الخلية الجرثومية (طفرة السلالة الجرثومية) إلى تغيير خاصية وراثية. أوميجا 7 هو حمض دهني غير أساسي أحادي غير مشبع له تأثيرات مضادة للالتهابات ومضادة للسمنة ومضادة لمرض السكر.
الأهداف: تقييم التأثيرات الوقائية المحتملة لأوميغا 7 ضد السمية الجينية للميثوتريكسات.ا
لطريقة: تم الحصول على مجموعتين رئيسيتين متساويتين من سبعين فأرًا ، وخمس مجموعات فرعية (كل واحدة من سبعة) تم تكوينها من هذه المجموعات على النحو التالي: المجموعة الأولى تلقيت البارافين السائل عن طريق الفم من أجل سبعة أيام متتالية. المجموعة الثانية: تلقيت البارافين السائل عن طريق الفم لمدة سبعة أيام متتالية ، تليها جرعة واحدة داخل الصفاق من الميثوتريكسات (20 مجم / كجم) في اليوم الثامن. المجموعة الثالثة: تلقيت أوميغا 7 (50 مجم / كجم) عن طريق الفم لمدة سبعة أيام متتالية ، تليها جرعة واحدة داخل الصفاق من الميثوتريكسات (20 مجم / كجم) في اليوم الثامن. المجموعة الرابعة: تلقيت أوميغا 7 (100 مجم / كجم) عن طريق الفم لمدة سبعة أيام متتالية ، تليها جرعة واحدة داخل الصفاق من الميثوتريكسات (20 مجم / كجم) في اليوم الثامن. المجموعة الخامسة: تلقيت أوميغا 7 (100 مجم / كجم) فموياً لمدة سبعة أيام متتالية. تم حقن المجموعة الرئيسية الأولى داخل الصفاق بـ 1 مجم / كجم من الكولشيسين ، ثم بعد ساعتين ، قُتلت جميع الفئران بسبب خلع العمود الفقري. تم استخدام خلايا نخاع العظام من المجموعة الرئيسية الأولى لقياس مؤشر الانقسام والانحرافات الصبغية ، واستخدمت خلايا نخاع العظام للمجموعة الثانية لقياس مظهر النواة الدقيقة. تم استخدام الحزمة الإحصائية للعلوم الاجتماعية (SPSS) واختبار ANOVA لمقارنة المجموعات.
النتائج: أدى علاج الفئران بأوميغا 7 إلى انخفاض كبير في انحراف الكروموسومات وزيغ النواة الدقيقة مع ارتفاع كبير في مؤشر الانقسام.
الخلاصة: لقد ثبت أن أوميغا 7 لها دور وقائي ضد السمية الجينية للميثوتريكسات.
الكلمات المفتاحية: انحراف الكروموسومات ، الميثوتريكسات ، مظهر النواة الصغيرة ، مؤشر الانقسام ، أوميغا 7.