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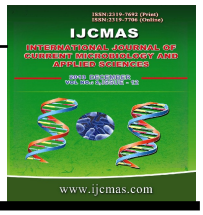


Original Research Article

Protective Effects of linseed Oil against Methotrexate Induced Genotoxicity in Bone Marrow Cells of Albino Mice *Mus musculus*

Galawezh Obaid Othman*

Department of Biology, Collage of Education, University of Salahaddin Erbil, KRG-Iraq
Corresponding author

Keywords

Linseed oil; chromosomal aberrations; protection; Methotrexate.

A B S T R A C T

The genotoxic actions of anti-neoplastic drugs can lead to the development of secondary cancers in patients in extended remission. One of the most attractive approaches to disease prevention involves the use of natural antioxidants to protect tissue against toxic injury. The present investigation was undertaken to study the possible protective effect of linseed oil against methotrexate induced chromosomal aberrations in single dose. The animals were randomly divided into four groups consisting of five mice. first group remain as control, second group was received single intraperitoneally injection of MTX (10 mg/kgb.w.) to induce genotoxicityas positive control also a group of Mice treated with linseed oil (0.1ml/kg b.w. /day) only. Last group was treated orally with linseed oil (0.1ml/kg b.w. /day) prior to an interperitoneal dose of methotrexate by three hours for fifteen days. Methotrexate have ability to induce various types of chromosomal aberrations such as (Chromosome break, Chromatid break, Centromeric break, fragments), The results explored that linseed oil possesses significant protective potential against Methotrexate by decreasing chromosomal aberration frequency in bone marrow cells.

Introduction

The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic diseases. There are several ways in which the action of mutagens can be reduced or prevented, Natural chemicals which act with DNA repair or with mutagen metabolism can be effective antimutagens (Ferguson, 1994). Genotoxicity refers to the capability of substances to damage DNA and/or cellular components regulating the fidelity of the genome (Eastmond.et.al.2009).The main problem posed by anticancer drugs is that they target not only the Tumor, but also other cells, thus causing the same damage to both abnormal and normal cells (Granados - Principal *et al*., 2010). Antineoplastic agent which acts as an antimetabolite of folic acid. Possesses immunosuppressant properties, Methotrexate competitively inhibits the

enzyme dihydrofolate reductase which is necessary for purine and pyrimidine synthesis and consequently prevents the formation of DNA and RNA (choudhury, *et al*., 2000).

The investigation of the inter-relation between free radicals and antioxidant dietary oils is a field of great interest for elucidating mechanisms of mutagenesis/carcinogenesis (Owen *et al*., 2000). Previous studies have shown that the mutagenic activity of food mutagens can be modulated by vegetable oils (Perez et al., 2002). Recently, it is very important to search for protective substances against mutagenic-carcinogenic agents. Which damage DNA and other cell targets (Ames and Gold, 1991).

Linseed oil, also known as flax seed oil, is marketed as a nutritional supplement. It contains unsaturated omega-3 fatty acid, linoleic acid (51.9-55.2%), which may be beneficial for reducing inflammation leading to atherosclerosis (Evangelista, 2004) for normal infant development Linseed oil is derived from the dried ripe seeds carcinogenesis, in two ways: by decreasing oxidative of the flax plant DNA damage and by decreasing cell division (Tuck and Hayball, 2002). Regular Linseed oil contains between 52 and 63 % alpha linoleic acid The linoleic acid has beneficial antioxidant activities (Turner, 2005) the present work was conducted for exploring the possibility of using flax seed (linseed) oil as a protective supplement against Methotrexate induced Genotoxicity in mice.

Materials and Methods

Animals

9week old male mice were used in this study. The mice were obtained from the animal House of Biology Department, Education College, Salahaddin University Erbil, Iraq and it kept under controlled conditions. The animals were randomly divided into four groups consisting of five mice. first group remain as control, second group was received single intraperitoneally injection of MTX (10 mg/kg b.w.) to induce genotoxicity as positive control also a group of Mice treated with linseed oil (0.1ml/kg b.w. /day) only. Last group was treated orally with linseed oil (0.1ml/kg b.w. /day) prior to an interperitoneal dose of methotrexate by three hours for fifteen days. For the analysis of chromosomal damage in metaphase cells, bone marrow preparations were prepared according to Evans method .The mice were injected subcutaneously with colchicines solutions (freshly prepared) due to the weight of each mouse. The injected mouse was left for (2.5-3) hours to collect the marrow cells in metaphase, then the mouse was killed by cervical dislocation, immediately the hind legs were dissected the bone marrow was extracted from femur and tibia by injecting the normal saline with small syringe and collecting in a centrifuge test-tube mixed thoroughly, then content centrifuged at (800 rpm for 10 min), then supernatant discarded and (5 ml) of fresh KCL solution which maintained at 37 °C was added slowly, the mixture was left for 15 min, in a water bath at 37 °C, 1ml of fixative added drop by drop from the side of centrifuge test-tube, the solution was mixed thoroughly again and centrifuged for the second time at 800 rpm for 10 min., supernatant discarded and 5 ml of fixative added drop by drop to the white cloud in the bottom which represented the pelleted marrow cells, then mixed and centrifuged at 800 rpm for 10 min, supernatant discarded and 3ml of fixative added slowly and left for 5

min. in room temperature. By using dropper, the mixture was dropped from three feet high over the clean moist slides. After drying in a drier the slides were stained with Geimsa stain for 10-15 minutes (Evans, 1964).

Result and Discussion

Methotrexate, a widely used anticancer drug, was tested for its cytotoxicity in mouse bone marrow after a single intraperitoneally treatment, and using the linseed oil as protective effect against methotrexate. The present finding indicated that linseed oil reduced the chromosome aberration in bone marrow cells as shown in figure (1) and (2) respectively. The results of the present study represented the protective effects of linseed oilin one dose (0.1 ml /kg body weight) on chromosome aberrations in bone marrow cells in albino mice induced by anticancer drag methotrexate after injected intraperitoneally at a dose (10 mg/kg body weight) asShows the in Table (1) significant effect ($p<0.05$) of linseed on chromosome aberration, was noticed when added before anticancer drags. This oil decrease the value of all types of chromosome aberration including (chromosome break, chromatid break, dicentric chromosome, pulverization) when compare to the negative control. The results clearly indicated that there was gradual decrease in the mean value of various types of chromosomal aberrations when compared with the group exposure to single dose of MXT 10mg/kg were injected intraperitoneally. So linseed oil protective leads to lowering the chromosome aberrations that happened by Methotrexate for example highest aberration was in chromosome beak was (17.00±0.800) in case of methotrexate while pretreated linseed oil lowerd it to (9.300±6.871) so on the other tretments.in this way linseed oil induced chromosomal aberrations at 0.1 ml/animal/day were not statistically significant when compared with untreated control, which indicated its non-clastogenicity. The effect of linseed oil on the reduction of total number of aberrations induced by methotrexate was statistically significant when compared with methotrexate alone. This study implies that treatment of linseed oil has a strong inhibitory role against the genotoxicity action of methotrexate. The statistical analysis of data showed that there was a gradual decrease in the frequency of various types of chromosomal aberrations in bone marrow cells of linseed oil and methotrexate treated animals.

Many Methods by which reduce or prevent the action of mutagens. Chemicals which interfere with DNA repair or with mutagen metabolism can be used as effective antimutagens (Ferguson, 1994).From the above result, we investigated the antigenotoxic properties of linseed oil towards a variety of genotoxic agents in bone marrow cells.

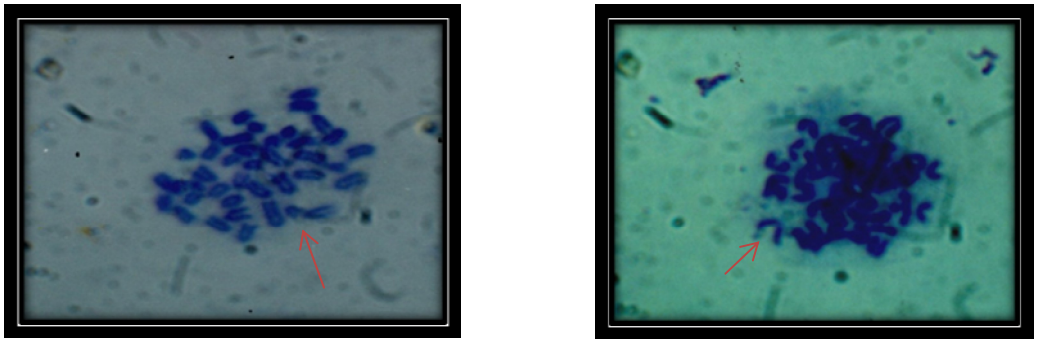
We showed that linseed oil displayed antigenotoxic activity when orally administered against methotrexate induced cytotoxicity and genotoxicity towards mouse somatic cells *in vivo*. Pretreatment of mice with linseed oil for fifteen days and simultaneously with a one dose of methotrexate significantly reduced the frequency of Chromosomal aberrations of bone marrow cells.

The antioxidant nature of linseed oil has been attributed to the presence of linoleic acid. The linoleic acid has beneficial

Table.1 Mean ± S.E for the effect of pretreated linseed oil and Methotrexate (MTX) intraperitoneally on chromosomal aberrations in male albino mice

Groups	Chromatid Break	Chromosome Break	Centromeric break	Fragment
Control	2.70±0.48	2.000±0.707	8.00±0.144	2.800±0.344
Linseed oil	2.00±0.400	1.00±0.879	7.00±0.144	3.100±0.048
10mg/Kg Methotrexate	11.10±0.850	17.00±0.800	14.80±0.213	6.600±0.472
Linseedoil + Methotrxate	3.10±0.623	9.300±6.871	8.8.00±0.827	0.600±0.578

Figure.1 Chromosomal aberration in bone marrow after administration MXT1.Chromatid gap With fragment 2.Centromericbreak



antioxidant activities. Also, it was clearly indicated that the prophylactic action of linseed oil against Cyclophosphamide-induced oxidative stress is due to its antioxidant effects.

In study by Metwally showed thatlinseed oil possesses protective action against carbendazim induced testicular toxicity in rat (Metwally *et al.*, 2011)

Our result is agreed with a study Showed that linseed oil, and grape seed oil have protective effect against Cyclophosphamide induced chromosomal And its mechanism for the protective effects of vegetable oils is that their phenolic compounds have antioxidant and antimutagenic properties in vivo (Evangelista *et al* ., 2006). The protection afforded by linseed oil might be due to the antioxidative action of its important constituents, the lignans, and glycosides of secoisolariciresinol as the major lignan, together with small amounts of matairesinol, isolariciresinol and pinoresinol. The lignan like

abnormalities. Similar results were also reported (Zaman and Salih, 2005). In a study by (Othman, 2002) on albino mice showed that a significant effect were found between vitamin C protective effect against Nicotine and vitamin c , was most effective to decrease all type of chromosomal aberration.in another study on plant system Black cumin oil has anti-mutagen effect in leading to lowering the effect of Ethyl Methyl Sulfonate and decrease the level of chromosome aberrations in Allium cepa root cells (Othman and Suleiman, 2012).

secoisolariciresinol and pinoresinol were reported to have strong antioxidant nature (Harper, *et al*., 1999). The antioxidant effects of linseed oil are probably ascribable to a combination of its high oleic acid content and its content of a variety of plant antioxidants, (Visioli and Galli, 1998; Wahle *et al*., 2004). On the basis of our results, we conclude that linseed oil has antimutagenic potential against methotrexate genotoxicity in vivo in bone marrow cells of albino mice. At the same time it exhibits a mild cytotoxic action;. However the mechanism by which it acts remains to be investigated in and further studies are necessary to clarify this point.

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