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Protective activity of *Coriandrum sativum* extract on methotrexate-induced genotoxicity in Swiss albino mice

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Abstract

Objective

Spice crops have been widely recognized for the diverse health benefits and important bioactive compounds synthesized in them. One of these plants is coriander (*C. sativum*). This annual plant has high commercial value, antibacterial, antioxidant, antihyperlipidemic, antidiabetic and neuroprotective activities, and is used in Mediterranean and European regions for its seeds and fresh leaves. Therefore, the goal of this research was to explore the protective mechanism of coriander extract in response to methotrexate (MTX)-induced chromosomal abnormalities and changes in mitotic index in mouse bone marrow cells.

Materials and methods

Twenty-four adult mice were randomly divided into four groups of six. The negative control group (group I) received distilled water orally for six consecutive days. The second group received an intraperitoneal (i.p.) injection of MTX (20 mg/kg body weight). The third group received *C. sativum* extract orally at a dose of 600 mg/kg body weight for six days. The fourth group was provided MTX (20 mg/kg, intraperitoneal) once, and C. sativum extract (600 mg/kg) for 6 days. Then, medullary cancer samples were analysed for chromosomal aberrations and the mitotic index in short term cultures by a conventional citogenetic technique.

Results

MTX-treated mice had significantly more chromosomal abnormalities and a lower mitotic index than controls. In contrast, the quantity of chromosomal abnormalities was markedly lower in C.sativum extract treated animals as opposed to control. Furthermore, a good protective effect was demonstrated in the group of animals treated with both MTX and C. sativum that might suggest that obtained C. sativum is able to alleviate the cytotoxic and genotoxic activity of MTX.

Conclusions

Based on the decrease of chromosomal aberrations and mitotic index observed after C. sativum treatment, it can be concluded that this extract is a good candidate for cytoprotection and genomic protection against damage induced by MTX. Therefore, the results of this research confirm the ability of C. sativum to be a hopeful protective chemopreventive agent for decreasing methotrexate-induced genotoxic effects.

Keywords: chromosomal aberrations, Coriandrum sativum, methotrexate, mice, mitotic index

Paper Type: Research Paper.

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Introduction

In recent years, significant attention has been paid to phytobiotics and medicinal plants due to their natural healing potential for replacing synthetic nutritional supplements in human and animal feeding (Amirteymoori et al., 2021). Essential oils, alkaloids, flavonoids, tannins, and phenolic acids are among the bioactive compounds of these natural products and have antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. (Mohammadabadi et al., 2025). As efforts are being made to reduce the use of synthetic chemicals and antibiotics, phytobiotics are one of the alternatives and are very suitable options for promoting health, increasing immunity and improving physiological performance in various organisms (Safaei et al., 2025). Compared to antibiotics, they have more advantages as natural antimicrobial growth promoters and can be used to improve zootechnical parameters, reduce the incidence of certain diseases, increase antioxidant defense, regulate cholesterol levels, support digestive enzyme activity, and promote overall liver function (Roudbar et al., 2015; Khezri et al., 2025). Phytobiotics have been shown to improve nutrient absorption, immune function, and metabolic efficiency, and they do so by modulating the composition of the gut microbiota (Vahabzadeh et

al., 2021). Also, by adding these bioactive plant compounds to the diet, it is feasible to increase feed intake, improve feed-to-muscle turnover, increase physical performance, and enhance physiological resilience (Vahabzadeh et al., 2020). Similarly, they can improve nutrient utilization leading to a decrease in stress markers, a better meat quality, and expression of genes involved in carcinogenesis as well as environmental impact energy (Mohammadabadi et al., 2024). Medicinal plants have been the source of traditional medicine in Alxora and many modern pharmaceuticals are synthesized directly from their plant's medicinal compounds. Hence the plant for treatment of diseases is a complementary, and in few cases an alternative. Coriandrum sativum (C. sativum), identified as coriander, is a herbaceous annual plant of the Apiaceae family. Commonly naturalised in the southern, arid zone of Australia It is grown as a crop for its many medicinal uses. The plant is native to Mediterranean countries and the Middle East, with its seeds, leaves, and other aboveground parts having been used for fever, inflammation, gastrointestinal disorders, and other conditions in the Ayurvedic system of medicine as well as in Unani medicine and Traditional Chinese Medicine (Chahal et al., 2017). The plant also comprises a range bioactive constituents including phenolic acids, flavonoids, sterols, and essential oils particularly linalool that are substantially responsible for its pharmacological properties. Its leaves are highly proteinaceous and also contribute to calcium, phosphorus, zinc, iron and vitamins (Mandal & Mandal 2015). All parts of the plant, seeds, fruits, leaves and roots are rich sources of diverse chemical constituents that contribute to its enormous healing properties. The most extensively investigated quality of C. sativum is its antioxidative capacity, ascribed to the scavenging influence of free radicals and involvement in oxidative imbalance mechanisms. Oxidative imbalance is involved in the aetiology of many chronic diseases like diabetes, cancer, neurodegenerative disorders and cardiovascular disease. C sativum extracts have been shown to attenuate lipid peroxidation and increase the activities of endogenous antioxidant enzymes; GPx, CAT, and SOD both in in vitro and in vivo investigations (Tang et al., 2013). Thus, including C. sativum in food products has been suggested to be a means of augmenting dietary antioxidants and reducing oxidative stress induced damage. Apart from its antioxidant potential, C. sativum possesses antibacterial, antimutagenic, antidiabetic, anticholesterolemic and anticancerous along with hepatoprotective and anxiolytic performance (Mahleyuddin et al., 2021; Nadeem et al., 2013). Other bioactive compounds, such as thymol, gallic acid and bornyl acetate also contribute to its anti-inflammatory, chemopreventive and neurorelaxant activities (Riella et al., 2012). On account of its diverse phytochemical composition and extensive range of therapeutic utility, C. sativum has garnered immense scientific attention as a potential defensive agent to counter the toxicity triggered by exposure to chemicals. Methotrexate (MTX) is a well-established antifolate chemotherapeutic and immunosuppressive drug that functions primarily by interfering with the

enzyme dihydrofolate reductase (DHFR), an enzyme involved in synthesizing nucleotides required for DNA replication and cell division (Tian et al., 2020). MTX is often used to treat numerous cancers, such as breast cancer (Bonadonna et al., 2008), testicular tumors, non-Hodgkin's lymphoma and acute lymphoblastic leukemia. Nevertheless, MTX is associated with severe cytotoxic and genotoxic side effects despite its clinical use. It has been observed that MTX caused a wide range of numerical and structural chromosomal abnormalities in albino mouse models such as polyploidy, end-to-end association, chromatid breaks, deletions fragments and ring chromosomes (Cunningham & Zygmunt; 1994; Alfy et al., 2016). These changes are usually dose-related, and increasingly apparent with longer exposures. MTX was also found to reduce the percentage of mitotic index and increase the incidence of micronuclei, suggesting deep cytostatic/genotoxic effects on bone marrow cells (Alfy et al., 2016). Moreover, MTX has been associated to developmental toxicity in a variety of vertebrate species (Pellizzer et al., 2004). Because of the increasing interest in natural antioxidants and their apparent ability to mitigate mutagenic agents by scavenging free radicals and boosting the cellular antioxidant defense system (Deepa & Anuradha, 2011), investigation on the defensive influence of phytochemicals to counteract MTX toxicity is important. Accordingly, the current investigation was undertaken to assess the protective role of C. sativum seed extract on methotrexate-induced chromosomal aberrations and cytotoxicity in albino mice.

Material and methods

Preparation of Coriandrum sativum seed extract: Fully developed seeds of C. sativum were procured from local market and cleaned properly. The seeds were dried and finely powdered. The powdered mass was cold macerated at 25 C° for 24 h and heated at 65 °C for 30 min to aid extraction. After measuring absorbance and/or fluorescence reference spectra, the mixture was filtered and kept in a dark-protected container at 4 °C pending use according to Kansal et al. (2011).

Animals: 24 healthy white Swiss mice; (Mus musculus) of both sexes each, 12–13-week-old and weighing 30-35 g were used in the study. The animals were maintained at conventional laboratory circumstances of a temperature-regulated room and a 12-hour light/dark cycle. They were grouped housed (five animals/cage) in acrylic cages with wood-shaving bedding. Commercial feed and tap water were available ad libitum within the entire period of research. All the animals were randomly placed into four groups (n = 6/group) as detailed below: Group 1 (Control group): The control set of mice received only distilled water orally for six consecutive days, Group 2 (MTX Group): A single intraperitoneal injection of MTX at a dose of 20 mg/kg body weight was given to mice (Padmanabhan et al., 2009), Group 3 (C. sativum group): C.

sativum seed extract has been given orally for subsequent six days to the normal mice at a dose level of 600 mg/kg body weight (Kansal et al.,2011; Donia,2019.), and finally in Group4(MTX +C. sativum group): Mice treated with MTX by (i.p.) injection of methotrexate followed by oral administration of C. sativum extract at a dose rate of hair oil perfumed worms per kg body weight daily as well for consecutively the six days respectively measurement from Kansal et al,(2011).

Mitotic index (MI) analysis: Mitotic index was determined by observation under a light microscope at 40× magnification of stained bone marrow slides. At least 1,000 cells (including both mitotic and non-mitotic ones) were scored for each animal. Mitotic index was calculated as follows:

$$\text{MI (\%)} = \left(\frac{\text{Number of dividing cells}}{\text{Total number of counted cells}}\right) \times 100$$

CA assay: Chromosomal aberrations in bone marrow cells were examined by the procedure as reported by Preston et al. (1987). In short, metaphase spalced was prepared and stained with G- staining technique adopted routinely in laboratory of clinical cytogenetics for detection of structural and numerical abnormalities using light microscope.

Statistical analysis: The data were analyzed using the SPSS. Data are presented as mean ± SE. Differences between groups were tested by one-way ANOVA, where significance was considered at $P \leq 0.05$.

Results

Influence of MTX and C. sativum extract on mitotic index in mice: A decrease in the MI was recorded both by marrow cells of treated with MTX mice, and exposure to the extracts. As shown in Table 1, the number of dividing cells in the MTX group (20 mg/kg) was meaningfully lower than that of the control group ($p \leq 0.05$), indicating this dose repressed BM proliferation powerfully via cytotoxic effect. However, treatment with C. sativum extract (600 mg/kg) only (MI compared to control $p \leq 0.05$) did not lead to a significant change as contrasted with the control value, suggesting that the extract is neither toxic nor antiproliferative under normal physiological conditions. Of more interest is the observation that C. sativum extract co-administration with MTX (600 mg/kg + 20 mg/kg) resulted in a marked elevation of MI as contrasted with the MTX-induced group ($p < 0.05$). This demonstrates that C. sativum prevented against the MTX-introduced mitotic arrest. The MI of the combination group was still lower relative to control, ($p \leq 0.05$), showing partial recovery of mitotic figures only. All of these would suggest that even as MTX effectively suppresses BM cell division, C. sativum co-administration modulates MTX cytotoxicity, owing to its antioxidant and cytoprotecting capacities.

Table 1. Effect of MTX and C. sativum extract on mitotic Index in mice bone marrow

Groups	Mitotic index (Mean ± SE)
Control group	7.93 ± 0.01*
Methotrexate (20 mg/kg)	2.87 ± 0.03*
C. sativum (600 mg/kg)	8.05 ± 0.02
C. sativum (600 mg/kg) + MTX (20 mg/kg)	6.06 ± 0.01*

* Significant variation at P≤0.05.

Effect of MTX and C. sativum extract on CAs in mouse bone marrow: Treatment with methotrexate was found to cause a marked increase in the prevalence of chromosomal aberrations (CAs) in mouse BM. MTX-treated mice showed increased structural abnormalities (chromatid gaps, fragments, breaks and ring chromosomes; $p \leq 0{\cdot}05$) when in contrast with those in control group as shown in Table 2. This is another evidence supporting the high genotoxicity of MTX. Treatment of mice with C. sativum extract only (600 mg/kg) did not result in a significant increase in chromosomal aberration frequency compared to the control group ($p \leq 0.05$), suggesting no geno-toxic effect at this dose level. Significantly less chromosomal aberrations in contrast with to the solo-MTX group were observed in the C. sativum + MTX co-treatment condition ($p < 0.01$). Although the values were still elevated compared with controls ($p \leq 0.05$), these findings indicated that the extract exerted a partial in vivo protective effect. This genotoxicity reduction could be due to some C. sativum phytochemicals, such as flavonoids and phenolic compounds, which have demonstrated antioxidant characteristics as well as antimutagenic and DNA-protective effects. These findings encourage the hypothesis that C. sativum may be used as a natural substance which could alleviate the cytogenetic damage induced by chemotherapeutic treatments including MTX.

Discussion

The bioactive principles present in essential oils and aroma extracts of herbs spices have biogenic activity like antibacterial, antifungal, antioxidant which are used by the food processing industries and pharmaceutical sectors extensively (Agnihotri et al. Besides the flavor of food, humans have for a very long time understood that foods can be used as an aid to health. In the current research, therefore, we assessed possible protective influence of Coriandrum sativum (C. sativum) extract against methotrexate (MTX)-induced reduction in mitotic index of mice and explored the cytotoxic impact of MTX on bone marrow cell proliferation. Consistent with the known antimetabolite and cytostatic mode of action of MTX, our results showed a considerable

decrease in the mitotic index for the treated group with respect to control. MTX is an inhibitor of dihydrofolate reductase, thus affecting folate metabolism/DNA synthesis and cell division with most susceptible tissues being those that are rapidly-growing, including the bone marrow (Howard et al., 2016).

Table 2. Effect of MTX and C. sativum Extract on CA in mouse bone marrow

Groups	Chro. Gap (Mean ± SE)	Chro. Frag (Mean ± SE)	Chro. Break (Mean ± SE)	Chro. Ring (Mean ± SE)	Total (Mean ± SE)
Control group	0.03 ± 0.02	1.06 ± 0.04	1.03 ± 0.06	1.00 ± 0.01	3.12 ± 0.07*
Methotrexate (20 mg/kg)	5.04 ± 0.05	6.94 ± 1.03	5.81 ± 0.03	7.02 ± 0.02	24.81 ± 1.13*
C. sativum (600 mg/kg)	0.08 ± 0.01	1.07 ± 0.03	1.03 ± 0.01	1.00 ± 0.01	3.18 ± 0.06*
C. sativum (600 mg/kg) + MTX (20 mg/kg)	2.03 ± 1.01	3.03 ± 0.04	2.07 ± 0.01	3.04 ± 1.02	10.17 ± 2.08*

* Significant variation at P≤0.05.

The significant decrease in mitotic activity we observed in MTX-treated mice coincides with previous reports of MTX-induced bone marrow toxicity involving chromosomal abnormalities, cell cycle arrest, and hematological depression (Fenech 2000). 1, 2 These unpleasant side effects have restricted high therapeutic dosing of MTX clinically, particularly with regard to its application as an immunosuppressant and cytotoxic agent. The significant decrease in mitotic index induced with MTX was highly inhibited when mice were co-treated with extract of C. sativum. The fact that mitotic index in the group treated with C. sativum and MTX did not reach baseline, does indicate that its protective effect is not enough to bring it back to a normal state. Extract alone did not significantly abrogate the mitotic index indicating its absence of intrinsic cytotoxicity at this concentration. These observations support earlier reports of C. sativum cytoprotective and antioxidative properties linked to its high flavonoids (e.g. quercetin), polyphenolic compounds (e.g., caffeic and ferulic acids) and essential oils, like linalool content (Savelev et al., 2003). Being a recognized antioxidant (Chahal et al., 2017), C. sativum could

provide protection by scavenging free radicals and activating endogenous antioxidant enzymes like GPx, SOD and CAT.

On the other hand, the results of this study also show that MTX significantly enhances frequencies of different types of chromosomal aberrations. The principal pathways through which MTX causes chromosomal instability are oxidative stress, inhibition of purine synthesis and disorganization of mitotic spindle function. MTX has also been showed to induce genomic instability in female mice, leading to chromosome misalignment of oocytes and decreased DNA methylation (Tian et al., 2020). These cytogenetic findings emphasize the need to appreciate MTX side effects, such as infertility, teratogenicity and secondary malignant neoplasms. Consequently, studying the effect of MTX on chromosomal integrity in albino mice is required in order to predict its genotoxicity and for devising strategies to reduce such effects. Earlier studies have demonstrated that inactivation of Dihydrofolate reductase by MTX is the major cause for chromosomal alterations followed by disruption of DNA synthesis and folate metabolism causing replication stress and chromosomal breakage. It can lead the appearance of abnormal morphologies, e.g. chromatid breaks, gaps and rings or numerical changes like polyploidy (Bonadonna et al., 2008). Because a higher concentration of MTX induces more chromosomal damage, severe cytotoxicity and suppressed mitotic activity might paradoxically reduce the number of abnormalities observed. MTX-induced genotoxicity is dose- and time-dependent. In addition, MTX promotes the production of reactive oxygen species (ROS) that increase oxidative stress-induced DNA damage and chromosomal instability. These effects are mediated by alterations in LOOs that were reported previously to result in an elevation in the number of chromosomal anomalies, and sister chromatid exchanges in human lymphocytes in vitro exposed to MTX (Rababa'h et al., 2021).

Genotoxicity Chromosomal anomalies in bone marrow cells were non-significant between the C. sativum and combined C. sativum + MTX group as compared with control group. By contrast, chromosomal anomalies were markedly higher in MTX-treated mice than in controls. Co-treatment of C. sativum extract with MTX resulted in significant decrease chromosomal aberrations compared with the MTX only, thereby indicating that the extract has a protective potential against MTX-induced genotoxicity (Table 2). These findings are in constituent with prior findings that MTX elevates the average of chromosomal anomalies and also the number of abnormal metaphases in mouse bone marrow (Alam et al., 2011). MTX inhibits dihydrofolate reductase (DHFR) and induces ROS, disrupting DNA synthesis and repair, resulting in chromosomal abnormalities, induction of micronucleus formation assays (MNF), and DNA strand breaks MTX exerts these main effects through inhibition of the enzyme DHFR involved in the reduction of dihydrofolate to tetra-hydrofolic acid a critical step for de novo nucleotide

biosynthesis. Some research confirms DNA protective and antioxidant effects of C. sativum. For example, ethyl acetate root extracts reduced H_2O_2-induced DNA damage significantly as evaluated in comet assays and contained high levels of phenolics (Tang et al., 2013). In addition, oral treatment with C. sativum extracts substantially reduced the occurrence of micronucleated PCEs induced by cyclophosphamide, suggesting reduced chromosomal damage in vivo (dos Santos et al., 2018). Furthermore, antimutagenic activity was also exhibited by C. sativum against metabolized aromatic amines (Cortés-Eslava et al., 2004). Antioxidants like metformin were also reported to decrease oxidative DNA damage and MTX-related genotoxicity. Collectively, MTX is found to be a potent CA inducer indicating the importance of monitoring genomic stability and secondly certain long-term protective strategies during MTX therapy (9). Taken together, available evidence indicates that C. sativum root is a potential candidate to treat the diseases related to cancer and oxidative stress. This potential is closely related to its antioxidant potential, which could prevent the advancement of diseases and can be used in treatment options, either through dietary supplementation or co-treatment with traditional drugs. These are also described earlier in the literature regarding traditional medicinal uses of C. sativum (Tang et al., 2013).

Conclusions: Overall, it can be concluded from the present findings that MTX; an anti-proliferative, a chemotherapeutic since long does produce significant cytotoxic and genotoxic effects in rapidly proliferating tissues through its marked inhibition of mitosis as well as markedly elevation value of chromosomal aberrations in bone marrow cells. By contrast, C. sativum extract at one dose tested appears a safe preparation because it did not demonstrate chromosomal integrity damaging or mitogenic effect in isolation. Of note, C. sativum extract co-administration significantly decreased the frequency of chromosomal anomalies and in part restored the mitotic activity of MTX-treated animals. These results indicated that C. sativum possesses antioxidant phytoconstituents with antigenotoxic and cytoprotective potential, facilitating genomic stability and abatement of oxidative stress. Thus, this work endorses C. sativum as a natural adjuvant in reducing bone marrow toxicity caused by MTX. Research is warranted to explain the molecular mechanisms behind these protective effects and determine their therapeutic implications for human.

Author Contributions

The author formulated the research idea, planned the genetic layout and coordinated all experimental procedures (including animal handling to cytogenetic analysis). All the data of this work were extracted, analyzed statistically and interpreted on

chromosome/micromorphology/meiosis by the author himself. Draft of manuscript was prepared and approved by the author.

Data Availability Statement

All the data regarding raw cytogenetic observation, mitotic index were produced via this study is maintained by the author and could be supplied to researchers upon request for good academic purpose.

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Ethical Considerations

All the animal procedures were performed as per institutional and national guidelines for laboratory animal care. All efforts were made to reduce the discomfort, stress and harm of experimental mice.

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Conflict of Interest

The author has no financial, academic or personal conflicts to disclose that could have influenced the design, conduct and reporting of this study.

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فعالیت محافظتی عصاره گشنیز بر روی سمیت ژنتیکی ناشی از متوترکسات در موش‌های آلبینوی سوئیسی

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چکیده

هدف: گیاهان ادویه‌ای به دلیل دارا بودن خواص درمانی متنوع و ترکیبات زیست‌فعال ضروری مشتق‌شده از آنها از زمان های قدیم مورد توجه بوده اند. یکی از این گیاهان گشنیز (C. sativum) است. این گیاه یکساله است، ارزش تجاری بالایی دارد، فعالیت‌های ضدباکتریایی، آنتی‌اکسیدانی، ضدهیپرلیپیدمی، ضددیابتی و محافظت‌کننده عصبی دارد و به دلیل دانه‌ها و برگ‌های تازه‌اش در مناطق مدیترانه‌ای و اروپایی مورد استفاده قرار میگیرد. لذا، هدف این مطالعه بررسی مکانیسم محافظتی عصاره گشنیز در برابر ناهنجاری‌های کروموزومی ناشی از متوترکسات (MTX) و تغییرات در شاخص میتوزی در سلول‌های مغز استخوان موش‌ها بود.

مواد و روش‌ها: بیست و چهار موش بالغ در چهار گروه شش تایی به طور تصادفی قرار گرفتند. به گروه کنترل منفی (گروه اول) شش روز متوالی آب مقطر را به صورت خوراکی مصرف کردند. گروه دوم یک تزریق داخل صفاقی MTX (i.p.) (۲۰ میلی‌گرم بر کیلوگرم وزن بدن) داشتند. به گروه سوم به مدت شش روز عصاره C. sativum به صورت خوراکی با دوز ۶۰۰ میلی‌گرم بر کیلوگرم وزن بدن داده شد. گروه چهارم دریافت کننده یک دوز داخل صفاقی MTX (۲۰ میلی‌گرم بر کیلوگرم) و به دنبال آن عصاره C. sativum (۶۰۰ میلی‌گرم بر کیلوگرم) به مدت شش روز بود. پس از اتمام آزمایش ها، برای ارزیابی ناهنجاری‌های کروموزومی و تعیین شاخص میتوزی با استفاده از روش‌های استاندارد سیتوژنتیک از نمونه‌های مغز استخوان استفاده شد.

نتایج: موش‌هایی که MTX دریافت کرده بودند نسبت به گروه کنترل ناهنجاری‌های کروموزومی بسیار بیشتر و شاخص میتوزی بسیار پایین‌تری را نشان دادند. در حالی‌که، حیوانات تیمار شده با عصاره C. sativum نسبت به گروه کنترل ناهنجاری‌های کروموزومی بسیار کمتر و شاخص میتوزی بسیار بالاتری را نشان دادند. همچنین در گروهی که هم با MTX و هم با C. sativum تیمار شده بودند اثر محافظتی خوبی را نشان دادند که میتواند نشان دهنده این باشد که C. sativum با اثرات سیتوتوکسیک و ژنوتوکسیک MTX مقابله کند.

نتیجه گیری: با توجه به مشاهده کاهش ناهنجاری‌های کروموزومی و افزایش شاخص میتوزی در اثر تیمار با C. sativum، می توان نتیجه گرفت که این عصاره پتانسیل خوبی برای محافظت سلولی و محافظت ژنتیکی در برابر آسیب ناشی از MTX را دارد. لذا، نتایج این پژوهش، از توانایی بالقوه C. sativum به عنوان یک عامل شیمی‌درمانی پیشگیرانه امیدوارکننده برای کاهش اثرات نامطلوب ژنوتوکسیک متوترکسات حمایت می‌کنند.

کلمات کلیدی: شاخص میتوزی، گشنیز، متوترکسات، موش، ناهنجاری‌های کروموزومی

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