

The Inhibitory Effect of Aqueous Ginger Extract on the Genotoxicity of Dexamethasone in Male Albino Mice

Aseel Raheem Al-Aamiri*¹

Abstract

Background: Many natural substances generated from plant crude extracts have recently been shown to protect against the harmful effects of a variety of pollutants. Ginger (*Zingiber officinalis*) is a widely used spice and medicinal herb.

Methods: To study the effect of aqueous ginger extract in inhibiting the genotoxicity of dexamethasone, we gave the first group dexamethasone (0.4 mg/kg) only. The second group was treated with an aqueous extract of ginger (50 mg/kg) only. The third group was treated with an aqueous extract of ginger followed by dexamethasone with a two-hour interval between doses. The last group was treated with dexamethasone and an aqueous ginger extract simultaneously. To perform genetic tests, we used mitotic index, chromosomal aberrations, and micronuclei tests.

Results: After the treatment with dexamethasone, chromosome aberration and micronuclei formation were induced; however, after treatment with an aqueous extract of ginger, chromosomal aberrations and micronuclei were significantly reduced in male mice. The aqueous extract of ginger did not exhibit cytotoxicity and showed high inhibitory efficiency against the toxicity and mutagenicity of dexamethasone.

Conclusion: The aqueous extract of ginger plays a promising role in protecting somatic cells from the cytogenetic effects of dexamethasone, and it reduces chromosomal aberrations and micronuclei in male albino mice.

Keywords: Chromosome Aberrations, Dexamethasone, Mitotic Index, Micronucleus, *Zingiber officinale*.

Introduction

Spices are now valued not only for their culinary qualities but also for their potential health advantages. Spices may offer health benefits due to their antioxidant properties, but their biological effects may also result from their ability to influence various cellular functions, such as cell division, drug metabolism, apoptosis, and differentiation (1). The World Health Organization (WHO) has highlighted the importance of herbal medicine, stating that a wide range of natural products possesses antioxidant, anti-carcinogenic, and anti-mutagenic properties that can be used as chemo preventive medicines (2). Spices and herbs have been used medicinally in

industrialized countries for centuries. For thousands of years, ginger (*Zingiber officinale*), a tropical and subtropical plant belonging to the *Zingiberaceae* family, has been cultivated. It originated in South-East Asia and later spread to other parts of the world, for use as a spice and as medicine (3). The ginger plant has a perennial tuberous root, or rhizome; its stems are erect, rounded, and covered in smooth leaf sheaths, reaching 2 to 3 feet in height. In many nations, the ginger rhizome is consumed as a fresh paste, dried powder, syrup-preserved slices, crystallized ginger (candy), or ginger-flavored tea (4).

Volatile oils and spicy phenolic chemicals

1: Department of Biology, College of Education, Al-Qadisiyah University, Al-Qadisiyah, Iraq.

*Corresponding author: Aseel Raheem Al-Aamiri; Tel: +96 407809345528; E-mail: aseel.alaamiri@qu.edu.iq.

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compounds, including gingerols, shogaols, and zingerone are significant active components of the ginger rhizome (5). The composition of ginger constituents varies depending on their geographic origin and whether the rhizomes are fresh or dried (6). The medicinal uses of ginger rhizome include weight control, immunomodulation, antigenotoxicity, antibacterial, antiarthritic, antioxidant, and anticoagulant effects (7).

Disrupting deoxyribonucleic acid (DNA) synthesis, cellular metabolism, and cell division is the main goal of anticancer drugs (8). Due to their mechanism of action, these drugs are likely to cause cytogenetic abnormalities and mutations. Therefore, anticancer medications are commonly used as mutagens in the majority of antimutagenic experiments (9). Dexamethasone is a synthetic glucocorticoid (GCs) used in clinical settings to treat diseases, brain tumors, and inflammatory conditions. Although dexamethasone is generally well tolerated at low doses, over 50% of patients experience side effects at high doses, including obesity, insulin resistance, muscular atrophy, and cognitive disorders. It is used to treat rheumatoid arthritis and bronchospasm, among other inflammatory and autoimmune diseases. Numerous studies have shown that treating humans and rats with high doses of dexamethasone in combination with corticosterone or cortisol add-on therapy improves mood, memory, and sleep quality (10,11).

The aim of this study is to investigate whether ginger has an antimutagenic effect against the genotoxic effects of the anticancer drug dexamethasone by using the mitotic index (MI), chromosomal aberration (CA), and micronucleus (MN) tests in the bone marrow cells of male albino mice.

Materials and Methods

Dose and Concentration of the Aqueous Ginger Extract (AGE)

The rhizomes of ginger were purchased from the market (Al-Diwaniyah, Iraq), chopped into pieces, and ground into a fine powder. Then, 125 g of the powder was macerated in one liter

of distilled water for 12 hours at 25 °C, then filtered. The filtrate was dried in the oven at 45 °C, powdered again, and stored at 4 °C (12).

Dose and Concentration of the Dexamethasone

Every glass ampoule (2 mL) contains dexamethasone sodium phosphate (equivalent to 8 mg dexamethasone), from AL-Forat Drug Store, MEDLAKPHARMA ITALI company was tested at a dose 0.4 mg/kg, and it was found that this concentration caused genotoxicity in mouse bone marrow. The drug solution was diluted with distilled water to achieve the desired dose and concentration of 0.01 mg/mouse (13).

Experimental Animals

All experiments involving mature and healthy albino Swiss male mice (*Mus musculus*) were **conducted using animals** obtained from the College of Veterinary Medicine at Al-Qadisiyah University, Iraq. At the beginning of the experiment, the mice weighed 25–30 g, and their age ranged from 11 to 12 weeks. The animals were kept under standard laboratory conditions, such as controlled temperature and light/dark cycles. They were housed in plastic cages with wood shavings for bedding with water and food available *ad libitum* throughout the experiment.

The animals were divided as follows:

1. Control group: Mice were given 0.2 mL of distilled water for six days.
2. Dexamethasone group: Mice were administered a therapeutic dose of dexamethasone drug (dissolved in distilled water) orally 0.4 mg/kg, 0.25 mL for six days.
3. Ginger (50 mg/kg) group: Mice were given an aqueous extract of ginger orally at 0.2 mL for six days.
4. Ginger then Dexamethasone group: Mice were given an aqueous extract of ginger (50 mg/kg, 0.2 mL orally), followed by dexamethasone (0.4 mg/kg, 0.25 mL orally) with a 2-hour interval between doses for six days.
5. Dexamethasone with Ginger group: Mice were given dexamethasone(0.4 mg/kg, 0.25

mL orally) with a 2-hour interval between doses for six days.

6. Dexamethasone then ginger group: Mice were given dexamethasone (0.4 mg/kg, 0.25 mL orally), followed by an aqueous ginger extract (50 mg/kg, 0.2 mL orally) with 2-hour interval between doses for six days.

A cytogenetic examination of bone marrow samples was performed. The slides were examined using light microscopy.

Chromosomal Preparation

The chromosomes were prepared using the direct technique according to (14) with the following modifications: Mice were injected with 0.25 mL of colchicine (125 g/mL) two hours before euthanasia. Then 5 mL of phosphate buffer saline (PBS) was carefully injected into the femur bone. The test tubes were centrifuged for 10 minutes at $1198 \times g$. As a hypotonic solution, 0.075 M pre-warmed (37°C) potassium chloride (KCl) was added to the tubes (5 mL total volume). The tubes were then incubated in a water bath at 37°C for 20 minutes, with periodic shaking. After incubation, the tubes were centrifuged again for 10 minutes at $1198 \times g$. The fixative solution was added drop by drop with constant shaking until the volume reached five mL.

Mitotic Index (MI) Test

The slides were examined under a light microscope at $40 \times$ magnification. A total of 1,000 cells, including both divided and non-divided cells, were counted. The percentage rate for mitotic cells was determined using the following equation:

$\text{MI}\% = (\text{Number of divided cells} / \text{Total number of cells}) * 100$ (16).

Chromosomal Aberrations (CA) Test

The prepared slides were examined under an oil immersion lens at $100 \times$ magnification, with the cells at the metaphase stage of division where chromosomal aberrations were visible; the percentage of these aberrations was calculated (14). Chromosomal aberrations is essential for investigating the effects that cause certain diseases (15).

Micronucleus (Mn) Test

The experiment was carried out following the technique adopted in (17). The femur bone was obtained after the animals were sacrificed. To collect the cellular content inside the test tube, the bone was flushed with one mL of heat-inactivated human plasma. The test tube was centrifuged at $300 \times g$ for 5 minutes. A thin smear was formed on a clean slide by gently mixing the cellular precipitate. The slide was left to dry for 24 hours at room temperature. The slides were fixed in absolute methanol for 5 minutes, then stained with Giemsa stain for 15 minutes, washed in distilled water (D.W.) and dried. For the micronucleus test, five slides were made for each animal. Under the oil immersion lens, the slides were inspected for the presence of micronuclei in least 1000 polychromatic erythrocytes (PCEs). The following equation was used to calculate the micronucleus index:

$\text{Micronucleus Index}\% = (\text{Number of micronuclei} / \text{Total number of PCE}) \times 100$ (18).

Statistical Analysis

Statistical analysis was performed using one-way ANOVA. A p-value of less than 0.05 was considered statistically significant. The results were presented as mean \pm standard error (SE).

Results

Comparing the control and dexamethasone group showed substantial differences, which are attributable to the toxic effect of dexamethasone, as it reduces the mitotic index (Table 1). Also, when we compare the interaction groups of ginger with dexamethasone, there is a substantial difference compared to the ginger group. These findings demonstrate that combining ginger with dexamethasone is more effective in enhancing the mitotic index of mice bone marrow. The results in Table 2 demonstrate that the number of structural and numerical aberrations increased after treatment with a therapeutic dose of the dexamethasone according to the study. Numerical chromosomal abnormalities were found to be

significant compared to the control group.

The chromosome abnormalities were classified into three main categories (Table 2). The first category was the numerical abnormalities, including polyploidy and aneuploidy. The second category was structural abnormalities including acentric fragments, breaks, ring chromosomes, and fragments of autosomal chromosomes. Polyploidy increased significantly, resulting in a marked rise in the total number of numerical chromosomal abnormalities. On the other hand, the findings show that there is no statistically significant difference in chromosomal aberrations between the ginger and control groups. In ginger with dexamethasone group, the number of structural and numerical chromosomal abnormalities was similar to the control group. In terms of structural and numerical chromosomal abnormalities, ginger treatment administered before and after the drug showed a significant difference when compared to the control group. It was obvious that the total number of

chromosomal abnormalities was reduced in ginger with dexamethasone group, the rate of reduction was (6.6 ± 3.24) compare with Dexamethasone group (31.8 ± 1.99) . As a result, the most efficient strategy to minimize chromosomal abnormalities is the combination of ginger with dexamethasone.

The number of micronuclei in mouse bone marrow rose significantly in dexamethasone group (Table 3). We detected no statistically significant difference in the number of micronuclei in the ginger group compared to the control group. The outcomes of the combination treatments with ginger and dexamethasone that received ginger before and after dexamethasone to the control group. When ginger was used with dexamethasone, the reduction was (2.98 ± 0.08) and was (6.85 ± 0.03) when ginger was used after the dexamethasone and (5.69 ± 1.84) when ginger was used before dexamethasone. As a result, it is clear that combining ginger with Dexamethasone is the most effective way to reduce the proportion of micronuclei.

Table 1. Effects of ginger and Dexamethasone treatments on mitotic index in bone marrow mice.

Groups	No. of examined cells	No. of divided cells	MI%
Control	2000	1770	*8.85± 0.01
Dexamethasone (0.4 mg/kg)	2000	626	*3.13± 0.12
Ginger (50 mg/kg)	2000	1648	8.24± 0.43
Ginger then Dexamethasone	2000	842	4.21± 0.63
Dexamethasone + ginger	2000	1664	*8.32± 0.52
Dexamethasone then ginger	2000	782	*3.91± 0.27

*Significant difference at $P \leq 0.05$. The values are presented as mean ± standard error (SE).

Table 2. Effects of ginger and Dexamethasone treatments on chromosomal aberrations in bone marrow male mice.

Groups	Chromosomes Aberrations						
	Structural aberrations				Numerical aberrations		
	Acentric Fragment	Breaks	Ring	Fragment	Polyploidy	Aneuploidy	Total
Control	0.02±0.12	1.32±0.43	1.16±0.03	0.00±0.00	0.00±0.00	0.38±0.81	*2.8±1.39
Dexamethasone (0.4 mg/kg)	6.21±0.19	4.93±1.01	6.8±0.03	5.74±0.65	5.32±0.06	2.71±0.05	*31.8±1.99
Ginger (50 mg/kg)	0.05±1.01	1.00±0.52	0.32±0.04	0.00±0.000	1.65±0.04	0.64±0.73	*3.6±2.34
Ginger then Dexamethasone	0.34±0.23	2.54±1.04	0.43±1.00	2.65±0.04	6.32±0.43	3.1±0.91	15.3±3.65
Dexamethasone +ginger	0.07±0.03	1.98±2.03	0.03±1.02	0.05±0.11	1.63±0.03	2.85±0.02	*6.6±3.24
Dexamethasone then ginger	1.43±0.01	2.94±2.01	1.97±0.09	3.32±0.90	7.12±0.94	2.71±0.01	19.4±3.96

*Significant difference at $P \leq 0.05$. The values are presented as mean \pm standard error (SE).

Table 3. Effects of ginger and Dexamethasone treatments on micronuclei in bone marrow male mice.

Groups	No. of examined cells	No. of micronuclei	MN
Control	2000	512	* 2.56±0.01
Dexamethasone (0.4 mg/kg)	2000	1462	*7.31±0.32
Ginger(50 mg/kg)	2000	544	* 2.72±1.02
Ginger then Dexamethasone	2000	1138	5.69±1.84
Dexamethasone +ginger	2000	596	*2.98±0.08
Dexamethasone then ginger	2000	1370	6.85±0.03

*Significant difference at $P \leq 0.05$. The values are presented as mean \pm standard error (SE).

Discussion

This study determined whether an aqueous ginger extract could protect somatic cells from the genotoxicity caused by dexamethasone in albino mice. The incidence of micronuclei and chromosomal abnormalities decreased significantly when ginger was combined with

dexamethasone. According to these results, ginger extract might have cytoprotective and antioxidant qualities that reduce the negative effects of dexamethasone when administered together. Dexamethasone, which are frequently used for their immunosuppressive

and anti-inflammatory effects, has been shown to cause genotoxicity in a number of animal models. Its genotoxic effects have been reported in a number of investigations, mostly due to the production of reactive oxygen species (ROS), which can cause mutations, chromosomal abnormalities, and DNA damage (19). Dexamethasone's genotoxic potential was confirmed in our investigation when it was administered to bone marrow cells of male albino mice, resulting in a significant increase in micronuclei development and chromosomal abnormalities. These findings are in agreement with research that demonstrated that Dexamethasone causes DNA fragmentation and oxidative stress, which results in genetic instability (20).

Studies have demonstrated that ginger (*Zingiber officinale*) protects against both physical and chemical genotoxicity or mutagenicity because the substances found in it, like shogaol and gingerol, are well-known for their strong anti-inflammatory and antioxidant properties. These substances have demonstrated the ability of ginger to act against free radicals, inhibit lipid peroxidation, reduce inflammation, and prevent oxidative DNA damage (21). In this investigation, the administration of aqueous ginger extract with dexamethasone treatment led to a significant decrease in chromosomal abnormalities and micronucleus formation and an increase in the mitotic index, similar to the control group, indicating that the antioxidant qualities of ginger are essential in reducing the genotoxic effects of dexamethasone.

Our results are in agreement with studies showing ginger's protective properties against DNA damage induced by a variety of genotoxic chemicals. For instance, ginger extract has been shown to reduce oxidative DNA damage in rats caused by several carcinogens (22). Additional confirmation of ginger's protective role against chemical-induced genotoxicity was provided by findings that ginger extract significantly reduced cyclophosphamide-induced chromosomal damage in mice (23). Ginger's protective effect is probably due to its capacity to alter the

antioxidant defense system. Ginger extract has been shown in numerous studies to enhance the activity of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD), which are essential for scavenging reactive oxygen species (ROS) (24). Ginger extract may prevent oxidative stress-induced DNA damage, which is a major contributing factor to dexamethasone's genotoxicity by strengthening the body's antioxidant defenses. In addition, ginger's preventive properties are further supported by research showing that it inhibits the activation of pro-inflammatory cytokines, which are frequently elevated in response to dexamethasone treatment (25). In this work, we evaluated the protective properties of ginger and the genotoxicity of dexamethasone using a validated *in vivo* model. Future research should look at the role of particular signaling pathways, such as the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which may mediate ginger's protective benefits and is known to control antioxidant responses (26). Moreover, a study demonstrates that *Zingiber officinale* effectively and dose-dependently inhibits doxorubicin-induced hepatotoxicity in male rats through its antioxidant properties (27). Nonetheless, a number of factors need to be considered. First, even though we observed protective effects of ginger, it is still unknown what precise chemical mechanisms by which ginger exerts this effect remain unknown. Additional research is required to confirm these findings in clinical settings and investigate ginger's potential as a natural protective agent in conjunction with conventional pharmaceutical therapies.

According to the study's findings, aqueous ginger extract significantly reduces the genotoxicity induced by dexamethasone in male albino mice. The frequency of micronuclei and chromosomal abnormalities was reduced, while the mitotic index increased when ginger was administered in combination with dexamethasone. Based on these findings, ginger's strong anti-inflammatory and antioxidant properties may be crucial in preventing the oxidative stress and damage

caused by dexamethasone. Nevertheless, further investigation is required to elucidate the molecular mechanisms underlying ginger's protective effects and to optimize its therapeutic application, especially in clinical settings involving humans. This study provides a foundation for future research on ginger's potential to mitigate glucocorticoid-induced genotoxicity and enhance patient safety during long-term dexamethasone therapy.

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Conflicts of Interest

The author declares that there are no conflicts of interest.

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