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The preventive effect of aqueous extract of *Cinnamomum zeylanicum* against cytotoxicity produced by cyclophosphamide in male white mice

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Abstract

Objective

Cinnamomum zeylanicum (true cinnamon) is a medicinal plant traditionally applied for its wide range of therapeutic properties, containing antioxidant, anti-inflammatory, antimicrobial, and cytoprotective effects. Recent investigations have highlighted its bioactive compounds, such as cinnamaldehyde and eugenol, which may play a task in modulating oxidative stress and cellular damage. Cyclophosphamide is a widely applied alkylating chemotherapeutic agent, particularly effective in treating different cancers and autoimmune conditions. However, its clinical utility is often limited due to its genotoxic and cytotoxic side effects, containing chromosomal aberrations and reproductive toxicity. Developing adjunct therapies to mitigate these side effects is of increasing interest. This research aims to evaluate the protective task of *C. zeylanicum* against cyclophosphamide-induced genotoxicity and cytotoxicity in male albino mice, potentially supporting its application as a natural chemoprotective agent.

Materials and methods

Thirty adult male albino mice were randomly divided into three groups (n = 10 per group). Group I served as the negative control and received distilled water. Group II was administered a single intraperitoneal dose of cyclophosphamide (20 mg/kg body weight). Group III received both *C. zeylanicum* extract (100 mg/kg body weight) and cyclophosphamide (20 mg/kg). The research assessed chromosomal aberrations in bone marrow cells, abnormalities in sperm head morphology, and the mitotic index to define the extent of genotoxic and cytotoxic effects.

Results

Mice treated with *C. zeylanicum* in combination with cyclophosphamide displayed a meaningful reduction in chromosomal aberrations and sperm head abnormalities compared to the group

receiving cyclophosphamide alone. Additionally, a notable enhance in the mitotic index was observed in the co-treatment group, denoting a protective and possibly regenerative effect on bone marrow cell proliferation.

Conclusions

The findings of this research demonstrate that *C. zeylanicum* possesses a protective effect against cyclophosphamide-induced genotoxic and cytotoxic damage in male albino mice. Its antioxidative and anti-inflammatory constituents may help preserve cellular integrity and enhance recovery following chemotherapeutic insult. These results suggest that *C. zeylanicum* could be considered as a complementary therapeutic agent to diminish the adverse side effects of cyclophosphamide. Further research, containing molecular analyses and clinical trials, is recommended to better understand its mechanisms of action and to evaluate its safety and efficacy in human subjects.

Keywords: chromosomal aberrations, *Cinnamomum zeylanicum*, cyclophosphamide, genotoxicity, sperm abnormalities

Paper Type: Research Paper.

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Introduction

Cyclophosphamide (CP) is one of the most widely applied and effective alkylating agents in the field of chemotherapy. It plays a critical task in the treatment of different malignant neoplasms and is also recognized as a potent immunosuppressive drug, often administered for autoimmune disorders and as supportive therapy in organ transplantation, especially bone marrow transplants (Emadi et al., 2009). Despite its proven efficacy in clinical oncology, cyclophosphamide's

therapeutic benefits are accompanied by meaningful drawbacks, primarily due to its toxic effects on normal, rapidly dividing cells. This includes bone marrow suppression, reproductive toxicity, gastrointestinal damage, and oxidative stress-related organ damage (Abd Elhalim et al., 2017).

CP is applied extensively to manage both hematologic malignancies—such as leukemia and lymphoma—and epithelial tumors, containing breast cancer, ovarian cancer, and small-cell lung carcinoma, where it serves as a cornerstone of combination chemotherapy regimens (Ahlmann & Hempel, 2016). Nevertheless, cyclophosphamide exerts genotoxic and cytotoxic effects by inducing DNA cross-linking, thereby impeding DNA replication and transcription. A major consequence of this mechanism is the induction of oxidative stress, which results in enhanced lipid peroxidation and the depletion of endogenous antioxidants like glutathione (Kocahan et al., 2017). This oxidative damage adversely affects different organ systems, containing the heart, liver, kidneys, and bone marrow, often manifesting clinically as anemia, leukopenia, and thrombocytopenia, thereby limiting the tolerability and continuation of chemotherapy in many patients (Abd Elhalim et al., 2017).

Due to these challenges, there is growing interest in identifying agents that can mitigate the side effects of cyclophosphamide without interfering with its therapeutic efficacy. One promising area of research involves natural compounds with antioxidant, anti-inflammatory, and anti-mutagenic properties. Numerous investigations have displayed that natural antioxidants can alleviate chemotherapy-induced toxicity by scavenging free radicals and enhancing cellular defense mechanisms (Habibi et al., 2015). Among these natural agents, medicinal plants are of particular interest, given their long history of use in traditional medicine systems and their relatively favorable safety profiles.

Cinnamomum zeylanicum (commonly known as true cinnamon) is a tropical evergreen tree from the Lauraceae family, native to Sri Lanka and widely distributed across South and Southeast Asia. It has been applied for centuries in culinary, cosmetic, and medicinal applications and is known by different names worldwide, containing *yook gway* (Chinese), *kaneel* (German), *canela* (Spanish), and *dalchini* (Hindi) (Aneja et al., 2009). The bark of *C. zeylanicum* is rich in bioactive compounds such as cinnamaldehyde, eugenol, trans-cinnamic acid, proanthocyanidins, kaempferol, and other flavonoids and phenolic acids. These constituents have demonstrated a wide range of pharmacological activities, containing antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Uma et al., 2009).

These properties are largely attributed to the plant's polyphenolic and volatile oil components. Cinnamaldehyde, for instance, has been reported to inhibit NF-κB signaling pathways, thereby reducing inflammation, while proanthocyanidins exhibit strong free radical scavenging activity. Moreover, kaempferol and cinnamic acid derivatives contribute to the

regulation of apoptosis and modulation of oxidative stress pathways. However, the bioavailability of these compounds can be affected by physicochemical and biological conditions—such as pH, temperature, and enzymatic activity—during digestion, which may influence their therapeutic potential.

Modern research has increasingly validated the traditional uses of *C. zeylanicum*, particularly its task in protecting against oxidative stress and chemically induced cytotoxicity. The plant's extract has been investigated in different in vivo and in vitro models, demonstrating potential to mitigate the adverse effects of drugs that induce genotoxic and reproductive toxicity. These protective effects are particularly relevant in the context of chemotherapeutic agents like cyclophosphamide, which are known to cause damage to rapidly dividing cells, containing bone marrow and spermatogenic cells.

Parallel to its medical applications in humans, *C. zeylanicum* and other phytobiotics are also being explored for their tasks in animal health and nutrition. In veterinary science and livestock production, medicinal plants have gained attention as natural alternatives to antibiotics (Hajalizadeh et al., 2019; Jafari Ahmadabadi et al., 2023). The use of phytobiotics in animal feed has been displayed to improve zootechnical parameters, suppress certain infectious diseases, and promote overall health by enhancing antioxidant defenses and modulating the immune response (Amirteymoori et al., 2021; Mohammadabadi et al., 2023).

Investigations have displayed that dietary supplementation with medicinal plants such as *C. zeylanicum* can enhance feed consumption, improve feed conversion ratios, and enhance carcass yield. In addition, these plants exhibit hypocholesterolemic effects, stimulate digestive enzymes, support liver function, and offer protective effects against oxidative damage and cytotoxicity (Safaei et al., 2022; Shokri et al., 2023; Mohammadabadi et al., 2024). For example, Vahabzadeh et al. (2020) and Shokri et al. (2023) demonstrated that the inclusion of such plants in animal diets contributes to improved growth performance and better health outcomes, further supporting their utility in both clinical and agricultural settings.

In recent years, there has been an escalating interest in developing natural, sustainable, and integrative approaches to disease prevention and treatment. The enhanced recognition of the adverse effects associated with synthetic drugs has spurred a return to plant-based therapies, which often offer multi-targeted actions with fewer side effects (Mohammadabadi et al., 2022). Phytobiotics, especially those derived from spices and herbs traditionally applied in both culinary and medicinal contexts, represent a growing field of research in pharmacognosy and integrative medicine (Shahsavari et al., 2022). Their broad spectrum of biological activities—containing antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer effects—

makes them valuable candidates in the management of complex diseases involving oxidative stress and inflammation (Shahsavari et al., 2023).

Therefore, *C. zeylanicum* presents itself as a compelling natural agent for investigation, particularly regarding its potential to counteract cyclophosphamide-induced cytotoxicity and genotoxicity. Understanding its protective mechanisms could provide insights into developing adjunct therapies that improve patient outcomes during chemotherapy, diminish drug-induced damage to healthy tissues, and enhance overall quality of life. The aim of this research is to investigate the protective effects of *Cinnamomum zeylanicum* against cyclophosphamide-induced genotoxicity and cytotoxicity in male albino mice. The research specifically evaluates chromosomal aberrations, sperm head morphology abnormalities, and changes in the mitotic index (Figure 1) of bone marrow cells as biomarkers of chemotherapeutic toxicity and cellular recovery.

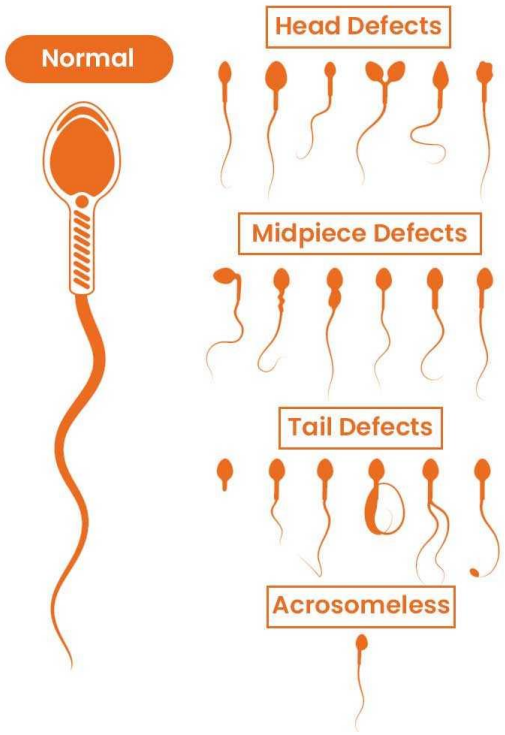

Figure 1. Comparison of the Mitotic Index in Bone Marrow Cells and Abnormal Sperm Head Morphology. The figure illustrates the differences in mitotic activity of bone marrow cells and the frequency of sperm head abnormalities between control and treated groups

Materials and methods

Solutions applied in the experiment: Cyclophosphamide was prepared at a concentration of 20 mg/kg body weight by dissolving 2 mg of the compound in 10 mL of sterile distilled water.

The solution was freshly prepared prior to each administration to ensure stability and efficacy. The selected route of administration was intraperitoneal (IP) injection, which is a commonly applied method for systemic drug delivery in rodents.

The bark of *Cinnamomum zeylanicum* was thoroughly cleaned, shade-dried, and ground into a fine powder using an electric grinder. An aqueous extract was obtained by subjecting the powdered bark to Soxhlet extraction for eight hours using distilled water as the solvent. After completion of the extraction cycle, the mixture was concentrated by evaporation and subsequently dried in a water bath at 50°C to obtain a semi-solid residue. The dried extract was stored in airtight containers at -20°C in a dark environment until use to preserve its phytochemical integrity.

For administration, the extract was reconstituted in distilled water and delivered via intraperitoneal injection at a dose of 100 mg/kg body weight. This dosage and route were selected based on previous investigations denoting its safety and biological activity in vivo.

Experimental animals: The research was conducted using mature, healthy male Swiss albino mice (*Mus musculus*), aged between 12 and 13 weeks and weighing approximately 25-30 grams. All animals were obtained from an accredited animal breeding facility and were allowed to acclimatize for one week before the start of the experiment.

The mice were housed in standard polypropylene cages (five animals per cage) with sterilized wood shavings as bedding material. Environmental conditions were maintained at a controlled temperature of 22 ± 2°C, with a relative humidity of 50–60% and a 12-hour light/dark cycle. The animals were provided ad libitum access to a standard commercial pellet diet and filtered tap water throughout the research period.

The mice were randomly assigned into three experimental groups, with 10 animals per group:

- Group I (Control Group): Received distilled water only via intraperitoneal injection.
- Group II (Cyclophosphamide Group): Received a single intraperitoneal dose of cyclophosphamide at 20 mg/kg body weight.
- Group III (Treatment Group): Received a single intraperitoneal dose of cyclophosphamide (20 mg/kg b.w.) along with an intraperitoneal dose of *Cinnamomum zeylanicum* aqueous extract at 100 mg/kg body weight.

The duration of the experiment was seven days, commencing from the day of compound administration. At the end of the experimental period, all animals were euthanized humanely in accordance with ethical guidelines for animal care and use. No mortality due to treatment was observed during the experimental course.

Chromosome aberration assay: To analyze chromosomal aberrations, each animal was administered an intraperitoneal injection of colchicine at a dose of 0.25 mL (10 mg/kg body

weight) 90 minutes prior to sacrifice. Colchicine was applied to arrest cells in the metaphase stage of mitosis, facilitating chromosomal analysis.

Following colchicine treatment, the animals were euthanized via cervical dislocation, and dissection was immediately performed to isolate bone marrow cells. The animal was positioned dorsally, and its limbs were secured with sterile pins. An incision was made along the abdominal midline, and the femurs were carefully excised under aseptic conditions.

Each femur was flushed with 5 mL of phosphate-buffered saline (PBS, pH 7.4) using a sterile syringe to collect bone marrow cells into centrifuge tubes. The cell suspensions were then centrifuged at 2000 rpm for 10 minutes to pellet the cells.

The supernatant was discarded, and 5 mL of pre-warmed (37°C) 0.075 M potassium chloride (KCl) solution was added to each tube to induce hypotonic swelling of the cells. The tubes were incubated in a water bath at 37°C for 20 minutes with gentle shaking at regular intervals to ensure uniform cell dispersion.

Following incubation, the tubes were centrifuged again at 2000 rpm for 10 minutes. The supernatant was removed, and a freshly prepared cold fixative solution (methanol:acetic acid, 3:1) was added dropwise while gently agitating the tube to avoid clumping. The total volume was brought up to approximately 5 mL. This fixation step was repeated three times to ensure proper chromosome preservation. A few drops of the final cell suspension were dropped onto pre-cleaned glass slides from a suitable height to allow adequate spreading of metaphase chromosomes. The slides were then air-dried on a hot plate at 50°C. Staining was performed using Giemsa stain. Slides were placed in a Coplin jar containing freshly filtered Giemsa working solution (5%) for 10–15 minutes. After staining, the slides were gently rinsed with distilled water, air-dried completely, and stored in labeled slide folders until microscopic examination.

Metaphase spreads were examined under a light microscope using an oil immersion objective (100× magnification). For each slide, 100 well-spread metaphase cells were analyzed to define the frequency and types of chromosomal aberrations. The scoring criteria included structural aberrations such as breaks, gaps, fragments, and exchanges. The results were recorded as a percentage of aberrant cells out of the total number of cells observed (Allen et al., 1977).

Mitotic index: The mitotic index (MI) is a key parameter in evaluating cell proliferation and is considered a critical prognostic indicator in different types of cancer. It provides insights into tumor aggressiveness, patient prognosis, and treatment response. Additionally, MI serves as a valuable marker in toxicological and pharmacological investigations for identifying the cytotoxic and anti-proliferative effects of chemical agents, containing potential anti-mitotic compounds.

To define the mitotic index, bone marrow slides prepared during the chromosome aberration assay were examined under a compound light microscope using high magnification (40×

objective). A total of 1,000 cells (both mitotic and non-mitotic) were counted per animal. Mitotic figures were identified based on standard cytological characteristics such as chromosomal condensation and metaphase plate alignment.

The mitotic index was calculated using the following formula, as described by Shubber et al. (1987):

(MI%) = ({Number of dividing (mitotic) cells}÷{Total number of cells observed})×100

A decrease in MI may indicate cytotoxicity or a suppressive effect on cell proliferation, whereas an enhance may reflect either regenerative activity or abnormal proliferative stimulation.

Sperm head morphology assay: To evaluate the genotoxic impact of treatments on male germ cells, sperm morphology analysis was conducted based on the protocol established by Wyrobek and Bruce (1975). At the end of the treatment period, the cauda epididymides were surgically removed from each animal. The tissues were finely minced in an isotonic physiological saline solution (0.9% NaCl) to release spermatozoa, and the suspension was filtered through a fine mesh to eliminate large tissue fragments. Smears were immediately prepared on clean glass slides and allowed to air-dry. The smears were then stained with 5% Eosin Y solution for 10 minutes, rinsed gently with distilled water, and left to air-dry completely. Stained slides were examined under a light microscope using oil immersion (100× objective) with a green filter to enhance contrast and visualize morphological details. For each animal, at least 1,000 spermatozoa were analyzed for morphological abnormalities, containing amorphous heads, pinheads, hookless, banana-shaped, and double-headed sperm. The frequency of abnormal sperm was recorded as a percentage of the total number of sperm cells examined.

Statistical analysis: All quantitative data were expressed as mean ± standard error (SE). Statistical comparisons between experimental groups were conducted using one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests, where applicable, to define the significance of differences between group means. The statistical analysis was performed using IBM SPSS Statistics v.20.0 software (IBM Corp, 2011). A p-value of ≤ 0.05 was considered statistically meaningful. All experiments were conducted with a sample size of 10 animals per group (n = 10), which provided sufficient statistical power to detect differences in cytogenetic and reproductive parameters.

Results and discussion

Effect of cyclophosphamide on chromosomal abnormalities: The analysis of bone marrow cells from male albino mice treated with cyclophosphamide revealed a meaningful enhance in structural chromosomal abnormalities compared to the untreated control group (Table

1). These findings clearly indicate the genotoxic potential of cyclophosphamide, which aligns with previous investigations reporting its ability to induce DNA damage due to its alkylating nature and production of reactive metabolites. Among the most frequently observed chromosomal aberrations in the cyclophosphamide-only group were chromatid breaks, ring chromosomes, deletions, and chromatid gaps-structural anomalies that are indicative of clastogenic effects and impaired chromosomal integrity. These abnormalities reflect cyclophosphamide's cytotoxic mechanism, which disrupts normal mitotic processes by interfering with DNA replication and repair pathways. Interestingly, the group treated with Cinnamomum zeylanicum extract alongside cyclophosphamide exhibited a meaningfully lower frequency of chromosomal aberrations compared to the group treated with cyclophosphamide alone. This suggests that C. zeylanicum possesses protective properties that can mitigate the genotoxic effects of cyclophosphamide. The reduction in chromosomal abnormalities in this co-treatment group implies that the phytochemical constituents of C. zeylanicum-particularly its polyphenolic compounds and essential oils—may exert antioxidant and anti-mutagenic activities, scavenging free radicals and enhancing DNA repair mechanisms. These findings are consistent with earlier reports highlighting the protective tasks of natural plant extracts against chemotherapeutic agent-induced cytotoxicity. The reduction in chromosomal damage supports the hypothesis that C. zeylanicum may serve as a natural adjuvant therapy capable of attenuating the harmful side effects of chemotherapy drugs like cyclophosphamide without compromising their therapeutic efficacy. Further molecular investigations are warranted to elucidate the precise mechanisms by which C. zeylanicum confers its genoprotective effects, containing its potential impact on oxidative stress markers, DNA repair enzymes, and cell cycle regulatory pathways.

Table 1. Average number of chromosomal aberrations in bone marrow cells of male mice treated with cyclophosphamide and *Cinnamomum zeylanicum*

Groups	Chromatid Break	Ring Chromosome	Deletion	Gap	Total
Control group	0.01±0.32	0.00±0.12	0.12±0.19	0.81±0.04	0.94±0.67
Cyclophosphamide group(20mg/ kg)	3.54±1.01	2.74±0.42	3.06±0.03	4.21±0.05	13.55±1.51*
Cyclophosphamide plus cinnamon group (20 mg/ kg+100 mg / kg)	0.54±0.21	0.53±1.03	0.31±0.04	1.18±0.32	2.56±1.60*

***The meaningful difference at P ≤ 0.05.**

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Effect of cyclophosphamide on the mitotic index: The results presented in Table 2 demonstrate a statistically meaningful decrease ($p \leq 0.05$) in the mitotic index of bone marrow cells in male mice treated with cyclophosphamide (20 mg/kg body weight) compared to the untreated control group. This reduction reflects the cytotoxic and anti-proliferative effects of cyclophosphamide, which is known to interfere with normal cell division by crosslinking DNA strands and impairing replication and mitotic progression. In contrast, the group that received a co-treatment of cyclophosphamide (20 mg/kg b.w.) and *Cinnamomum zeylanicum* extract (100 mg/kg b.w.) exhibited a notable improvement in the mitotic index. The mitotic activity in this group approached levels similar to the control group, suggesting that *C. zeylanicum* plays a protective task in preserving the proliferative potential of bone marrow cells. The observed enhance in the mitotic index in the combination group indicates that *C. zeylanicum* may help counteract the mitotic inhibition caused by cyclophosphamide. This protective effect is likely due to the bioactive constituents of *C. zeylanicum*, which possess strong antioxidant properties that mitigate oxidative damage and support the maintenance of cellular homeostasis. These findings support earlier investigations that suggest phytochemicals, particularly those rich in polyphenols and flavonoids, can modulate the effects of chemotherapeutic agents and protect healthy tissues from their adverse effects. The restoration of mitotic activity in bone marrow cells following *C. zeylanicum* administration further highlights its potential as an adjuvant treatment to alleviate chemotherapy-induced cytotoxicity.

Table 2. Average mitotic index in bone marrow cells of male mice treated with cyclophosphamide and *Cinnamomum zeylanicum*

Groups	No. of mice	Mitotic index (Mean ±SD)
Control group	10	3.42±0.65
Cyclophosphamide group(20mg/ kg)	10	1.33±0.08*
Cyclophosphamide plus cinnamon group (20 mg/ kg+100 mg / kg)	10	3.91±0.04*

*** The meaningful difference at $P \leq 0.05$.**

Effect of cyclophosphamide on sperm head morphology: The results presented in Table 3 and Figure 2 indicate a statistically meaningful enhance in the frequency of sperm head abnormalities in male mice treated with cyclophosphamide (20 mg/kg b.w.) compared to both the control and the co-treatment groups. The observed morphological abnormalities included

hammer-shaped sperm, sperm without a hook, banana-shaped sperm, and sperm with two heads. These types of malformations are indicative of genotoxic stress and defective spermatogenesis. Treatment with *Cinnamomum zeylanicum* extract (100 mg/kg b.w.), when administered alongside cyclophosphamide, led to a marked reduction in the frequency of these sperm abnormalities. The morphology of sperm in this group was closer to that of the control, suggesting that *C. zeylanicum* exerts a protective effect on male germ cells. The enhanced abnormalities observed in the cyclophosphamide-only group are consistent with the known mutagenic and cytotoxic effects of alkylating agents, which interfere with DNA integrity and cell division during spermatogenesis. On the other hand, the phytochemical components of *C. zeylanicum*, particularly polyphenols and cinnamaldehyde, are known for their antioxidant and anti-inflammatory properties, which likely play a task in mitigating these damaging effects. These findings align with previous investigations reporting that natural antioxidants can ameliorate reproductive toxicity induced by chemotherapeutic agents. The results support the hypothesis that *C. zeylanicum* has the potential to safeguard male reproductive health under conditions of drug-induced stress.

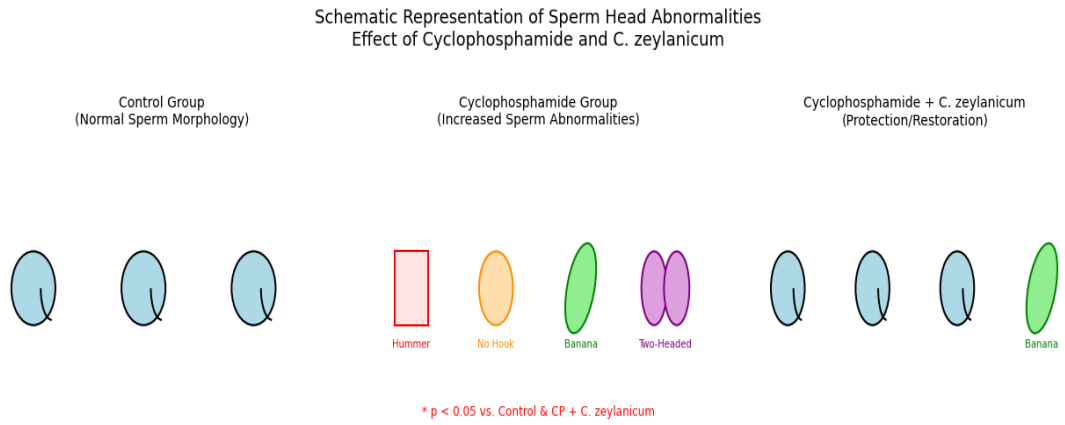

Figure 2. Effect of cyclophosphamide on sperm head morphology

Cyclophosphamide (CP) is one of the most commonly applied chemotherapeutic agents for the treatment of different cancers. However, due to its non-selective mechanism of action, it is equally toxic to both healthy and malignant cells. CP is metabolized in the liver to form active compounds such as phosphoramide mustard and acrolein, which are responsible for its cytotoxic effects. While these metabolites interfere with DNA replication in cancer cells and suppress tumor growth, they also induce mutagenic and genotoxic effects in normal cells, potentially leading to secondary malignancies and impaired cellular functions (Hayatsu et al., 1988; Ghobadi et al., 2017; Iqubal et al., 2019).

Table 3. Average frequency of sperm head abnormalities in male mice treated with cyclophosphamide and *Cinnamomum zeylanicum*

Groups	hummer sperm	sperm without a hook	Banana sperm	sperm with two heads	Total
Control group	0.01±0.38	0.00±1.94	1.19±0.42	0.37±0.12	1.57±2.86
Cyclophosphamide group (20 mg/ kg)	3.51±0.63	3.42±2.12	3.04±0.32	4.31±0.05	14.28±3.12*
Cyclophosphamide plus cinnamon group (20 mg/kg+100 mg/kg)	1.05±0.12	1.64±0.62	1.32±0.01	1.73±1.03	5.74±1.78*

***The meaningful difference at P ≤ 0.05.**

The cytotoxic mechanism of CP primarily involves the formation of DNA adducts and interstrand cross-links through alkylation, particularly at the N7 position of guanine bases. This interferes with the normal function of DNA and RNA, resulting in strand breaks, cell cycle arrest, and apoptosis (Sharma et al., 2012). Moreover, CP and its reactive metabolites tend to interact with nucleophilic centers in nucleic acids and proteins, leading to excessive generation of reactive oxygen species (ROS), oxidative stress, and widespread cellular damage. These effects are especially detrimental to rapidly dividing cells, such as those in the bone marrow and reproductive organs.

In this research, administration of CP resulted in meaningful enhances in chromosomal abnormalities in bone marrow cells, reduction in the mitotic index, and pronounced morphological abnormalities in sperm heads, confirming the genotoxic and cytostatic potential of the drug. These findings are consistent with previous investigations displaying that alkylating agents like CP induce clastogenic effects, mitotic suppression, and reproductive toxicity (Kaina, 2004; Lemes et al., 2017; Margiana et al., 2022). Interestingly, the co-administration of *Cinnamomum zeylanicum* (C. zeylanicum) extract with CP displayed remarkable cytoprotective effects. This plant, widely applied in traditional medicine systems such as Ayurveda and Traditional Chinese Medicine, has demonstrated a broad spectrum of pharmacological activities. Among the approximately 300 species in the genus *Cinnamomum*, *C. zeylanicum* (true cinnamon) has been studied extensively for its rich content of bioactive compounds containing cinnamaldehyde, eugenol, linalool, and different polyphenols (Uma et al., 2009).

These phytochemicals possess well-documented antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory properties. In our findings, the extract of *C. zeylanicum* meaningfully mitigated CP-induced chromosomal aberrations, restored mitotic activity, and diminished the frequency of abnormal sperm morphology. This suggests that *C.*

zeylanicum may exert its protective effects by scavenging ROS, stabilizing cellular membranes, and possibly enhancing DNA repair mechanisms. Our observations align with recent research denoting that natural products rich in antioxidants can counteract chemotherapy-induced genotoxicity and support normal cellular functions (Arif et al., 2023; Bashar et al., 2022; Zaman et al., 2023). The restoration of the mitotic index in the co-treated group suggests that *C. zeylanicum* not only diminishes DNA damage but may also promote proper mitotic progression by modulating cell cycle regulators.

CP treatment resulted in a notable enhance in structural chromosomal aberrations, such as chromatid breaks, ring chromosomes, and deletions (Table 1), highlighting its mutagenic potential. These aberrations stem from CP's interference with the structural integrity of the genome during mitosis. In contrast, the group receiving both CP and *C. zeylanicum* displayed a meaningful reduction in these abnormalities. This suggests that the extract's antioxidant constituents may neutralize ROS generated by CP metabolism, thereby preserving chromosomal stability and enhancing the DNA repair response.

As displayed in Table 2, CP exposure meaningfully diminished the mitotic index, denoting a strong inhibition of cell proliferation. This effect likely arises from CP-induced DNA lesions that trigger cell cycle checkpoints and apoptosis. However, co-treatment with *C. zeylanicum* restored the mitotic index to near-normal levels, further supporting its task in counteracting the cytostatic effects of CP. These results suggest that *C. zeylanicum* may facilitate DNA repair or prevent excessive damage, thus allowing cells to resume normal mitotic activity.

CP meaningfully enhanced the incidence of abnormal sperm forms, such as banana-shaped, hookless, and double-headed sperm (Table 3; Figure 2), denoting substantial genotoxic damage during spermatogenesis. These deformities can compromise fertility and are indicative of DNA damage in germ cells. Notably, co-treatment with *C. zeylanicum* extract markedly diminished the frequency of these abnormalities, supporting its protective task in reproductive health. The antioxidant properties of the extract likely contributed to the preservation of germ cell integrity and proper morphological development.

Conclusions: Although *Cinnamomum zeylanicum* is widely recognized as a culinary spice and a component of traditional medicine, the present research suggests that it also possesses meaningful potential as a source of bioactive compounds for clinical applications. Specifically, its protective effects against cyclophosphamide (CP)-induced cytotoxicity were evident in reducing chromosomal aberrations, normalizing the mitotic index, and minimizing sperm head abnormalities in male albino mice. The observed mitigation of CP's harmful effects is likely attributable to the antioxidant properties of phytochemicals present in the *C. zeylanicum* extract.

These compounds may neutralize reactive oxygen species and support cellular repair mechanisms, thereby preserving genomic and reproductive integrity. The findings of this research highlight the potential of *C. zeylanicum* as a natural, adjunctive agent in chemotherapy protocols to diminish genotoxic side effects. Further investigations, containing phytochemical profiling and molecular pathway analyses, as well as well-designed clinical trials, are warranted to fully explore its therapeutic efficacy and safety in human subjects.

Author Contributions

Aseel Raheem Mardan Al-Aamiri designed the research, provided experimental samples, analysed the data, wrote the paper and approved the final manuscript.

Data Availability Statement

The data can be provided by the corresponding author on reasonable request.

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Ethical Considerations

All procedures involving animals were performed in compliance with institutional ethical standards and national regulations for the care and use of laboratory animals.

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Conflict of Interest

The authors declare no conflicts of interest.

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اثر پیشگیرانه عصاره آبی دارچین سیلان (Cinnamomum zeylanicum) در برابر سمیت سلولی ناشی از سیکلوفسفامید در موش‌های نر سفید

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چکیده

هدف: دارچین سیلان (Cinnamomum zeylanicum) یک گیاه دارویی است که به طور سنتی به دلیل خواص درمانی گسترده‌ای از جمله اثرات آنتی‌اکسیدانی، ضد التهابی، ضد میکروبی و محافظت سلولی مورد استفاده قرار می‌گیرد. مطالعات اخیر ترکیبات زیست‌فعال آن مانند سینام‌آلدئید و اوژنول را برجسته کرده‌اند که ممکن است در تنظیم استرس اکسیداتیو و آسیب‌های سلولی نقش داشته باشند. سیکلوفسفامید، یک داروی شیمی‌درمانی آلکیله‌کننده رایج است که به‌ویژه در درمان انواع سرطان‌ها و بیماری‌های خودایمنی مؤثر است، اما استفاده بالینی از آن به دلیل عوارض جانبی ژنوتوکسیک و سایتوتوکسیک، از جمله ناهنجاری‌های کروموزومی و سمیت تولیدمثلی محدود شده است. توسعه درمان‌های کمکی برای کاهش این عوارض مورد توجه روزافزون قرار گرفته است. این مطالعه با هدف بررسی نقش محافظتی دارچین سیلان در برابر سمیت ژنی و سلولی ناشی از سیکلوفسفامید در موش‌های نر آلبینو انجام شد تا احتمال استفاده از آن به عنوان یک عامل شیمی‌محافظ طبیعی را بررسی کند.

مواد و روش‌ها: سی موش نر بالغ آلبینو به‌صورت تصادفی به سه گروه (n=10) تقسیم شدند. گروه اول (کنترل منفی) آب مقطر دریافت کرد. گروه دوم یک دوز درون‌صفاقی از سیکلوفسفامید (۲۰ میلی‌گرم به ازای هر کیلوگرم وزن بدن) دریافت نمود. گروه سوم همزمان عصاره دارچین سیلان (۱۰۰ میلی‌گرم/کیلوگرم) و سیکلوفسفامید (۲۰ میلی‌گرم/کیلوگرم) دریافت کرد. ناهنجاری‌های کروموزومی در سلول‌های مغز استخوان، ناهنجاری‌های مورفولوژی سر اسپرم، و شاخص میتوز برای تعیین میزان سمیت ژنتیکی و سلولی مورد ارزیابی قرار گرفتند.

نتایج: موش‌هایی که همزمان با سیکلوفسفامید، عصاره دارچین دریافت کردند، کاهش قابل توجهی در ناهنجاری‌های کروموزومی و ناهنجاری‌های سر اسپرم نسبت به گروه دریافت‌کننده تنها سیکلوفسفامید نشان دادند. همچنین، افزایش چشمگیری در شاخص میتوز در گروه درمان ترکیبی مشاهده شد که نشان‌دهنده اثر محافظتی و احتمالاً بازسازنده بر تکثیر سلول‌های مغز استخوان است.

نتیجه‌گیری: یافته‌های این مطالعه نشان داد که دارچین سیلان دارای اثر محافظتی در برابر آسیب ژنتیکی و سلولی ناشی از سیکلوفسفامید در موش‌های نر آلبینو می‌باشد. ترکیبات آنتی‌اکسیدانی و ضد التهابی آن ممکن است به حفظ یکپارچگی سلولی و بهبود روند بازسازی پس از درمان شیمی‌درمانی کمک کنند. این نتایج نشان می‌دهد که دارچین سیلان می‌تواند به عنوان یک عامل درمانی مکمل برای کاهش عوارض جانبی سیکلوفسفامید در نظر گرفته شود. پیشنهاد می‌شود تحقیقات بیشتری، شامل مطالعات مولکولی و کارآزمایی‌های بالینی، برای درک بهتر مکانیسم اثر آن و بررسی ایمنی و کارایی آن در انسان انجام شود.

واژگان کلیدی: دارچین سیلان، سمیت ژنی، سیکلوفسفامید، ناهنجاری اسپرم، ناهنجاری کروموزومی

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