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Protective Effects of Ginger Extract against the Toxicity of Cyclophosphamide on Testes: An Experimental Laboratory-Based Study

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ABSTRACT

Background: *Cyclophosphamide (CP) is a widely used medication in chemotherapy and can cause oxidative stress. Oxidative stress can affect testicular functions by reducing the sperm motility and concentration, changing the sperm morphology, and increasing DNA fragmentation in sperm. Ginger is one of the most widely used spices in various foods and is used as an herbal medicine in many countries due to its antioxidant effects. We aim to study the protective effects of ginger against CP-induced testicular toxicity in rats.* ***Objectives:*** *This study was conducted to investigate the role of ginger in preventing cyclophosphamide-induced adverse effects on the testicular histology of CP-treated male rats.* ***Methods:*** *The study was performed on 30 male albino rats with body weights of 300-350 g. The animals were divided into the following three groups (10/cage): Group 1 (control, untreated group), Group 2 (CP group, received a single dose of CP at 100 mg/kg^{-1} BW intraperitoneally), and Group 3 (CP+ginger, received ginger extract orally at 500 mg/kg for 35 days after CP injection). The morphological and histological structures of the testes were compared in the different groups of rats.* ***Results:*** *The CP-treated group showed a disorganized germinal epithelium compared with those of the controls. The CP+ginger-treated group showed a significant recovery of the organization of the germinal epithelium and the cellular attachments. Caspase-3-positive cells were significantly higher in the CP group and had remarkably lower levels in the CP+ginger-treated group. A reduction in the diameter of the seminiferous tubules and the destruction of connective tissue were observed in the CP-treated group; these changes were improved in the CP+ginger-treated group.* ***Conclusion:*** *Ginger extract can protect reproductive functions against CP-induced toxicity in rats.*

Keywords: Ginger extract, Antioxidant, Cyclophosphamide, Rat, Testes

BACKGROUND

Ginger rhizome is a flowering plant, and the rhizome is ginger root, or simply ginger, which is used worldwide as a spice and folk medicine. The antioxidative and androgenic activities of ginger have been reported in animal models [1]. The characteristic fragrance and flavor of ginger result from volatile oils that compose 1%-3% of the weight of fresh ginger. It also contains acids, resins, vitamin C, and folic acid. In addition, it contains moderate amounts of vitamin B6 and dietary minerals, including magnesium and manganese, but low in nutrient content [2].

Ginger has a strong antioxidant effect and may either relieve or rule out the generation of free radicals. It is considered an important herbal medicine that only has a few negative side effects if consumed in reasonable quantities [3]. The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective effects against radiation toxicity [4,5] and a number of toxic agents, such as carbon tetrachloride and cisplatin [5].

The inhibition of xanthine oxidase activity that is responsible for the generation of reactive oxygen species, such as superoxide anions, has been documented with gingerol [6]. The levels of superoxide dismutase and catalase enzymes, which are important components of enzymatic antioxidative defenses, were significantly stimulated in the liver tissues of rats that were fed ginger at all levels [7].

Cyclophosphamide (CP), also known as cytophosphane, is a medication that is used as a chemotherapy treatment and to suppress the immune system. It is used extensively as an antineoplastic agent for the treatment of various cancers, multiple sclerosis, systemic lupus erythematosus, and other benign tumors [8]. As an immune suppressor, it is used in the treatment of nephrotic syndrome and following an organ transplant. CP treatment is associated with oligospermia and azoospermia, as well as biochemical and histological alterations in the testis and epididymis of humans and rats [9-11].

MATERIALS AND METHODS

Chemicals

CP was purchased from Baxter Oncology GmbH, Frankfurt, Germany.

Ginger

Tablets (500 mg) that were produced by Arab Co. Pharmaceuticals (El Asher Men Ramadan City, Egypt) and Medical Plant MEPACO (Nasr City, Cairo, Egypt) were tested. The tablets were ground into a fine powder. After being milled, the ginger powder was macerated in distilled water to be a ginger extract [12].

Animals

In this study, 30 healthy, adult, male Wistar albino rats (300-350 g, 3 months old) were used. They were obtained from an animal house at the College of Pharmacy, Prince Sattam Bin Abdulaziz University, KSA. They were maintained under controlled standard animal housing conditions (temperature: 26°C-28°C; photoperiod: 12 hours natural light and 12 hours dark; and humidity: 80%-90%) with access to food and water ad libitum at the Animal Care Facility at the Department of Clinical Pharmacy at Prince Sattam bin Abdulaziz University. The pellet diet consisted of 23% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, and 55% nitrogen-free extract (carbohydrates). The rats were kept under observation for approximately 2 weeks before the start of the experiments to allow for adaptation.

Ethical Considerations

All procedures that were performed in this study that involved animal models were in accordance with the ethical standards of the Institutional Ethics Committee of Prince Sattam bin Abdulaziz University and the Animal House Committee (IRB, PSAU-2017 ANT 1/34PI).

Experimental Design

The rats were randomly divided into three groups of 10 as follows: Group 1: The control group received a single intraperitoneal injection of isotonic saline solution (1 ml). Group 2: The CP group received a single dose of CP (100 mg/kg^{-1} BW) intraperitoneally. One hundred milligrams of CP was chosen for our study, but before starting our study, we injected 100 mg/kg^{-1} BW of CP into four rats to ensure significant histological changes in the testis. The dose was chosen based on previous studies [8,13]. Group 3 (CP+ginger) received ginger extract orally 500 mg/kg per day for 35 days after a cyclophosphamide injection (100 mg/kg^{-1} BW) intraperitoneally.

Histological and Histochemical Studies

After 1 month, the rats from the control and treated groups were anesthetized by intraperitoneal injection with 1% pentobarbital sodium (0.4 ml/100 g, ip). Then, the rats were sacrificed by cervical dislocation. The testes were removed and washed with ice-cold normal saline (0.9%) to remove the blood. The testicular samples were taken for histological

and histochemical studies. The specimens were prepared via fixation in 10% neutral buffered formalin and Carnoy's fluid. For the histological study, the paraffin sections were stained with Harris's hematoxylin and eosin (H and E) [14]. For the detection of collagen fibers, the paraffin sections were stained using Mallory's trichrome stain [14]. For the histochemical study, the paraffin sections that were 5 µm thick were prepared and stained with Feulgen stain [14]. Later, the stained sections were examined via light microscopy and were photographed. All the detected variations between the three groups from the microscopic findings were scientifically discussed.

Immunohistochemical Study

Sections of testes were deparaffinized with xylene, followed by antigen retrieval by heating in citrate buffer (10 mM, 20 minutes). This was followed by endogenous peroxidase blocking in 3% H_2O_2 for 10 minutes and incubation with the anti-caspase-3 antibody (1:100; Abcam, Ab4051). After washing the slides with phosphate-buffered saline, the sections were incubated with the appropriate secondary antibodies at room temperature for 1 hour, followed by detection with 3-amino-9-ethylcarbazole, a chromogen. The slides were mounted with a paramount aqueous mounting medium.

Statistical Analyses

The data are presented as the mean ± SEM (standard error of the mean). Additionally, the statistical analyses were carried out using ANOVA; SPSS-PC, version 24.0, and a p-value of <0.05 were considered statistically significant.

RESULTS

Histological Results

The testicular sections from the control adult albino rats showed normal morphology, and all structures were arranged in a normal pattern (Figure 1). The histological sections from the CP-treated group exhibited an irregularity of the seminiferous tubule (ST) structures with a significant decrease in the diameters compared with those of the control group (Figure 2A and 2B). The germinal epithelium was disorganized and showed abnormal cellular attachment. Spermatogonia were the major cell type observed. Multinucleated cells were also observed in the CP-treated group (Figure 2B). The third group (CP+ginger) showed a significant recovery of the ST diameters in comparison with those from the rats treated with CP (Figure 2C and 2D). The interstitial connective tissue showed marked destruction of the connective tissues with the subsequent widening of the interstitial tissue (IT) spaces compared with those from the control group (Figure 3A and B). The group that was treated with ginger extract showed a considerable recovery of the interstitial connective tissue to normal levels and a significant increase in the amount of the collagen fibers compared with those from the CP group (Figure 3A and 3B).

Immunohistochemical Results

In the CP group, a marked increase in the number of caspase-3 positive cells was observed in the STs in comparison with those from the control group (Figure 4A and 4B). An increase in the number of caspase-3-positive cells was also observed in the STs in the third group (CP+ginger) in comparison with those from the controls (Figure 4C and 4D). The mean diameters of STs, numbers of pyknotic and caspase-3-positive cells are given in Table 1.

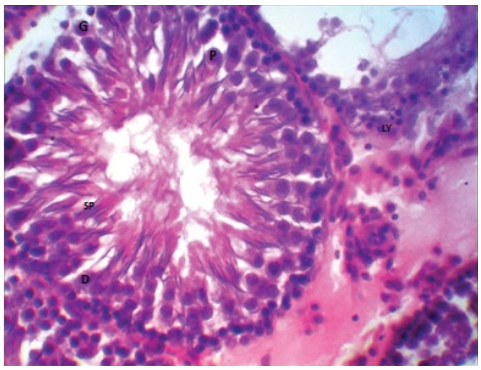

Figure 1 A light photomicrograph of a section of a rat testicular tissue from the control group shows the normal association of the germ cells and the normal architecture of the IT. G=Spermatogonium, P=Spermatocytes, D=Spermatid, SP=Sperm, S=Sertoli cells, Ly=Leydig cells. H and E staining (400x)

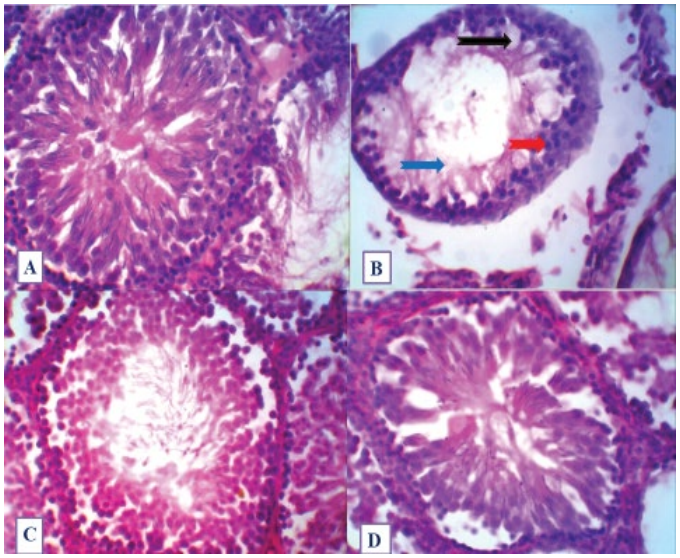

Figure 2 (A) A light photomicrograph of a section of a rat testicular tissue from the control group. The STs have an ordinary shape. The ST epithelium is structurally intact and shows a normal association with germ cells. (B) A light photomicrograph of a section of rat testicular tissue from rats that were treated with cyclophosphamide. The STs have irregular shapes, and the germinal epithelium is disorganized. The depletion of germ cells, pyknotic germ cells (black arrow), and karyolysis (blue arrow) can be seen. The giant cell formation with two or three nuclei (red arrow) is seen in the lumen of the irregularly shaped ST. (C and D) A light photomicrograph of a section of a rat testicular tissue from rats that were treated with ginger extract. The STs have a partial recovery to the normal structure. H and E staining (400x)

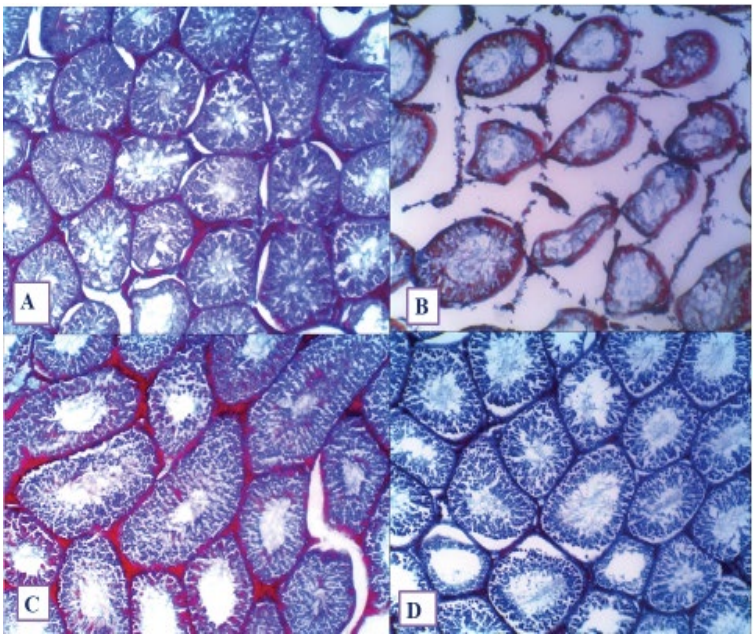

Figure 3 (A) A light photomicrograph of a section of a rat testicular tissue from the control group shows the normal distribution of collagen fibers in the IT around the STs. (B) A light photomicrograph of a section of a rat testicular tissue from rats that were treated with cyclophosphamide shows a marked reduction of collagen fibers in the IT around the STs and causes an increase in the diameter of the interstitial spaces. (C and D) A light photomicrograph of a section of a rat testicular tissue from rats that were treated with ginger extract shows a similar distribution of collagen fibers in the IT around the STs in comparison with those from the control group. Mallory's trichrome staining (200x)

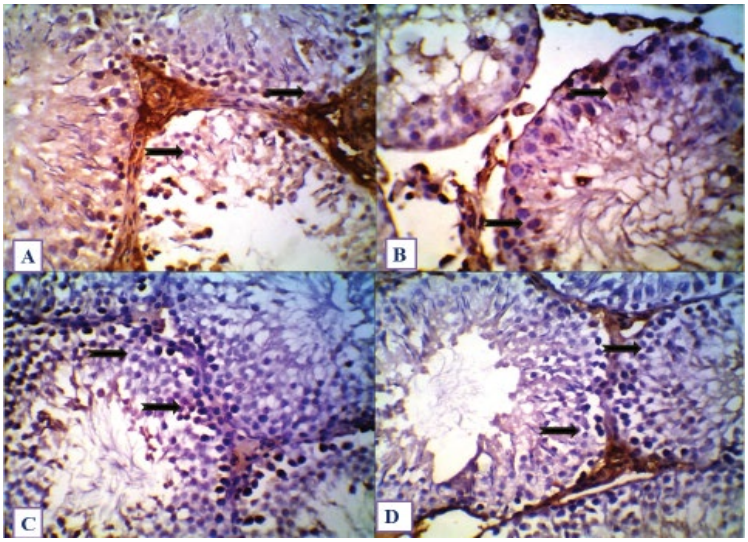

Figure 4 (A) A light micrograph of testicular tissue of a rat from the control group. The ST germ cells show the mild expression of caspase-3 with immunostaining (black arrows). (B) A light micrograph of testicular tissue of a rat treated with CP. The ST germ cells show the marked expression of caspase-3 with immunostaining (black arrows). (C) A light photomicrograph of a section of a rat testicular tissue from rats that were treated with ginger extract shows the mild expression of caspase-3 with immunostaining (black arrows) in the ST germ cells. D) A light photomicrograph of a section of a rat testicular tissue from rats that were treated with ginger extract shows the moderate expression of caspase-3 with immunostaining (black arrows) in the ST germ cells. Caspase-3 immunostaining (400x)

Table 1 Mean ± SD diameters of the seminiferous tubules, number of pyknotic and caspase 3 +ve cells in different study groups

Parameters	Diameters of the Seminepherous Tubules	Myknotic Cells	Caspase-3+ve Cells
Study Groups			
Group 1 (Control)	178.11 ± 13.92	29.073 ± 1.48	12.00 ± 1.94
Group 2 (CP)	122.61 ± 32.17*	15.08 ± 4.02*	36.81 ± 2.78*
Group 3 (ginger+CP)	189.78 ± 10.67**	24.75 ± 6.28**	15.00 ± 3.31**
*Significantly different from the control group ($p<0.05$); **Significantly different from CP group ($p<0.05$)			

DISCUSSION

This study was designed to investigate the protective effects of ginger extract on cyclophosphamide-induced oxidative stress in rat testes. It is an established fact that cyclophosphamide is used in chemotherapeutic regimens. On the one hand, it has a positive implication against cancer, but at the same time, it puts the body under oxidative stress and reproductive deformities. The deleterious effects of CP have been well documented, and some agents, such as Phyllanthus fraternus Webster, have been used to protect the testicular structure of CP-treated mouse testes [15]. One study reported the protective effects of ginger on the sperm characteristics and epididymal morphology in rats treated with CP [16]. However, this study tested the combined effects of ginger and pumpkin seed extracts, and it is not clear if the protective effects were greater because of the ginger extract or the pumpkin seed extract. In this continuation of the previous work, we conducted this study to determine the direct effects of ginger extract on the histological changes of rat testes.

Our results showed that there was a deterioration of testicular structures, especially in the diameter of STs. The injection of ginger extract significantly improved the histology of the testes in rats. Caspase-3 is an apoptotic marker of programmed cell death. In our study, the number of caspase-3-positive cells was significantly higher in the CP-treated group, while it was lower in the ginger-treated groups. The effects of ginger on testicular histology are scarcely reported. Vitamin C has also shown protective effects against CP-induced rat testicular toxicity [17]. Likewise, Diallyl disulfide also improved testicular functions in rats treated with CP [18]. Others have reported that the long-term use

of CP as a chemotherapeutic agent can reduce the body weight and damage the reproductive organs in male rats. This can lead to atrophy of the testes, epididymides, and consequently, fertility impairment [18-20].

It has been documented in various experimental studies that effective anticancer therapies with cytotoxic drugs, such as CP, are limited by their side effects, including reproductive toxicity [8]. Our findings support the results of Ilbey, et al. [8], who reported irregular and diminished STs that contained only a few germ cells in the CP-induced group and that ginger has both antioxidative and androgenic activities in animal models [1].

However, the results of the current study are in line with the study of Zahedi, et al. [21], who explained that the administration of ginger can overcome the reproductive toxicity of gentamicin. Similarly, Shalaby, et al. [22] declared that the oral administration of the ginger extract at 250 mg/kg and 500 mg/kg body weight for 65 days lead to the improvement of spermatogenesis in diabetic male rats. The deleterious effects of A1C13 have also been improved with ginger administration.

Limitations and Recommendations

The findings of this study should be translated to humans with caution. Furthermore, the use of specific concentrations of other antioxidants is recommended in future studies. These results further suggest that studies should use ginger as a drug to protect patients from the side effects of chemotherapy. To attain better results, further investigations should be carried out on this subject. However, comprehensive chemical and pharmacological research are required to determine the exact mechanism of ginger on the testis and to identify the active ingredient responsible for this effect.

CONCLUSION

The present study has demonstrated that ginger possesses antioxidant activities and reduces the side effects of CP on testicular structures. The results of this study may advocate the use of ginger by patients receiving anticancer therapy. However, this speculation warrants randomized, controlled trials on a large human population.

DECLARATIONS

Authors' Contributions

This work was performed as a collaboration among all of the authors. Ali Hassan A. Ali and Sameer Al-Ghamdi participated in the study design and wrote the first draft of the manuscript. Ghanem G Alanazi, Muath A Alsomait, and Abdulaziz N Alaskar collected and processed the samples. Abdulmohsen K. El-Enazi, Hisham M Alashqar, and Gulfam Ahmad participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

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Availability of Data and Materials

The data are available upon request from the authors.

Ethics Approval

The study was approved by the Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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