

Short Communication

Effects of *Ocimum tenuiflorum* on Induced Testicular Degeneration by Filgrastim in Wistar Rats

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Abstract

Since the creation of man on earth, herbal remedies have been used as an invaluable, safe, and available natural source of medicine for the treatment of several dysfunctions in living animals and human beings. The pharmacological properties of these herbs are commonly known to include analgesic, antidiabetic, antispasmodic, hepatoprotective, reproductive, and cardioprotective potentials. Various nutritional, environmental, and physiological factors can affect the male reproductive system. Several herbal remedies are reported to target the testis at the spermatogenesis and hormonal level. Most of the chemotherapy drugs used for treating cancers cause adverse effects on male reproductive functions. Filgrastim is used for the treatment of cancer in patients suffering from neutropenia. *Ocimum tenuiflorum* is one of the herbal remedies used as a natural antioxidant substance for protecting the body organs against the toxic effects of chemotherapy drugs. Therefore, the current study was designed to investigate the possible productive effects of the *O. tenuiflorum* against adverse effects of filgrastim on testicular tissues in male Wistar rats. In total, 40 adult male rats were selected and randomly divided into four groups (n=10). Group 1 was treated with intraperitoneal administration of filgrastim at a dose of 30.83 µg/kg/day for 1 week. Group 2 received *O. tenuiflorum* suspended in corn oil and administered by gavages at 20 mg/kg/day for 1 week. Group 3 was intraperitoneally injected with filgrastim at a dose of 30.83 µg/kg/day for 1 week and treated with *O. tenuiflorum* at similar manure to group 2. Finally, group 4 was treated with placebo (0.9% saline solution). The results showed that filgrastim administration leads to the degeneration of spermatozoa and germ cells in the testicles of rats. The results of the current study showed that *O. tenuiflorum* has some ameliorating effects on the testicles and fertility of the rats which were treated with filgrastim. Finally, the recorded data showed that *O. tenuiflorum* has protective effects on testis tissues and reproductive functions in male rats.

Keywords: Neutropenia, Antioxidant, Testicular

1. Introduction

Cancer treatments have different side effects that are coordinated through the use of different chemical substances (1, 2). One of the routine drugs in cancer therapy is called filgrastim. filgrastim is a medication used to treat low neutrophil count. Low neutrophil

counts may occur due to HIV/AIDS following chemotherapy or radiation poisoning or unknown reasons (3). The filgrastim is a protein used for stimulates that activates the proliferation and differentiation of neutrophil precursors (3), which are considered hematopoietic myeloid growth factors (4).

In the last years, research has found that filgrastim decreases the fertility performance and reproductive functions of patients who used it under myelosuppressive conditions for increasing the neutrophil (4). Furthermore, testicular degeneration was commonly encountered in cancer patients who used filgrastim. Moreover, the researchers have illustrated that it disrupts the concentration of follicle-stimulating hormone and luteinizing hormone and hence, destroys and damages the Leydig and Sertoli cells of testicular tissue. All the previously-mentioned troubles caused by chemotherapy drugs encourage us to use natural substances and herbal remedies to reduce the adverse effects of these chemical drugs, such as filgrastim (4, 5).

Other studies have suggested that the use of natural and herbal products, such as lycopene and Curcuma, as an antioxidant, can reduce and ameliorate the toxicity of chemotherapy drugs and chemical substances (6-9). The results of a study conducted by Saber et al. showed that the use of filgrastim causes testicular degeneration (10). In the aforementioned study, this degeneration resulted in a decrease in the testosterone level and finally suppression of the spermatogenesis process (10).

The *Ocimum tenuiflorum* is a very rare herb called the "queen of herbs" which has been used widely in the field of science from ancient times to modern research due to its high number of medicinal properties (11). At the same time, different researchers and studies proved that *O. tenuiflorum* has unique medicinal properties (11) which are used as antioxidants in the treatment of breast cancer (12) and hyperlipidemia (12). A researcher believes that *O. Tenuiflorum* also has antibiotic properties (13).

Therefore, the current study aimed to investigate the possible protective effects of *O. tenuiflorum* against histological degeneration induced by the usage of filgrastim in Wistar albino rats.

2. Materials and Methods

2.1. Filgrastim

The filgrastim was purchased from Al-Faiha Company in Najaf, Iraq.

2.2. *Ocimum Tenuiflorum*

The *O. tenuiflorum* leaves were purchased from the College of Dentistry in the Islamic University in Najaf, Iraq. After proper washing with double distilled water, the leaves were left to completely dry. Afterward, the leaves were crushed to powder, then the *O. tenuiflorum* powder was dissolved and mixed in 750 mL of double distilled water and then concentrated by using a rotary evaporator and stored in the refrigerator at -20 °C until use.

2.3. Animal and Experimental Design

In total, 40 adult male rats with an average weight of 200-300 g were selected and randomly divided into four groups (n=10). Group 1 was treated with intraperitoneal administration of filgrastim at a dose of 30.83 µg/kg/day for 1 week. Group 2 received *O. tenuiflorum* was suspended in corn oil and administered by gavages at 20 mg/kg/day for 1 week. Group 3 was intraperitoneally injected with filgrastim at a dose of 30.83 µg/kg/day for 1 week and treated with *O. tenuiflorum* at similar manure to group 2. Finally, group 4 was treated with placebo (0.9% saline solution) (10). After 40 days of the initiation of the experiment, all the animals were scarified by cervical dislocation (14) and the testicles were taken for histological evaluation (7).

2.4. Morphological Analysis

The morphological and histopathological evaluation was performed as previously described by Taib, Budin (15). The testes in both groups were treated with 10% buffered formalin solution and a routine histological procedure was conducted. All the testicular sections were stained with hematoxylin and eosin (H & E) stains and monitored for morphological changes under 10X and 40X magnifications. A section of the testes was cut into small pieces (1 mm³), treated with 2.5% glutaraldehyde 0.1 N phosphate-buffered saline at room temperature for 1 h, and post-fixed with osmium tetroxide for another hour. The testes tissue was dehydrated in 70%, 90%, and 100% (twice) acetone for 5 min each, and then in 1:1 acetone to resin ratio for 5 min; finally, it was embedded in epoxy resin. Ultrathin slices (90 nm) were observed using a

transmission electron microscope, Tecnai G2 (FEI, USA), at 100 kV(15).

2.5. Statistical Analysis

The recorded data were normally distributed. The differences between the treated and control groups were statistically evaluated using an independent Student's t-test. All the data were expressed as mean \pm SD, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

The main objective of the present study was not only to determine the adverse effect of filgrastim but also to identify how *O. tenuiflorum* protects the testicular tissue during the use of filgrastim. The results of the present study illustrated the effect of filgrastim on the testicular tissue which is depicted in Figure 4. The Histopathological section of the animals in group 1 is clearly visualized in Figure 4. The adverse effect of filgrastim on spermatozoa and germ cells is shown in Figure 4.

These findings are in agreement with those of the previous study conducted by Saber et al. (10) which indicated that the filgrastim causes testicular degeneration. Comparison of the histopathological sections obtained from group 1 (Figure 3) and group 4 (Figure 1) illustrated no histological changes in the testis section due to degenerative effects of filgrastim in the control group. Moreover, it was found that in the control group (group 4), all the testicles function were in normal stages of spermiogenesis with clarity of lamina propria and lumen (16, 17).

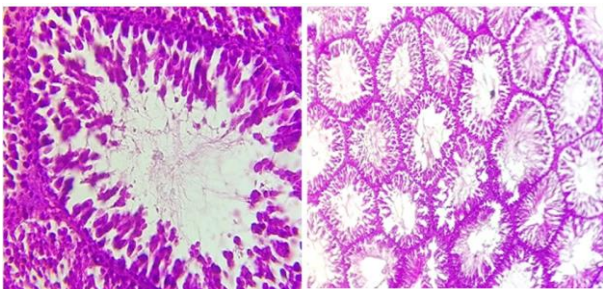


Figure 1. Histopathological section of the control group. The histological section of the tests shows the stages of spermatogenesis with clarity of lamina propria and lumen. The section is stained with H&E stain. The section is captured with a 20 \times magnifier scale.

Figure 2 illustrates the ameliorating effect of *O. tenuiflorum* on the testis and fertility of the rats which was previously approved by Joseph, Jaja (18). Similar to the recorded data in the current study, they showed that *O. tenuiflorum* was associated with an increase in sperm quality and mitigation of iron-induced testicular toxicity via modulation of redox imbalance. Findings of other studies suggested the beneficial effects of using *O. tenuiflorum* with chemotherapeutic drugs (19). The histological section of the current study in group 2 (Figure 2) showed the positive effects of *O. tenuiflorum* in the case of sperm count inside the cavity with clarity of myoid cells, compared to the control (Figure 1).

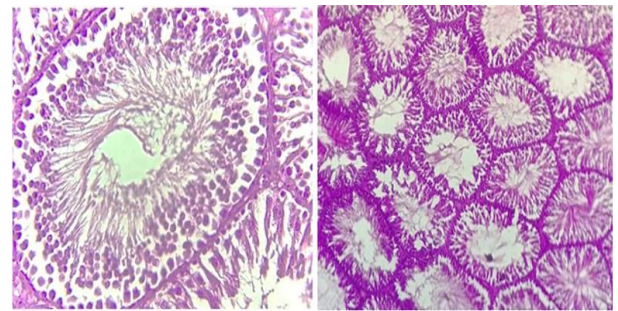


Figure 2. Histopathological section of *Ocimum tenuiflorum* group. The histological section of tests shows an increase in the number of sperms inside the cavity with the clarity of myoid cells. The section was stained with H&E stain and captured with a 20 \times magnifier scale.

Figure 3 shows the experimental group, which received *O. Tenuiflorum*, after the induction of testicular degeneration by the filgrastim. Its histological section showed response with the possibility of distinguishing the stages of sperm formation, but it shows the presence of damage to the lamina propria. On the other hand, comparing the results between group 3 (Figure 3) and 1 (Figure 4) revealed a significant increase at the normal spermatogenic stage and sperm cell as well as the normal section of histological strength in group 3, compared to group 1. In addition, we can see normal Leydig and Sertoli cells in group 3.

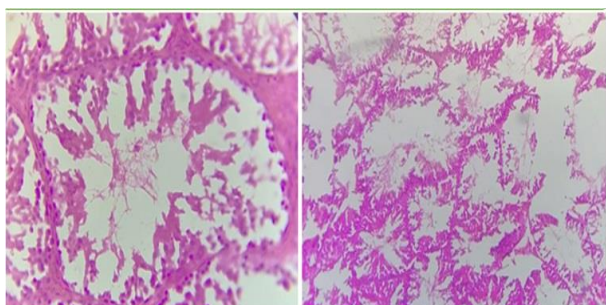


Figure 3. Histopathological section of the experimental group. The histological section shows a response with the possibility of distinguishing the stages of sperm formation, but it shows the presence of damage to the lamina propria. The section was captured with a 20× magnifier scale.

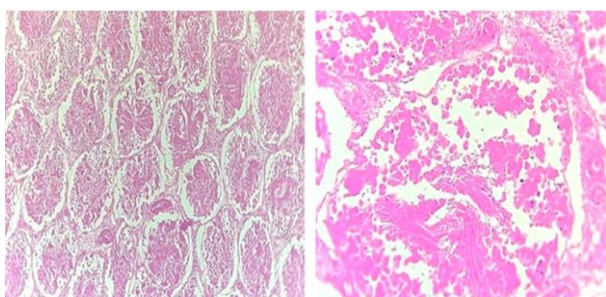


Figure 4. Histopathological section of induction group. The histological section of the tests shows tissue damage with the inability to distinguish the stages of sperm formation or Sertoli cells. The section was captured with a 20× magnifier scale.

In conclusion, the results of the present study revealed that filgrastim has negative effects on the testicular tissue as it causes testicular degeneration. On the other hand, the protective effects of *O. tenuiflorum* against adverse effects of the filgrastim on the male reproductive organ are well established.

Authors' Contribution

Study concept and design: G. S. T. A. and G. S. B.

Acquisition of data: H. F. A.

Analysis and interpretation of data: M. A. A.

Drafting of the manuscript: A. M. B. A.

Critical revision of the manuscript for important intellectual content: G. S. B. and H. F. A.

Statistical analysis: G. S. T. A.

Administrative, technical, and material support: : G. S. T. A. and G. S. B.

Ethics

All the procedures in this study, including animal husbandry, handling, and scarifying were performed according to the guidelines instructed by the Animal Ethics Committee of the University of Kufa, Najaf, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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