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

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Abstract

Since the creation of the man on the earth the herbal remedies have been used as an invaluable, safe, and available natural sources of medicines for treating several dysfunctions in living animals and human being. Pharmacological properties of these herbs are commonly known as 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 caused adverse effects on men reproductive functions. Filgrastim used for treatment of cancer in patients suffering from neutropenia. *Ocimum tenuiflorum* is one of the herbal remedies used as a natural antioxidant substance for protective the body organs against toxic effects of chemotherapy drugs. Thus the current study was designed to investigate the possible productive effect of the *Ocimum tenuiflorum* against adverse effects of Filgrastim on testicular tissue in male Wistar rats. Forty adult male rats randomly divided into 4 groups (n = 10). Group 1 was treated with Filgrastim intraperitoneally at a dose of 30.83 µg/kg/day for one week; group 2 received *Ocimum tenuiflorum* suspended in corn oil and administered by gavages at 20 mg/kg/day for one week; group 3 were intraperitoneally injected with Filgrastim at a dose of 30.83 µg/kg/day for one week treated with Ocimum tenuiflorum at a similar manure to group 2; group 4 treated with a placebo
(0.9% saline solution). The results showed that Filgrastim administration obviously lead to the degeneration of spermatozoa and germ cell in the rats' testicles. The results of the current study showed that Ocimum tenuiflorum has some ameliorate effects on the testicles and fertility of the rats which have treated with Filgrastim. Finally, the recorded data showed that *Ocimum tenuiflorum* has protective effects on testis tissue 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 with HIV/AIDS, following chemotherapy or radiation poisoning, or be of an unknown cause (3). The Filgrastim is a protein used for stimulates and activated the proliferation and differentiation of neutrophil precursors (3), which are considered as hematopoietic myeloid growth factors (4). In the last years, the research found that Filgrastim causes decreased performance of fertility and reproductive of patients that were used under myelosuppressive conditions for increasing the neutrophil (4). Furthermore, testicular degeneration was commonly encountered in cancer patients who used Filgrastim, moreover, the researcher illustrated that disrupts the concentration of FSH and LH hence lead to destroy and damage on Leydig and Sertoli cells of testicular tissue. All troubles caused by chemotherapy drugs that were previously mentioned 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 suggested that the use of natural and herbal products, such as lycopene and Curcuma, as an antioxidant against 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). As a consequence of this degeneration testosterone level decreased and finally the spermatogenesis process was suppressed (10).

The *Ocimum Tenuiflorum* is an herb, the scantest called “queen of herbs” which has been used widely in the field of science from ancient times to modern research because of its large number of medicinal properties (11). At the same time, different researchers and studies proved *Ocimum*
*Ocimum tenuiflorum* has unique medicinal properties (11) which are used as antioxidants in breast cancer (12) and in the treatment of hyperlipidemia (13). Some researcher believes that *Ocimum Tenuiflorum* also has antibiotic properties (14).

Thus, the aim of the current study was to investigate the possible protective effects of *Ocimum tenuiflorum* against histological degeneration induced by used Filgrastim in Wistar albino rats.

2. Material and methods

2.1. Filgrastim

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

2.2. *Ocimum tenuiflorum*

The *Ocimum tenuiflorum* leaves was purchased from the college of dentistry in the Islamic university in Najaf. After proper washing with double distilled water let the leaves to completely dry. There after the leaves crushed to powder, then the *Ocimum tenuiflorum powder* was dissolved and mixed in 750 mL of double distilled water then concentrated by using a rotary evaporator and stored in the refrigerator at -20 °C until use.

2.3. Animal and experimental design

Forty adult male rats with average weigh between 200-300 g randomly divided into 4 groups (n = 10). Group 1 was treated with Filgrastim intraperitoneally at a dose of 30.83 µg/kg/day for one week; group 2 received *Ocimum tenuiflorum* suspended in corn oil and administered by gavages at 20 mg/kg/day for one week; group 3 were intraperitoneally injected with Filgrastim at a dose of 30.83 µg/kg/day for one week treated with Ocimum tenuiflorum at a similar manure to group 2; group 4 treated with a placebo (0.9% saline solution) (10). After 40 days of the initiation of the experiment all the animals were scarified by cervical dislocation (15) and testicles was taken for histological evaluation (7)

2.4. Morphological analysis

The morphological and histopathological evaluation was performed as previously described by Taib, Budin (16). The testes in both groups were treated with 10% buffered formalin solution and a routine histological procedure was conducted. All 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 mm$^3$), treated with 2.5% glutaraldehyde 0.1 N PBS at room temperature for one hour and post-fixed with osmium tetraoxide for another hour. Testes tissue was dehydrated in 70, 90 and 100% (twice) acetone for five minutes each, followed by 1:1 (acetone: resin) for five minutes and then embedded in epoxy resin. Ultrathin slices (90 nm) were observed using a transmission electron microscope, Tecnai G2 (FEI, USA), at 100 kV (16).

2.5. Animal Ethics

All the procedure in this study including the animal husbandry, handling and animal scarifying were done according to the guidelines instructed by the university Animal Ethics commute.

2.6. 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 data are expressed as the mean±SD, with significant values at p<0.05.

3. Result and Discussion

The main objective of the present study was not only to determine the adverse effect of Filgrastim but also how *Ocimum tenuiflorum* protects the testicular tissue during use of Filgrastim. The result of present study illustrated the effect of Filgrastim on the testicular tissue which is depicted in figure 3. The Histopathological section of the animals in group 1 is clearly visualized in figure 3. The adverse effect of Filgrastim on spermatozoa and germ cells obviously showed on figure 3, these findings are in agreement with the previous study conducted by Saber et al. (10) which was indicated that the Filgrastim causes testicular degeneration. Comparing the histopathological sections obtained from group 1 (Figure 3) and group 4 (Figure 1) illustrated that histological change in testis section due to degenerative effects of Filgrastim in control group did not see and in the control group (group 4) all the testicles function were in normal stages of spermiogenesis with clarity of lamina propria and lumen (17, 18).
Figure 1. Histopathological section of control group

Histological section of 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 20× magnifier scale.
The result on the figure 2 illustrated the ameliorate effect of *Ocimum tenuiflorum* on the testis and fertility of the rats which was previously approved by Joseph et al. (19). Similar to the recorded data in the current study they showed that *Ocimum tenuiflorum* was associated with increase sperm quality and has mitigated iron-induced testicular toxicity via modulation of redox imbalance. Other study suggested the beneficial effects of using *Ocimum tenuiflorum* with the chemotherapeutic drugs (20). The histological section of the current study in group 2 (Figure 2) showed the positive effects of *Ocimum tenuiflorum in case of* sperm count inside the cavity with clarity of myoid cells compared with control on Figure 1.

![Histopathological section of *Ocimum tenuiflorum* group](image)

**Figure 2.** Histopathological section of *Ocimum tenuiflorum* group

Histological section of tests showing an increase in the number of sperms inside the cavity with clarity of myoid cells. The section is stained with H&E stain. The section is captured with 20x magnifier scale.
The result on the figure 4 showed the treatment group with *OcimumTenuiflorum* after induction of testicular degeneration by the Filgrastim which the 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 other hand, comparing the results between the group 3 (Figure 4) and group 1 (Figure 3) revealed a significant increased on the normal spermatogenic stage and sperm cell as well as the normal section of histological strength in group 3 compare with group 1. In addition we can see normal Leydig and Sertoli cells in group 3.

![Histopathological section of induction group](image)

**Figure 3.** Histopathological section of induction group

Histological section of tests shows tissue damage with the inability to distinguish the stages of sperm formation or Sertoli cells. The section is captured with 20x magnifier scale.
Figure 4. Histopathological section of treatment 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 is captured with 20x magnifier scale.

In conclusion, the present study recorded that Filgrastim has negative effect on the testicular tissue since cause's testicular degeneration. On the other hand, the protective effects of *Ocimum tenuiflorum* against adverse effects of the Filgrastim on male reproductive organ are well established.

References:

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