

Protective Effect of *Ocimum Tenuiflorum* Against Negative Effect Filgrastim on Sperm Parameters of Male Rats

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Abstract: Filgrastim is an anticancer drug used for the treatment of cancer patients that suffering neutropenia, on the last years found filgrastim has an adverse effect and wreak lesion on different organs as testis that attributed to formation reactive oxygen species. In general, the last studies suggested to used natural substance beside the anticancer drug for decreased the reactive oxygen species as well as curcumins and lycopene. The *Ocimum tenuiflorum* generally used as antiinflammation and antioxidant, thus our study aimed for evolution the productive effect of *Ocimum tenuiflorum* against filgrastim. In the study 40 male rats was used and divided in 4 equal groups which the 1st group was intraperitoneal injected of 30.83 mcg/kg/day of Filgrastim for one week, the 2nd group that treated with of *Ocimum basilicum* suspended in corn oil and administered by gavage at 20 mg/kg for 2 week, while the 3rd group that rats was intraperitoneal injected of 30.83 mcg/kg/day of Filgrastim for one week beside that treated with *Ocimum tenuiflorum* group and finally the 4th group treated with placebo, than, after 40 days of experiment, evaluation of sperm parameters. In conclusion our result proved that *Ocimum tenuiflorum* has productive effect against adverse of filgrastim.

Keywords: sperm, infertility, Filgrastim, *Ocimum tenuiflorum*, and antitumor.

The general technicality of chemotherapy drugs is not only destroying the cancer cells; however, it was also causing toxicity in healthy cells (Oun et al 2018) overwhelmingly on the cells with high replication rate as germ cells (Grimison et al 2010). Filgrastim was first approved used in 1991 (Cornes and Krendyukov 2019) medication for the treatment of cancer patients that suffering neutropenia due to receiving myelosuppressive chemotherapy (Dale et al 2018). Additionally, the last clinical experiments illustrated that filgrastim decreased febrile neutropenia in cancer patients and subsequently reduced neutropenia incidence (Gascón et al 2016). The last studies provide that Filgrastim has severe side effects as splenic rupture, allergic reactions, and reproductive impairment (Sari et al 2013). Furthermore, the cells previously injured by repeated exposure to antitumor agents and Filgrastim lead to increasing the risk of toxicity of neutrophil products which drives the production of reactive oxygen species (ROS) (Vilaplana et al 2016, Bustani and Baiee 2021). Filgrastim has an upsetting effect on the male and female reproductive system furthermore, has a negative consequence on the sperm parameters (Saber et al 2021). Studies suggested to used multiple antioxidants and natural source substances as productive and ameliorative for decreased the adverse effect of chemotherapy drugs such as Cardamomum (Almeer et al 2021) and Curcumin

(Alibraheemi et al 2021).

Ocimum tenuiflorum generally known as Tulsi and is widely cultivated in different regions of the world, which in the last decade using as a medicinal drug (Deogade and Prasad 2019). Generally used as antiasthmatic, antipyretic, analgesic, antioxidant, and anti-inflammatory (Saxena et al 2012; Iqbal et al 2020). Studies investigated that *Ocimum tenuiflorum* have properties on redox imbalance, and glucose dysmetabolism in oxidative mediated testicular toxicity (Lawal et al 2019) as well as from previously established studies that overall effects of extract of *Ocimum tenuiflorum* in patients with stress were found to be significant increase in sperm parameters than patients don't use *Ocimum tenuiflorum* (Saxena et al 2012).

Thus, the current study aims to clarify the effect of Filgrastim on the sperm parameters in Wistar rats and evaluate the protective effect of *Ocimum tenuiflorum* against the adverse effect of Filgrastim.

MATERIAL AND METHODS

Filgrastim;

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

***Ocimum tenuiflorum*;**

The fresh leaves of *Ocimum. basilicum* were purchased from herbalist and transport to laboratory of Faculty of veterinary, university of Kufa, Iraq, The leaves were washed with distal water then that dried and crushed to powder hence to utilized as mixer, since 100 g was dissolved in 750 ml water and concentrated it by use a rotary evaporator after one 1 hour and stored in refrigerator until the experiments.

Animal and experimental design

Forty adult male rats used in the present study. Which that was purchase from the animal housed of Faculty of sciences, university of Kufa, Najaf, Iraq. The rat's weight was average between 250 and 350. The rats divided randomly in four equal group and hosed in clean cages in the animal house at the Faculty of veterinary, University of Kufa. and the animals were maintained at about two weak for adaptation before starting the experiment. The experimental design included four groups; 1st group (Filgrastim group) since that rats was intraperitoneal injected of 30.83 mcg/kg/day of Filgrastim for one week (Saber et al 2021), the 2nd group (*Ocimum tenuiflorum* group) since that treated with of *Ocimum basilicum* suspended in corn oil and administered by gavage at 20 mg/kg for 2 week (Olofinsan et al 2021) , while the 3rd (Filgrastim and *Ocimum tenuiflorum* group) since that rats was intraperitoneal injected of 30.83 mcg/kg/day of Filgrastim for one week beside that treated with *Ocimum tenuiflorum* group and finally the 4th group (control group) treated with placebo. After 40 days of experiment period all animals of each group were scarified (Mohammadi et al., 2014) and tail of epididymis of testis was taken for measuring the sperm fertility parameters via measured

the following parameters (Sperm general motility%, Sperm individual (progressive) motility %, sperm viability %, acrosomal damage % of sperm, sperm concentration (spm/ml), testis was taken for histopathology examination (Bustani and Baiee 2021, ALIBRAHEEMI et al., 2021)

Sperm parameters

For measured the general and progressive motility percentage of sperm must be placed 10 μ l of the epididymal semen on a warm slide and cover-slid and evaluated by microscope under 400 \times magnifications (Bustani and Baiee et al. 2020). Moreover, sperm viability, morphology and acrosomal integrity evaluation by calculate 200 staining sperm by eosin nigrosine as described by Kaka et al (2015).

Statistical analysis

In the present study use SPSS program (version 23.0) was used to analyze the statistical results. The mean and standard error of data were calculated for the continues variables. A p-value of less than 0.05 was considered as the least statistical significance.

RESULT AND DISSOCIATION

According to a table -1, the outcomes of the result showed that the value of general and progressive motility decreased significantly in the Filgrastim group lower than treatment groups and controls due to adverse effect of on the testicular tissue as recorded by several authors (Saber et al., 2021) which illustrated causes reduction in glutathione and increases ROS activity in various tissues especially soft tissue and testis tissue thus lead to decreased in spermatogenesis as well as causes damage the stage of spermatation (Baiee et al 2020; Lee et al 2020), furthermore, that approbate the result in decreased of sperm motility. Serval study conceive that decreased in the sperm motility instructs to increased in the ROS or in the zink arrived to sperm across testicular tissue (Alonge et al 2019; Weiden et al 2020; Saber et al 2021). Current study proved that Filgrastim causes decreased in sperm viability due to increase in the ROS during spermatogenesis and spermatation showed in table-1 as showed in previous study since proved that toxic substance causes degeneration and damage to rats sperm during spermatation or spermatogenesis stage (Mima et al 2018).

Table 1: Effect of Ocimum tenuiflorum on Motility and Viability against Filgrastim

Groups	Parameters		
	General Motility	Progressive Motility	Viability
Filgrastim group	45.00 \pm 4.00 b	40.00 \pm 4.00 b	26.67 \pm 6.69 b
Ocimum tenuiflorum group	88.00 \pm 1.53 a	83.67 \pm 0.67 a	85.33 \pm 0.60 a
Filgrastim and Ocimum tenuiflorum group	83.33 \pm 1.67 a	78.33 \pm 1.67 a	81.00 \pm 2.65 ac
Positive control grope	73.33 \pm 3.33 a	68.33 \pm 3.33 a	64.33 \pm 1.36 c

Means with the same letters in the same column are not significantly difference

The result in Table 2 showed adverse effect of Filgrastim on the concentration of sperm and morphology integrity which decreased significantly when compared with treatment gropes and control. The Filgrastim effect included causes raised in ROS concentration and free radical molecular which has adverse effect on the sperm parameters and testis as well as Filgrastim causes elevated leukocyte concentration, thus causes relapsing sperm morphology which several studies believe that is an engagement between sperm morphology and leukocyte (Morikawa 2020).

Table 2: Effect of *Ocimum tenuiflorum* on Acrosome, Morphology and Concentration and Viability against Filgrastim

Groups	Parameters		
	Acrosome Defect	Morphology integrity	Concentration
Filgrastim group	1.88 ± 0.11 a	90.83 ± 0.73 a	0.38 × 10 ⁹ a
<i>Ocimum tenuiflorum</i> group	0.83 ± 0.15 b	95.17 ± 1.01 b	1.23 × 10 ⁹ b
Filgrastim and <i>Ocimum tenuiflorum</i> group	1.03 ± 0.10 c	95.50 ± 0.50 b	1.18 × 10 ⁹ b
Positive control grope	0.85 ± 0.12 a	95.67 ± 0.17 b	1.12 × 10 ⁹ b

Means with the same letters in the same column are not significantly difference

On other hand, current study showed productive effect of the *Ocimum tenuiflorum* on the sperm parameters which the result in table 1, showed that was no significant different between control and *Ocimum tenuiflorum* group and treatment grope (Filgrastim and *Ocimum tenuiflorum* group) in all sperm parameters as well as *Ocimum tenuiflorum* has ameliorated on sperm parameters due to its antioxidant properties (Thokchom et al 2020). Several studies proved that *Ocimum tenuiflorum* has several positive effects on testis against toxic substances (Olofinsan et al 2021), adverse effect of drug (Deogade et al 2019) and chemotherapy (Rana et al 2017). As well as *Ocimum tenuiflorum* has ameliorated effect on sperm parameters which that agreed with our results (Angkha et al 2020)

CONCLUSIONS

Interestingly, it was observed that the *Ocimum tenuiflorum* has promoted and productive effect against adverse effect of Filgrastim on testis and sperm parameters.

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