

Ameliorative and Protective Effect of *Armoracia Rusticana* Against Filgrastim Sperm Parameters and Serum Reproductive Hormones of Adult Male Rats

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Abstract:

Filgrastim is an anticancer drug used for the treatment of cancer patients suffering neutropenia. In the last years, studies proved that filgrastim has an adverse effect and wreaked lesions on different organs as testis attributed to the formation of reactive oxygen species. In general, previous studies suggested using natural substances besides the anticancer drug to decrease reactive oxygen species such as curcumins and lycopene. The *Armoracia Rusticana* is a folk medicinal herb used in different medical fields which has an antioxidant effect on the cells. Thus, the present study aimed to investigate the possibility of using the *Armoracia Rusticana* as protection and ameliorative the effect against the adverse effect of Filgrastim on mature albino rats. Moreover, the present study evaluated the sperm parameters and serum reproductive hormones. Finally, the current investigation deduced that *Armoracia Rusticana* has ameliorative and protective against the advisers Filgrastim and can be used synergistically.

Keywords: Fertility, *Armoracia Rusticana*, Filgrastim, sperm parameters, and Adult Male Rats.

Introduction

In the last decades, several factors that affected human life for that reason caused a reduction in reproductive, maternal health, and fetal and child (Alabedi et al., 2021; Bustani et al., 2022). Anticancer and chemotherapy drugs have been the most attention of researchers in the last years (Ghosh, 2019; Moore et al., 2016). The general technicality of chemotherapy drugs is not only destroying the cancer cells but also causing toxicity in healthy cells (Bustani et al., 2022). Chemotherapy as filgrastim has an adverse effect on different organs such as the spleen (Barrios & Poletti, 2005), testis (Alabedi et al., 2021), and liver (Namjou et al., 2019). Previous studies proved that filgrastim has an adverse effect on the reproductive parameters (Saber et al., 2021) and hormonal disturbance since that effect on the level of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) (Baldo, 2014; Saber et al., 2021). Generally, the chemotherapies cause an increase in the formation of reactive oxygen species (ROS) in the cells, subsequently decreasing the cellular function such as spermatogenesis, secretion of hormones, and other organ function. Testicular degeneration is one of the most harmful effects of the filgrastim. The use of filgrastim leads to changes after ischemia and adverse effects of reactive oxygen species, and decreased sperm parameters (El-Shafei & Hassan, 2021). The modern studies suggested used the natural antioxidant drove from the plants and fruits to the reduce the adverse effect of chemotherapies such as Curcumin (Bustani et al., 2022), lycopene (Al-Mousaw et al., 2022), and Ocimum

tenuiflorum (Alabedi et al., 2021) since proved protective and ameliorative effect against the different negative environment and drug effect. the *Armoracia Rusticana* is a perennial herb widely used as a condiment and a horseradish peroxidase source. In the last years, the use of *Armoracia Rusticana* has spread in the scientific community as an anticancer (Nguyen et al., 2013), anti-inflammatory (Marzocco et al., 2015), and antioxidant (Calabrone et al., 2015). Thus our study aimed to use *Armoracia Rusticana* as ameliorated and protective of the level of the reproductive hormones (testosterone, FSH, and LH) and semen parameters against the adverse effects aires by using the filgrastim as an anticancer.

Material and Method: -

Animal: -

Generally, this study used forty adult male rats (Albino strains), with rats' body weight 200–250 g. The rats were purchased from the Baghdad research center and hosed in international cages in the faculty of density medicine, the Islamic university under the universal condition.

Experimental Design

The animals were divided into four equal groups, each with ten rats; the first group was designated as (control group), the second as (Filgrastim group), the third as (*Armoracia Rusticana* group), and the fourth as (*Armoracia Rusticana* group) in two different concentrations (4mg/kg and 8mg/kg) sequentially. The first group was given a placebo, while the second, third, and fourth groups received an intraperitoneal injection of Filgrastim 0.5 ml/kg/day for one week after the trial (Saber et al., 2021). Aside from Filgrastim, the third group was given *Armoracia Rusticana* extract suspended in maize oil and given by gavage at 4 mg/kg for 10 days. (Alabedi et al., 2021; Javaid et al., 2021). Finally, a fourth group was treated with *Armoracia Rusticana* extract suspended in maize oil and delivered by gavage at 8 mg/kg for 10 days only, in addition to Filgrastim. After 41 days of testing, all animals in each group were sacrificed. (Bustani & Baiee, 2021) and the tail of the epididymis of the testis was extracted to measure the sperm fertility parameters using the following parameters (general and individual sperm motility percentage, viability percentage of sperm, acrosomal damage % of sperm and sperm concentration (spm/ml) (Ali Hameed et al., 2021).

Preparation of drugs and samples:

1- Drug

Filgrastim was purchased from medicines stores of Al-Faiha Company.

2- *Armoracia rusticana* plants

The *Armoracia Rusticana* was collected from the Faculty of Pharmacy's Garden of Medicinal Plants. The cut-up air-dried plant material was extracted with methanol or water at 65°C. This procedure was repeated five times. A vacuum rotatory evaporator was then used to filter and concentrate the hot extract solution. The final extracts were stored at 4°C in the dark until tested.

3- Semen sample

Bilateral tests and epididymitis of sacrificed animals were taken at various intervals throughout the experiments from anesthetized animals using intramuscular (IM) injections of Ketamine 90 mg/kg B.W. and Xylazine 40 mg/kg B.W. A sample was utilized to measure the following parameters: -

- a- Semen Morphology
- b- Acrosomal integrity
- c- Concentration
- d- General and progressive motility
- e- Viability

4- Blood sample

By using the disposable syringe, blood samples were taken from each rat and flowed into a gel tube for serum separation. After centrifuging the blood sample (3000 rounds/15 minutes), serum was obtained and stored in the freezer until the serum sample was utilized to measure the following parameters.

- 1- Follicle-stimulating hormone
- 2- Luteinizing Hormone
- 3- Testosterone

5- Statistical analysis

Graphpad prism 8 was used to conduct a statistical analysis of the experimental outcomes. T-test and one-way analysis (ANOVA) were employed to determine the significance of differences between groups and within times. The data were expressed as mean standard errors (SE), with a statistical significance of (P value 0.05). LSD was used to see if there was a substantial difference in the treatment methods. (Swift, 1997).

Result and discussion

The data obtained from table 1 illustrated the negative effect of the filgrastim on the hormonal level, which is a significant increase in the FSH and LH of the filgrastim group compared by the control and decreased significantly of levels of testosterone compared by the control group. Previous studies illustrated that filgrastim has an adverse effect on the determination of Follicle-stimulating hormone, Luteinizing Hormone, and testosterone which Saber et al. (2021) found significantly decreased the serum testosterone levels compared with normal groups (Saber et al., 2021) as well as in the current study, increased in the FSH and LH concentrations was observed in the Filgrastim-treated rats in comparison with the control group, and this disagrees with previous articles (Al-amery et al., 2022; Namjou et al., 2019) In addition, it was discovered that in guinea pigs, LH levels increased but FSH and LH levels remained unchanged, whereas, in ram lambs, both FSH and LH levels increased (Aponte et al., 2019).

On the other hand, the table-1 showed the significant protective effect of two treatment concentration groups of *Armoracia Rusticana* extraction on the hormone levels of FSH, LH, and testosterone, mainly used at 8 mg/kg for ten days; furthermore, the previous studies demonstrated that natural antioxidant sources and natural medicinal plants have a protective effect against the adverse effect of the most chemotherapeutic drugs as

cyclophosphamide since the researcher showed the lycopene is having a defensive impact against the cyclophosphamide that induced testicular toxicity in male rats (Al-Mousaw et al., 2022). Moreover, the studies showed that extraction of *Ocimum Tenuiflorum* has a positive result during the use of the filgrastim as a chemotherapies drug (Alabedi et al., 2021).

Table-1: Effect of *Armoracia Rusticana* on hormonal level of Follicle-stimulating hormone, Luteinizing Hormone and testosterone against Filgrastim

Groups	FSH (mIU/ml)	LH (mIU/ml)	Testosterone(ng/ml)
Control	5.07 ± 0.08 a	4.57 ± 0.116 a	4.20 ± 0.12 a
Filgrastim	10.7 ± 0.27 b	16.5 ± 0.90 b	1.20 ± 0.04 b
T1	7.2 ± 0.12 c	9.2 ± 2 c	3.7 ± 0.2 a
T2	5.9 ± 0.12 a	5.2 ± 0.2 a	4.4 ± 0.1 a

Control: control group treated with a placebo

Filgrastim: Intraperitoneal injection signal dose of 200 mg/kg

T1: *Armoracia rusticana* extraction administered by gavage at 4 mg/kg for 10 days

T2: *Armoracia rusticana* extraction administered by gavage at 8 mg/kg for 10 days

The result in the table-2 illustrates the sperm parameters, including morphology, acrosomal integrity, and concentration. The result proved the negative effect of the filgrastim on the sperm quality as in agreement with previous suites, which proved that using the filgrastim as chemotherapies causes damage in the sperm cells during spermatogenesis, furthermore decreasing the sperm parameters (Alabedi et al., 2021; Barrios & Poletti, 2005; Bustani et al., 2022; Namjou et al., 2019; Saber et al., 2021). The result showed that sperm morphology, acrosomal integrity, and concentration decreased significantly from the control group.

On the other hand, the result showed the protective effect of the *Armoracia Rusticana* extraction in both concentrations of 4 mg/kg and 8 mg/kg for ten days since the parameters in table-2 are showed that the T2 group (8 mg/kg) is superior significantly on than other groups. Scientists suggested using that used the natural substance as an antioxidant and synergistic drug with chemotherapy (Al-amery et al., 2022; Bustani et al., 2022) and toxic drugs (K Abdulameer et al., 2022). The last studies explain the antioxidant effect of the *Armoracia Rusticana* against stress that is induced by different environmental influence (Calabrone et al., 2015; Marzocco et al., 2015; Nguyen et al., 2013). As well as studies have shown that *Armoracia Rusticana* has antimicrobial properties (Manuguerra et al., 2020).

Table-2: Effect of *Armoracia Rusticana* on the sperm parameters (Sperm Morphology, Acrosomal integrity, and concentration) against Filgrastim.

	Sperm Morphology	Acrosomal integrity	Concentration
Control	99.2 ± 1 a	98.5 ± 0.5 a	12 × 50 × 10 ⁶ a
Filgrastim	90.4 ± 0.5 b	89.5 ± 0.5 b	5.1 × 50 × 10 ⁶ b
T1	97 ± 0.5 c	93 ± 1 c	10.9 × 50 × 10 ⁶ c
T2	98.4 ± 0.5 ac	97 ± 0.5 a	13.5 × 50 × 10 ⁶ a

Control: control group was treated with a placebo

Filgrastim: Intraperitoneal injection signal dose of 200 mg/kg

T1: *Armoracia rusticana* extraction administered by gavage at 4 mg/kg for 10 days

T2: *Armoracia rusticana* extraction administered by gavage at 8 mg/kg for 10 days

Moreover, the results in figure-1 showed a significant effect on the *in vitro* evaluation of sperm parameters, including motility in both general and progressive assessment and viability of sperm as augmenting the previous studies (Al-amery et al., 2022; Saber et al., 2021). The treatment groups with both concentrations of *Armoracia Rusticana* showed significant improvement and enhanced sperm motility and viability.

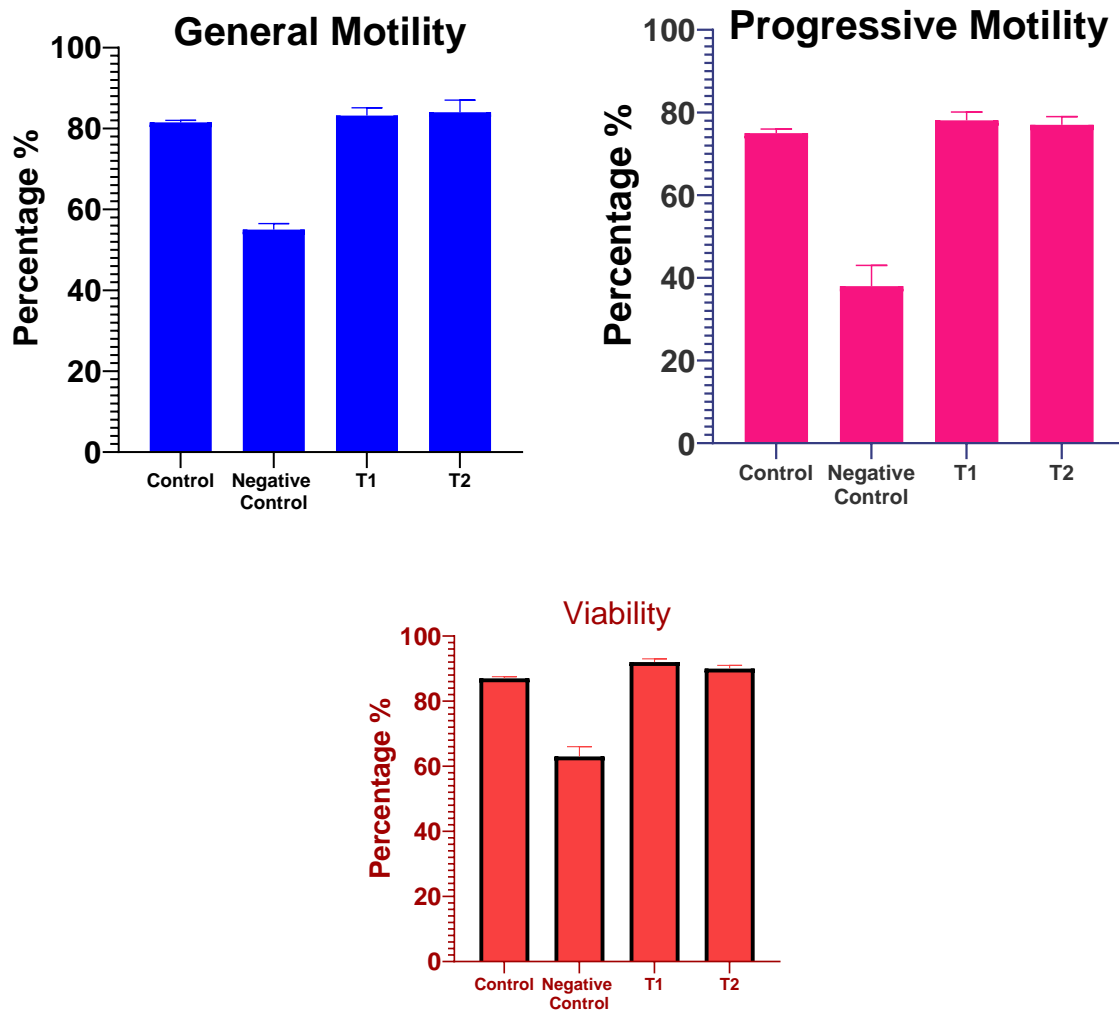


Figure-1 : Effect of *Armoracia Rusticana* on the sperm parameters (Sperm viability, sperm motility and concentration) against Filgrastim

Control: control group was treated with a placebo

Filgrastim: Intraperitoneal injection signal dose of 200 mg/kg

T1: *Armoracia rusticana* extraction administered by gavage at 4 mg/kg for 10 days

T2: *Armoracia rusticana* extraction administered by gavage at 8 mg/kg for 10 days

Conclusion: -

The present study recorded that Filgrastim harms the hormonal levels FSH, LH, and testosterone since it causes testicular degeneration as well as illustrated the productive effect of *Armoracia Rusticana* against advises the development of the Filgrastim.

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