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Original Article

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## Effect of savory and yarrow on spermatogenesis in adult mice undergoing chemotherapy Cyclosporine

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### Abstract

Spermatogenesis process during which the male germ cells are produced at each stage of the disorder, it can lead to infertility. The aim of this study was to evaluate the effect of fennel and yarrow on spermatogenesis in adult micereceiving chemotherapy is cyclosporine. For this study, 32 male Wistar rats with an average weight of 20 ± 200 g were randomly divided into four groups (control, cyclosporine A, cyclosporine and aqueous extracts of yarrow, cyclosporine and aqueous extract of fennel). Three groups of mice at a dose cyclosporine day / kg / mg40 dissolved in half ml sesame oil, received daily. The other two groups, water extracts of yarrow and fennel with a dose of day / kg / mg 150 received. After 21 and 45 days, all rats were euthanized, the testis and epididymis were separated in the next stage of sperm characteristics, morphological changes and morphological index were Trzya by spermatogenesis. Administration of cyclosporine A significant reduction in the number and motility of sperm, as well as a significant increase in the amount of dead and abnormal sperm has caused. If the plant extracts of yarrow and fennel is to improve these parameters. The results of this study show that cyclosporine A by oxidative stress can damage the testes provide, while the aqueous extract of yarrow and fennel with scavenging free radicals and oxidative processes can be reduced the adverse effects of this drug.

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**Keywords:** Oil, Fennel, Yarrow, Cyclosporine, Spermatogenesis.

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## 1. Introduction

Cyclosporine A is a neutral cyclic peptide molecule has a molecular weight of 1201 Dalton eleven amino acids with hydrophobic properties is formed. Effect of cyclosporine A as one of the most original and exclusive immunosuppressive drugs, prevention and reduction of T-cell-cell interactions of the immune response in the body. CSA pharmacodynamic studies have shown that the effect of immunohistochemical immunosuppressant focused at the molecular level on T lymphocytes and T-lymphocytes inhibit transcription of genes that ultimately lead to the creation of antigen-antibody complex becomes also prevents the synthesis of interleukin, especially interleukin 2 to stimulate cell activity lymphocyte helper and cytotoxic lymphocyte cells and interferon gamma synthesis on the other hand prevents that disconnection between T cells and macrophage the cell (Granelli-Piperno, 1990). Like many other medicinal plants all over the world for treatment and prevention of some of the diseases on adjustable use. But the effects of a number of them have been studied scientifically. Medicinal herbs, therapeutic methods useful in both modern and traditional systems is *Thymus serpyllifolius* on two types of summer and winter savory. Summer Savory Winter Savory withstand more cold and moisture and has a rapid growth and high emissions. Savory summer annual species, native to southern Europe and parts of North America. Winter savory kind of hard stems and woody perennial that is native to Europe and North Africa and a more limited use. Summer savory as a medicinal plant, traditionally as a stimulant, carminative, mucus, stomach tonic, anti-diarrhea and anti-infection sexual enhancer used (Emami et al., 2004; Sefidkon et al., 2005). Yarrow is a plant belonging to the Asteraceae that since ancient times it has been used to treat various diseases. New scientific studies, while confirming some of the therapeutic effects of yarrow, rather than in rows of useful medicinal plants have been preserved. Flower and herb yarrow, essential oil is the most important compounds include *β*-pinene, alpha-pinene, beta-pinene, limonene and linalyl acetate. Studies conducted in 1993 by Kastner and his colleagues have shown that the plant extract has anti-inflammatory and anti-edema, which are sesquiterpene lactones present in the plant are attributed (Kastner et al., 1993). The plant mainly as an anti-bleeding drug, stimulating regeneration, wound healing, analgesic and spasmolytic been consumed (Benedek et al., 2007), as well as anti-tumor properties reported for one of its derivatives (Tozyo et al., 1994). Traditional tribes of northern Europe and North America as a contraceptive drug, a stimulant used abortion and menstrual stimulant treatment (De Laszlo and Henshaw, 1954). The aim of this study was to evaluate the effect of fennel and yarrow on spermatogenesis in adult mice receiving chemotherapy is cyclosporine.

## 2. Materials and methods

### 2.1. Extraction savory

First aerial dried, then crushed and milled to extract oil from water distillation and used Clevenger apparatus. 100 grams of dried plant dry weight and a 1-liter round-bottom flask and then poured some water (about two-thirds of balloons) to add and balloons attached to the device Clevenger distillation for 4 hours do. After oil extraction, dewatering work done by anhydrous sodium sulfate and dark in a closed container away from light and can be kept in the refrigerator.

### 2.2. Preparation of extract

Yarrow plant flower grow natural areas around the city of Urmia collected and verified after 10 days in the laboratory temperature and away from direct sunlight, drying and grinding were. The infusion of the dried plant ingredients by heating them for 30 minutes three times in water at 70 ° C was prepared. The resulting liquid after filtration, the solvent removal device in a vacuum and at a temperature of 56 ° C and was concentrated to the initial volume 12/1. The final concentration of the extract and drying, and storage at -20 ° C was dissolved in distilled water immediately after use (Cavalcanti et al., 2006).

### 2.3. Animals

In this study, 32 Wistar male rats in the weight range 20 ± 200 g were used for testing.

#### **2.4. Determine the effective dose animals**

Male mice two months after birth and are more than capable of producing maximum daily sperm and fertilize the female rats. Sexual maturation in female rats also occurs 50-70 days after birth. Previous studies on cyclosporine A study shows that treatment with cyclosporine dose mg / kg / day 40 impairs fertility in rats. Therefore, in this study, the concentrations used.

#### **2.5. Group of animals**

32 adult male rats were divided into 4 groups of 8 and under medication regimen were as follows:

- 1) Control group: only normal saline via gavage for 45 days were administered.
- 2) fennel extract + cyclosporine and Group 3
- 3) Group Yarrow + cyclosporine
- 4) pharmaceutical drug cyclosporine.

#### **2.6. Histological evaluation**

Before the start of the trial and treated, adult male rats for 14 days in the breeding and keeping laboratory animals were grouped together and will adapt to the environment and environmental stress on test results does not affect. After half a day of treated rats at day 45, 21 and the other half were euthanized by cervical intervertebral displacement. Then the tail of the epididymis is used to assess the quantitative and qualitative factors.

#### **2.7. Evaluation of sperm**

##### **2.7.1. Sperm count**

Hemocytometer was used to count the number of sperm. 10 ml of the diluted solution of sperm (1 to 20) on conventional glass slides, and the number of sperm per milliliter using the formula  $d * 5000 * n$  were determined by optical microscope with magnification of 400 times, where n is the number of sperm count and d photos containing sperm suspension is diluted (Zambrano et al., 2005).

##### **2.7.2. Assess the viability and morphology**

To evaluate the percentage of morphologically abnormal sperm Ngrvzyn eosin staining was used. The detection of dead sperm in the staining on the principle that damage to the plasma membrane, the color of the sperm are permeable. So those sperm that each of the parts of the head, neck or tail was painted as sperm were dead. In the same way that a remnant cytoplasmic sperm morphological abnormalities were also other, were considered as abnormal sperm. 200 sperm per sample at 400 times magnification were examined and the results were expressed as a percentage.

### **3. Results**

The results of the various parameters of sperm.

#### **3.1. Results sperm count Apy-Dydym**

The number of sprm- Hay Apy-Dydymy in the groups treated with drugs during 45 Santymvn significantly at  $p < 0.01$  relative to the control group or the control. sprm-Ha number of drug combination therapy with savory or yarrow Santymvn significantly at  $p < 0.01$  in comparison with the number of 45-day drug treatment Santymvn increased and the impact of the drug alone in reducing the number of sprm-Ha more and consumption of savory or yarrow with the drug, the drug's effect in reducing sperm count reduced Santymvn-up.

#### **3.2. Results of the live sperm**

Results showed that the effect of experimental treatments sprm-Hay percent live at 1 percent, significantly ( $p < 0.01$ ), respectively. Santymvn sperm viability with the drug for 45 days minimum, and the number of live sperm in the control group compared with the percentage in most treatments were significantly ( $p < 0.01$ ). The number of live sperm in the epididymis in the group treated with the drug during 45 Santymvn significantly at  $p < 0.01$

compared to control group, all treatments reduced sperm live Yaft.drdsd drug combination therapy with savory Santymvn or yarrow significantly at  $p < 0.01$  in comparison with the number of 45-day drug treatment and the effect of this drug increased Santymvn alone in reducing the percentage of live sperm is more or yarrow and fennel to the drug consumption, drug effects Santymvn in reducing the limit reduced sperm viability.

### 3.3. The results of the counting of immature sperm-Hay

Results showed that the effect of experimental treatments immature sperm at 1 percent, significantly ( $p < 0.01$ ) the percentage of immature sperm with drugs Santymvn for 45 days maximum, and percent control, compared with the percentage of immature sperm in most treatments was significantly lower ( $p < 0.01$ ). The Nmvdar3-3, immature sperm-Hay the groups treated with drugs during 45 Santymvn significantly at  $p < 0.01$  compared to control group and all was obtained. The immature sperm Santymvn drug combination therapy with savory or yarrow significantly at  $p < 0.01$  as compared to 45% in treatment is the use of yarrow and fennel fell Santymvn drug day with drug Santymvn, percent of sperm Savory fell immature so that the group receiving the drug in 45 days, with a significant difference with the control group ( $p > 0.05$ ).

### 3.4. Results of sperm-Ha with damaged chromatin

Results showed that the effect of experimental treatments affected Asprm-Hay percent at 1 percent, significantly ( $p < 0.01$ ), respectively. The sperm with damaged chromatin Santymvn drug for 45 days maximum, and the percentage of sperm with damaged chromatin control compared to their numbers in all treatments was significantly lower ( $p < 0.01$ ). The Nmvdar4-3, the sperm with damaged chromatin in the groups treated with drugs during 45 Santymvn significantly at  $p < 0.01$  compared to control group and all was obtained. Asprm-Hay percent with damaged chromatin associated with drug use Santymvn with savory or yarrow significantly at  $p < 0.01$  in comparison with the number of 45-day drug treatment decreased the consumption of yarrow and fennel Santymvn with drug Santymvn, percent of sperm with damaged chromatin were reduced.

## 4. Discussion

The production of reactive oxygen species (ROS) in the male reproductive system because of severe toxic effects on sperm quality and performance MvzvzyNdvlvzhy has become important in (Saleh and Agarwal, 2002). Studies have shown that 45-25% of infertile men have high levels of ROS in samples of their semen (De lamirande and Gagnon, 1992) but Baydkhatr that the small amounts of sperm for fertilization ROS for reaction chromosome mobility and Zryft access is necessary (Agarwal et al., 2004). The term oxidative stress when used as the oxidant of antioxidant surpass (Sies, 1993). Sperm are especially sensitive to damage caused by oxidative stress because they have plasma screens with high levels of unsaturated fatty acids are linked by dual inhibition of enzymes in the cytoplasm there are a small amount (Vernet et al., 2004). Several studies have shown that among the different cell types found in semen, sperm abnormal and immature leukocytes main sources of ROS production form (Aitken and West, 1990; Garrido et al., 2004).

The results of this study showed that the percentage of sperm with damaged chromatin immature sperm treated with cyclosporine A, a significant increase compared to the control group at  $p > 0.05$  showed. Menon and colleagues Seethalakshmi study and research on the reproductive system in male rats with different doses of cyclosporine reported that the effect of cyclosporine testes of rats, showed degenerative changes and decreased sperm count and motility and subsequent high doses of this Infertility treatment were observed (Seethalakshmi et al., 1987). In another study in 1990 by Seethalakshmi on rat testis and testicular function under the effect of fertility and very low doses of cyclosporine. They have suggested that cyclosporine reduces sperm count and motility also decreased 50% and 60% of infertility gets (Seethalakshmi et al., 1990).

Masuda study in 2003 showed that cyclosporine A reduces the number of spermatozoa, spermatids deformity, increase the remaining bodies, severe degenerative changes in the seminiferous ducts and increasing degeneration and abnormal sperm counting round within two weeks after taking lead (Masuda et al., 2003). The results of this study show that administration of cyclosporine A significant reduction in the number and motility of sperm, as well as a significant increase in the amount of dead and abnormal sperm has caused. If the plant extracts of yarrow and fennel is to improve these parameters.

Traditional tribes of northern Europe and North America yarrow as a contraceptive drug, a stimulant abortion and menstrual stimulant used (De Laszlo and Henshaw, 1954). Today, yarrow modern medicine orally as anti-

inflammatory, antispasmodic, lowering blood pressure, reducing fever, urinary tract disinfectant and locally as balms and moisturizing the skin is used. In the past, this plant medicine as anti-inflammatory, diuretic, binding rule has been used (Ahero, 1975).

Savory herb used in traditional medicine has therapeutic uses, such as analgesic and anti-viral infection and also property (Abed et al., 1999), anti-inflammatory, anti-bacterial and anti-fungal, anti-spasmodic and anti-diarrhea and vasodilatory (Sanchez de Rojas et al., 1996). In study of Takzare the effect of different concentrations of the extract was tested on 32 rats showed that the comparison between the experimental group and control group, the groups that received high doses of the extract changes such as thickening of the basement membrane tube seminiferous epithelium germ cell depletion, congestion in the testes have been shown (Takzareof Mortazavi et al., 2012). Dalsenter study the effect on sperm count and abnormal extract in rats has been investigated. In this study, although there was no significant difference between the control and treatment of abnormal shapes, but there was no significant difference between the two groups (Dalsenter et al., 2004).

## 5. Conclusion

Finally, it can be stated that cyclosporine A structural damage in the testes of the performance through the balance of Aksydasyvn- resuscitation can cause oxidative stress, causing reproductive toxicity is male reproductive system, the Create partial protection against the adverse effects of cyclosporine in the male reproductive system and can be used as an alternative to improve functions such as cyclosporine therapy in transplant patients is simultaneously used.

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