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Evaluation of the Hypophysial-Testicular Axis and Testis Tissues after Matricaria Chamomilla Consumption in Rats

Hossein Khastar[1], Mehrnoush Ghaemmaghami[2], Leili Hatami[3*]

[1] Dept. of Physiology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.
[2] Dept. of Midwifery, School of Nursing & Midwifery, Shahroud University of Medical Sciences, Shahroud, Iran.
[3] Dept. of Basic Science, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.

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Abstract

Background: *Matricaria chamomilla is an ancient herbal drug widely consumed to sexual system improvement. The aim of this research was to study the effect of Matricaria chamomilla hydroalcholic extract on the pituitary-testis axis and testes tissues of male rats.*

Methods: *Mature male rats were randomly assigned to two groups: the Matricaria group (100 mg/kg of Matricaria chamomilla extract via feeding tube once daily for 8 weeks) and the control group (1 ml of distilled water orally for the same period). After 8 weeks, animal were evaluated for blood levels of testosterone, estrogen, FSH, and LH. Fertility indices like sperm count and the weight of the reproductive organs were assayed. In addition, testes histological assessments were done.*

Results: *Consumption of Matricaria caused elevation in blood testosterone, FSH an LH. Spermatogonia, Spermatocyte, Spermatid, Spermatozoa, Leydig and Epididymal spermatozoa reserve count and reproductive organs weight were increased too.*

Conclusions: *These findings suggest that Matricaria chamomilla extract improve pituitary-testis axis and spermatogenesis in rats.*

Keywords: Matricaria chamomilla hydroalcholic extract, Hypophysial-testicular axis, Spermatogenesis.
***Corresponding to:** L Hatami, **Email:** hatlail@yahoo.co.uk
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Introduction

Infertility is often a complex problem in medical sciences and affects 6.1 million U.S. couples. Male infertility is the inability to produce enough healthy and active sperms.[1,2] Due to the side effects of synthetic drugs in modern medicine, the use today of traditional medicine, such as acupuncture and herbal drugs, is considered in the treatment of infertility.[3,4] In recent years, researchers have concentrated on the effects of different herbal drugs on the fertility of laboratory animals, and their results have provided valuable information. Many studies have been designed on the hypophysial-gonadal axis and testicular tissue using plant extracts. Extracts such as carrot seed[5] and vajicaranrasayana herbs[4] have beneficial effects on the hypothalamic-pituitary-gonadal system and spermatogenesis.

Chamomilla is one of the plants used in traditional medicine for its various health benefits. The consumption of German chamomilla (Matricaria chamomilla or Matricaria recutita), of the Asteraceae family, as a herbal drug, dates back many years in many countries. Chamomilla is used for treatments such as the relief of pain, morphine withdrawal syndrome, and cancer.[6-8]

Chamomilla extract consists of approximately 120 chemical compositions including Chamazulenes, flavonoids, Coumarins, Apigenin, and Bisabolol. According to scientific research, components of chamomilla extract have anti-inflammatory, antibacterial, and antioxidant activities.[9] Chamomilla has flavonoid antioxidants that are effective in neutralizing reactive oxygen metabolites (ROMs). ROMs affect the cell membrane and lead to membrane lipid peroxidation and to malondialdehyde production elevation.[10,11] ROMs cause lipid peroxidation in the sperm membrane, which is associated with decreasing the sperm motility and damaging the membranous parts. Antioxidants are compounds that inhibit the formation of free radicals and lipid peroxidation, protect the sperm cell from damage by the free radicals, and improve sperm qualities and finally fertility parameters.[2,12]

According to traditional medicine, chamomilla is used as a sexual-power stimulant. Chamomilla is a relatively cheap, widely available, accepted, and effective herbal drug. Because there were no scientific reports about its effects on the reproductive system and considering the widespread application of medicinal plants for the treatment of diseases with lower side effects in contrast to the currently used medicines, this study was designed to assess the effects of Matricaria chamomilla hydroalcoholic extract on the pituitary-testis axis and testes tissues of male rats.

Materials and Methods

Male Sprague–Dawley rats weighing 220-260 g were housed under controlled environmental conditions (24 ± 2°C and 12-h light/dark cycle) and allowed free access to standard rat chow and tap water. Animal care was in compliance with the guidelines of the Animal and Human Ethical Committee of Shahroud University of Medical Sciences.

To prepare the whole plant Matricaria chamomilla hydroalcoholic extract, German chamomilla was collected from the Baghiyatallah Agricultural Research in autumn. Plants were dried at 25°C in the shade. For extraction after grinding the dried plants, they were kept in a container with 300 g/L of 80% ethanol for 48 h.After filtering, the ethanol solution was removed by rotary machine and obtained extracts were dissolved in distilled water.[13]

The animals were randomly divided into two groups: 1- control group (n=11) and 2- the matricariagroup (n=11). The Matricaria group received 100 mg/kg/day of body weight (1 ml)

chamomilla extract orally for eight weeks and the control group was administered 1 ml of distilled water, orally, daily. The rats' weights were measured at the beginning and end of the experiment. Finally blood was collected from the left ventricle with ether anesthesia. The samples collected were centrifuged for 15 min at 3000 rpm and serum samples were kept at −20°C for measurement of serum levels of estrogen, testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Hormonal measurements were performed based on using the laboratory methods radioimmunoassay kit (Kavoshyar Co.). The testicles were removed and weighed after the isolation of the surrounding connective tissues. After preparation of tissue slices, the number of sertoli cells, leydig cells, spermatogonia, primary spermatocytes, and spermatids was determined by counting cells in the visual field by randomly preparing smears. For each group, 20 slides and for each slide, three seminiferous tubes, were randomly selected. The epididymis was also removed to investigate the sperm. The removed epididymis was laid in saline at 37°C for 20 min to determine the amount of sperm stored in the epididymis by light microscope at a magnification of 400×.

The results are expressed as means±standard errors. Unpaired Student t-test was used for comparison, and statistical significance was determined as $P<0.05$.

Results

Chamomilla extract effects on body weight and testicular weight: Figure 1 shows that oral administration of the chamomilla extract increased the body weight of animals in the treatment group compared with the control group. Figure 2 shows that the weight of the testes was significantly increased compared with the control group.

Chamomilla extract effects on sperm count: Sperm counts increased in the Matricaria group compared to the control group (Table 1).

Chamomilla extract effects on hormone levels: Serum levels of LH and testosterone in the Matricaria group were significantly increased. No significant difference in estrogen levels was observed (Table 2).

Chamomilla extract effects on testicular tissue changes: Histological results showed that both groups were normal in appearance, shape, and distribution of tubules. However, according to Table 2, the number of spermatogonia, primary spermatocytes, spermatids and sperm was increased in the Matricaria group. The number of leydig cells was also increased in the Matricaria group (Figure 3).

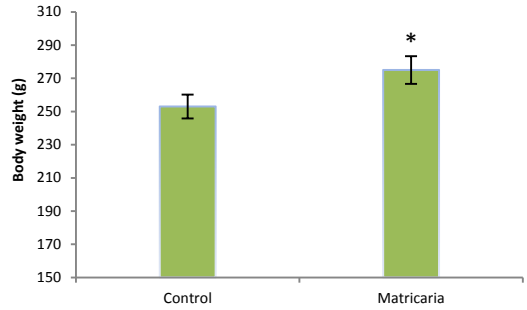

Figure 1. Rat body weight (Mean±SEM). Note: * Denotes P<0.05 versus control group

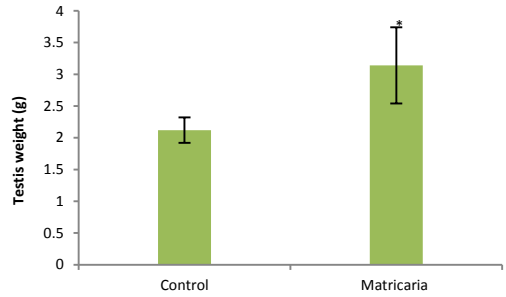

Figure 2. Rat testes weight (Mean±SEM). Note: * Denotes P<0.05 versus control group

Table 1. Histological indices of testes and epididymal spermatozoa reserve

	Spermatogonia	Spermatocyte	Spermatid	Spermatozoa	Sertoli	Leydig	Epididymal spermatozoa reserve
Control	60.56 ± 3.14	69.55±3.09	251.10±11.56	161.57±9.29	14.94±1.86	23.22±1.22	199.08±9.17
Matricaria	*71.22±4.49	*79.91±5.61	*299.15±19.86	*188.69±13.08	16.05±1.71	*28.70±2.19	*230.95±17.38

Results are expressed as Means±SEM. Note: *Denotes *P*<0.05 versus control group

Table 2. Serum testosterone, estrogen, FSH and LH levels (Mean±SEM).

	Testosterone (ng/ml)	Estrogen (pg/ml)		FSH (miu/ml)	LH (mIU/ml)
Control	139.86±6.01	15.1±2.52	24.19±2.59	211.54±9.22	
Matricaria	*161.56±12.41	17.11±3.68	*33.67±3.88	*245.71±16.84	

Results are expressed as Means±SEM. Note: * Denotes *P*<0.05 versus control group

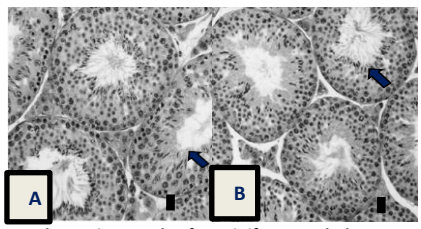

Figure 3. Photomicrograph of seminiferous tubules. A: Control group and B: Matricaria group. Scale Bar = 200 µm (H&E×200)

Discussion

In the present study, the influence of the matricariachamomillahydroalcoholic extract on the pituitary-gonadal axis and testicular tissue of adult rats was evaluated. Chamomilla plant has several different compounds, such as Chamazulenes, flavonoids, Coumarins, Apigenin and Bisabolol. The results of this study indicate that the chamomilla extract led to changes in the structure and function of the testes and pituitary-gonadal axis of rats.[14]

Matricaria chamomilla hydroalcoholic extract consumption caused a significant increase in the body and testes weight and this weight gain probably was the result of testosterone elevation. Previous studies have shown that testosterone has a direct effect on sertoli cells, released tubal fluid, and several proteins, such as growth factor and transferrins that have roles in feeding and dividing sexual cells and finally in sperm production. Thus, considering the important role of testosterone in spermatogenesis, it is clear that the increase in this hormone causes increase in the number of sperm.[14,15]

Testosterone and LH levels were increased followed by MatricariaChamomillahydroalcoholic extract consumption. Because the testosterone was secreted by leydig cells in response to the stimulation by LH from the pituitary gland, the probable mechanism of the testosterone elevation after treatment is a result of the effect of MatricariaChamomillahydroalcoholic extract on anterior pituitary gonadotroph cells and on increasing LH production.[14,15] On the other hand, a negative feedback mechanism of testosterone controls the secretion of LH from the anterior pituitary and chamomilla may indirectly increase the secretion of the gonadotropin releasing hormone (GnRH) from the hypothalamus. This is followed by an increase in LH secretion from the anterior pituitary gland, resulting in an increase in testosterone. However, it is likely that more time is needed for the mechanism of the pituitary-testicular negative feedback,[16] and further studies here are needed.

Matricaria Chamomilla hydroalcoholic extract has no effect on the secretion of FSH. This hormone is secreted from the anterior pituitary and directly affects the sertoli cells and plays a major role in starting the process of spermatogenesis.[17,18] Studies have shown that FSH secretion is independent of GnRH.[19] Studies in recent years have shown that many mutations occur in the LH receptors on the leydig cells, but the FSH receptor mutations rarely occur by the sertoli cells.[20,21] However, further studies are warranted.

ROMs are produced in the daily body reactions and increased ROM levels can be associated with abnormality of sperm development, function, and fertilizing capacity.[2] Chamomilla has flavonoids and phenolic compounds that are effective antioxidants to neutralize the ROMs.[9] ROMs decrease antioxidant enzymes of tissues and antioxidant consumption improves the antioxidant defense system of body.[22,23]One of the possible consequences of the effect of chamomilla on increasing the sperm count may occur due to the presence of antioxidants in the extract. It was demonstrated that the decrease in antioxidant enzyme activity reduced the sperm count and administration of the antioxidant increased the total number of sperm.[24]

Research indicates that phytoestrogens in plant extract are capable of binding to the estrogen receptor, creating a negative feedback on decrease in LH and testosterone levels.[25,26] Phytoestrogens found in chamomilla act similar to the female sexual steroids. Karbalaei et al. showed the chamomilla estrogenic effects on the testes and on reduction of the spermatogenesis, with a concentration of 400 mg kg chamomilla.[27,28] In many medicinal plants, the dosage of extract can have an important role in its effects. Some dosages may have agonist effects but others may have antagonist effects. According to reports of Nusier et al.,[28] after rosemary consumption, the number of sperm was reduced, while Heydari et al.[29] showed that low dosage has no effect on the spermatogenesis.

Due to the high phytoestrogenic factors in chamomilla and the increase of LH after chamomilla extract in the present study, it can be concluded that at this dosage (50 mg/kg/day), phytoestrogenic effect is not very strong, and no significant changes in serum estrogen levels in this study confirm this results.

In traditional medicine, chamomilla has effects on increase in sex power. According to our findings, MatricariaChamomillahydroalcoholic extract had androgenic effects and increased androgen dependent factors. In addition, increases in sperm count and testes weight gain were seen. Generally, chamomile has beneficial effects on male sex indices. However, further research is required to determine the exact mechanisms of each component of chamomiles extracts.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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