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Original Article

Effects of Hydroalcoholic Extract of *Matricaria chamomilla* on Serum Testosterone and Estradiol Levels, Spermatozoon Quality, and Tail Length in Rat

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Abstract

Background: *Matricaria chamomilla* (chamomile) is a herb used to treat various human illnesses. The present study was conducted to investigate the effects of chamomile extract on spermatozoon quality, serum levels of estradiol and testosterone, and sperm tail length in male adult rat.

Methods: Male Sprague-Dawley rats received extract of chamomile (400 mg/kg once daily, orally) during an 8-week period, while the control animals received water. After this period, the animals were sacrificed and the blood samples were obtained. The serum levels of testosterone, estradiol, and the number, motility, and morphology of spermatozoon were assessed. The spermatozoon tail length was assessed by a rapid stereological method.

Results: The body weight, and weight, and volume of the testis in the control and experimental rats did not change significantly. Serum testosterone level was decreased (~76%, P<0.005) and the serum estradiol level was increased (~16%, P<0.04) in the experimental animals. The spermatozoon count and motility were decreased in the experimental group but spermatozoon morphology did not show significant changes. The mid-piece and total tail length were reduced in the experimental group (~22%, P<0.001).

Conclusion: *Matricaria chamomilla* extract can decrease spermatozoa count and motility, spermatozoon tail length, serum testosterone level and increase serum estradiol level in male adult rat.

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Keywords ● *Matricaria chamomilla* ● sperm ● estradiol ● testosterone ● Stereology

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Introduction

Matricaria chamomilla (chamomile) is a herb used to treat various human illnesses since the ancient time.[1] The main constituants of chamomile are aminoacids, polysaccharides, fatty acids, essential oil, mineral elements, flavonoids and other phenolic compounds.[2] It has been reported that phytoestrogens are one of the components of chamomile.[3] Phytoestrogens are a group of naturally occurring non-steroidal

plant compounds. Because of the structural similarity between phytoestrogens and estradiol, they have the ability to cause estrogenic and/or antiestrogenic effects.

Various studies have demonstrated the health benefits of phytoestrogens in various conditions including vasomotor symptoms,[3] and postmenopausal health risks.[4-6] The compounds have also anticarcinogenic, neuroprotective, cardioprotective,[7] and bone formation promoting properties.[8,9] From a functional view, the effects of chamomile are similar to estrogen and progesterone hormones.[10-13] On the other hand, in males, estrogen is present in low concentrations in blood, but it can be found in high concentrations in seminal fluid. It is well proven that male reproductive tissue expresses estrogen receptors.[14]

The main functions of male reproductive system are sex hormone and seminal fluid production. Spermatozoon is a haploid cell which as male gamete ejaculates to join the ovum of a female to form a zygote. Spermatozoon has a tail or flagellum. The tail of a spermatozoon plays a role in the propulsive velocity of the cell and thus its ability to achieve fertilization. The total spermatozoon tail contains mid-piece, principal piece, and end piece. Motility is mainly based on the energetic (the mid-piece), and kinetic components (length of the spermatozoon's tail).[15]

Although the biochemical and hormonal properties of chamomile have been studied,[1-3,10-13] the effects of this herb on spermatozoon have received less attention. We aimed to evaluate the effect of chamomile on the serum levels of estradiol and testosterone and sperm quality in male rats. In addition, the structural changes of spermatozoon's tail were also investigated.

Materials and Methods

Plant Material

Chamomile flowers were collected in spring 2008 from Yasuj province (Yasuj, Iran). The identity of the plant was approved by a herbalist. The voucher specimen (No. 1387-1) was deposited in the central herbarium of Shiraz University of Medical Sciences.

Preparation of Hydroalcoholic Extract

The plant flower parts were left to dry and protected from direct sunlight. They were powdered and the hydroalcoholic extract was obtained by the percolation method. One hundred grams of the powdered chamomile was put into percolator and 1200 ml of 50% ethanol was added to the powder during three days. The resulting solution was collected and the solvent was evaporated. Twenty-nine grams of a semi-solid extract was obtained from 100 g of chamomile powder. The extract was mixed with normal saline to achieve appropriate concentration.[16]

Animals and Treatment

All animal experiments were approved by the Animal Ethics Committees of Shiraz University of Medical Sciences. Twenty male Sprague-Dawley rats weighing 250-300 g were provided by the laboratory animal center of Shiraz University of Medical Sciences.

The animals were kept in a constant humidity and temperature. The rats were distributed randomly into two groups. The control group received water and the experimental group received chamomile extract (400 mg/kg/day, orally via a stainless steal feeding needle).[17] The gavage was done for 8 weeks. The spermatogenesis cycle is about 48-56 days in rats.[18] On the last day of the treatment, the animals were weighed, euthanized, and blood samples were obtained from abdominal aorta. The testes were removed and weighed. The testes volumes were estimated according to the method described by Scherle.[19]

Hormone Assay

One-milliliter blood samples were obtained and the sera were separated by centrifugation and stored at -20ºC for the subsequent hormone assays. Serum testosterone and estradiol levels were measured by radioimmunoassay.

Sperm Collection

One centimeter of the vas deferens distal to the cauda epididymis was cut and placed in medium to allow sperm to “swim out”. Hank's balanced salt solution was used as a medium and was warmed at 37°C to avoid distortion of the sperm.[20,21]

Spermatozoon Count

Each semen sample was spread on a hemocytometer and the heads of the spermatozoa were counted manually using optical microscope. Between 300 and 400 spermatozoa were counted and the data were expressed as the total number of spermatozoa/ml in each rat.[20,21]

Spermatozoon Motility

The spermatozoon suspension was placed on a slide. The slides were evaluated with a microscope in 10 microscopic fields and

200–300 spermatozoa were analyzed at a final magnification of ×1000. The assessment of the motile spermatozoon fraction was defined as the mean number of motile spermatozoa multiplied by 100 divided to the total number of the spermatozoa. The motility of each spermatozoon was graded as follows:

I. Rapid progressive motility
II. Slow or sluggish progressive motility
III. Non-progressive motility
IV. No motility

If more than 50% of spermatozoa were graded as III or IV, it was considered as abnormal motility.[20,21]

Spermatozoon Morphology

Spermatozoa were classified as normal and abnormal. The abnormality was defined as a variety of head and tail anomalies including blunt hook, banana-head, amorphous, pin-head, two-head, two-tail, small head, and bent tail spermatozoa**.** The spermatozoon suspension prepared for analysis was placed on a slide and air dried. The sample was stained with Eosin Y. The slides were evaluated with a microscope in ten microscopic fields, and spermatozoa were analyzed at the final magnification of ×400. The normal morphology spermatozoon fraction was defined as the mean number of normal spermatozoa divided to the total number of spermatozoa multiplied by 100.[20,21]

Stereological Study

The spermatozoon samples prepared for morphology assessment were used for stereological estimating. A video-microscopy system, composed of a microscope (Nikon E-200, Japan) linked to a video camera, a computer, and a monitor was used to determine the mean spermatozoon tail length at a final magnification of ×2000. A ×60 oil immersion objective with numerical aperture of 1.4, was used to achieve a better recognition of the tail. The length was estimated according to the stereological methods for estimating the length in two-dimensional space.[22-24] According to the rules for systematic random sampling, the microscopic fields were sampled. Briefly, the microscope stage was moved in an equal interval along the X- and Y-direction of the microscope stage, using the stage micrometer of the microscope. Between 100 and 150 spermatozoa were sampled on each slide. To achieve an acceptable precision, it has been advised at least 5 specimens in each group undergo the analysis and a 100-200 probe interaction (e.g. sampling of 100-200 spermatozoon's head by counting frame, or 100-200 intersections of Merz grid with spermatozoon's tail) should be considered.[25-27]

In each sampled microscopic field, a test system including two elements was superposed on the image on the monitor. The first component was the unbiased counting frame (figure 1). In this frame, if a spermatozoon's head lay inside the frame and did not touch the forbidden lines (left and inferior borders of the frame), it was sampled. After calculating the magnification, the counting frame size was 83µm× 52µm. Another component was a rectangle (with an area of 53 µm multiplied by 26 µm) that a Merz grid was inside it. Merz grid is

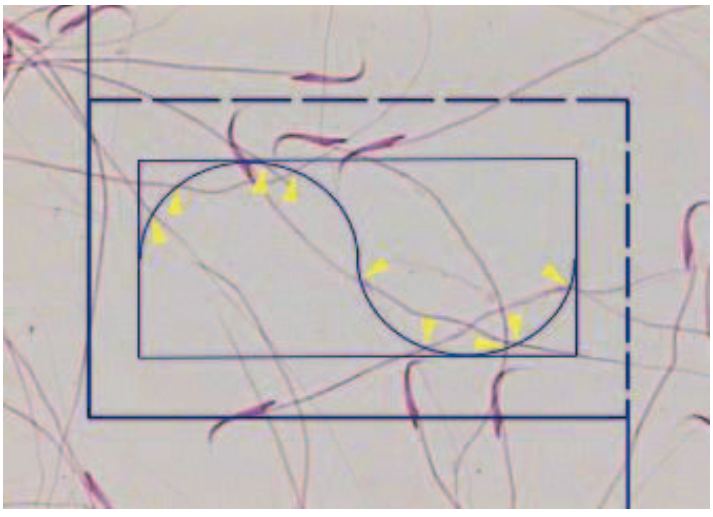

Figure 1: This figure shows the spermatozoa heads and tails in a microscopic field. To estimate the tail length, a test system consisted of two components was superimposed on the image. The first was an unbiased counting frame (the large frame) with acceptance (dotted) and forbidden (bold) lines. If the spermatozoa head lie inside the frame and did not touch the exclusion lines, they were sampled (here three spermatozoa). Another component was a rectangle with a Merz grid inside it (the curve with two semicircles). The arrow heads show the intersections between the Merz grid and the tails.

a curve which consists of two equal semicircles (figure 1). The following formula was used for estimating the mean spermatozoon tail length:[22-24]

ΣL (total tails) = (π/2). (a/l). (1/asf).ΣI

L (tail) = ΣL/ΣN, where "a/l" was the Merz grid constant which was obtained dividing the area of each basic tile by length of semicircles. Within this tile, there were two semicircles of length of π.d., (simple formula for estimation of the perimeter of a circle), where "d" was the diameter of the circle and "asf" was the area sampling fraction. The "asf" was computed by dividing the area of the rectangle by the area of the unbiased counting frame. "ΣI" was the total intersections of the tails (mid-piece or total tail) with the semicircles (figure 1). "ΣN" was the total number of the counted spermatozoa in the unbiased counting frame in all fields.

Statistical Analysis

SPSS software version 11.5 was used for the analysis. The data were reported as mean±SD. Statistical comparisons between the groups were performed using Mann-Whitney U test. P values<0.05 were considered as significant.

Results

Body and Testes Weight and Volume

The body weight and the testes weight and volume of the control and the treatment groups did not change significantly (table1).

Serum Levels of Testosterone and Estradiol

Serum testosterone level was lower (~76%) in the treatment group in comparison with the control group (P<0.005). The serum level of estradiol was significantly higher (~16%) in the treatment group in comparison with the control rats (P<0.04; table 1).

Spermatozoon Count

The spermatozoon count was decreased significantly (~42%; P<0.002) in the treatment group compared with the control group (table 1).

Spermatozoon Motility

The percentage of the motile spermatozoa in the treatment group showed a significant reduction in comparison with the control group (P<0.001; table 1).

Spermatozoon Morphology

The normal spermatozoon morphology did not show any significant difference in the treatment group compared with the control group (table 1).

Spermatozoon Tail Length

The mid-piece length in the treatment group was reduced (~22%; P<0.001) in comparison with the control group (table 1). The total length of the spermatozoon tail was also reduced (~22%) in the treatment group (P<0.03) in comparison with the control group (table 1).

Discussion

We described the effects of chamomile extract on the spermatozoa count, motility, morphology, tail length, and serum levels of estradiol and testosterone in adult male rats. Herbal and natural products represent one of the most popular alternative treatments by people. Many of the natural products have hormonal activity.[12,28-34] In our study, the effects of chamomile extract on male reproductive system in rats may be caused by the hormonal effects. The spermatozoa count and motile spermatozoa decreased after the 8-week treatment with chamomile extract. It has been shown by Das and colleagues,[35] that high dose of flavonoid-rich seed extract of Vitex negundo decreased the spermatozoa count and motility in rats.

We found that a high concentration of chamomile extract decreased the serum level of testosterone and increased the serum estradiol level in adult male rats. This may be due to the estrogenic activity of phytoestrogens in chamomile extract. This finding is in agreement with Rosenberg Zand and co-workers,[10] who

Table 1: Mean±SD of the factors assessed in the control and treatment groups

Factors	Control (n=10)	Treatment (n=10)	P value
Body weight (g)	332±43	341±26	0.5
Testis weight (g)	1.4±0.17	1.5±0.19	0.5
Testis volume	1.4±0.15	1.5±0.17	0.2
Serum levels of testosterone (ng/ml)	2.1±1.3	0.5±0.5	0.005
Serum levels of estradiol (pg/ml)	11.7±1.6	13.6±2.0	0.04
Sperm count (×10^6/ml)	40.2±14.8	23.2±8.6	0.002
Motility (%)	76.6±5.5	65.2±5.9	0.001
Morphology (%)	93.5±3.7	92±5.3	0.5
Mid-piece (μm)	37.1±7.9	28.7±6.3	0.001
Total length of sperm tail (μm)	109.2±8.8	84.6±10.8	0.03

showed that high concentrations of chamomile extract possessed a weak estrogenic and progestational activity in cultured supernatant of breast cancer tissue. Tarrago-Castellanos and others,[36] showed that other herbs such as coumestrol (a phytoestrogen that can be found in high concentrations in the dietary elements of cattle) decreased testosterone levels in rats. In addition they observed that the phytoestrogen had an inhibitory effect on spermatogenesis. Tamir and colleagues,[37] showed that the plant extracts might exhibit different activities over a concentration range as agonist or antagonist, suggesting an estrogen receptor-mediated effect at low concentration and an estrogen receptor independent effect at higher concentration. Qin and co-workers,[38] showed that flavonoids from Semen Cuscutae increased testosterone and LH secretion in vitro and in vivo in rats. It should be noted that in the study of herbal extracts, we cannot attribute the observed biological effects to a particular constituent, because many other compounds are present in the plant extracts.

The tail of a spermatozoon is one of the important parts that plays a role in the swimming ability of the cell and consequent fertilization. Our results showed that the length of all parts of the spermatozoon tail was influenced by chamomile extract. The decreased spermatozoon motility may be caused by the effects of chamomile extract on both energetic and kinetic components of the spermatozoon tail. The tail of spermatozoon was estimated by a rapid stereological method. It is a simple method without the need of digital tracing of the tail on the images of spermatozoon at light or electron microscopic studies. Rønn and colleagues,[22] have reported that in estimation of the length of neurite using the stereological method, the developing process of the neurons was approximately five times less time-consuming than the conventional method using the digital tracing of the neurite images. They showed that the method was both faster and less laborious in comparison with the conventional methods.

In the present study, we did not use any software to provide the geometrical grids like rectangular frame or semicircles. In the rapid stereological method, it is possible to draw the frames and Merz grid (compose of two semicircles) on an ordinary transparent paper and superpose them on the live or captured spermatozoon images on the monitor. The method takes 5-10 minuets for each sample analysis and can be used complementary to the routine sperm evaluation.

Conclusion

Matricaria chamomilla extract can decrease spermatozoa count and motility, spermatozoa tail length, and serum testosterone level and increase serum estradiol level in adult male rats.

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Conflict of Interest: None declared

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