The effects of Matricaria Chamomilla extract during neonatal period of rats on pituitary-gonadal hormone axis and changes in testicular tissue of male progenies

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Abstract

German Chamomile (Matricaria Chamomilla) is from the Astraceae family. This plant has been used in traditional medicine such as analgesic, antispasmodic and anti-inflammatory drugs and for treating skin diseases and so on. In this study, the effects of using German chamomile's hydroalcoholic extract during the neonatal period were evaluated on pituitary-gonadal hormone axis and changes in testicular tissue of male rat progenies. Forty female mature virgin rats from the Wistar race, in the weight range of 180-200g and age range of 90-100 days, were used. After childbirth, samples were divided into four groups (ten mice per group) including control, placebo, and two experimental groups. Control group did not receive injections. Placebo group was injected with 0.5cc of normal saline daily during the lactation period. Experimental groups received 50mg/kg and 100mg/kg of hydroalcoholic extract obtained by soaking method. Injections were done intraperitoneally every day during the lactation period. At the end of the period (24 days), blood samples were taken from heart, serum was separated and the amount of testosterone, FSH, and LH were measured. Also, testis tissue slides were prepared and colored using eosin-hematoxylin method and studied histologically. Results showed that the extract increased FSH not significantly whereas increased LH, testosterone and also male sexual cells including spermatogonia, spermatocytes, spermatids, Sertoli cells, and Leydig cells significantly ($P<0.05$).

Key words: Chamomile, hydroalcoholic extract, neonatal, pituitary gonadal axis, testis tissue, testosterone, LH, FSH

Introduction

The use of herbs is as old as human creation. By studying the ancient tribes, we find that medicinal plants have been used as medicine, pesticides, detergents, paints and so on. Some extant chemical compounds in plants have a complex structure that it is impossible to synthesize in the laboratory or is possible only by spending a lot of time and money. After facing problems such as water, air and soil pollution which have been caused by chemical factors and also side effects of chemical drugs which often appear after a few generations, the use of nondestructive matters was proposed so that herbal drugs were used more than 7% in industrialized countries (Zargari, 2008, Zaman 1989).

Due to adverse effects and side effects of chemical drugs, using medicinal plants has been considered of late. Many studies have been conducted about the effects of various plants on fertility of laboratory mammals which have presented valuable results (Parandin et al. 2011).

Fertility is one of the most important issues in medicine. The most common reason for men's infertility is their inability in producing male sexual hormones and sufficient active healthy sperms (Kumar and Kant Singh. 2015). Spermatogenesis in the testis is carried out under the control of secreted testosterone and secretion action of testes is controlled itself by hypothalamic-pituitary-testicular axis (Ramaswamy and Weinbauer. 2014).

Chamomile (Matricaria Chamomilla) from Astraea family has been proposed in traditional medicine because of its different properties. It is a fragrant plant which grows in lawns and gravel courts. Chamomile has a green white stem, and small hairy leaves with narrow irregular cuts (Esmaeili et al. 2007). The origin of this plant is from different parts surrounding the Mediterranean Sea but is now found in Europe, Moderate regions of Asia, and even in America. Chamomile is used in traditional medicine as a pain reliever and anti-depression drug (Viapiana et al. 2016). Chamomile is also used for treating many human diseases such as hay fever, inflammation, muscle spasms, menstrual disorders, insomnia, ulcers, digestive disorders, rheumatic pain, and hemorrhoids (Srivastava et al. 2010). Also, scientists have reported positive effects of chamomile on clinical and laboratory symptoms of polycystic ovaries (Zafari Zangeneh et al. 2010).

Chamomile contains flavonoids such as apigenin and luteolin, volatile oils such as bisabolol chamazulene, and sesquiterpene, lactones including matricarin, mucilage contains polysaccharides, capric and nonilik ethers amino acids, fatty acids, phenolic acids, and other compounds (Johari et al. 2015). Previous studies have shown that extant compounds in chamomile's extract have anti-bacterial, anti-inflammatory properties and anti-oxidant activity. This plant is full of flavonoids which have effective anti-oxidants for neutralizing oxygen radicals (Hatami and Estakhr. 2013).

Free oxygen species are capable of lipid peroxidation in sperm membrane which is followed by reduced mobility and damages to membrane parts of sperm. Anti-oxidants are compounds which prevent formation of free radicals and peroxidation of lipids, protect sperm cells from free radicals and improve sperm quality and fertility parameters (Maneesh and Jayalskhmi. 2006). Medicinal plants have positive effects on fertility increment, hormonal imbalances, sexual dysfunction and have been considered from ancient times.

Chamomile is dry and warm according to traditional medicine of Iran, and has been used as a sexual stimulant. Chemical studies on this plant have shown large amounts of anti-oxidants (Hatami and Estakhri. 2013).

Since the efficacy of herbal medicines must be proven in clinical trials, and because few have studied the effects of chamomile's extract on male reproductive activity and testicular function, this study was carried out to investigate the effects of German chamomile's extract during the neonatal period of rats on pituitary-gonadal hormone axis and changes in testicular tissue of male progenies.

Materials and Methods

The study was conducted in the animal nest of Islamic Azad University- Falavarjan Branch (2016). Forty virgin mature female rats from Wistar race, in the weight range of 180-200g and age range of 90-100 days were used as parents. To adapt to the environment, samples were kept under 22 to 26 ° C, 40-60% humidity and natural photo period with free access to water. Also, 10 adult male Wistar rats were used for mating.

At first, 100 micrograms of estradiol valerate was dissolved in olive oil and injected intramuscularly to synchronize the ovulation time of rats. After 42 hours, 50 micrograms of progesterone was injected intramuscularly (Hosseini et al. 2013). Six hours later, vaginal smears were taken from rats using swab moistened with saline. Immediately after spreading the sample on a slide, 96% ethanol was added to stabilize them and they were dried in the air. Then, slides were colored using Gimsa solution which was diluted at a ratio of 1 to 20 (Jamil et al. 2013).

According to the proportions and morphology of leukocytes and epithelial cells, estrus cycle stages were determined. So that in proestrus stage nucleated epithelial cells were dominant, in estrous phase, horn cells without nuclei and during the next stage Metestrus, the same percentage of horn cells, epithelial cells and leukocytes were observed. In diestrus stage leucocytes were dominant (Hubscher et al. 2005, Marcondes et al. 2002).

Microscopic observations showed that rats had been synchronized at Estrous stage. Rats were divided into four members' groups with a male rate for mating and kept for one night. By observing vaginal plug day zero of pregnancy was designated and then male rats were separated and samples were divided into four groups (10

rats in each group) including control, placebo, and two experimental groups. Control group received no treatment. Placebo group received 0.5 cc of normal saline for 24 days as injection stress. Experimental groups received 50 and 100 mg/kg weight of hydroalcoholic extract intraproteonal every day during location.

Herbal samples were prepared from Isfahan Agricultural Research Center and the extract was prepared using soaking method.

Male and female progenies were separated from day 24 which is the end of lactation and were kept until maturity (two months). After that, male progenies were anesthetized by intraperitoneal injection of 0.7 mg/kg ketamine 10 % and blood samples were taken from the heart. For separation of serum, special test tubes were used. Samples were centrifuged for 15 minutes (300 cycles/ minute).

Then, serum was separated from clot and the amount of FSH and LH hormones were measured using electrochemiluminescence (ECC- SIMENS) whereas testosterone was measured using Elisa method (state fax 2100). Also, testis tissues were placed in formalin 10%. Then, some slides were taken from every tissue sample and after dehydration, clarification, parraffinization, molding and preparation of tissue sections by microtome stages, slides with 5 micrometer thickness were prepared, colored by eosin-hematoxylin method and studied using light microscopy.

Obtained data were analyzed using SPSS. One-way analysis of variance and Tukey's mean comparison test were used at 5% probability level.

Results

Pituitary- gonadal axis hormones

Variance analysis results showed that Follicle stimulating hormone (FSH) of experimental groups (50 and 100 mg/kg of extract) were increased in proportion to control and placebo groups during neonatal period but not significantly (Table 1).

Luteinizing hormone (LH) of experimental groups (50 and 100 mg/kg of extract) were increased in proportion to control and placebo groups during neonatal period. The increment was not significant for 50 mg/kg but was significant for 100 mg/kg (Table 1).

Testosterone was increased significantly ($P<0.05$) by experimental groups during neonatal period (Table 1).

Testis tissue

Microscopic studies of testis sides did not show significant differences between various groups. In these samples, tissue appearance was normal with somniferous tubules which had spermatogenesis cells and tubular connective tissue. It must be mentioned that better appearance of experimental groups shows stimulating effect of chamomile on spermatogenesis.

Average and standard deviation of spermatogonia are presented in Table 2 (page 122). Statistical analysis showed significant increases in experimental groups in proportion to control group.

Statistical analysis showed significant increases in the number of spermatocytes in the experimental groups in proportion to control group. (Table 2)

Table 2 shows significant increase in spermatid number of experimental groups in proportion to control group during neonatal period ($P<0.05$).

The number of Sertoli cells was increased significantly in experimental groups (Table 2)

The number of Leydig cells showed significant increases in experimental groups in proportion to control group. (Table 2)

Table 1: Comparison the mean serum level of LH, FSH hormones and testosterone in the groups treated with HEG

Hormones / Groups	FSH(mIU/dl) Mean ±SD	LH(mIU/dl) Mean ±SD	Testosterone (ng/dl) Mean ±SD
Control	0.927 ± 0.0356	0.858 ± 0.0476	0.402 ± 0.0447
Placebo	0.894 ± 0.0222	0.858 ± .0518	0.381 ± 0.0446
experimental group1	0.959 ± 0.0517	0.908 ± 0.0567	0.699 ± 0.0886*
experimental group2	1.002 ± 0.1023	1.011 ± 0.0543*	0.869 ± 0.0384*

*Shows significant difference from control group ($P<0.05$)

Figure 1: Cross-section of testis, control group (10X)

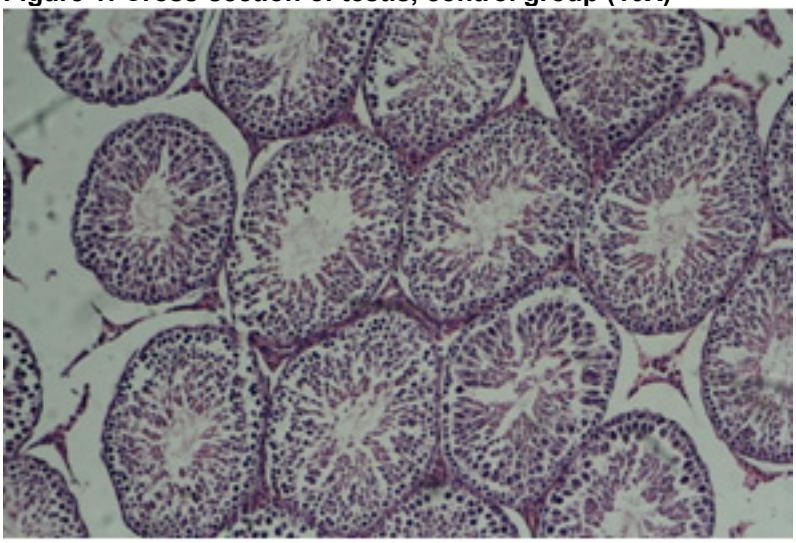

Figure 2: Cross-section of testis, placebo group (10X)

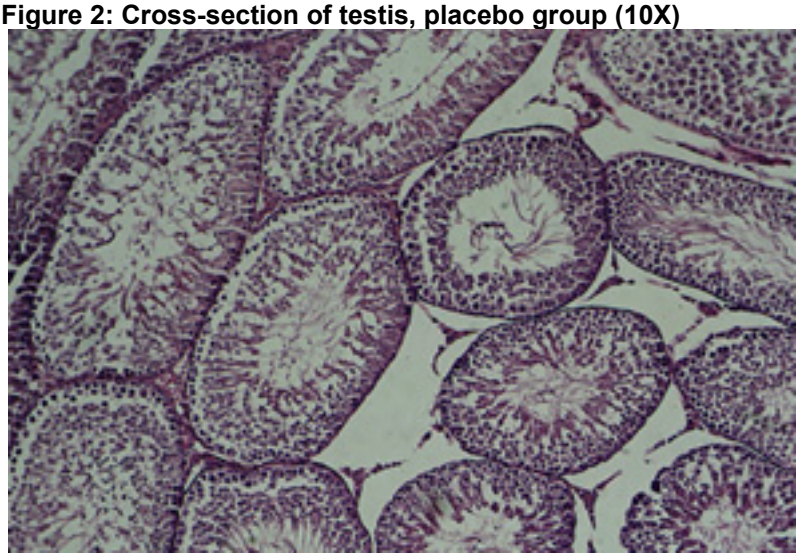

Figure 3: Cross-section of testis, first experimental group (10X)

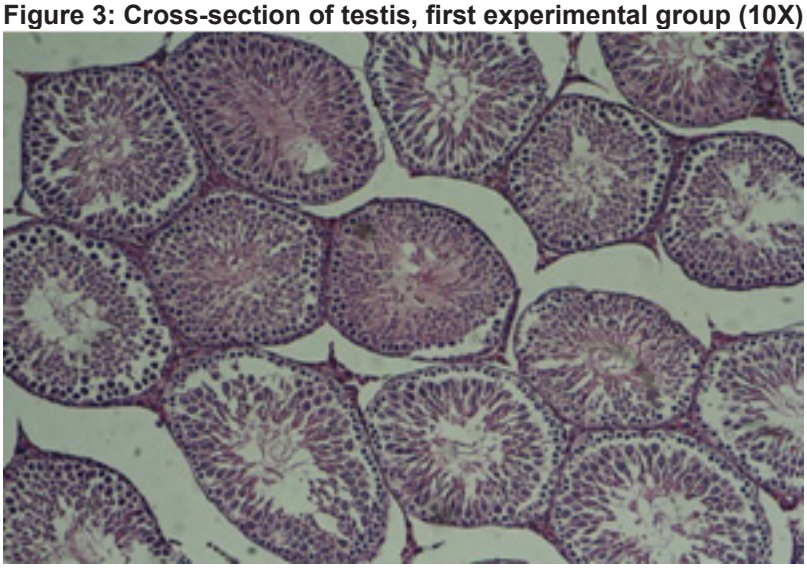

Figure 4: Cross-section of testis, second experimental group (10X)

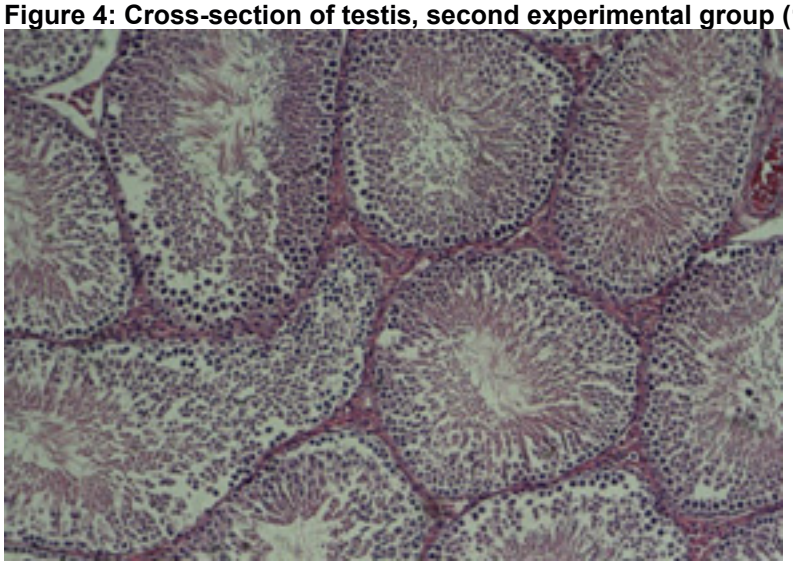

Table 2: the number of lineage sex cells in the groups treated with HEG in comparison to control

Cells Groups	Spermatogonia cells	Spermatocytes cells	Spermatid cells	Sertoli cells	Leydig cells
Control	67.740 ± 3.1081	80.280 ± 2.5503	162.21 ± 3.626	16.330 ± 0.3466	26.970 ± 0.4762
Placebo	65.700 ± 4.0393	84.520 ± 2.6448	166.16 ± 3.795	17.280 ± 0.8561	27.270 ± 0.8820
experimental group1	78.790±3.6309*	93.710 ± 3.0090*	182.74 ± 5.591*	18.920 ± 0.7843*	29.060 ± 1.2686*
experimental group2	85.520 ± 3.1818*	98.450 ± 3.0222*	192.47 ± 6.444*	20.310 ± 1.0203*	30.720 ± 1.3522*

*Shows significant difference from control group (P<0.05)

Discussion

Results of this study showed that hydro alcoholic extract of chamomile did not change testis structure of rats. However, the amount of spermatogonia, spermatocytes, spermatids, Sertoli and Leydig cells were significantly increased by the extract. Also, FSH amount was increased but not significantly and LH was significantly increased by 100 mg/kg group. Testosterone was increased significantly in both experimental groups.

Also, the number of spermatogonia, spermatocytes, spermatids, Sertoli and Leydig cells were increased in experimental groups dose dependently.

The increment may be because of extant compounds in chamomile which affect hypothalamic-pituitary- testis axis and increased mentioned hormones. This axis itself can be affected by various controlling factors (negative and positive). Previous studies have shown that testosterone plays an improvement role in nourishing the dividing sexual cells by direct effect on Sertoli cells, secretion of seminiferous tubules liquid and various proteins such as growth factor and transferrin (Carlson. 2012).

In Hatami and Estakhr's study (2013), the number of spermatogonia, spermatocytes and spermatids were increased by chamomile extract. In that study, FSH amount of the treatment group was not significantly different from the control group and LH and testosterone hormones were significantly increased by the extract which is in agreement with our results.

Capuzzoa et al. (2014) reported that anti-oxidant power chamazulene in chamomile was much more than ascorbic acid (vitamin C).

Chamomile is rich of flavonoids and phenolic compounds which are effective antioxidants for neutralizing oxygen free radicals (Pekka et al. 1996). Antioxidants are probable mechanisms of chamomile's effects on sperm increment.

Crocetin comes from phenolic compounds of chamomile's extract (Karbalaydoust. 2009). This matter is used for storing sperm under very low temperatures because of its anti-oxidant effects (Henkel. 2005). Also, anti-oxidant properties of phenolic compounds eliminate free radicals and affect sperm relating factors (Gill-Guzman et al. 2001).

Acharya et al. (2008) showed that reduced activity of antioxidant enzymes decreased the number of sperm but following administration of antioxidants total number of sperm was increased.

Chamomile extract contains phytoestrogen compounds which are from prolactin secretion stimulating factors. Prolactin increment causes down-regulation of luteinizing hormone (LH) in Leydig cells, reduction in enzymes involved in steroidogenesis and finally testosterone reduction which cholesterol is its synthesis prerequisite. Also, phytosterols of chamomile's extract reduce steroidal hormones such as testosterone by reducing cholesterol amount (Wilson and Foster. 2003, Hannana et al. 2003, Shingo et al. 2015).

Johari et al. (2015) studied the effects of chamomile's extract on serum concentrations of testosterone and gonadotropins in male rats and reported that chamomile reduced the amount of testosterone but didn't affect gonadotropins and announced that phytoestrogen existence was the reason for testosterone reduction. These results are opposed to our results which can be due to dose difference or consumption time of extract at maturity or neonatal periods.

Since free radicals are produced in daily reactions of body and affect reduction of sperm number and its mobility (Gill-Gursman et al. 2001) and due to the fact that laboratory animals experience stress because of living in closed spaces, chamomile has probably had positive effects on spermatogenesis because of its anti-oxidants including chamazulene (a powerful anti-oxidant).

Chamomile with its anti-oxidant properties can improve the process of making sperm plus increase in sexual hormones.

Conclusion

According to results, existence of antioxidant reduces negative effects of phytoestrogens on performance of pituitary-gonadal axis and spermatogenesis process but more studies are needed.

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