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The Effects of a Hydroalcoholic Extract of *Matricaria chamomilla* Flower on the Pituitary-Gonadal Axis and Ovaries of Rats

Habibollah Johari [1*], Esfandyar Sharifi [2], Masume Mardan [2], Farshid kafilzadeh [3], Vahid Hemayatkhah [3], Hossen Kargar [3], Nasim Nikpoor [4]

[1] Department of Biology, Darab Branch, Islamic Azad University, Darab, IR Iran
[2] Department of Biology, Kazeron Branch, Islamic Azad University, Kazeron, IR Iran
[3] Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, IR Iran
[4] Kish University of Medical Sciences, Kish, IR Iran

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ABSTRACT

Background: Chamomile plant extracts contain phytoestrogen compounds. These compounds act as agonist or antagonist estrogen receptors and as aromatase enzyme inhibitors, thus affecting the level of steroid hormones. In traditional medicine, chamomile is used to relieve menstrual pain and as a housing drug.
Objectives: The aim of this study was to investigate the effects of a hydroalcoholic extract of *Matricaria chamomilla* flowers on the pituitary-gonadal axis and on the ovaries of rats.
Materials and Methods: In the present study, 45 female Wistar rats were divided into 5 groups of 9: the control, sham, and experimental groups I, II, and III; rats in the experimental groups I, II, and III received intraperitoneal injections of 10, 20, and 40 mg/kg of a hydroalcoholic extract of chamomilla flowers for 14 days, respectively. Twenty-four hours after the last treatment, blood samples were obtained from the heart, centrifuged, and then the sera were evaluated for determining the concentration of gonadotropins, estrogen, and progesterone by radioimmunoassay. In addition, the ovaries were removed and fixed, and ovarian sections were studied stereologically.
Results: No significant changes in body weight were detected for the different groups, except experimental group III, which showed a decrease. Furthermore, varying the amount of chamomilla extract had no effect on the amount of the luteinizing hormone and follicle-stimulating hormone. In experimental group I that received 10 mg/kg chamomilla extract, the serum concentration of estrogen showed a significant decrease, while that of progesterone showed a meaningful increase. The mean number of secondary follicles and corpora lutea were not significantly different for the different groups, but a significant decline was observed in the mean number of primary and graafian follicles in the experimental group treated with 20 and 40 mg/kg hydroalcoholic extract of the chamomilla flower.
Conclusions: The phytoestrogen present in the hydroalcoholic extract of chamomilla causes a decrease in the serum level of estrogen.

Implication for health policy/practice/research/medical education:
Changes due to phytoestrogen and coumarin compounds present in the hydroalcoholic extracts of chamomile could affect serum concentration of estrogen and progesterone. These hormonal changes cause a decrease in the number of ovarian follicles.

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* *Corresponding author:* Habibollah Johari, Derartment of Biology, Islamic Azad University of Darab, Islamic Azed University avenue, P.O.Box: 74817-83143, Darab, IR Iran. Tel: +98-7313334266, +98-9177311566, *E-mail:* Johari@Iaudarab.ac.ir, hjowhary@yahoo.co.uk

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1. Background

From ancient times, medicinal plants have been valuable and important. Galen used chamomile to cure recurrent fevers. In the 1980s, European specialists dis-

covered that 70% of the individuals suffering from migraines can be cured of their headaches by consuming 2–3 chamomile leaves daily (1). The composition of plants have been confirmed to be compatible with the body's natural structure. In traditional medicine, chamomile was used to relieve menstrual pain and as a housing drug. Chamomile is a highly aromatic annual plant with a height of 20–40 cm. Various species (German, Roman, and cow chamomile) of the plant have different features(2, 3). This plant has 1% extract. The blue color of the extract results from a lipophenolic compound called camazolin (4). Chamomile contains compounds such as coumarin phytoestrol, flavonoids, and acrolin (5-7). High concentrations of coumarin are transformed to 3- and 4-coumarin epoxide in rats, which is toxic to the kidneys and lungs and leads to death. However, in humans, coumarin is transformed and metabolized to a less toxic compound called 7-hydroxy coumarin (8, 9). Camazolin exerts its anti-inflammatory activity by controlling the synthesis of leukotriene B4 in granulocytes and neutrophils, as well as the oxidation of arachidonic acid (10, 11). The flavonoids in chamomile have anti-viral, anti-allergic, anti-cancer, and anti-oxidative effects (12, 13). One of these flavonoids is apigenin, which influences and inhibits its DNA synthesis in estrogen-dependent and non-estrogen-dependent cancer cells of the breast (5). Flavonoids prevent the oxidation of low-density lipoprotein (LDL), and therefore prevent the formation of atherosclerotic plaques (14). Chamomile reduces the adverse effects of chemotherapy, increases the effects of medication, and can cause miscarriage in pregnant women (15). Coumarin increases venous blood flow and reduces the permeability of capillaries. Coumarin is transformed to dicoumarol; dicoumarol causes blood thinning and prevents the formation of vitamin K, leading to internal and external hemorrhage (8, 9).

2. Objectives

The aim of this study was to evaluate the effect of hydroalcoholic extracts of chamomile on the changes in ovarian tissue and on the production of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and progesterone. The results of this research can be used in fertility treatment centers.

3. Materials and Methods

Forty-five female rats with a mean weight of 200 ± 10 g were purchased from the animal house of the Azad Islamic University of Kazeroon and randomly divided into 5 groups of 9 rats (1 control group, 1 sham group, and 3 treatment groups [I, II, III]). All groups were housed at a temperature of 25 ± 2°C and were exposed to 12 hours of light and 12 hours of darkness. Food and water were provided to the rats without restrictions. Treatment groups I, II, and III received 10, 20, and 40 mg of hydroalcoholic extract of chamomile per kg (mg/kg per day) of body weight, respectively. The control group did not receive any extract and the sham group received equivalent amounts of physiologic serum. For 14 days, hydroalcoholic extract of chamomile was intraperitoneally injected using an insulin syringe. Chamomile samples were obtained from the Agricultural and Natural Research Center of Fars province. Identification of genus and species samples was done by plant taxonomy experts from the College of Science/ Shiraz University. Hydroalcoholic extracts of chamomile were obtained using the method of Erdemoglu *et al.* (16). Before the start of the experiment, the estrous cycles of the rats were synchronized by injecting 100 μg of estradiol valerate dissolved in 0.2 mL olive oil followed with an injection of 500 mg of progesterone dissolved in 0.02 mL of olive oil 42 hours later. To confirm cycle synchronization, a vaginal smear was prepared from all animals. To prepare the vaginal smear, the vagina was washed with physiologic serum several times and spread onto a slide. The slides were examined under light microscopy after Giemsa staining (17, 18). One day after the last treatment, all rats were weighed, anesthetized by ether, and blood samples were taken directly from the heart. In order to separate sera, blood samples were centrifuged at 5000 RPM for 10 min, and then the serum levels of LH, FSH, estrogen and progesterone were determined by radioimmunoassay using specific rat kits (Specteria, Finland) (19, 20). For histological studies, the ovaries were removed and placed in Bouin's solution. After stepwise dehydration in increasing concentrations of ethanol, samples were cleared using xylene, and routine histological processes were performed. Five micrometer sections were prepared and stained with hematoxylin and eosin. The number of corpora lutea and of primary, secondary and Graafian follicles in each group were determined (17, 18, 21).

3.1. Statistical Analysis

The data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) 16 software (SPSS Inc. Chicago, IL). The mean concentrations of hormones and ovarian cell counts in the various groups were compared with those in the control groups by performing analysis of variance and *t*-tests. P values less than 0.05 were considered statistically significant.

4. Results

No significant differences were observed in the body weights for the different groups, except experimental group III (dosage of 40 mg/kg), which showed a decrease in body weight (*Table 1*). Moreover, the results of the assays to determine hormone levels showed that different amounts of chamomile extract had no effect on serum FSH and LH concentrations. The serum estrogen concentration in the group receiving 10 mg/kg of extract decreased, while the progesterone concentration in this group increased. The number of primary and Graafian ovarian follicles reduced in the groups receiving moderate and high amounts of chamomile extract (*Table 2)* (*Figures 1-4*).

Table 1. Mean Body Weight and Levels of Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estrogen, and Progesterone of Rats in the Experimental and Control Groups

	Control, Mean ± SD	Sham, Mean ± SD	10 mg/kg, Mean ± SD	20 mg/kg, Mean ± SD	40 mg/kg, Mean ± SD
Body weight, g	206.9 ± 0.98	206.3 ± 1.09	205 ± 1.94	203.2 ± 1.08	201.6 ± 0.82 [a]
FSH, mIU/mL	0.17 ± 0.06	0.25 ± 0.98	0.24 ± 0.12	0.25 ± 0.04	0.24 ± 0.07
LH, mIU/mL	0.19 ± 0.05	0.18 ± 0.07	0.18 ± 0.05	0.18 ± 0.04	0.19 ± 0.04
Progesterone, pg/mL	66.4 ± 27.6	63.1 ± 26.02	100.3 ± 24.9 [a]	72.7 ± 15.08	78.4 ± 19.6
Estrogen, pg/mL	126.2 ± 46.38	131.9 ± 32.04	99.8 ± 21.03 [a]	124.7 ± 22 .5	121.1 ± 32.01

[a] Indicates a significant difference between the control and experimental groups ($p \leq 0.05$)

Table 2. Mean Number of Primary, Secondary, and Graafian Follicles and Corpora Lutea in the Ovaries of Rats in the Experimental and Control Groups

	Control, Mean ± SD	Sham, Mean ± SD	10 mg/kg, Mean ± SD	20 mg/kg, Mean ± SD	40 mg/kg, Mean ± SD
Primary follicles	5.8 ± 2.1	3.4 ± 0. 2	4.5 ± 1.3	4 ± 0.5 [a]	3.4 ± 1.5 [a]
Secondary follicles	4.6 ± 1.1	2.9 ± 0.5	4.3 ± 0.09	4 ± 0.7	4 ± 1.3
Graafian follicles	0.6 ± 0.24	0.3 ± 0.23	0. 4 ± 0.24	0 [a]	0 [a]
Corpora lutea	4.5 ± 0.6	4.2 ± 1.2	4.3 ± 0.6	4.1 ± 1.3	4 ± 1.2
Estrogen, pg/mL	126.2 ± 46.38	131.9 ± 32.04	99.8 ± 21.03 [a]	124.7 ± 22 .5	121.1 ± 32.01

[a] Indicates a significant difference between the control and experimental groups ($P \leq 0.05$).

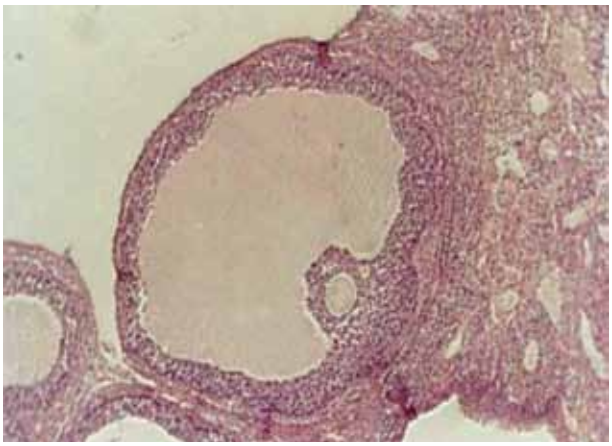

Figure 1. Graafian Follicle of A Rat Belonging to the Control Group (Hematoxylin and Eosin Staining; ×40)

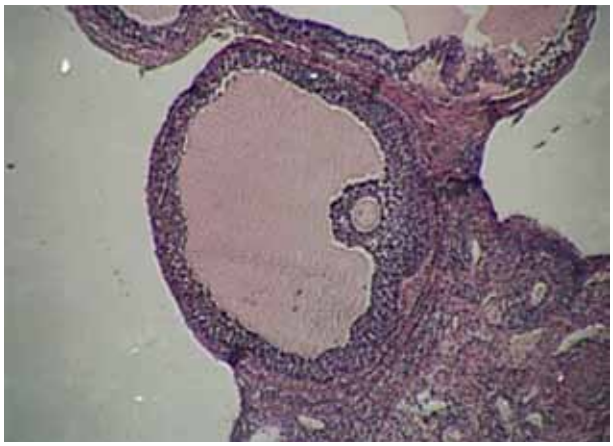

Figure 2. Graafian Follicle of a Rat Belonging to Experimental Group I (Hematoxylin and Eosin Staining; ×40)

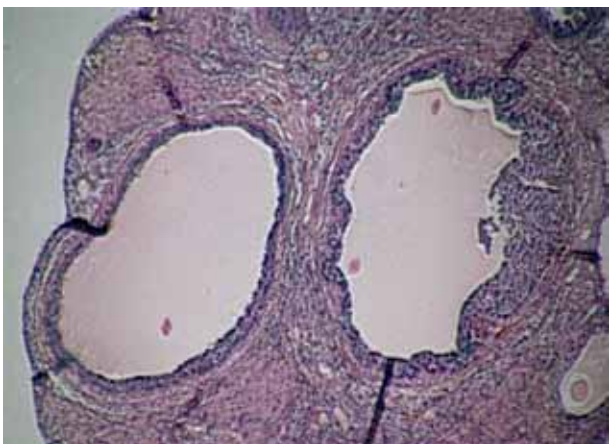

Figure 3. Graafian Follicle of a Rat Belonging to Experimental Group II (Hematoxylin and Eosin Staining; ×40)

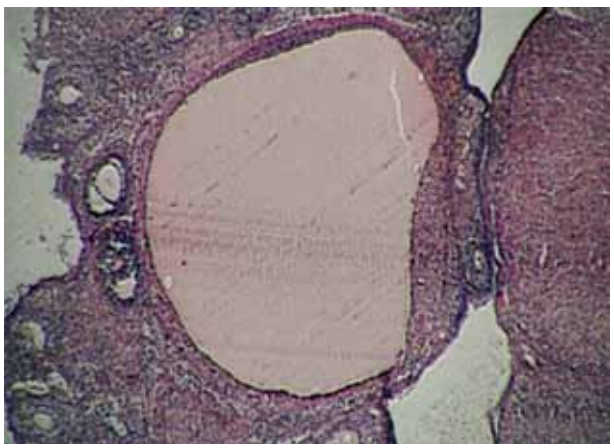

Figure 4. Graafian Follicle of a Rat Belonging to experimental Group III (Hematoxylin and Eosin Staining; ×40)

5. Discussion

The control and experimental groups showed differences in weight loss; however, significant difference was recorded in the group that received the maximum dose of the plant extract. Hydroalcoholic extracts of chamomile contain compounds called phytoestrogens, which are secondary metabolites produced in various types of plants, that stimulate biological responses (6). Phytoestrogens are transported by lipoproteins in the plasma of rats. These compounds reduce cholesterol and LDL by 10% and 20%, respectively. Plant sterols also stimulate lipid decomposition in rat cells and reduce and control cholesterol absorption. Therefore, rats treated with the alcoholic extract of chamomile showed weight loss (6, 22). The phytosterols in chamomile extracts increase dihydroepiandrostandione, which is produced in small amounts in the liver. The increase in this compound leads to an increase in lipid oxidation and controls lipid absorption; this explains the weight loss in treated rats (9, 23, 24). Hydroalcoholic extracts of chamomile also contain ascorbic acid, which reduces weight, prevents weight gain, and reduces cholesterol level (9, 25). Based on our results and considering the thermogenic effects of progesterone (increase in the rate of basal metabolism), the increase in serum progesterone concentration in the experimental groups might have caused an increase in basal metabolism and led to weight loss in the experimental groups (23, 26).

No significant difference was observed in the serum concentrations of FSH and LH in the control and experimental groups. Hydroalcoholic extracts of chamomile contain phytoestrogens, which have estrogenic characteristics and can act as agonists or antagonists of estrogen receptors (26). One of the phytoestrogen compounds is called genestin, and studies indicate that this compound does not influence the secretion of LH from the pituitary gland (27). The moderating characteristic of phytoestrogens leads to an increase or decrease in estrogen levels, which influences gonadotropin production. The number of estrogen receptors on the surface of the hypothalamus are moderated; therefore, the amount of gonadotropin-releasing hormone (GnRH) and the amount of gonadotropins produced in the pituitary gland does not change. The phytoestrogenic characteristic of chamomile extract causes the increase or decrease of estrogen and GnRH, and therefore, levels of FSH and LH in the pituitary gland are not affected (28, 29).

Estrogen levels were reduced in the experimental groups, but the reduction was significant only in the group that received the lowest dose of the herbal extract. This might be because phytoestrogens regulate aromatase and the activity of important enzymes in the biosynthesis of estrogen. This enzyme is also present in the pituitary gland, and its concentration differs among people. Compared to women, men show more expression of the mRNA encoding this enzyme in the pituitary gland of men (30). This enzyme transforms androgens to estrogens in tissues. Therefore, when phytoestreogenic compounds control this enzyme, they prevent this transformation and estrogen production is reduced. Chamomile extracts contain coumarin compounds, which can reduce estrogen. Coumarin chlorophenyl, benzyl, and methoxyl are 3 important functional groups that pay a role in regulating aromatase (31). Phytoestrogens reduce the activity of cytochrome p450, thus inhibiting the transformation of cholesterol to pregnenolone, and hence, reduce the amount of estrogen (32). The serum concentration of progesterone increased in the experimental group injected with 10 mg/kg of chamomile extract. This increase can inhibit granulosa cell division and consequently, decrease estrogen secretion. Phytoestrogens inhibit the progesterone metabolizing enzyme 20-alphahydroxy steroid dehydrogenase, which deactivates progesterone and transforms it to 3-α 5-α tetrahydroprogesterone. Some of the phytoestrogenic compounds that control this enzyme are 3-alpha7-hydroxy flavon, 3- and 7-dihydroxyflavone, and flavones. These compounds bind to the active site of the progesterone metabolizing enzyme (between 308 leucine and 227 tryptophan) and prevent the transformation to 3-α 5-α tetraprogesterone and thus, the amount of this hormone increases (33, 34).

Finally, phytoestrogens prevent estrogen-induced follicle production and ovule maturity by binding to estrogen receptors. When phytoestrogens occupy estrogen receptors, follicle production is controlled, and the number of primary follicles decreases. Since secondary follicles and graafian follicles are produced by primary follicles, a decrease in the number of primary follicles results in a decrease in the number of secondary and graafian follicles. The obstruction of estrogen receptors by phytoestrogens prevents the growth of follicles and hence, reduce the number of corpora lutea (35, 36). Changes due to phytoestrogen and coumarin compounds present in the hydroalcoholic extracts of chamomile could affect serum concentration of estrogen and progesterone. This causes a decrease in estrogen levels. A decrease in estrogen levels causes increased progesterone secretion via feedback mechanisms. Finally, these hormonal changes cause a decrease in the number of ovarian follicles.

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