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Effect of *Zataria Multiflora* Extract Oil on
Menstrual Cycle in Women

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Abstract

Zataria multiflora Boiss is a plant from Lamiaceae (Mint Family). It is traditionally used for its medicinal effects such as its anti-inflammatory, antibacterial, antiviral, and antifungal properties. It has a phytoestrogen which belongs to flavonoid groups. Pervious studies showed that the smelling the extracted oil of this plant accelerates sexual maturity in mice. In this experiment I investigated the effect of smelling this oil on women's menstrual cycle which was not reported in the literature before. For this purpose I examined this oil on 15 single girls. These girls smelled the oil for four months. There were two control groups in this experiment. Analyzing the data from experimental group and control groups showed that smelling this product could bring about meaningful changes in experimental group A ($P=0.012$, $P<0.05$) compared to the control groups ($P=0.119$, $P>0.05$ for group B). This study provides the first hand evidence for the hypothesis that the smelling of *Zataria multiflora* extract oil shortens menstrual cycle. The mechanism of its action is not clearly known yet and is open for future researches.

Introduction

Nowadays researchers pay special attention to herbal products to produce harmless drugs from their ingredients. Most of the herbal products are to be used through oral route. In this study I focused on an herbal product to be used through olfaction. I deemed olfaction was an easier way of administration of a drug to the body for dealing with certain gynecological problems like prolongs menses. For this purpose, I used *Zataria multiflora* in my study.

Zataria multiflora Boiss belongs to plant kingdom, Tracheophyta phylum, Magnoliopsida class, Lamiales order, Lamiaceae family (Mint Family)^{1,2}, *Zataria* genus. Its specific epithet is *multiflora* Boiss, but its botanical name is *Zataria multiflora*³.

This plant is indigenous to Iran^{1,4}, Pakistan and Afghanistan² and it traditionally is deemed to be useful as antiseptic^{2,4,5}, antispasmodic², anti-inflammatory, antibacterial, antiviral⁶, antioxidant potential⁶⁻⁸, antinociceptive^{9,10}, antifungal, and anti-*Candida*⁴. Khosravi demonstrated that subcutaneous administration *Z. multiflora* stimulates cellular immunity in rabbits¹¹.

The composition of the oil of *Zataria multiflora* Boiss was studied by gas liquid chromatography (GLC), column chromatography (CC), NMR, and GLC/MS¹². The result of these studies shows that there are these elements in it: flavonoids², thymol¹³, carvacrol¹²⁻¹⁴, zatrinal, oleanolic acid, betulic acid¹³, rosmarinic acid^{9,13,15}, monoterpenoids, sesquiterpenoids, p-cymene^{12,16}, γ -terpinene^{9,13,17}, dihydroxyaromadendrane, luteolin, α -tocopherolquinone¹⁶, β -sitosterol, stigmasterol, hexadecanoic, linalool¹³ and gamma-terpinene¹².

Phytochemical screening supported the presence of flavonoids in *Z. multiflora*². Flavonoids are phenolic compounds and are structurally similar to steroid hormones, particularly estrogens. Estrogenic effects of flavonoids were first discovered during the investigation of the infertility in Australian sheep eating red clover which has flavonoid compound¹⁸. *Vitex agnus-castus* is another plant that contains flavonoids as active phytoestrogen¹⁹. The extract of this plant is used for improving menstrual disorders such as amenorrhea, dysmenorrhea, and the menstrual cycle irregularities²⁰. Because of *Z. multiflora* estrogen like structures¹⁸, I thought that perhaps flavonoids are the effective compounds which can affect the reproductive function in mice and human.

Flavonoids also have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidant²¹, and antinociceptive effects²². In experimental conditions, flavonoids show a useful effect in colon inflammation²³. The anti-inflammatory effects of flavonoids are probably related to prevention of neutrophil infiltration, absence of IL-1 and decrease of prostaglandin production²⁴.

Below, I will briefly mention some of the properties of the compounds found in *Zataria multiflora* with their proven effects:

Both rosmarinic acid and thymol have the anti-*Candida* activity⁴. In addition, both thymol and carvacrol in *Z. multiflora* oil have antibacterial and antifungal effects^{15,25}.

Rosmarinic acid is a natural phenolic compound with two phenolic rings, both of them have the two ortho-position hydroxyl groups²⁶. It has also antibacterial, antiviral, antioxidant, anti-inflammatory⁶, antimutagenic potentials²⁶.

Luteolin has inhibitory effects on TNF- α -induced IL-8 production in the intestinal epithelial cells. The existence of interleukin IL-8 is essential for initiation and maintenance of inflammatory responses²⁷.

Thymol is the phenolic structure and like other phenolic compounds has redox potential. So it can adsorb and neutralize free radicals and breakdown peroxides. Therefore thymol plays the role of an antioxidant and anti-inflammatory agent in human cells²⁸.

Carvacrol, the other *zataria*'s compound, seems to inhibit the growth of bacterial pathogens. The combination of carvacrol and cymene, the compound with similar structure and function, can enhance the antimicrobial action of carvacrol^{29,30}.

Oleanolic acid and ursolic acid are triterpenoid compounds which are present in many medical herbs such as *Zataria*. They have anti-inflammatory effects³¹.

As it is mentioned, flavonoids are phytoestrogen¹⁸. Flavonoids are categorized, according to their chemical structure, into flavonols, flavones, flavanones, catechins, anthocyanidins, chalcones, and isoflavones³².

Among flavonoid groups, isoflavones which include genistein, daidzein, O-demethylangolensin, and equol³³, have similar structure to estrogen and bind to estrogen receptors³⁴⁻³⁶ and modulate sex hormone levels in blood stream in women³⁷.

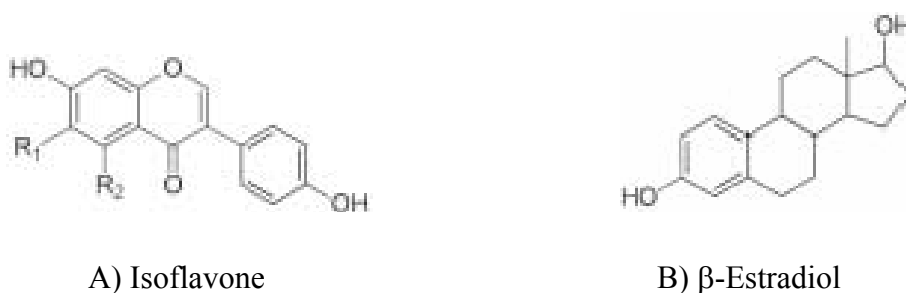


Figure 1. (A) General structure of Isoflavones³⁸. (B) The structure of β-Estradiol, the main estrogen that is secreted from ovaries³⁹. Both of them have aromatic structure and two hydroxyl groups.

Because of alleviating menopausal symptoms and improving menstrual cycle disorders by using the plant estrogens, phytoestrogens, are the interesting subjects that are recently noticed. Study in ovariectomized female mice showed that the injection of soy equol cause an increase in the weight of uterine and vaginal epithelial thickness⁴⁰. The results of another study on female monkeys suggested that using high doses of soy isoflavones, genistein, daidzein and equol in short-term did not contribute to increased uterine or breast cancer risk⁴¹. In postmenopausal women, soy isoflavones which were given in their diet showed slight effect on the level of plasma sex hormones and no effect on vaginal or endometrial tissues⁴². In another research, it is mentioned that soy phytoestrogen decreases some menopausal symptoms such as hot flashes⁴³.

According to Bazzyar and Charkhzarin, the smelling of *Zataria multiflora*'s extract oil has a remarkable effect on precocious puberty in mice⁸⁸ by affecting ovaries⁸⁹ and it is shown that mice become pregnant sooner compared to the control group⁸⁸. Although the effective compounds of *Zataria* which caused these effects is not quite known, but studies showed that one kind of phytoestrogens, i.e. isoflavones, must be present among its compounds.

Two major soy isoflavones, i.e. genistein and daidzein can bind to both mammal estrogen receptors with higher affinity for ERβ⁴⁰. There are two receptors for estrogen (ER) in neural regions, ERα and ERβ that are isomers of each other. The distribution pattern of these estrogen receptors is different in some brain regions of mammals⁴⁴. In cerebral cortex, globus pallidus, horizontal and vertical limbs of the diagonal band of Broca, substantia nigra and ventral tegmental area, raphe nuclei, locus coeruleus, suprachiasmatic nucleus, olfactory bulb⁴⁵, and hippocampus⁴⁶ ERβ predominates^{45,46}. Also ERβ is observed by antibody staining

in the bed nucleus of the stria terminalis and neurons within the vomeronasal neuroepithelium⁴⁶.

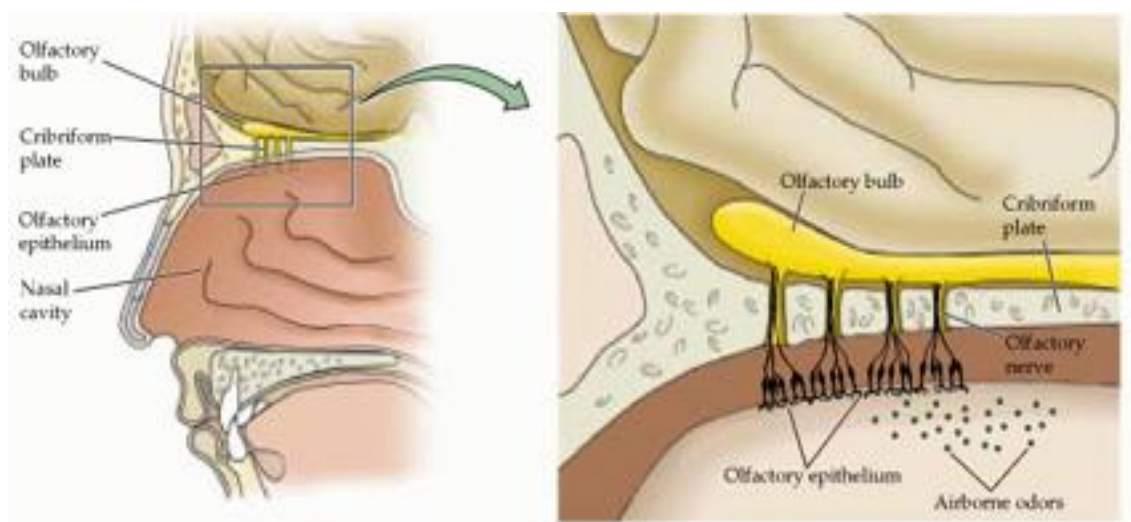
ER α is major receptor in the cortical amygdaloid nucleus, medial preoptic area in the vomeronasal organ (VNO)⁴⁷, and the arcuate and ventromedial nuclei of the hypothalamus, where play important role in regulation of reproductive functions both in mouse and human^{46,48}. But in other regions such as medial amygdala, the anteroventral periventricular nucleus of the preoptic area and hypothalamus both ER α and β are present^{44,45}.

Although ER β exists in olfactory bulb which is a part of main olfactory pathway and receive information from olfactory epithelium, but the existence of the ERs in the olfactory epithelium has not been reported yet⁴⁶.

The olfactory system in mammals is composed of main olfactory system and vomeronasal system⁴⁹. Main olfactory system receives and detects a large variety of volatile odors⁵⁰⁻⁵² and aromatic chemicals⁵³ and has an important role in the regulation of the reproductive processes^{54,55}. Below I briefly review the olfactory pathway in human.

Olfactory receptors are bipolar neurons with non-motile cilia⁵⁶⁻⁵⁸ and unmyelinated axon⁵⁹ that are located in a mucous membrane in the interior and the roof of the nasal cavity⁵⁷.

Figure 2: location of olfactory receptors in nasal cavity and relationship between olfactory epithelium and olfactory bulb⁵⁹.



These receptors are proteins with seven transmembrane hydrophobic domains⁵⁷, an extracellular region that is different in each receptor and one intracellular region that interact with odorant specific G-protein.

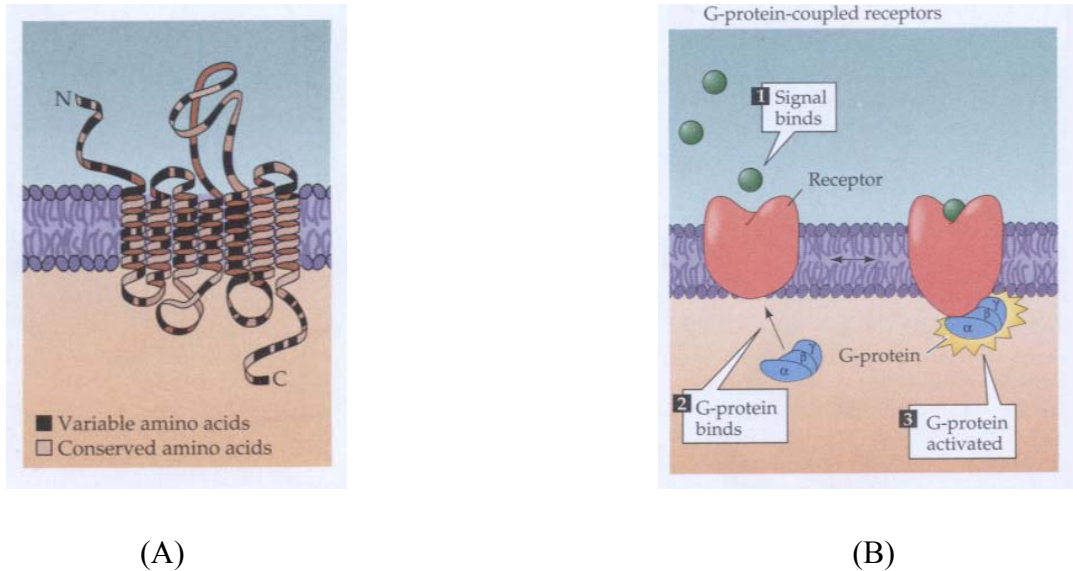


Figure 3. (A) The seven transmembrane olfactory odorant receptor. (B) G-protein coupled receptors⁵⁹.

Binding odorants to the receptor, activates G-protein and it in return binds and phosphorylates the adenylate cyclase to generate cyclic AMP (cAMP) from ATP^{57,58,60}. Then the cAMP binds to a cation- selective channel and opens it⁶¹, so Na (+) and Ca (2+) enter through the channel into cilia and depolarize neuron⁶⁰. Increase Ca ion concentration in the cell opens Ca-gated Cl (-) channels and leads to depolarization of olfactory receptor by exiting chloride ion from the cell, so that an action potential gets generated in the axon hillock region⁵⁹.

Odorant information from several thousand olfactory neurons converge into a few glomeruli in olfactory bulb^{54,58,62} and synapse to dendrites of the mitral cells, granular cells and tufted cells in glomeruli^{58,63}.

Axons from mitral cells project directly to the olfactory cortex. These axons project from the olfactory bulb to the three-layered pyriform cortex in the temporal lobe, olfactory tubercle, the lateral division of the entorhinal cortex⁶⁴, and amygdala. Some neurons in pyriform cortex and amygdala project their axons to several thalamic and hypothalamic nuclei and to the hippocampus⁵⁹.

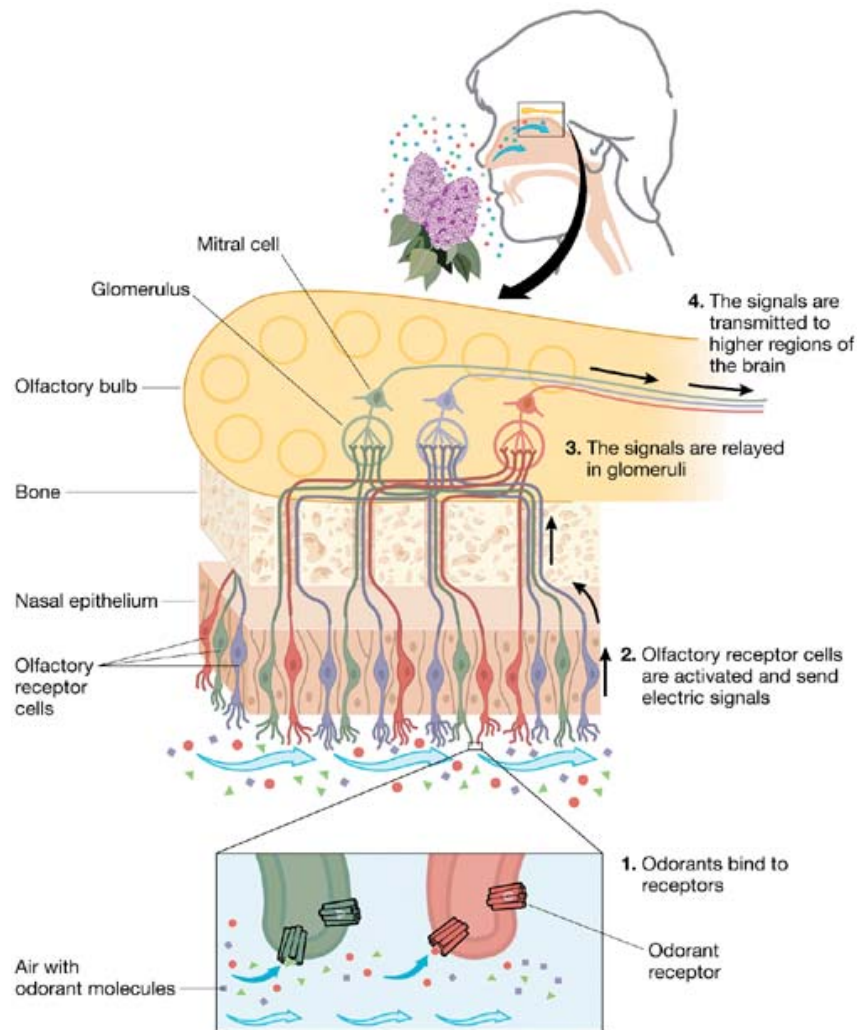


Figure 4. Olfactory pathway from epithelium to olfactory bulb. Axons from olfactory epithelium project to mitral cells in the bulb glomeruli and from there to olfactory cortex⁶⁵.

Sensory inputs from limited olfactory neurons in the main olfactory epithelium go to GnRH neurons in hypothalamus through the primary olfactory cortex and modulate their activity⁵⁰. Some studies suggest that main olfactory inputs are responsible for modulating the GnRH secretion by olfaction stimulation^{50,66}.

Some researchers believe that vomeronasal system (VNS) is responsible for detecting sexual pheromones and controlling social and reproductive behavior in mammals but the exact mechanism of encoding these signals is not known^{67,68}.

Bipolar vomeronasal receptor neurons send their input to the accessory olfactory bulb and synapse with mitral, granular and tufted cells. This information from accessory olfactory bulb arrives to the medial nucleus of the amygdala (MeA), then innervates to the medial preoptic nucleus, the ventromedial nucleus and the ventral premammillary nucleus of

hypothalamus where the GnRH secretion is controlled^{66,69}. Inputs of this system arrive to the areas containing GnRH-expressing neurons, but there are not any anatomical or functional connection between these cells and VNS⁵⁰.

Although estrogen receptors are not found in the main olfactory epithelium (MOE), there are different types of neurons in the medial area of this epithelium, in zones 2 and 3 of MOE, that express the guanylyl cyclase receptor instead of the adenylate cyclase⁵⁰. Inputs of these neurons seem to arrive to gonadotropin-releasing hormone (GnRH) neurons which are distributed in some areas of the brain such as rostral hypothalamus, anteroventral periventricular nucleus⁴⁸, medial preoptic area, septum and diagonal band of Broca, via main olfactory system, including all major subdivisions of limbic system and primary olfactory cortex. Chemosensory inputs from these regions probably modulate the function of GnRH neurons⁵⁰ and induce them to release GnRH⁴⁸.

Gonadotropin-releasing hormone (GnRH) system is part of reproductive system that regulates and controls the reproductive behavior and function⁴⁸ by secreting GnRH into the bloodstream⁷⁰.

The gene that code GnRH is located on chromosome 8p⁷¹. Secretion of GnRH in the portal vessel leads to releasing gonadotropin hormone from anterior pituitary gland and it return stimulates steroids secretion from the ovary, the concentration of these steroid hormones control the secretion of GnRH from hypothalamus in negative feedback pattern. Although GnRH neurons do not express estrogen receptors themselves, but NO-ergic neurons in the preoptic area and arcuate/median eminence express both estrogen receptors protein and nitric oxide synthetase (NOS)⁷².

It seems that estrogen influences GnRH secretion by stimulating the synthesis of NO in NO-ergic neurons and diffuse it through the cell membranes to bind to its receptor in the cytoplasm of neighboring neurons(GnRH neurons), because NO is a gaseous neurotransmitter. In the target cell, NO binds to the iron of hemoproteins, guanylyl cyclase and cyclooxygenase, which catalyze the production of cyclic GMP as second messenger that lead to releasing GnRH from the neuron terminal by exocytosis of secretory granules⁷².

Some other factors directly or indirectly influence the synthesis and secretion of GnRH from the terminal part of their neurons. In addition to estrogen, noradrenaline stimulate the GnRH releasing by activating the adrenergic receptor on NO-ergic neurons.

Also oxytocin⁷² and Mn²⁺ in high concentration control gonadotropin secretion by inducing NO signaling pathway⁷³. Kisspeptin is a protein coded by Kiss1 gene and binds to GPR54, a G-protein-coupled receptor⁷³. Kiss1- expressing neurons are located in the forebrain⁷⁴ and its receptor is expressed by GnRH neurons and hypothalamic neurons that control the GnRH neurons secretion^{74,75}. The reaction between kisspeptin and its receptor induce the GnRH neurons to secrete GnRH, also estradiol can stimulate these receptors and modulate the kisspeptin effect⁷⁵.

In mammals, both the olfactory and GnRH neurons originate in the olfactory placode^{48,76} from neuroblast cells⁵³. These cell lines not only have the olfactory properties and respond to odors, but also show the neural property and produce GnRH and are sensitive to sex steroids in human fetus⁵³.

After creation and during the development of olfactory system⁷⁶, GnRH neurons migrate along olfactory and vomeronasal nerves^{76,77} through the cribriform plate and nasal septum to the septal-preoptic area to arrive to median eminence of hypothalamus^{53,76}. GnRH neurons migration is controlled by some factors such as the interactions between neuropilins and their ligands, semaphorins, for regulating axonal pathfinding⁷⁶, and also existence of Kal protein is essential for this purpose^{53,71}. This protein is coded by *KAL1* gene which locates on chromosomes 8 and X^{71,78}. If this protein defectively gets expressed, GnRH neuron migration and secretion, and also the ability to smell will become defective^{53,76}. These factors arise from olfactory mesenchyme⁷⁸ and bulb⁷¹. In addition to median eminence of hypothalamus and medial preoptic area, GnRH neurons are present in the mucosa of the nasal cavity along the *nervus terminalis*⁷⁹. But the role of these neurons in regulating reproductive function is not clear.

GnRH neurons in the median eminence secrete GnRH into the pituitary portal vessels⁷⁰. This hormone regulates the synthesis and secretion of luteinizing hormone (LH) and the follicular-stimulating hormone (FSH), two gonadotropin hormones, from anterior pituitary gland. In women LH and FSH in turn regulate the secretion of steroids, estrogen and progesterone, from ovary into bloodstream. These hormones control the female reproductive organs such as uterus, and ovary during the menstrual cycle⁵⁰.

Menstrual cycle is divided to five phases according to the changes in ovary and uterus. During follicular phase, the thickness of uterus increases, and in ovary some follicles

begin developing and after several days one or occasionally two follicles become dominant and the rest atrophy and die⁸⁰.

During an ovulation cycle, an egg gets released from a dominant follicle. If the egg is not fertilized within about a day of ovulation, it will die⁸⁰.

Luteal phase is a phase after ovulation. What is left out of the dominant follicle when the egg is released is called corpus luteum which secretes progesterone and helps in thickening of endometrium for potential implantation of an embryo. The last phase is called ischemic phase. If implantation does not occur within two weeks after ovulation, corpus luteum will die. It causes sharp drops in levels of both progesterone and estrogen hormones and shedding of uterus lining^{39,81}. The drop in the serum level of these hormones signals GnRH secretion by negative feedback, so that another cycle begins⁵⁰.

In summery, menstrual cycle is under the control of the hormonal^{82,83} as well as neuronal system⁸⁴. In addition to these factors, some environmental characteristics⁸³ can also influence the pattern of menstrual cycle, for example smoking which decreases the length of cycles⁸⁵. Also menstrual cycle can be affected by olfaction⁸⁶. For example women living together at different stages of menstrual cycle tend to synchronized menses⁸⁷. It seems that human female produces pheromone compounds⁵³ that have potential for the above mentioned menstrual synchrony, as well as social regulation of ovulation⁸⁶ by olfaction.

In my dissertation, I tried to investigate whether the smelling of the extract oil of *Z. multiflora*, which is shown to accelerate the puberty in mice⁸⁸, has any effect on menstrual cycle in human women. For this purpose I tested this oil on 15 girls for 4 months and then I analyzed the changes by statistical method, *t*-test.

Material & method

The extracting apparatus which I used was made of a 4000 ml balloon for boiling the mixture of herbal leaves and water, and a cylindrical tube that liquefied the produced steam. The extracted oil was sucked out by a pipette which had a narrow bended top attached to safety pipette filler.

For preparation of *Z. multiflora* extracted oil, I used ten Kgs of dried herb obtained from the city of Shiraz. About 60g of the *Z. multiflora*'s leave were weighed and boiled in

2500-3000 ml of water at a time for extracting the oil for each experiment. Thus the mass ratio of leaves to water was 1 to 50 and the volume ratio was 1 to 3. Experimentally I found using less than the optimal 60 grams of leaves at a time were not conducive to enough oil for a few experiments, and using more leaves used to prevent the proper flow of steam from the balloon outlet. I used to boil the mixture for 3 - 4 hours to obtain 3-3.5cc of oil, and then I reduced water to 2000 ml so the mass ratio changed to 1:40 and the amount of extracted oil obtained was about 4 or 5 cc.

The fresh extracted oil of *Z. multiflora* is light yellow and it has a pungent odor. After about 1 week, the intensity of this odor decreases and its color becomes darker. If bare skin comes into contact with the oil, it gets irritated and red. As the oil had a strong eroding effect on plastic and metal containers, I used to keep it in glass bottles with thick plastic lid.

For my main project, the first step was finding some volunteers. So, I consulted a statistician about the number of samples. He suggested that between ten and twenty was enough for this purpose. Also he said that it was not necessary to have equal numbers of people in experimental and control groups.

For 4 months I gave the oil to one candidate girl to smell it as a pretest for investigating any possible problems. I had told the girl that smelling the oil was for investigating any possible effect of it on improving the skin problems such as acne. I found no problems as I observed her at the end of four months.

I tried to distract volunteers from realization of my purpose of experimentation by exposing them to some irrelevant questions during the experiment. Later I was told that it was not an ethical act to have told lies to candidates and had not informed them of possible risks of the test and did not have a written consent letter from them. I was thinking at the time that perhaps they would not cooperate with me if they knew this oil could probably shorten their menstrual cycle or could have other risks. I apologize for telling lies, and not considering the research ethical norms.

Then I asked a number of single girls between 18-30 from Tehran and Karaj to cooperate with me in this project. 58 girls initially accepted to enter this experiment and I divided them into three groups (A, B, and C) randomly, but in the course of the project some of them left. Groups A, B, and C began with 22, 15, and 15 volunteers but ended with 14, 9, and 11.

Freshly extracted oil was divided and put into 14 bottles for 14 volunteers and was given to them on weekly basis. Each bottle was labeled with a code number, the name of the volunteer, and the name of her group. The names of volunteers were registered on the bottles and in a notebook.

The volunteers were instructed to smell the oil about 6 times every day and register the time of smelling in certain tables (Appendix1) that were prepared for this purpose. I weekly retrieved the filled tables and would give them a new one. This table was given to remind them to smell the oil. Also they recorded the first and the last day of their menstrual cycle each month. So, it was possible to compare the length of their menstrual cycle before and after the experiment to realize whether there was a change in the timing of the menstrual cycle.

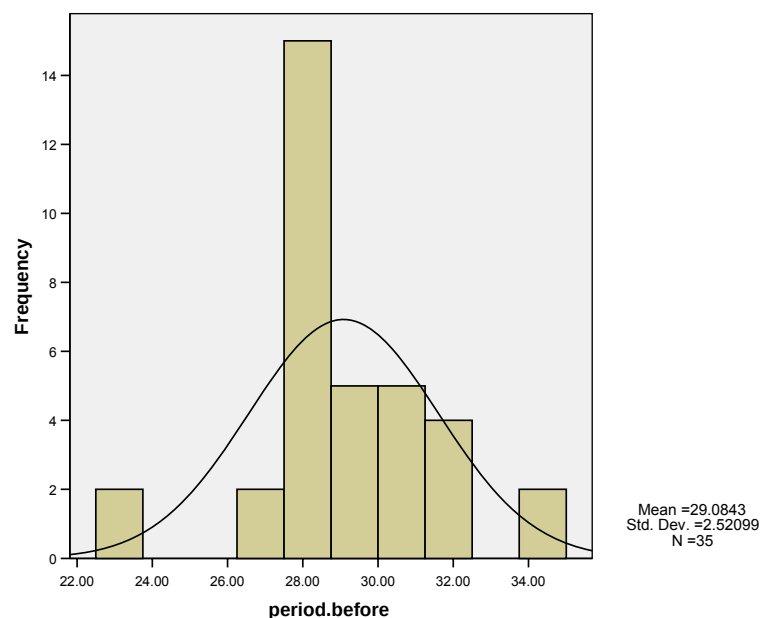
Several factors such as emotional stress, suggestion, taking in some medicine, and other environmental factors can influence menstrual cycle. Generally the whole factors in researches involving human being can not be kept under strict control as in lab experimental model of research, so I tried to control some of them for example the role of suggestion, and anxiety. I prepared two questionnaires with the help of a sociologist. The first questionnaire (Appendix 2) asked about some personal information such as playing any sports, taking any special drugs and the timing of their menstruation. Also there were some questions in that appendix like the type daily meal intake to distract the volunteers from getting any idea of what I intended to do. During the experiment also I questioned volunteers about the effect of smelling this extract on feeling any emotional tranquility or the change they possibly noticed on skin tenderness in order to mislead them from knowing the main purpose of the experiment.

The second questionnaire was an anxiety test which was developed by Cattell⁹⁰ (Appendix3). This test contains two parts, one part from question 1-20 is about the hidden anxiety and second part from question 20-40 is questions about evident anxiety. The level of anxiety was determined by adding the grades of these two parts. I used it to determine the amount of general anxiety of volunteers.

Groups B and C were used as my control groups. Group B smelled water, and the group C smelled nothing. These two groups also filled the questionnaires like group A.

I suggested group B that the compound which they would smell was a diluted form of the substance under my experiment, so it was odorless, but probably would shorten their menstrual cycle. This was to check the effect of suggestion on menstrual cycle. If the menstrual cycle of group B would not vary, any changes in group A cycles could be more assuredly attributed to the effect of smelling the oil. I did not explain to members of group C about the purpose of this experiment but they just recorded their menstrual cycle data for me.

Below is a bar diagram based on the data obtained before beginning of the experiment from members of three groups about the duration of their menstrual cycle. The distribution frequency of the cases shows that they follow a standard normal distribution curve. That allowed me to use the *t*-test for statistical analysis of the results.



Results

In this study the effect of smelling *Z. multiflora* essential oil on menstrual cycle of women was tested for four months. I used the *t*-test for comparison of two groups to analyze my results.

In tables one to four, I showed the duration of menstrual cycles of candidates in three groups A) experimental, which smelled *Zataria* extract, B) control that smelled water, and C) naive which smelled nothing. In these tables, each number in the first vertical column indicates one candidate and the horizontal row shows duration of menstrual cycle in mentioned months of experiment. For example the duration of menstrual cycle between months 1 and 2 in case 1 of group A, was 28 days. The first table corresponds to the time before starting the smelling for three groups, and the rest of tables, two to four, show the duration of the menstrual cycle of the subjects after smelling experiment for each group separately.

Table 1. The duration of menstrual cycle before the starting experiment in three groups.

Case	Duration 1-2 (group A)	Duration 2-3 (group A)	Duration 1-2 (group B)	Duration 2-3 (group B)	Duration 1-2 (group C)
1	28	27	31	22	28
2	33	31	30	29	32
3	29	33	27	28	31
4	32	30	27	28	30
5	28	28	33	31	29
6	32	32	30	28	29
7	31	16	30	29	35
8	29	28	21	25	27
9	29	28	28	29	32
10	27	28			30
11	20	38			29
12	30	29			
13	29	28			
14	30	24			
15	34	36			

Table 2. The duration of menstrual cycle in group A members.

Case	Duration 3-4	Duration 4-5	Duration 5-6	Duration 6-7	Duration 7-8	Duration 8-9
1	26	28	28	26	25	26
2	28	28	32	32	28	
3	30	21	25	29	27	
4	31	32	27	27	31	
5	28	26	26	26	25	*
6	32	37	32	18		
7	27	28	28	26		
8	29	28	25	25	26	
9	28	26	27	26	25	27
10	26	28	25	25	25	26
11	48	17	23	27	24	24
12	31	30	29	29		
13	29	29	27	26	27	
14	26	26	25	30	27	
15	32	41	33	29	30	

Table 3. The duration of menstrual cycle in group B members.

Case	Duration 3-4	Duration 4-5	Duration 5-6	Duration 6-7	Duration 7-8
1	32	29	31	28	29
2	29	30	29	29	
3	28	27	29	29	
4	28	27	25	27	
5	32	28	35	33	
6	23	33	32	30	
7	29	31	30	31	
8	29	23	27	20	
9	29	29	28	29	

Table 4. The menstrual cycle length of group C members.

Case	Duration 3-4	Duration 4-5	Duration 5-6	Duration 6-7
1	28	28	28	*
2	30	31	33	
3	30	31	32	
4	30	31	30	
5	28	29	28	
6	31	25	27	
7	35	36	33	
8	28	29	27	27
9	27	29	30	
10	26	27	27	
11	29	28	28	29

The averages of group A cycle lengths are mentioned in table 5-A, and for group B are recorded in table 5-B. These data are used for next steps such as calculating the correlation coefficient and confidence interval.

Case	Average before	Average after
1	27.5	26.5
2	32	29.6
3	31	26.4
4	31	29.6
5	28	26.2
6	32	29.75
7	23.5	27.25
8	28.5	26.6
9	28.5	26.5
10	27.5	25.83
11	29	27.17
12	29.5	29.75
13	28.5	27.6
14	27	26.8
15	35	33

(A)

Case	Average before	Average after
1	26.5	29.8
2	29.5	29.25
3	27.5	28.25
4	27.5	26.75
5	32	32
6	29	29.5
7	29.5	30.25
8	23	24.75
9	28.5	28.75

(B)

Table 5. The average of menstrual cycle length before and after the experiment for group A , and group B. The condition of each person, before and after the smelling oil is compared with herself.

Below I assume that *Zataria* extract oil had no effect on menstrual cycle and these data from group A was obtained by chance (null hypothesis, H0).

Table A. Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	period.before	29.2333	15	2.69832	.69670
	period.after	27.9027	15	1.99893	.51612

		N	Correlation	Sig.
Pair 1	period.before & period.after	15	.751	.001

Table C. Paired Samples Test

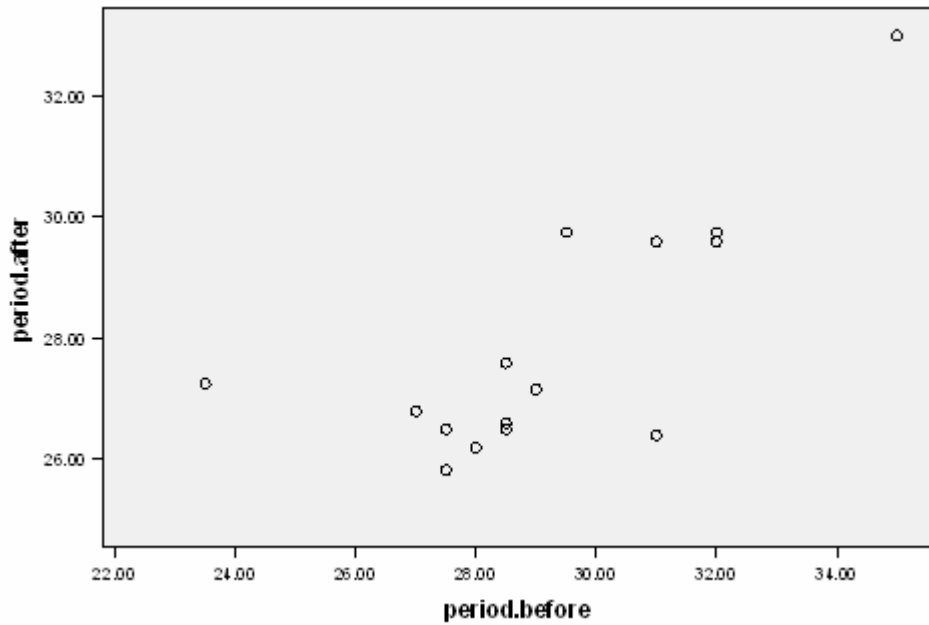
		Paired Differences				t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower Upper			
Pair 1	period.before - period.after	1.33067	1.78049	.45972	.34467 2.31667	2.895	14	.012

P value in the last column of table C is 0.012, meaning that the results are way beyond the chance level ($P < 0.05$) This table also showed that shortening of menstrual cycle was between 0.3-2.3 days with 95% confidence interval.

Also the 1.3 difference between the mean values before and after of smelling indicates that the smelling of this extract had a definite effect in menstrual cycle and could bring about a change about 1.3 days (from 29.23 to 27.92) i.e. The duration of menstrual cycles became around 27.92 days after experiment instead of 29.

The scatter diagram for data of group A demonstrates how most cases of group A after smelling *Zataria* fall beneath the bisector line.

Diagram 1. Scatter diagram for experimental group



For group B, the null hypothesis explains that the water has no effect on menstrual cycle. The results of the accounts are recorded in three tables in continue. According to P value in the last table, 0.119, which is more than significant level, the null hypothesis is proved, so smelling water has no effect on menstrual cycle. Also the amount of changes in this cycle in group B was between -1.62 and 0.22 days that is not meaningful.

Table D. Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	period.before	28.1111	9	2.48468	.82823
	period.after	28.8111	9	2.08997	.69666

Table E. Paired Samples Correlations

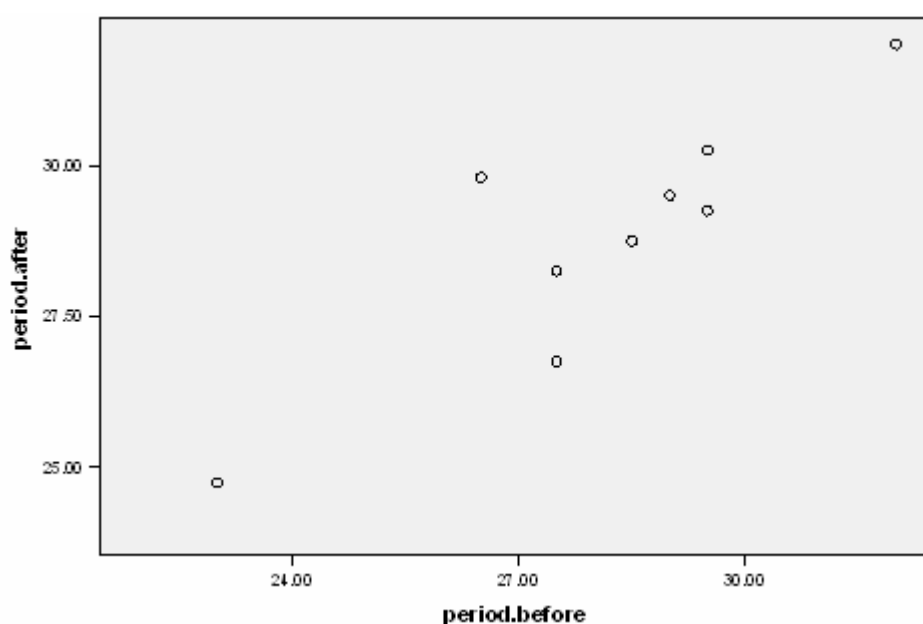
		N	Correlation	Sig.
Pair 1	period.before & period.after	9	.875	.002

Table F. Paired Samples Test

		Paired Differences				t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower Upper			
Pair 1	period.before - period.after	-.70000	1.20442	.40147	-1.62580 .22580	-1.744	8	.119

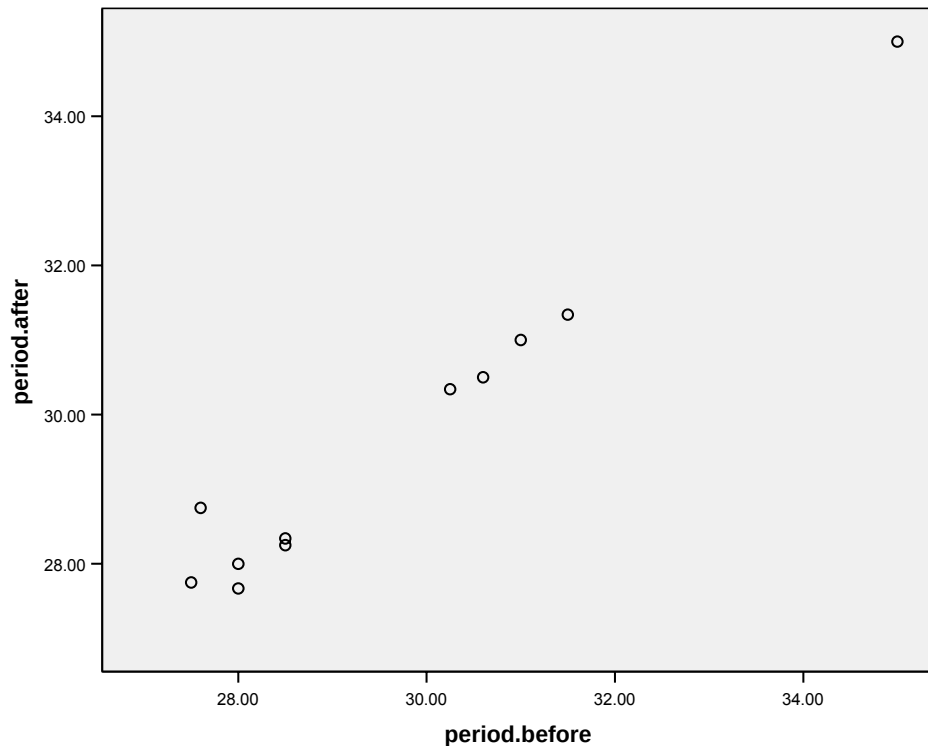
Duration of menstrual cycles in most of the cases of group B, was similar before and after the experiment because as it is shown in scatter diagram 2 most of cases are located around the bisector line. It supports that smelling water had no effect on menstrual cycle length of group B.

Diagram 2. The scatter diagram for control group who smelled water.



Similar to group B, the results of group C indicated that there were no significant changes in duration of this group's menstrual cycle. P value in this case is 0.72 which is more than significant level 0.05 and also the difference between the mean before and after experiment was very little (differences mean=0.04). Below is the diagram which demonstrates the subjects are mostly scattered in the bisector line supporting the above mentioned results.

Diagram 3. The scatter diagram for group C which smelled nothing.



Analysis of questionnaire answers before beginning of the experiment showed that the menstrual cycles in 9 members of group A, 6 persons in group B, and 10 persons in group C were regular and in the rest, it was not. Cases 2, 3, 7, 11, and 15 in group A had irregular and fluctuating menstrual cycles before the experiment. During this experiment cases 2, 7, and 14 showed approximately a regular pattern, while cases 11 and 15 had regular cycles pattern just in the last months. The case number 5 in group A was taking cyproterone compound and the case number 1 in group C was on marvellon pill for regulating their menstrual cycle (shown by red star in tables A & C).

The results of anxiety test are summarized in table 6. 6 cases out of 35 had less anxiety and 8 girls were indifferent, but in general all of them were in normal range and no case had extreme anxiety. The averages for the hidden anxiety in groups A, B, and C were 17, 16, and 18 and the averages for evident anxiety in these groups were 12.75, 13, and 13.6, respectively.

Table 6. The results of anxiety test.

Women	The results interpretation	Group A	Group B	Group C
0-14	Capable of doing the responsibilities that come up in stressful conditions.	—	—	—
15-18	Capable of doing the responsibilities that come up in stressful conditions.	—	—	—
19-23	Probably indifferent and without motivation. Capable of doing the responsibilities that come up in stressful conditions.	4	2	2
24-27	Normal for anxiety	1	1	2
28-31	Normal for anxiety	4	3	4
33-36	Normal for anxiety	2	—	1
37-40	Normal for anxiety	—	3	—
41-44	Neurotics that have less anxiety. Normal for anxiety	4	—	2
45-49	Averaged anxiety. The average mark of neurotics.	—	—	—
50-53	Extreme anxiety. Need to psychological consultation or curve.	—	—	—
54-80	Very high level of anxiety. Neurotics anxiety	—	—	—

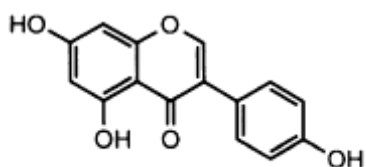
Performance of anxiety test and questionnaire and explanation of the expected research outcome to group C showed that anxiety, suggestion and chance did not have any significant effect on results in this experiment.

Discussion

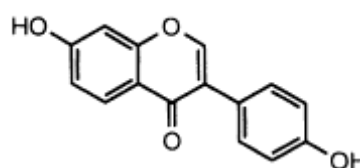
Zataria multiflora Boiss is a plant that is orally used as antiseptic^{2,4,5}, antispasmodic², anti-inflammatory, antibacterial, antiviral⁶, antioxidant⁶⁻⁸, antinociceptive^{9,10}, and antifungal⁴ medicine. Previous studies on *Zataria* showed that it has flavonoids as in *Vitex agnus-castus* (VAC) plant¹⁹. Flavonoids are considered as natural phytoestrogen in VAC which proved to have positive effect on improving menstrual disorder²⁰. Knowing about VAC, I suspected

that flavonoids were the effective compound in *Zataria* too, and that it might influence menstrual cycle. On the basis of their structures, flavonoides are divided into several categories; one being the isoflavones which have four subtypes of phytoestrogens: genistein, daidzein, O-demethylangolensin, and equol³³.

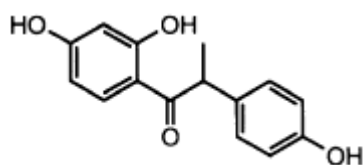
Figure5. The types of isoflavonoid^{33,35}.



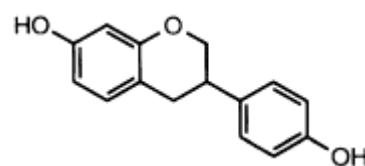
Genistein



Daidzein



O-Demethylangolensin



Equol

Recently researchers tested this hypothesis that phytoestrogens alleviate menopausal symptoms. Most studies are done on soy isoflavonoides that include genistein, daidzein and its metabolite equol. One of these studies investigated the effect of injecting and dietary equol on the reproductive organs in mice. Thirty day old females in this study were then ovariectomized for histological examination. The experimented animals were categorized into two groups, one of these groups was fed with phytoestrogen-free diet but received daily injections of equol with 4, 8, 12, and 20 mg/ (kg body weight), and the other group was given equol in their diet (500 - 1000 ppm for 12 days). At the end, experimental animals were killed for weighting their uterus and investigating vaginal epithelial thickness. Also the concentration of equal in their serum was measured. Analysis of the results showed that in injected animals, equol concentration was increased from 1.4 before the experiment, to 7.5 μ M after that. But increasing in the weight of uterus was significant only with 12 and 20 mg/

(kg body weight) of equol injections. In other group that was given dietary equol, its concentration was 5.9 and 8.1 μM and the uterine weight did not significantly increase. Also, increase in dietary and injected doses of equol led to increase in vaginal **epithelial** thickness. So, it was shown that equol acted as estrogen agonist in this study. Its effect was stronger by injecting route compared to oral feeding⁴⁰.

For evaluating the risk of isoflavone and equol on uterine and breast cancer, a research was performed on 30 adult female menopausal cynomolgus macaques. Monkeys were equally divided to three groups. No drug was in the diet of control group, IF group diet was supplemented with isoflavones (genistein, daidzein) and equol was added to the diet of EQ group for 28-30 days. Measurement of the concentration of serum isoflavonoid four hours after feeding showed that it was significantly higher in IF and EQ groups than control group. The major isoflavonoid in the serum was equol in both IF and EQ groups. Analysis of the data by SAS statistical package method showed that there were not proliferative lesions in breast and uterus in any one of the three groups. The results of this study suggested that these phytoestrogens did not contribute to increased uterine or breast cancer risk⁴¹.

Another study investigated the hormonal effect of soy isoflavones in the postmenopausal women. For this purpose three different concentrations of isoflavones were given to 18 postmenopausal women. Blood samples were collected on days 1, 36, 64, and 92 for analyzing the levels of estrogens, androgens, gonadotropins, and sex hormone binding globulin (SHBG). Also endometrial biopsies were performed at the first and the end of the experiment. Analysis of the obtained data by Statistical Analysis System and paired *t*-test method showed that the low and high concentration of isoflavonoid had small but significant effect on reducing the levels of estrone-sulfate, estradiol, and estrone but just a little rise in SHBG. In vaginal cytology or endometrial biopsy, significant changes were not observed. These data suggested the slight effect of isoflavons on plasma hormones in postmenopausal women⁴². Another study showed that consuming 50mg/day isoflavonens for 12 weeks on 177 postmenopausal women reduced hot flashes. However there was not enough information to definitively prove that isoflavens were the causing agent⁴³.

If *Zataria* contains isoflavonoids like what is in soy, it can also be tested as an herbal drug for alleviating menopausal symptoms. Because of the limitation in the equipments in our laboratory, I could not separate compounds such as thymol, cavacrol, rosmarinic acid,

and flavonoids and their subsets in *Zataria*, so I investigated the effect of the whole *Zataria* leaf extract on volunteers.

Old medical books are full of the effects of this plant in human when taken orally. Two researches, Baziyar & Charkh Zarin, showed that smelling the extracted oil of *Zataria* accelerates the precocious maturity and makes an earlier pregnancy in mice to become possible.

Baziyar worked on BALB/c mice and divided them to experimental (n=10), and control groups (n=21). In experimental and control groups, she placed pregnant females in separate cages. In the cages of experimental, but not in controls, she hanged a cotton pad and soaked it with 8-10 drops of fresh *Zataria* extract twice a day. So the born mice in these cages were exposed to *Zataria* immediately after birth. The average time for genital pore opening and reaching to puberty in female mice is normally about 35 days and the first childbirth time is about 12-14 weeks. She repeated the experiment 10 times and noted the time of genital pore opening and the first childbirth in both experimental and control groups. She found that the average time for puberty and child birth in controls was similar to normal, but in experimental cases the average time of childbirth was between the 10-11th weeks. She suggested that this extract accelerated puberty and childbirth in mice about 1/5⁸⁸.

Charkh Zarin study concentrated on the histological examination of ovarian tissue which confirmed early maturity of ovarian follicles after exposure to *Zataria*⁸⁹. For this purpose, she repeated Baziyar's work at first and kept the experimental (n=29) and control (n=27) pregnant females to separate cages and the experimental were exposed to *Zatria* oil. But she used *Mus musculus* species of mouse, which its genital pore opens about the 40th days and the first childbirth is in the 13th weeks after birth. She reported the same results as Baziyar's. In control group the average time for genital opening and the first childbirth was very close to normal but in experimental group average time for genital opening was at day 37 and the first childbirth was at 11th weeks. She continued her experiment by killing one female from both control and experimental groups in different ages, 9 times on days 8, 12, 18, 22, 25, 28, 30, 35, and 40. She fixed their ovarian tissues, sectioned them, stained the sections and studied them and took some microscopic photos. The results of histological investigation showed that the follicles in experimental animals were matured sooner and the number of these follicles was more than in controls⁸⁹.

Mouse is used as a common animal model in physiological and other biological researches. Because mouse and human are mammals and their reproductive system, expect for small differences such as the duration of maturity and menstrual cycle is similar, so may be the extracted oil of *Zataria* plant could influence the sexual cycle in human the same way as in mouse. If the findings of this study support my hypothesis, *Zataria* may be considered as a potential drug for improving female menstrual cycle abnormalities.

The results of my project showed that smelling of *Zataria* extract led to 0.3-2 days shortening of menstrual cycle length in experimental ($P=0.012$, $P<0.05$) with 95% confidence interval while there were not any meaningful changes in control groups ($P=0.119$, $P>0.05$). The result of my experiment supported the effect of *Zataria* extract on woman's reproductive system like the reports of Bazyar (1998) and Charkh Zarin (2004) about the quickening effect of this extract on mice sexual maturity.

Although the statistical analysis of my work supported that the smelling of the extracted oil shortens menstrual cycle length in women, I did not have the equipments to analyze the sexual hormonal changes in the candidates' blood or microscopically see the alteration of vaginal tissue or ovarian follicles to prove my hypothesis physiologically or histologically.

As mentioned earlier, *Zataria* extract contains flavonoids, a group of phytoestrogens which have an estrogen like structure. Estrogen is a steroidal hormone. Some researchers believe that steroids, in the form of odor, like other pheromones are detected by accessory olfactory receptors and by the vomeronasal organ^{67,68} but others suggest that main olfactory receptors are responsible to detect them^{50,66}. Some studies mention that although pheromones and similar compounds are detected by accessory olfactory system in mammals, but this system has not significant function in human because its receptor genes are expressed in only %8 of people⁵⁹.

Menstruation is a part of menstrual cycle which is controlled by both hormonal and nervous system. Hormonal system controls menstrual cycle by a negative feedback hierarchical system. Decreasing estrogen level in the circulation causes the secretion of GnRH from GnRH neurons, in the median eminence of hypothalamus and this in turn induces the secretion of gonadotropin hormone from pituitary gland. Gonadotropin induces synthesis and excretion of steroidal hormones, estrogen and progesterone, from ovary. Increasing these hormonal levels in the blood leads to the stopping of GnRH from

hypothalamus. The whole thing is controlled indirectly by some parts of the brain such as the limbic system and hippocampus nuclei. Environment can influence this system. So question is at what level the molecules of the *Zataria* extract can influence menstrual cycle pattern?

In the Kal disease, when the KAL1 gene is defectively expressed, both the olfaction and GnRH secretion become defective^{53,76}. In addition to median eminence, GnRH neurons are distributed in several areas of brain such as medial preoptic area of hypothalamus and the mucosa of the nasal cavity⁷⁹.

Estrogen is one of the molecules that indirectly induce the secretion of GnRH because neurons which stimulate GnRH neurons to release GnRH have estrogen receptors. This inducing effect is studied about the estrogen in the blood circulation but the relationship between inhaled phytoestrogens and menstrual cycle is not researched well.

Conclusion

In spite of some deficiencies that existed in this experiment such as absence of the same condition for all volunteers and the lack of physiological and histological studies on them, the results of my project showed that smelling of *Zataria multiflora* extracted oil in long term (from two to four months in this experiment) significantly influenced menstrual cycle in women. The extract smelling caused a decrease in menstrual cycle length of 0.3- 2 days in 15 volunteers. ($P= 0.012$, $P<0.05$).

I did not study the mechanism of action of *Zataria* oil on olfactory or reproductive system. My study needed histological or physiological verification about the changes in serum level of sexual hormones or on the changes in cornified cells of vaginal epithelium to prove my hypothesis more strongly. If the existence of flavonoids and isoflavonoids and their subsets get proved in *Zataria* extract, these substances might be used as a possible drug for improving menopausal symptoms or menstrual disorders.

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Appendix

Appendix1: *The time table of smelling each day.*

Please smell this essential oil 6 times each day and register the time of smelling exactly in the cells of this table.

Saturday						
Sunday						
Monday						
Tuesday						
Wednesday						
Thursday						
Friday						

Please pay attention to below points.

1- Do not allow it touch to your skin.

2- After smelling, close bottle's lid and keep it in cold condition and far from the exposing to sun light.

3- I retake it next week.

Thank you for cooperation.

Appendix 2: The questionnaire about personal characteristics.

Table1- Determine your family condition.

Num	The members of your family (Parents, brothers, sisters) Ambiguous question	Single/Married	Infectious disease (ambiguous question)	In girls and women: the condition of menstruation (ambiguous question)
1				
2				
3				
4				
5				
6				
7				

1. Do you take the especial medicine or comforting drugs? Yes ☐ No ☐

If your answer is yes:

A-What is the name of your medicine? And why do you take it?

B- How long do you take it?

2. Did you have caught cold 3 months ago? Yes ☐ No ☐

A- If your answer is yes, what kind of drugs do you take?

B- How many did you have caught cold?

3. What kind of foods do you usually use?

A- Using milk and dairy product daily.

B- Eating fruits and vegetables daily.

C- Using dried nuts or seeds continually.

D- Consumption of protein compounds regularly.

E- Using carbohydrate compounds a lot.

F- Eating fast foods continually.

4. Do you exercise regularly? Yes ☐ No ☐ Occasionally ☐

A- If your answer is yes, where do you exercise?

At home ☐ In the park ☐ In a team or club ☐

B- How much do you do it each day?

Appendix 3: Catell anxiety test

Name:

Family Name:

Date:

Sex:

Other Information:

In the following pages, you will see 40 questions. These questions talk about the problems that most of people face in their daily life. To know yourself better, we recommend that you answer to all questions with utmost honesty.

To know how to answer, two examples are given. There are three choices for each question and a box for each choice. First read the question carefully, and then choose the choice that describes your situation most. Finally mark the appropriate box with "x".

Example 1:

I like walking....	Yes	To some extent	No
	☐	☐	☐

Choose the choice in the middle only when you cannot choose "Yes" or "No". We recommend that you use this choice seldom.

Example: In the evening, I prefer ...

A. To talk to someone.

B. To go to the cinema.

A	?	B
☐	☐	☐

Please note that "B" always comes after "A". Do not forget that you can choose the middle choice only when it is impossible to choose "A" or "B".

Please pay attention to the following points:

1. Answer all questions. Mark only one choice for each question. Your answer will be kept confidentially.
2. Do not waste time by any confusion feeling. After reading the question, answer it immediately based on the way you think at the moment not your last week thoughts or your usual thoughts. You may have answered the same kind of questions before, but now you have to answer them according your present feelings.

Most people answer these questions in 5 minutes and fewer in 10 minutes. The moment you finish all questions, go straight to the following texts.

1. I lose my interest in people and entertainments very fast. True ☐ ? ☐ False ☐
2. Even if people do not have positive point of view to me, I won't lose my self-confidence. True ☐ ? ☐ False ☐
3. Before I say a word, I think about it to make sure what I am going to say is true. Yes ☐ ? ☐ No ☐
4. I tend to envy people. Occasionally ☐ Seldom ☐ Never ☐
5. If I could have been born again: A ☐ ? ☐ B ☐
A. I would have organized it in a completely different way.
B. I would like to have the same life.
6. I consult with my parents in all issues. Yes ☐ ? ☐ No ☐
7. It is very difficult for me when others say "No!" to me; even if I know what I want is impossible. True ☐ ? ☐ False ☐

8. I doubt the honesty of people who are friendlier to me than I expect.

Yes ☐ ? ☐ No ☐

9. Any time my parents told me something and asked me to follow:

A. They were always logical.

A ☐ ? ☐ B ☐

B. They were often illogical.

10. It seems I need my friends more than they need me.

Seldom ☐ Occasionally ☐ often ☐

11. I am sure I can confront a sudden problem.

Seldom ☐ Occasionally ☐ often ☐

12. When I was a kid, I used to be afraid of dark.

Very often ☐ Occasionally ☐ Never ☐

13. Sometimes people tell me that I show my enthusiasm in my behavior and speech.

Yes ☐ ? ☐ No ☐

14. If others misuse my compassion and kindness:

A. I will get hurt and I cannot forgive it.

Yes ☐ ? ☐ No ☐

B. I will forget it very fast and forgive it.

15. People's criticisms of me will hurt me more than they may help me.

Very often ☐ Occasionally ☐ Never ☐

16. It usually happens that I get angry with people.

True ☐ ? ☐ False ☐

17. I will get anxious exactly as the time I want to have something and I do not know what I want.

Seldom ☐ Occasionally ☐ Very often ☐

18. Sometimes I ask myself: "Are people really interested in what I am saying?"

True ☐ ? ☐ False ☐

19. I have not ever had unknown feelings like vague pains, digestive disorder, and heard my heart beating...

True ☐ ? ☐ False ☐

20. Discussing with some people, I get upset so much that I cannot talk without effort.

Occasionally ☐ Seldom ☐ Never ☐

21. When I do something, I use more energy since I do things with a feeling of stress.
True ☐ ? ☐ False ☐
22. I do things in a way that I do not forget things and details. True ☐ ? ☐ False ☐
23. Whatever the problems and obstacles, I always show perseverance and follow my former goals. Yes ☐ ? ☐ No ☐
24. In difficult situations, I lose my way. Yes ☐ ? ☐ No ☐
25. Sometimes I have nightmares. Yes ☐ ? ☐ No ☐
26. When I face a problem, I always feel I have enough energy. Yes ☐ ? ☐ No ☐
27. Sometimes I feel without any reasonable cause or due to a force I have to count things. True ☐ ? ☐ False ☐
28. Most of the people have strange minds though they do not like to accept it.
True ☐ ? ☐ False ☐
29. When I do something wrong in front of some people, I can forget it very fast.
Yes ☐ ? ☐ No ☐
30. I feel I am bad tempered and I do not like to see anyone: A ☐ ? ☐ B ☐
A. To some extent
B. Often
31. When things are not done orderly, I feel like crying.
Never ☐ Seldom ☐ Occasionally ☐
32. Even among people, sometimes I feel lonely and worthless. Yes ☐ ? ☐ No ☐
33. I wake up in the middle of night and because of troubled thoughts I can sleep again with difficulty. Often ☐ Occasionally ☐ Never ☐
34. Whatever the problem, I usually keep the appearance unchanged.
Yes ☐ ? ☐ No ☐
35. Sometimes without any reason I feel guilty or regretful. Yes ☐ ? ☐ No ☐
36. My nerves are so sensitive that some sounds such as the noise made by open and closing a door hurts me and I cannot tolerate it.
Often ☐ Occasionally ☐ Never ☐
37. If I get angry for any reason, usually I become calm again very fast.
True ☐ ? ☐ False ☐

38. When I remember something difficult I have to do, I usually become anxious and sweat. Yes ☐ ? ☐ No ☐

39. When I want to sleep, I usually can fall asleep in a few minutes. Yes ☐ ? ☐ No ☐

40. When I think about things that I was interested in them previously and for a short time, I feel very anxious. Yes ☐ ? ☐ No ☐

Please check to ensure you have answered all questions.

آزمون اضطراب کتل

نام و نام خانوادگی... تاریخ... جنس... سن... سایر اطلاعات...

در صفحاتی که به دنبال می آید 40 سؤال خواهید دید. این سؤالات مشکلاتی مطرح می کنند که اکثر مردم در زندگی خود با آنها روبرو می شوند. برای اینکه خود را بهتر بشناسید توصیه می کنیم به همه سؤالات با صداقت کامل پاسخ دهید.

برای آشنایی با شیوه پاسخگویی دو سؤال نمونه ذکر شده است. در مقابل هر سؤال سه پاسخ و در کنار هر پاسخ یک مربع وجود دارد. ابتدا سؤال را به دقت بخوانید بعد پاسخی انتخاب کنید که با وضعیت شما بیشتر سازگاری دارد. آن گاه در داخل مربعی که در زیر کنار پاسخ شما قرار دارد * بگذارید.

مثال اول. من قدم زدن را دوست دارم. ☐ بلی ☐ به مناسبتهایی ☐ خیر

تنها زمانی علامت * را در مربع وسط بگذارید که نتوانید پاسخ بلی یا خیر بدهید. توصیه می شود که از پاسخ وسط کمتر استفاده کنید.

مثال دوم. من ترجیح می دهم عصر...

A. با کسی گپ بزنم. B. به سینما بروم. ☐ A ☐ ؟ ☐ B

تعدادی از سؤالات ایجاب خواهد کرد تا یکی از دو پاسخ A و B را انتخاب کنید. پاسخ B همیشه در سمت چپ یا بعد از پاسخ A قرار دارد. یادتان باشد که پاسخ وسط را تنها زمانی انتخاب کنید که انتخاب یکی از دو پاسخ A و B واقعاً غیر ممکن باشد.

به نکات زیر توجه کنید.

1- به همه سؤالات پاسخ دهید. برای هر سؤال تنها یک پاسخ انتخاب کنید. پاسخهای شما کاملاً محرمانه خواهد بود.

وقت خود را به تردید و دودلی نگذرانید. پس از آنکه سؤال را خواندید بلافاصله به آن پاسخ دهید: بر اساس آنچه هفته گذشته فکر می کردید یا معمولاً فکر می کنید. ممکن است قبلاً به سؤالاتی از این نوع پاسخ داده باشید اما به این سؤالات باید بر اساس آنچه حالا احساس می کنید پاسخ دهید.

اکثر مردم در مدت پنج دقیقه و تعداد کمی نیز در مدت ده دقیقه به این سؤالات پاسخ می دهند. به محض اینکه به همه سؤالات پاسخ دادید مطالب بعدی را بخوانید.

1- علاقه من به مردم و سرگرمیها خیلی زود عوض می شود. ☐ صحیح ☐ ؟ ☐ غلط

2- حتی اگر مردم نظر خوبی نسبت به من نداشته باشند آرامش کامل خود را از دست نمی دهم.

☐ صحیح ☐ ؟ ☐ غلط

3- قبل از اظهار نظر تأمل می کنم تا مطمئن شوم آنچه می گویم درست است. ☐ بله ☐ ؟ ☐ خیر

4- تمایل دارم حسادت کنم. ☐ گاهی ☐ به ندرت ☐ هرگز

5- اگر قرار بود زندگی خود را دوباره از سر بگیرم:

A- آن را به صورت کاملاً متفاوتی سازمان می دادم.

- B- دلم می خواست همین زندگی را داشته باشم. ☐ A ☐ ؟ ☐ B
- 6- در همه مسائل مهم با والدین خود مشورت می کنم. ☐ بله ☐ ؟ ☐ خیر
- 7- شنیدن پاسخ مه برایم بسیار دشوار است حتی اگر بدانم آنچه می خواهم غیر ممکن است. ☐ صحیح ☐ ؟ ☐ غلط
- 8- در صداقت افرادی که با من رفتار دوستانه تر از حد انتظار دارند تردید می کنم. ☐ صحیح ☐ ؟ ☐ غلط
- 9- والدین من هر وقت دستور می دادند و می خواستند که من اطاعت کنم: ☐ A- همیشه منطقی بودند. B- اغلب غیر منطقی بودند. ☐ A ☐ ؟ ☐ B
- 10- به نظر می رسد که نیاز من به دوستان بیشتر از نیاز آنها به من است. ☐ به ندرت ☐ گاهی ☐ اغلب
- 11- مطمئن هستم که می توانم در مقابل یک مشکل ناگهانی مقاومت کنم. ☐ به ندرت ☐ گاهی ☐ اغلب
- 12- وقتی بچه بودم از تاریکی می ترسیدم. ☐ اغلب ☐ گاهی ☐ هرگز
- 13- گاهی مردم می گویند که من هیجان خود را به آسانی در اعمال و گفتار نشان می دهم. ☐ بله ☐ ؟ ☐ خیر
- 14- اگر مردم از محبت و مهربانی من سوء استفاده کنند: ☐ A- ناراحت می شوم و کینه به دل می گیرم. B- به زودی فراموش می کنم و می بخشم. ☐ A ☐ ؟ ☐ B
- 15- انتقادهای مردم بیشتر از آنچه به من کمک کنند ناراحت می کنند. ☐ اغلب ☐ گاهی ☐ هرگز
- 16- زیاد اتفاق می افتد که خیلی زود عتیبه مردم عصبانی می شوم. ☐ صحیح ☐ ؟ ☐ غلط
- 17- درست مثل زمانی که چیزی بخواهم و ندانم که چه می خواهم احساس نگرانی می کنم. ☐ به ندرت ☐ گاهی ☐ اغلب
- 18- گاهی از خود می پرسم: آیا مردم واقعاً به آنچه می گویم علاقه مندند؟ ☐ صحیح ☐ ؟ ☐ غلط
- 19- هرگز دچار احساسهای نامعلوم مثل دردهای مبهم اختلالات اضمه شنیدن صدای قلب و... نشده ام. ☐ صحیح ☐ ؟ ☐ غلط
- 20- هنگام بحث با برخی مردم آن قدر ناراحت می شوم که به زحمت حرف می زنم. ☐ گاهی ☐ به ندرت ☐ هرگز

21- وقتی کاری انجام می دهم بیشتر از دیگران انرژی مصرف می کنم زیرا توأم با اضطراب کار می کنم.

صحیح □ ؟ □ غلط □

22- شیوه ای به کار می برم که حواس پرتی نداشته باشم و جزئیات را فراموش نکنم.

صحیح □ ؟ □ غلط □

23- موانع و دشواریها هر چه باشند همیشه پشتکار نشان می دهم و اولین هدفهای خود را دنبال می کنم.

بله □ ؟ □ خیر □

24- در موقعیتهای دشوار برانگیخته می شوم و راه خود را گم می کنم.

بله □ ؟ □ خیر □

25- گاهی خوابهای آشفته می بینم.

بله □ ؟ □ خیر □

26- وقتی خود را در مقابل یک مشکل همیشه احساس می کنم که به قدر کافی انرژی دارم.

بله □ ؟ □ خیر □

27- گاهی احساس می کنم که به اجبار و بدون دلی قانع کننده ای باید چیزهایی بشمارم.

صحیح □ ؟ □ غلط □

28- اکثر مردم ذهن عجیب و غریبی دارند هر چند دوست ندارند آن را قبول کنند.

صحیح □ ؟ □ غلط □

29- وقتی در میان جمع مرتکب یک رفتار ناشایسته می شوم می توانم آن را به زودی فراموش کنم.

بله □ ؟ □ خیر □

30- احساس می کنم که بداخلاق هستم و نمی خواهم کسی را ببینم:

A- به مناسبتهایی. B- اغلب

31- وقتی کارها خوب پیش نمی رود گریه ام می گیرد.

هرگز □ به ندرت □ گاهی □

32- حتی در بین جمع گاهی احساس تنهایی و بی ارزشی می کنم.

بله □ ؟ □ خیر □

33- نصف شب بیدار می شوم و به علت اشتغالات ذهنی به دشواری می توانم دوباره بخوابم.

اغلب □ گاهی □ هرگز □

34- دشواریها هر چه باشد معمولاً ظاهر امر را حفظ می کنم.

بله □ ؟ □ خیر □

35- گاهی بدون علت احساس گناه یا احساس پشیمانی می کنم.

بله □ ؟ □ خیر □

36- اعصاب من به قدری خراب است که برخی صداها مثل غرچ و غروج کردن در برابم تحمل می شود و ناراحت می کند.

اغلب □ گاهی □ هرگز □

37- اگر به علتی ناراحت شوم معمولاً خیلی زود آرامش خود را باز می یابم. صحیح □ ؟ □ غلط □

38- وقتی به یاد یک کار دشوار می افتم که باید انجام دهم معمولاً لرزه بر اندامم می افتد یا عرق می کنم.

بله □ ؟ □ خیر □

39- وقتی می خواهم بخوابم معمولاً خیلی زود به خواب می روم در عرض چند دقیقه.

بله □ ؟ □ خیر □

40- وقتی به چیزهایی فکر می کنم که قبلاً و به مدت خیلی کم به آنها علاقه مند بودم گاهی حالت اضطراب یا

برانگیختگی بیش از حد به من دست می دهد. بله □ ؟ □ خیر □

سؤالات را دوباره بررسی کنید تا مطمئن شوید به هم آنها پاسخ داده اید.