

Original Article

The Effect of Hydroalcoholic Extract of Leaves of Vitex on the Gestation Indices of Male Rats

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Abstract

Background: Phytoestrogens are some plant compounds with estrogenic biological effects which are found in many nutritional sources as soybean, flaxseed, and sesame. Vitex agnus-castus, also called Vitex, owns phytoestrogen properties. Studies have shown that phytoestrogens have different impacts on the gestation process and reproduction indices.

Objective: The present study was aimed to investigate the effects of Vitex extract on the gestation indices in the male rat as well as studying its histological properties in the rat testicles.

Materials and Methods: The hydro-alcoholic extract of Vitex (in three doses of 165, 265 and 365 mg/kg), vehicle (normal saline) and the hydro-alcoholic powder of soybean (120 mg/kg) were respectively given to understudy, vehicle and positive control groups for 49 days. After weighing the rats in the 1st and 49th days, the blood samples of all groups were taken and tested for estradiol levels, testosterone, FSH and LH. Moreover, such reproductive indices as sperm count, sperm motion, and prostate and testicle weight were studied and samples were collected for histological studies.

Results: Prescription of the hydro-alcoholic extract of Vitex (in three doses of 165, 265 and 365 mg/kg) did not change the rat's weight, significantly (P-value= 0.06). Hormonal studies reduced the progesterone, LH, and FSH compared to the vehicle group, significantly (P-value<0.05). In addition, the amount of estradiol was significantly more than the vehicle group and the most effect was observed at a dose of 365 mg/kg (P-value=0.02). Histological studies showed a reduction in existing spermatozoa in the seminiferous ducts.

Conclusions: This study had shown that the Vitex extract had inhibiting effects on the gestation indices in male rat and due to its destructive effects on the testicle tissues, more studies were required.

Keywords: Phytoestrogen, Vitex, Fertility index, Spermatozoa

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Introduction

Human beings have long been using medicinal plants and traditional medicine for healing and curing many diseases. This procedure has been going on without really knowing the positive or negative impacts these plants might have had on different body organs (Uniyal, Singh, Jamwal, & Lal, 2006).

Vitex has been widely studied for a long time. Vitex (*Vitex Agnus – cactus L.*) from the family of Verbenaceae contains phytoestrogen with Alkaloid and flavonoid compounds. Phytoestrogen is plant compounds with estrogenic biologic properties which are similar to the 17 beta-estradiol in form and action and also have some effects similar to estrogens (Karunamoorthi, Ramanujam, & Rathinasamy, 2008).

Phytoestrogens are classified into three basic categories of lignans, iso-flavonoids, and coumestans. They are found in many nutritional sources as soybean, sesame, kiwi and flaxseed. The biological and pharmacological activity of these Phytoestrogens has been affirmed in different studies (Branca & Lorenzetti, 2005).

Vitex is mainly cultivated in Central Asia, Mediterranean, and tropical regions. It is known with different names as mountain pepper, nausea or *Vitex Pseudo-Negundo* (Hauskn.) in Iran. In addition, due to its suppression of sexual desire in women, it is also called the chaste tree and monk pepper (Ramezani, Nasri, & Bahadoran, 2008). In the history of this plant, it was being spread on the bed by the wife of soldiers who had gone to war in order to decrease their sexual desire (Daniele, Coon, Pittler, & Ernst, 2005; Ramezani et al., 2008). Studies have shown that Vitex owns anaphrodisiac, sudoriferous, diuretic, analgesic and appetizing properties. In 2000, Burger et al. demonstrated that the plant extract has been very effective in balancing and regulating the sex hormones in women and also in reducing the premenstrual stress syndrome (PMS)

(Iheji, 2016; van Die, Bone, Burger, Reece, & Teede, 2009).

This plant is used in the treatment of benign prostatic hyperplasia and inhibition of prostate cancer cells (Adelson, Loprinzi, & Hershman, 2005). Moreover, it is reported that the Vitex extract has had good impacts on women suffering from progesterone deficiency during the luteal phase due to Hyperprolactinemia in a 3 months treatment period (Daniele et al., 2005; He et al., 2009).

The chemical compounds of Vitex extract contain many active compounds such as alkaloid vitexin, flavanols derivatives, and Kaempferol and quercetin which are mainly composed of casticin (Karunamoorthi et al., 2008).

The Vitex leaves contain different types of glycosides like vitexin and vitexinin, different types of flavonoids like casticin, orientin, iso-vitexina, alkaloid viticin, different types of iridoids like aucubine, agnoside, and different types of steroids like A4-3-Keto Ester (Hoberg, Meier, & Sticher, 2000). The iridoids of aucubine and agnoside were separated from the plant leaves up to 6%. Several studies demonstrated that phytoestrogens reduce the Spermatogenesis and gestation index (Dehghani, Panjehshahin, & Vojdani, 2010; Najafizadeh, Dehghani, Panjeh Shahin, & Hamzei Taj, 2013). Thus, the present study is aimed at investigating the effect of a hydro-alcoholic extract of *Vitex agnus – castus L.* leaves on the gestation indices of male rats.

MATERIALS AND METHODS

Experimental animals:

Male sprague-dawley rats (180-240 g) were bought from Iran University and used in the present study. During the extract gavage, they were kept in the animal room of Iran University under controlled conditions with respect to light (12 h light and 12 h darkness),

temperature (18 to 22 °C), humidity (45 to 50%) and free access to food and water. The rats were fed with prepared food tabs provided from Pars Poultry & Livestock Company.

Plant Extract Preparation:

Collecting and drying the plant:

The leaves of *Vitex* collected from the herbarium of pharmacognosy department of Faculty of Pharmacy in Tehran Medical Science University (approved by the faculty with identification code: 126194) were used in the present study. Leaves were dried in standard conditions far from the sunlight, humidity, microbial pollution and in appropriate air conditions. They were firstly cleaned of any pollutant and then powdered using an electric mill.

Extraction method:

A percolation method was used for extract preparation. After being powdered, the *Vitex* leaves were put in a container to which 80% ethanol hydro-alcoholic solvent was added and regularly mixed for 72 h. The mixture was then filtered and the obtained extract was filtered once more for usage. This process was repeated one more time. The obtained extract was dried using a rotary machine and prepared to be fed to animals in doses of 165, 265 and 365 mg/kg of the body weight.

Experimental groups:

After determining the doses of extract, the experiment was conducted by gavaging the hydro-alcoholic extract of *Vitex* in three doses of 165, 265 and 365 mg/kg of body weight to 5 groups of 6 rats each.

The first group (vehicle group): 1 cc normal saline was daily given to the rats as a vehicle for 49 days.

The second group (soybean positive control group): 1 cc hydro-alcoholic soybean powder with a dose of 120 mg/kg was daily given to the rats for 49 days.

The third group (experimental group 1): 1 cc extract with a dose of 165 mg/kg was daily

given to the rats for 49 days.

The fourth group (experimental group 2): 1 cc extract with a dose of 265 mg/kg was daily given to the rats for 49 days.

The fifth group (experimental group 3): 1 cc extract with a dose of 365 mg/kg was daily given to the rats for 49 days.

Animal and pharmacological studies:

Sampling:

All rats were weighed before the first dose prescription and 24 h after the last extract prescription. Their blood samples were taken through the tail vein (under anesthesia with ether) at the 49th day and then centrifuged (1500 round for 20 min). The blood serum was kept at 20 °C to be red for measuring testosterone and estradiol hormones, FSH and LH using Radio Immuno Assay method. Rats were anesthetized with ether and then dissected in 49th day and their reproductive organs, including the left testis, epididymis, seminal vesicles and left prostate were separated for further investigations.

Total count of normal epididymal sperm:

After the preparation of the sperm-containing solution, one drop was put on the neubauer slide and the normal sperms in squares related to white globule (16 cells) were carefully counted.

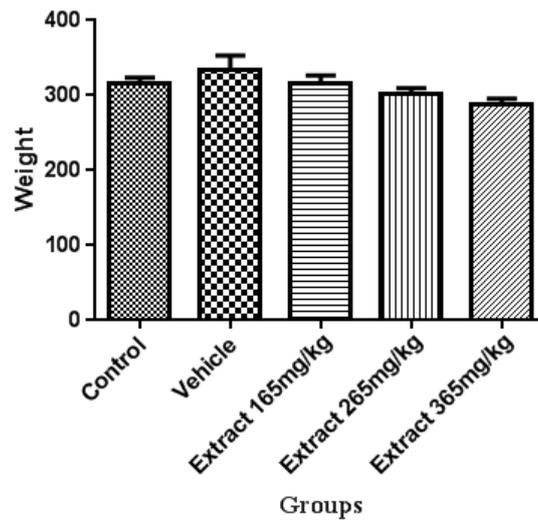
Statistical Analysis

The results of hormonal investigations and the number of sperms, testicles' weight among experimental and control groups were studied in the form of Mean \pm SEM. One-way variance (ANOVA) and Tukey tests were conducted for studying the significance of the difference between the experimental and control groups. The P-value was considered as P<0.05. Diagrams were drawn based on the results obtained from PRISM software.

RESULTS

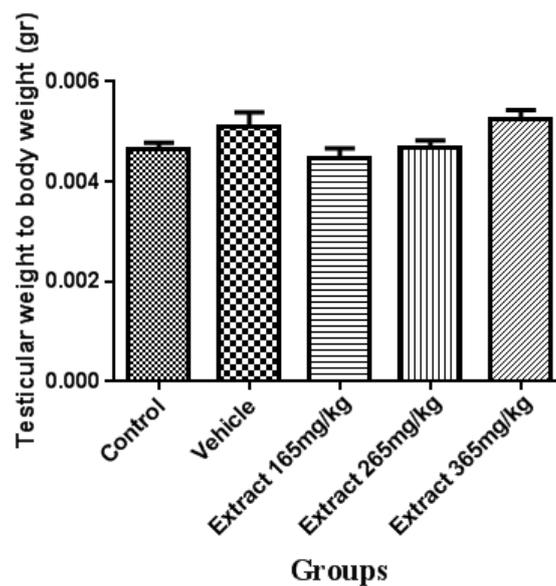
The effect of Vitex hydro-alcoholic extract on the rats' weight:

As it is shown in Figure 1, no significant difference was observed between the vehicle, control and plant extracts regarding the parameter mentioned.

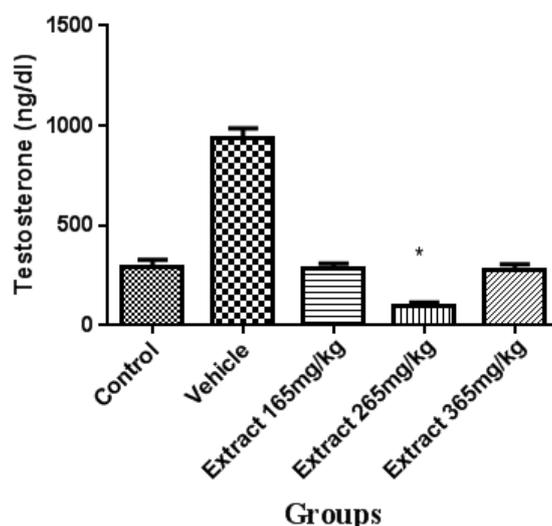
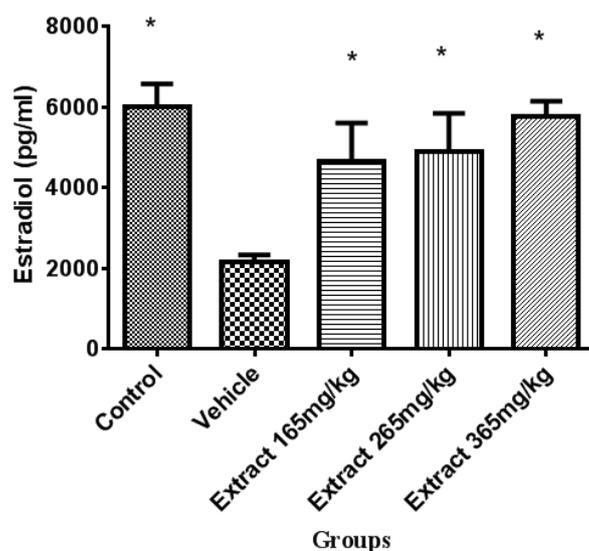


The effect of Vitex hydro-alcoholic extract on the ratio of the weight of the left testicle to the rats' weight:

As it is shown in Figure 2, no significant difference was observed among the control, vehicle and plant extracts regarding the above parameter.



The effect of Vitex hydro-alcoholic extract on rats' estradiol, testosterone, LH and FSH hormones: Given the Figure 3-6, there were significant differences in progesterone, LH and FSH hormones among the control, vehicle and plant extracts regarding the above parameter. Moreover, the estradiol level increased significantly compared to the vehicle group which was observed at a dose of 365 mg/kg (Figure 3).

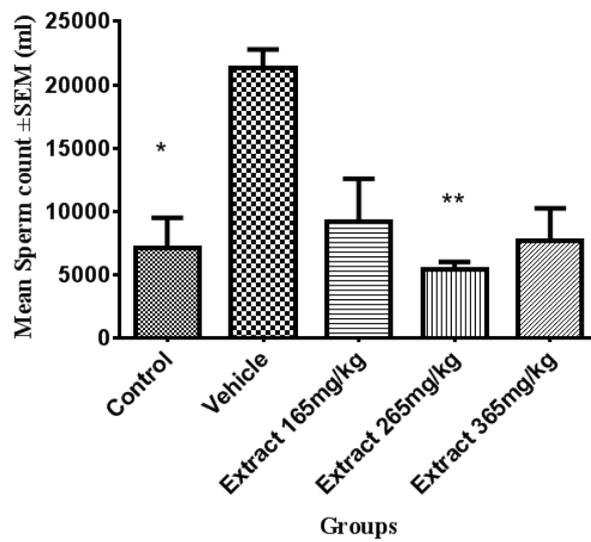
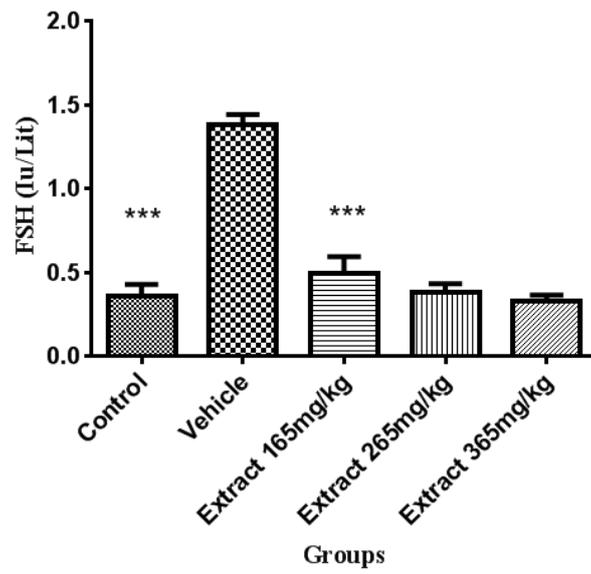
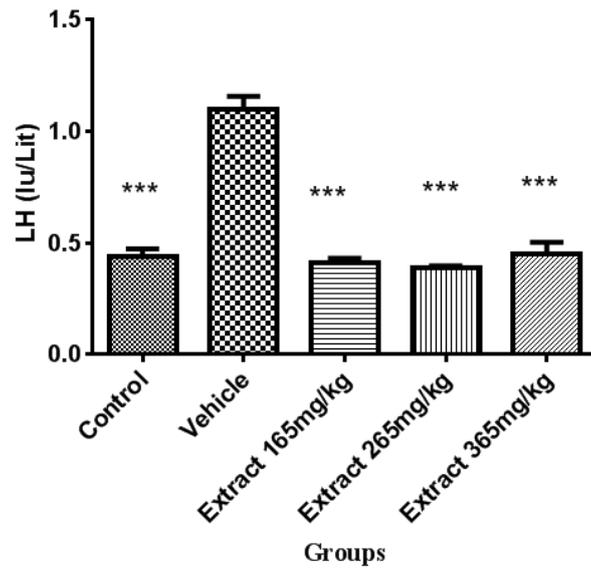


The effect of Vitex hydro-alcoholic extract on the sperm count:

Figure 7 shows that there was a significant difference among the control, vehicle and plant extracts on the number of sperms of epididymis compared to the control group.

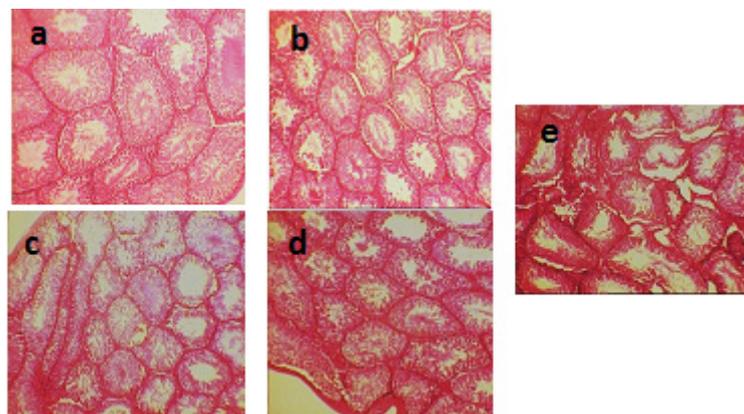
The effect of Vitex hydro-alcoholic extract on histological studies:

In the histological studies of Vitex on the tissues of the testicle, the spermatozoon cells in the center of seminiferous tubules were decreased in line with an increase in the extract dose. There were also some acidophilic grounds in seminiferous duct centers indicating necrosis.



Interstitial capillaries and interstitial cells were observed.

In line with the increase of extract dose in experimental groups, the thickness of the surrounding walls of the seminiferous ducts was increased and an irregularity was observed in their forms. For instance, the duct's walls were destroyed in some parts and completely deformed from its natural state for the dose of 365 mg/kg. (Figure 8)



DISCUSSION

Findings of the present study indicated that the Vitex caused the reduction in some gestation indices including the number of sperms, the level of testosterone, testicle's weight. It has also led to the increase in estradiol hormone in male rats.

In traditional medicine, Vitex has been used for pain relief and swollen uterus. It has also been recommended that its vapor or consumption seemed to be useful in reducing sexual desire (Ramezani et al., 2008).

The present results among the experimental groups indicated no change in the rats' weight. Thus, it was concluded that the prepared extracts in prescribed doses have not influenced the body's metabolism.

The results obtained by comparing the estradiol levels showed significant difference among the control groups. However, no significant difference was observed in relation to the soybean group as a positive control; whereas, the latter showed a significant increase in relation to the control group. In a study conducted in 2013, the effect of olive

phytoestrogens on the male rats' spermatogenesis was investigated. The estradiol level had increased insignificantly. In another study in which the effect of kiwi phytoestrogens on the male rats' spermatogenesis was investigated, the estradiol level had increased ((Dehghani et al., 2010; Najafizadeh et al., 2013).

In addition, the comparison of testosterone level results, among five groups, indicated a significant reduction. Maximum decrease was observed in the dose of 265 mg/kg of the hormone level. In this regard, Weber et al. and Roberts et al. yielded similar results in their study on the effect of phytoestrogens on testosterone (Price et al., 2000; Weber, Setchell, Stocco, & Lephart, 2001)

Prescription of Vitex extract significantly reduced the number of sperms in all three doses. Also, Dehghani et al. and Nasri had reported similar results (Nasri, Oryan, Haeri Rohani, Amin, & Yahyavi, 2004)

No significant decrease was observed in the seminal vesicle and left testicle for all three prescribed doses of the extract of Vitex fruit. Sprando studied the effect of flax seed phytoestrogen on the seminal vesicle and testicle weight loss (Sprando et al., 2000). It

should be mentioned that the phytoestrogen used in the present study was of genistein type in lignans Vitex, which is similar to the findings of Najafizadeh in 2013. (Dehghani et al., 2010; Najafizadeh et al., 2013).

Similar to the soybean group, the prescription of Vitex extract reduced their LH level in all three doses, significantly. In this regard, the two studies reported the same result that unsaturated fatty acids of this plant can negatively affect the male gestation (Ohno, Nakajima, Inoue, Nakazawa, & Nakajin, 2003).

The findings suggested that different effects of phytoestrogen on the male reproductive system could be due to the estrogenic and anti-estrogenic effects. It meant that phytoestrogens could act through estrogen receptors with agonistic and antagonistic properties depending on the phytoestrogen type and the target organ. For instance, isoflavones are weak estrogen agonists which are bound to estrogen receptor less than estradiol (Mueller, Simon, Chae, Metzler, & Korach, 2004; Whitten, Lewis, Russell, & Naftolin, 1995) While, the body's estradiol level is low in order to bind to the receptor, Isoflavones indicate more agonistic properties. On the other hand, their anti-estrogenic properties depend on the relative concentrations of internal phytoestrogens and estrogens. It could be that when the level of internal estrogen was high, phytoestrogens made the estradiol receptor unavailable to estradiol. Moreover, genistein can show both estrogenic and anti-estrogenic activity (because of its competition with estradiol to bind to the protein. Pharmacological studies suggested that the present compounds in the Vitex extract were specifically bounded to beta estrogen receptors in the heart, veins, bone and bladder (Wuttke, Jarry, Christoffel, Spengler, & Seidlova-Wuttke, 2003).

Although it was shown that the three doses (165, 265 and 365) of Vitex extract have decreased the spermatogenesis, LH and FSH hormones, and testosterone level and increased the estradiol level, its textural effects must still

be studied. Because the extract had an effect on the testicle tissue and causes the destruction of tissues and defragmentation of seminiferous tubules at high doses.

CONCLUSION

Findings of the present study indicated that hydro-alcoholic extract of Vitex had decreased the gestation indices of male rats, which was probably due to its phytoestrogen effects.

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REFERENCES

- Adelson, K. B., Loprinzi, C. L., & Hershman, D. L. Treatment of hot flushes in breast and prostate cancer. *Expert Opin Pharmacother* . 2005; 6(7), 1095-1106.
- Branca, Francesco, and Stefano Lorenzetti. Health effects of phytoestrogens. *Forum Nutr*. Karger Publishers, 2005; 100-111

- Daniele, C., Coon, J. T., Pittler, M. H., & Ernst, E. Vitex agnus castus. *Drug Saf.* 2005; 28(4), 319-332.
- Dehghani, F., Panjehshahin, M., & Vojdani, Z.. Effect of hydroalcoholic extract of caraway on thyroid gland structure and hormones in female rat. *Iran J Vet Res.* 2010; 11(4), 337-341.
- He, Z., Chen, R., Zhou, Y., Geng, L., Zhang, Z., Chen, S., Lin, S. Treatment for premenstrual syndrome with Vitex agnus castus: A prospective, randomized, multi-center placebo controlled study in China. *Maturitas.* 2009; 63(1), 99-103.
- Hoberg, E., Meier, B., & Sticher, O. Quantitative high performance liquid chromatographic analysis of diterpenoids in agni-casti fructus. *Planta Med.* 2000; 66(04), 352-355.
- Iheji, C. U. Anti-oxidant Properties of Some Lesser Known Fruits and Vegetables Consumed by families in South East Zone of Nigeria. 2016.
- Karunamoorthi, K., Ramanujam, S., & Rathinasamy, R. Evaluation of leaf extracts of Vitex negundo L.(Family: Verbenaceae) against larvae of Culex tritaeniorhynchus and repellent activity on adult vector mosquitoes. *Parasitol Res.*2008;103(3), 545-550.
- Mueller, S. O., Simon, S., Chae, K., Metzler, M., & Korach, K. S. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor (ER) and ER in human cells. *Toxicol Sci.* 2004; 80(1), 14-25.
- Najafizadeh, P., Dehghani, F., Panjeh Shahin, M., & Hamzei Taj, S. (2013). The effect of a hydro-alcoholic extract of olive fruit on reproductive argons in male sprague-dawley rat. *Int J Reprod Biomed (Yazd).* 2013; 11(4), 293-300.
- Nasri, S., Oryan, S., Haeri Rohani, A., Amin, G., & Yahyavi, H. The effects of vitex agnus castus L. extract on gonadotrophines and testosterone in male mice. *Iranian Int J Sci.* 2004;5(1), 25-31.
- Ohno, S., Nakajima, Y., Inoue, K., Nakazawa, H., & Nakajin, S. Genistein administration decreases serum corticosterone and testosterone levels in rats. *Life Sci.* 2003; 74(6), 733-742.
- Price, V. H., Roberts, J. L., Hordinsky, M., Olsen, E. A., Savin, R., Bergfeld, W., Pappas, F. Lack of efficacy of finasteride in postmenopausal women with androgenetic alopecia. *J Am Acad Dermatol.* 2000; 43(5), 768-776.
- Ramezani, M., Nasri, S., & Bahadoran, H. The Effects of Vitex agnus castus Total Extract on Spermatogenesis of Balb/C Mice. *Armaghane danesh.* 2008; 13(3), 35-44.
- Sprando, R., Collins, T., Black, T., Olejnik, N., Rorie, J., Scott, M., O'Donnell, M. The effect of maternal exposure to flaxseed on spermatogenesis in F 1 generation rats. *Food Chem Toxicol.*2000; 38(4), 325-334.
- Uniyal, S. K., Singh, K., Jamwal, P., & Lal, B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. *J Ethnobiol Ethnomed.* 2006; 2(1), 1.
- van Die, M. D., Bone, K. M., Burger, H. G., Reece, J. E., & Teede, H. J. Effects of a combination of Hypericum perforatum and Vitex agnus-castus on PMS-like symptoms in late-perimenopausal women: findings from a subpopulation analysis. *J Altern Complement Med.* 2009 ;15(9), 1045-1048.
- Weber, K., Setchell, K., Stocco, D., & Lephart, E. Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. *J Endocrinol.* 2001; 170(3), 591-599.
- Whitten, P. L., Lewis, C., Russell, E., & Naftolin, F. Potential adverse effects of phytoestrogens. *J Nutr.* 1995; 125(3), 771S.
- Wuttke, W., Jarry, H., Christoffel, V., Spengler, B., & Seidlova-Wuttke, D. Chaste tree (Vitex agnus-castus)—pharmacology and clinical indications. *Phytomedicine.* 2003; 10(4), 348-357.