The Effects of Vitex ugnus-castus L. Extract on Fertility of Male Rats

Naji T, Rabie Nezhad Ganji N*, Ramezanloo F, Kheirvari Khezerloo J, Daustany M

***Abstract*—** Vitex agnus-custus L [Verbenaceae] in the category of flavonoids which is native phytoestrogen compounds in the middle east and southern Europe and in many countries it has medical use, , but it is not a herb which contains estrogenic compounds and does not impact directly on the ovaries and it seems by effecting on hypothalamic-pituitary axis apply its own effect. This herb cause decreases in releasing FSH and LH and prolactin from the pituitary and also it decrease LH, FSH and testosterone in male rat and due to reduction of testosterone it effects on spermatogenesis as well and reduces it.

In this study for 48 days to experimental groups 1, 2 and 3 the extract of ethanol extract of vitex agnus-castus leaves at doses of 165, 265 and 365 mg/kg and divided into the 4 group as a sham group equivalent volume and duration of administration of the extract in experimental groups, the carrier and group 5 as a positive control of soybean at a dose of 120 mg/ kg were gavaged. After 49 days, the reproductive parameters such as sperm count, weight of testis, epididymis and levels of estradiol and testosterone, FSH and LH were measured and then histological studies were conducted.
Administration of Vitex extract caused a significant reduction in the number of sperms. Serum factors indicate significant changes in the normal testicular function.

***Index Terms*—** Vitex ugnus-castus L, Fertility, Male Rats**.**

I. Introduction

Vitex agnus-castus [Verbenaceae] is a deciduous tree or a large shrub that is native to Europe but also widely distributed in the Southern United States. The fruits of V. agnus-castus [chaste berry, VAC] have a long history [over 2000 years] of use as an herbal medicine. Currently, the fruit extract is used as a dietary supplement for estrogen hormone imbalance which can produce menstrual cycle disorders and premenstrual syndrome [PMS], such as cyclical mastalgia, and corpus luteum insufficiency [1,2] as well as for alleviating menopausal symptoms such as hot flashes [3].

Tahereh Naji (PhD) is with the Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology , Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS). (e-mail: t.s.naji@yahoo.com).

Nima Rabie Nezhad Ganji (*corresponding author) is with Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology , Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran(IAUPS).
(email: nimarabienezhadganji@gmail.com)

Fereshteh Ramezanloo is with Department of Medicine, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

Jamil Kheirvari Khezerloo is with Department of Biochemistry, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS). (email:miladkheirvari@yahoo.com)

Mahya Daustany is with the Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS). (e-mail: monafras@yahoo.com).

The classes of phytochemicals that have been reported in VAC fruits include essential oils [4,5], flavonoids [6,7], iridoids [8], and diterpenoids [9–10], as well as a diterpene lactam, vitexlactam A [11]. Under investigation for safety and efficacy at the UIC/NIH Center for Botanical Dietary Supplements Research, preliminary screening of a methanolic extract of VAC showed it contained ligands for the estrogen receptor [ER] [3]. Since at that time, there had been no literature reports of estrogenic constituents of VAC, an ER binding assay was used to guide phytochemical. The epididymis is one of the most important components of the mammalian male reproductive system. It is only during transit through the epididymal luminal microenvironment that spermatozoa undergo maturation and acquire progressive motility and the ability to fertilize oocytes [12]-[15]. The mammalian epididymal duct can be subdivided into four morphological regions: the initial segment, the caput, corpus and cauda epididymidis, all of which are essential for sperm maturation [16],[17].

II. Material And Method

In this laboratory experimental study, Vitex agnus castus was purchased from the Pharmacognosy herbarium of School of Pharmacy, Tehran University of Medical Sciences and was identified by experts of the sector of University of Medical Sciences. The leaves were dried in room temperature avoiding from direct sunlight and then ground into a fine powder. Adult male Sprague-dawely rats, weighing 240-180 g of were used in this study. They were randomly housed in polyethylene cages access to food and water in a room with controlled temperature [18-24 °C] and a 12 h light–dark cycle. Humidity of 45 to 50 animals were provided and they were fed the pills provided from the company of Pars livestock and poultry. In this study for 48 days to experimental groups 1, 2 and 3 the extract of ethanol extract of *vitex agnus-castus* leaves at doses of 165, 265 and 365 miligrams per kilogram and to the group 4 as a sham group equivalent volume and duration of administration of the extract in experimental groups, the carrier and group 5 as a positive control of soybean at a dose of 120 mg/ kg were gavaged. After 49 days, the reproductive parameters such as sperm count, weight of testis, epididymis and levels of estradiol and testosterone, FSH and LH were measured and then histological studies were conducted. Statistical significance was evaluated by one-way analysis of variance (ANOVA) and following that using Turkey test. Differences with $P<0.05$ were considered significant.

III. Results

Figure I show the fertility of epididymal spermatozoa cells in response to different doses of *Vitex ugnus-castus L*. Extract. Our findings show that viability of *Vitex* extract

http://dx.doi.org/10.17758/EAP.EAP816237

caused a significant reduction in the number of sperms. Serum factors indicate significant changes in the normal testicular function.

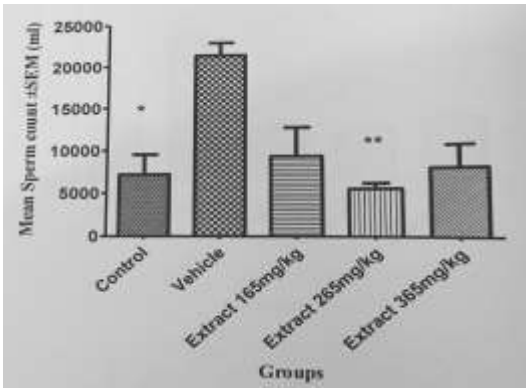

Fig I. fertility of epididymal spermatozoa cells in response to different doses of Vitex ugnus-castus L. Extract

IV. Discussion

Human and animal studies have determined *Vitex* to be safe for most women of menstruating age, but they note that it should not be used during pregnancy [18]. In mammals, the epididymis is responsible for sperm transport, concentration, storage, and maturation. Sperm maturation involves the acquisition of forward motility and fertilizing ability. Identification and characterization of differentially expressed genes in epididymis have provided us additional insight into the mechanisms of sperm maturation. Our previous study demonstrated that several epididymal proteins, such as Bin1b [19], HongrES1 [20], and Defb15 [21], are essential in sperm motility, capacitation, and male fertility, respectively. In the present study, we identified an epididymis-specific gene, *Spink13* and demonstrated its physiological function in regulation of acrosome reaction. The biological role of *Spink13* in the sperm acrosome and how its down-regulation causes the fertility reduction remains to be elucidated. One possibility is that SPINK13 functions as a protease inhibitor necessary for the regulation of critical proteases involved in early signaling events during fertilization, which is consistent with the hypothesis that the regulated serine protease activity might be the key of sperm maturation [22]. Furthermore, the fact that *Spink13* is evolutionarily conserved from rodent to human and has no expression in testis indicates that it is a putative target for post-testicular male contraception. The *SPINK13* protein has the characteristic signature of serine proteases inhibitors consisting of an N-terminal signal peptide followed by a Kazal domain, but its serine protease inhibitory activity is still unclear, rat *spink13* showed potent inhibitory effect on acrosin proteolytic activity using the gelatin substrate film method. It is very likely that other target proteases might exist in addition to acrosin. The active *spink13* recombinant protein after heterologous expression with proper folding will be a key step in further studies. However, the inhibitory target specificity by SPINK13 is achieved primarily through temporal and spatial restrictions, such as the site of expression, membrane anchorage, and the microenvironment of epididymis, thus complicating the identification of *in vivo* target proteases of SPINK13 using conventional protease inhibitor assay. It is generally known that gene expression in the epididymis is driven by androgens signaling through the androgen receptor; many gene expressions in the epididymis have been demonstrated to be regulated by androgen [23.24]. This study shows that androgen also regulates the *Spink13* gene. Androgen and its receptor form a complex that interacts with androgen response elements in androgen-responsive genes and regulates their expression. Considering the high homology of rat *Spink13* to its mouse homologue, we referred to the results of ChIP-seq in mouse caput epididymis [25] to predict 12 potential androgen receptor-binding sites in intergenic and intronic regions .Most of these androgen receptor-binding sites harbored conserved androgen response element motifs across rat and mouse based on whole genome alignment. Thus, it is possible that androgen-androgen receptor acts directly on *Spink13* gene to regulate its gene expression. It is noteworthy that the decline of Spink13 mRNA is not very sharp in response to the rapid decrease of androgen level. We speculate other regulation mechanisms are also involved, such as mRNA stability. Three different nociceptive tests [formalin test, acetic acid-induced writhing response, and tail immersion test] were employed for evaluation of possible peripheral and central effects of the *Vitex agnus-castus* essential oil. Using these methods, it was revealed that subcutaneous injection of EOVAC produced antinociceptive effects in the rats. Subcutaneous injection of formalin 2.5% into the ventral surface of right hind paw produced a biphasic pattern of nociceptive responses in rats. Each phase of formalin test has different mechanisms of nociception. The first phase is produced by direct simulative effect of formalin on myelenated and unmyelenated nociceptive afferent fibers, mainly C fibers which corresponds to acute nociceptive neurogenic pain. This phase of formalin pain is more sensitive to opioidergic agent's effect. The second phase of formalin test is associated with release of several inflammatory mediators and excitatory amino acids such as glutamate and aspartate causing an inflammatory type of nociception and is very sensitive to anti-inflammatory actions of non-steroid anti-inflammatory drugs as the cyclooxygenase inhibitor. Several studies suggested that the chemicals or drugs that act as analgesic via activation of central mechanisms of analgesia can inhibit both phases of formalin test whereas peripherally acting drugs can inhibit only the late phase. Our results showed that EOVAC significantly reduced licking and biting behaviors in the both phases of formalin test and increased latency time in the tail immersion test at various time points post-treatment in rats. These findings suggested that the analgesic activity of EOVAC is mediated by both peripheral and central antinociceptive mechanisms in these models of nociception. In the present study, intraperitoneal injection of morphine (10 mg/kg) produced an inhibitory effect on both phases of formalin pain and also increased latency time in the tail immersion test. Moreover, pretreatment with naloxone (a non-selective opioid receptors antagonist), at a dose that did not produce any significant effect on the formalin pain or thermal pain responses, completely prevented analgesia induced by EOVAC on both phases of the formalin test as well as tail immersion test. These results indicated that at least part of the antinociceptive effect observed from EOVAC is due to activation of endogenous opioidergic system. Researchers reported that different fractions of *Vitex agnus-castus* extract act as an agonist of μ and δ but not κ opioid receptors. Opioidergic activity exhibited by VAC may be one of the important mechanisms of action of VAC in reduction of pain and treatment of PMS syndrome. In the

present study, it was revealed that pretreatment with atropine (1 mg/kg) significantly prevented the analgesic effect of EOVAC (50 mg/kg) in the formalin test as well as tail immersion test. These results suggest an acetylcholine muscarinic receptors involvement in the analgesic effect induced by EOVAC. Cholinergic system has an important role in the pain modulation. EOVAC contains some of terpenes such as (-)-Linalool and α-phellandrene that produce analgesia via activation of cholinergic system. Our results showed that the EOVAC and piroxicam (non-selective cyclooxygenase inhibitor) as a positive control significantly reduced writhing response. This model of visceral nociception is a typical model of inflammatory pain and also accepted as a screening method for the assessment of analgesic and/or anti-inflammatory properties of new compounds and chemicals. Acetic acid promotes the release of prostaglandins, serotonin, and histamine in the peritoneal fluids. Therefore, the current results suggested that the EOVAC significantly produced inhibitory effect in this inflammatory model of pain, and this effect may be related to its suppressive effect on the biosynthesis pathway of pro inflammatory substances or reduction of endogenous pro-inflammatory substances release. We can conclude that the EOVAC produced analgesic effect in these models of nociception and this effect seems to be mediated by activation of endogenous opioidergic system and muscarinergic receptors of cholinergic system. In addition, part of antinociceptive activity of Vitex agnus castus essential oil may be due to its anti-inflammatory effect.

V. Conclusion

Vitex extract can reduce the number of sperms in the epididymis and damage to the testes, which the regularly usage can lead to infertility.

Acknowledgment

We appreciate all who helped us to exert the present study. This paper reports a part of results selected from research work carried out by Fereshteh Ramezanloo supervised by Dr. T . Naji at Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

References

[1] Merz PG, Schrödter A, Rietbrock S, Gorkow C, Löw D. Prolactin secretion and tolerance during treatment with an Agnus castus extract [BP1095E1] - Effect on prolactin secretion. Phytopharmaka in Forschung und Klinischer Anwendung. 1995:93–97.

[2] Webster DE, Lu J, Chen SN, Farnsworth NR, Wang ZJ. Activation of the mu-opiate receptor by Vitex agnus-castus methanol extracts: Implication for its use in PMS. J Ethnopharmacol. 2006;106:216–221.

[3] Liu JH, Burdette JE, Xu HY, Gu CG, van Breemen RB, Bhat KPL, Booth N, Constantinou AI, Pezzuto JM, Fong HHS, Farnsworth NR, Bolton JL. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. J Agric Food Chem. 2001;49:2472–2479.

[4] Sorensen JM, Katsiotis ST. Variation in essential oil yield and composition of Cretan Vitex agnus-castus L. fruits. J Essent Oil Res. 1999;11:599–605.

[5] Senatore F, Napolitano F, Ozcan M. Chemical composition and antibacterial activity of essential oil from fruits of Vitex agnus-castus L. [Verbenaceae] growing in Turkey. J Essent Oil-Bear Plants. 2003;6:185–190.

[6] Wollenweber E, Mann K. Flavonols from fruits of Vitex agnus-castus. Planta Med. 1983;48:126–127.

[7] Hirobe C, Qiao ZS, Takeya K, Itokawa H. Cytotoxic flavonoids from Vitex agnus-castus. Phytochemistry. 1997;46:521–554.

[8 Görler K, Öhlke D, Soicke H. Iridoid derivatives from Vitex agnus-castus. Planta Med. 1985;50:530–531.

[9 Hoberg E, Orjala J, Meier B, Sticher O. Diterpenoids from the fruits of Vitex agnus-castus. Phytochemistry. 1999;52:1555–1558.

[10] Ono M, Nagasawa Y, Ikeda T, Tsuchihashi R, Okawa M, Kinjo J, Yoshimitsu H, Nohara T. Three new diterpenoids from the fruit of Vitex agnus-castus. Chem Pharm Bull. 2009;57:1132–1135.

[11] Li S, Zhang H, Qiu S, Niu X, Santarsiero BD, Mesecar AD, Fong HHS, Farnsworth NR, Sun HD. Vitexlactam A, a novel labdane diterpene lactam from the fruits of Vitex agnuscastus. Tetrahedron Lett. 2002;43:5131–5134.

[12] Cornwall GA. New insights into epididymal biology and function. Hum Reprod Update. 2009;15:213–27.

[13] Jones RC. To store or mature spermatozoa? The primary role of the epididymis. Int J Androl. 1999;22:57–67.

[14] Bedford JM, Nicander L. Ultrastructural changes in the acrosome and sperm membranes during maturation of spermatozoa in the testis and epididymis of the rabbit and monkey. J Anat. 1971;108:527–43.

[15] Orgebin-Crist MC. Sperm maturation in rabbit epididymis. Nature. 1967;216:816–8.

[16] Visconti PE, Moore GD, Bailey JL, Leclerc P, Connors SA, et al. Capacitation of mouse spermatozoa. II. Protein tyrosine phosphorylation and capacitation are regulated by a cAMP-dependent pathway. Development. 995;121:1139-50.

[17] Yanagimachi R. New York: Raven Press; 1994. The Physiology of Reproduction.

[18] Donald J, Brow ND. Vitex agnus castus clinical monograph. Herbal Research Review; 1994.

[19] Zhou C. X., Zhang Y. L., Xiao L., Zheng M., Leung K. M., Chan M. Y., Lo P. S., Tsang L. L., Wong H. Y., Ho L. S., Chung Y. W., Chan H. C. [2004] An epididymis-specific β-defensin is important for the initiation of sperm maturation. Nat. Cell Biol. 6, 458–464.

[20] Zhou Y., Zheng M., Shi Q., Zhang L., Zhen W., Chen W., Zhang Y. [2008] An epididymis-specific secretory protein HongrES1 critically regulates sperm capacitation and male fertility. PLoS One 3, e4106.

[21] Zhao Y., Diao H., Ni Z., Hu S., Yu H., Zhang Y. [2011] The epididymis-specific antimicrobial peptide β-defensin 15 is required for sperm motility and male fertility in the rat [Rattus norvegicus]. Cell Mol. Life Sci. 68, 697–708.

[22] Cesari A., Monclus Mde L., Tejón G. P., Clementi M., Fornes M. W. [2010] Regulated serine proteinase lytic system on mammalian sperm surface. There must be a role. Theriogenology 74, 699–711.e1–5.

[23] Courty Y. [1991] Testosterone and corticosterone co-regulate messenger RNA coding for secretory proteins in the epididymis of the lizard [Lacerta vivipara]. J. Reprod. Fertil. 91, 292–300.

[24] Moore H. D., Curry M. R., Penfold L. M., Pryor J. P. [1992] The culture of human epididymal epithelium and in vitro maturation of epididymal spermatozoa. Fertil. Steril. 58, 776–783.

[25] Hu S., Yao G., Guan X., Ni Z., Ma W., Wilson E. M., French F. S., Liu Q., Zhang Y. [2010] Research resource. Genome-wide mapping of in vivo androgen receptor binding sites in mouse epididymis. Mol. Endocrinol. 24, 2392–2405.

[26] Hobson J. P., Netzel-Arnett S., Szabo R., Réhault S. M., Church F. C., Strickland D. K., Lawrence D. A., Antalis T. M., Bugge T. H. [2004] Mouse DESC1 is located within a cluster of seven DESC1-like genes and encodes a type II transmembrane serine protease that forms serpin inhibitory complexes. J. Biol. Chem. 279, 46981–46994.

[27] Takano N., Matsui H., Takahashi T. [2005] TESSP-1. A novel serine protease gene expressed in the spermatogonia and spermatocytes of adult mouse testes. Mol. Reprod. Dev. 70, 1–10.