INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EFFECT OF ALCOHOLIC EXTRACT OF *VITEX AGNUS* SEEDS ON SOME FUNCTION OF FEMALE REPRODUCTIVE SYSTEM

[1]Huda F. Hasan, [2]Zena M. Fahmi Qaragholi, [3]Oday K. Luaibi [3]*Noor A. Abdull-Wahed
[1]Dept. of Vet. Physiological and Pharmacological, Collage of Vet. Medicine, University of Baghdad.
[2]Dept. of Pharmacognosy and Medicinal Plants- College of Pharmacy-University of Baghdad.
[3]Dept. of Vet. Internal and Prevent Medicine, Collage of Vet. Medicine, University of Baghdad.[3]*Veterinarian, B.V.S.M. Baghdad- Iraq.

ABSTRACT
Vitex species has been used for hundreds of years to regulate the function of the reproductive organs in animal, *Vitex* extract have been used in traditional medicine to treat premenstrual tension, used to prevent uterine fibroids and menopausal symptoms and menorrhagia. In this giving study seeds of this plant was extracted by alcoholic extract 70% ethanol, twenty mice were divided into two groups, the first group was treated by daily dose of 365mg/kg body weight of crude *Vitex agnus* seeds extract and the second group was considered as control and treated by distilled water, the period of treatment was 30 days given orally. Results of body weight for group treated with crude *Vitex agnnus* seeds extract showed no significant difference ($P<0.05$) when compared with the control group. While, result of the fertility index and the number of embryos for the group treated with crude *Vitex agnnus* seeds extract showed a significant increase ($P<0.05$) as compared with the control group. Moreover, results of LH and estrogen hormone for treated group showed significant increase ($P<0.05$) when compared with control group. In addition, result of histology of the ovaries of the treated group showed multible follicles and uteruses of the treated group showed cellular debris within the glandular endometrium with high density of vascularization. In summary, the crude extract of *Vitex agnnus* extract has an important role in the regulation of LH and estrogen hormones and it showed an obvious ability in the enhancement of fertility in female mice.

KEY WORDS: *Vitex agnnus* seeds, reproductive system and fertility.

INTRODUCTION

There are some evidence that suggests that complementary and alternative medicine has found increased utilization among animals infertility treatment. (Jonina and Stavros,1999). *Vitex* or chestberry is one of *theverbenaceae* family comebacks to (order Lamiales, class Magnoliopsida, and division magnoliophyta) native to the Mediterranean and central Asia (Jonina and Stavros,1999). It can be grown as a large, deciduous, multistemmed shrub with small and narrow leaves, green on top. It produces long spikes of lavender flowers (Edward *et al*., 1996). *Vitex* has been used for hundreds of years to regulate the function of the reproductive organs in animals (Christie and Walker, 1998). Phytomedicinal preparations containing vitex extract have been used in traditional medicine to treat premenstrual tension (Loch, *et al*., 2001), and used to prevent uterine fibroids, menopausal symptoms and menorrhagia. However, its mechanism of action has not been established (Loch,*et al*., 1991). Characteristic constituents of the *vitex agnus-castus* leaf include essential oils, glycosides, flavonoids and also labdanditerpenoids, rolundifuran, vitexilactone which have high binding affinity to dopamine receptors (Hoberg, *et al*., 1999) The aim of this study is to investigate the potential effect of *Vitex* alcoholic extract on reproductive organ of female mice.

MATERIALS AND METHODS

Extraction of *Vitex agnus* seeds: (200) gm of *Vitex agnus* seeds were obtained from the local market and classified by Prof. Dr. Ali Al- Musawi, a plant classification specialist, college of Science- University of Baghdad. The plant material was air-dried under the shade. Then grinded in to fine powder. Later, was boiled with 1.5 liter of 70% ethanol in a magnetic stirrer for eight hours. After that, the crude extract was obtained after evaporating the solvent in an incubator. The crude extract was stored in a refrigerator for biological studies (Maridass and John, 2008). A yield of 257 ml/gm of the extract powder was obtained.

Preparation of stock solution from crude *Vitex agnus* seeds extract: (365) mg of the dried extract was dissolved in distilled water; volumes were completed to (10) ml. 0.1 ml of the stalk was given to each (10) gm B.W (Gholamali and Kobra, 2012).

Experimental Animals: Twenty albino Swiss mice weighting (28-30) gm were obtained from the animal house of Biotechnology Research Center-AlNahrain University- Baghdad-Iraq. Mice were placed in plastic cages 30x10x10 cm. Standard rodent diet (commercial feed pellets) and tap water was freely available. Housing conditions were maintained at 28±2c° and light /dark cycle (14/10 hours). The litter trays of the animal cages were changed every 7 days.

Experimental designs Twenty mice were divided into two groups 10 mice in each one); **First group**: Treated daily with (365) mg/kg body weight of crude *Vitex agnus* seeds extract given orally by stomach tube for (30) days. **Second group (Control)**: Treated by distilled water given orally by stomach tube for (30) days.

Induction of estrus cycle and Vaginal smear: After isolation of the females for a period of (4-5) days from

males (synchronization), the grouped females are exposed to male; the majority is stimulated into estrus with a high percentage occurring on the third day (Whitten, 1956), Vaginal smear was taken to ensure and detect estrus cycle and vaginal smear was obtained by inserting a sterilized loop gently into the vagina and allow it to touch vaginal wall by rolling the loop smoothly. Smears then were spread on a clean slide and fixed on flame before staining with 1% aqueous methylene blue for 3 – 5 min, stained smears were then washed with tap water, air dried and examined under a light microscope to determine estrous cycle phases (Humason, 1997).

Collection of blood sample: Mice in estrous phase were anaesthetized and blood was collected after euthanasia, then the blood was centrifuged at 3000 rpm for 10 minutes for preparation of sera which were stored at (-20°C) until use for hormonal determination.

Parameter used in this experiment

- **Body weight:** for each mouse the BW was recorded at the beginning and end of the experimental work, using a regular balance.
- **Fertility index:** parameters were calculated according to (Reshu, and Patwant , 2007):
  Fertility index= Total numbers of females pregnant/ Total numbers of females mated x 100.
- **Hormones determination:** Determination the baseline of circulating serum levels of estrogen, FSH and LH , the quantitative analysis was done in clinical laboratory of Radio Active Isotope.
- **Numbers of embryos:** this was measured by pushing tris buffer by insulin syringe into orifice of uterus after attachment orifice of each horn this occurred inside glass (Muhannad , *et al*., 2013)
- **Histological study:** the groups were taken the parts from uterus and ovaries after killed it. These samples were taken for histological study and these were kept in 10% formalin solution until the time of sections (Luna, 1968). The sections were worked in the dental medicine college, university of Baghdad.
- **Statistical analysis** The ready program SAS from the SAS institute (2001) was used in statistical analysis for study the effect of different treated in adjective studies and the significant between medium was compared with less significant LSD.

RESULTS:

Results in table (1) showed no significant difference ($P<0.05$) of body weight for group treated with *Vitex agnus* seeds crude extract as compared with control group while the results of fertility index and number of embryos for groups treated with V*itex agnus* seeds crude extract showed significant increase ($P<0.05$) as compared of control group.

TABLE 1: The effect of *Vitex agnus* seeds crude extract on body weight (g), Fertility index (%) and Number of embryos.

Parameters	mice treated with *Vitex agnnus* seeds crude extract	mice treated with distilled water
Body weight	34.629a	33.732a
Fertility index	97±1.8a	84±1.2b
Number of embryos	7± 0.43a	4±0.21b

*Data taken as mean ±SE

The results in table (2) showed significant increase ($P<0.05$) of LH and estrogen hormones for group treated with crude *Vitex agnnus* seeds extract as compared with control group with no significant deference in FSH hormone.

TABLE 2: The effect of *Vitex agnus* extract on (LH, FSH and estrogen) hormone.

Hormones	Group treated with *Vitex agnnus*	Group treated with distilled water
LH (mIU /ml)	1.99±0.12a	0.63±0.14b
FSH (mIU /ml)	0.49±0.14a	0.56±0.10a
Estrogen(P.mol/L)	7020±532.22a	5360.0±627.64b

*Data taken as mean ±SE

The histological changes

The histological section of ovary mice treated with alcoholic extract of *vitex agnus* seeds , showed by multible follicles during estrus cycle figure(1) .

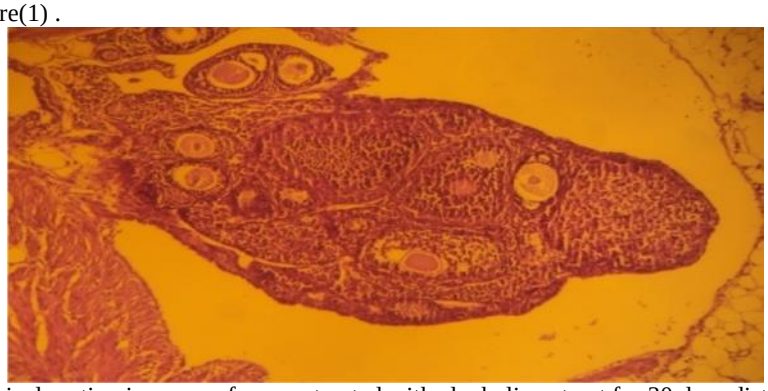

FIGURE 1: Histological section in ovary of mouse treated with alcoholic extract for 30 days distingushed by multible follicles during estrus cycle. (H & E X 400).

The histological section of uterus in group treated with alcoholic extract showed cellular debris within the glandular endometrium with high density of vascularization Figure (2).

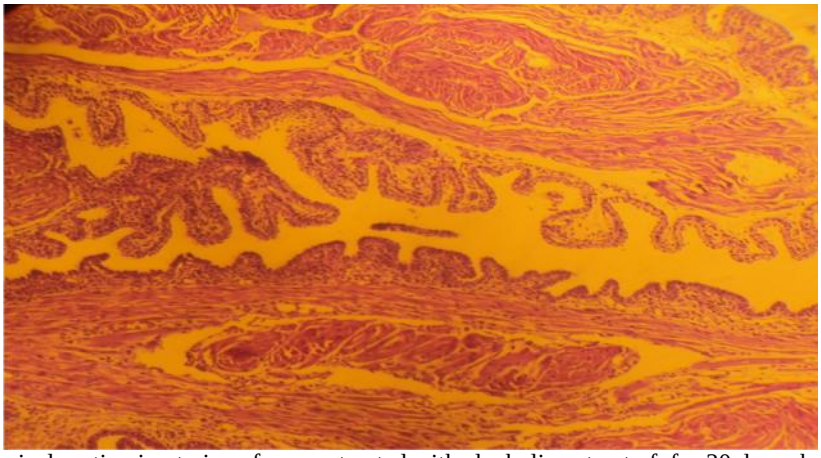

FIGURE 2: Histological section in uterine of mouse treated with alcoholic extract of for 30 days showed cellular debris within the glandular endometrium with high density of vascularization during estrus cycle. (H&EX400).

DISCUSSION

After 30 days of daily oral treatment with *Vitex agnus* crude extract the result of body weight might attributed to the nutrient of extracts might which have allowed proper utilization of the nutrients in the normal level this result agreed with result reported by (Akhondzadeh, 2000). The result of fertility index for group treated with *Vitex agnnus* extract this might be attributed to *Vitex* increases fertility by helping regulate hormonal and menstrual balance. *Vitex* is a key ingredient in pregnant in animals (Milewicz,*et al*., 1993), the result of numbers of embryos for extract group showed significant increase as compared with control this result can be explained by *Vitex* stimulates and stabilize the reproductive hormones involved in ovulation and assists in restoring overall hormonal balance, cycle balance, and menstrual regularity, and increase fertility (Bergmann,*et al*., 2000).The increase in LH hormone for group treated with *Vitex agnnus* extract this might attributed to *Vitex* works by acting on the hypothalamus and pituitary gland, which in turn secrete hormones or send signals to other parts of the body to trigger the production of reproductive hormones, *Vitex* has been shown to help increase the level of luteinizing hormone (or LH) while gently suppressing the secretion of FSH follicle stimulating hormone (Christie and Kora, 1997), while the increase in estrogen hormone for extract treated group might be attributed to *Vitex agnus castus* showed estrogen-like effects , it contains high percentage of phytoestrogens which are plant sterols that are similar to estrogen in structure and function (Honari,et al., 2012). The multiple follicles in ovary tissue section of mice treated with alcoholic extract might attributed to V*itex agnus castus* may be used to stimulate the hormones involved in ovulation and marked improvement earlier ovulation, achieved pregnancies" also induce maturation of the ovarian follicle and the healthy development of reproductive tissues and has the effect in stimulating and normalizing pituitary gland function, *Vitex* may be very effective in promoting normal hormonal function, regular and frequent ovulation (Bergmann, *et al*., 2000). The cellular debris within the glandular endometrium with high density of vascularization in uterus of mouse treated with alcoholic extract might attributed to *Vitex agnus* increase estrogen hormone lead to stimulates the growth of the uterine lining, causing it to thicken during the preovulatory phase of the cycle. It is well establishedthat estrogen is directly responsible for the growth and development of reproductive organs. In synergy with FSH, estradiol stimulates granulose cell proliferation during follicular development (Telefo,*et al*., 1998).

REFERENCES

Akhondzadeh, S.H. (2000) Iran's encyclopedia of medicinal plants.1st ed. Iran: Arjomand press;;1.1: 51.

Bergmann, J., Luft, B., Boehmann, S., Runnebaum, B., Gerhard, I. (2000) The efficacy of the complex medication Phyto-Hypophyson L in female, hormone-related sterility. A randomized, placebo-controlled clinical double-blind study. *Forsch komplementarmed klass naturheilkd.* 7 : 190-9.

Christie, S., Walker, A. (1997) Vitex agnus castus, A review of its traditional and modern therapeutic use, current use from a survey of practitioners. The European Journal of Herbal Medicine; 3:29 -45.

Christie, S., Walker, A.F. (1998) *Vitex agnus-castus:* a review of its traditional and modern therapeutic use. The European Journal of Herbal Medicine , 3(3): 29- 45.

Edward, F., Gilman, Dennis, G. Watson (1993) *Vitex agnus castus*Alba: Alba, Chaste tree. Institute of food andagriculture sciences; University of Florida, Gainesville FL 32611.

Gholamali, J., Kobra, A. I. (2012) Effect of *Vitex agnus-castus* fruits hydroalcoholic extract on sex hormones in rat with induced polycystic ovary syndrome (PCOS)16(1):62-69

Hoberg, E., Orjala, J.E., Meier, B., Sticher, O. (1999) Diterpenoids from the fruits of *Vitex agnus- castus*. Photochemistry ,52: 1555-1558.

Honari, I. N., Pourabolli, E., Hakimizadeh, A., Roohbakhsh, A., Shamsizadeh, R., Vazirinejad, M.R., Rahmani, H.A., Rezazadeh, P., Yazdian, M., Allahtavakoli (2012) Effect of *Vitex agnus* extraction on anexiety like behaviors in ovariectomized rats Vol. 14, No. 5.

Humason, G.L. (1997) Animal Tissue Techniques. 5th ed., the Johns Hopkins University Press, Baltimore and London , 361 – 378.

Jonina, M.S., Stavros, T.K. (1999) Parameters influencing the yield and composition of the essential oil from *Vitex agnus – castus* fruits.Plantamedica, 66: 245- 250.

Loch, E., Boehlert, K.J., Peelers, M. (1991) The treatment of menstrual disorders with *Vitex agnus- castus*tincture. Derfrauenarzt, 32 (8): 867-870.

Loch, E.G., Selle, H., Boblitz, N. (2001) Treatment of premenstrual syndrome with a phyto pharmaceutical formulation containing *Vitex agnus-castus*. Journal of woman's health and gender based medicine, 9:315- 320.

Luna, L.G. (1968) Manual of histologic staining methods of the armed .Institute of pathology.3rd., McGraw-Hill Boocompany, N.Y.,Toronto.London, Sydney; 12-31

Maridass, M., and John De Britto, A. (2008) Origin of Plant Derived Medicines. *Ethnobotanical Leaflets*., **12**, 373

Milewicz, A., Gejdel, E., Sworen, H., Sienkiewicz, K., Jedrzejak, J., Teucher, T., Schmitz, H. (1993) *Vitex agnus castus* extracted in the treatment of luteal phase defect due to latent hyperprolactinemia. *Arzneimittelforschung* 43 752-756.

Muhannad, A.A., AlBayaty, Farid J. AL-Tahan and Huda F.Hasan (2013) Influence of proteins extract from *helianthus annuus L*.seeds on blood volume of reproductive organs in pregnant mice.VOL 6-6-03

Reshu, M. and Patwant, K. (2007) Antifertility effect of meliaazedarachlinn (dharek) seed extract in female albino rat.Indian Journal of experimental biology. 45 : 853-860.

SAS institute. SAS/ATAT (2001) Guide for personal computers version, 9th Ed. SAS instytiteinc; cary NC,USA.

Telefo, P.B., Moundipa, P.F., Tchana, A.N., Tchouanguep Dzickotze C., Mbiapo, F.T. (1998) Effects of an aqueous extract of *Aloe buettneri, Justicia insularis, Hibiscus macranthus, Dicliptera verticillata* on some physiological and biochemical parameters of reproduction in immature female rats. *J Ethnopharmacol*; 63: 193-200.

Whitten,W. K. (1956) Modification of the oestrous cycle of the mouse by external stimuli associated with male. J. Endocrinol. 13:399-404.