

The Effects of *Vitex agnus castus* L. Extract on Gonadotrophines and Testosterone in Male Mice

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(received: 4/4/2003 ; accepted: 22/9/2003)

Abstract

Usually, the side effects of synthetic drugs appear after a long time of their usage. For that reason, these years, diseases treatment by herbal drugs is very interested. In this study, regarding to the mentioned purposes, we evaluate the effects of *Vitex agnus castus* L. (Vit.) fruit extract on the activity of Pituitary- Gonadal axis in Balb/C male mice. LD50 of the extract was obtained 1650 mg/kg. Regarding to the LD50 of the extract, the following doses (65, 165, 265, 365, 465 mg/kg) were injected intraperitoneally for 30 days in 5 groups of animals. After the last injection, we collected the blood samples and measured FSH, LH and testosterone by Radio Immuno Assay (RIA) technique. Vitex extract decreased the FSH, LH and testosterone levels comparing to the control and sham groups. The optimum dose was obtained 365 mg/kg. These results suggest that *Vit.* fruit extract has an anti-androgenic effect and probably acts through the Hypothalamic-Pituitary- Gonadal (HPG) axis.

Keywords: *Vitex agnus castus, LD50, FSH, LH, Testosterone.*

Introduction

Vit. is a member of the Verbenacea family, native to the Mediterranean and central Asia (Jonina, 1999). It is a shrub with finger- shaped leaves and slender violet flowers. The fruit has a spicy pepper like aroma and taste. The dried ripe fruits are used medicinally. *Vit.* has a long history of use, first mentioned in the writings of Hippocrates in the 4th century BC. (Burch, 1999).

It is mentioned by the Greek physician, Dioscorides, as a beverage taken to lower libido. According to the Greek historian, Pliny, Vit. fruit strewn on the beds of soldier,s wives faithfulness while their husbands were at battle. (Blumenthal, 2000)

It has been used to treat menstrual problems, Pms (premenstrual syndrome), fibroid cysts, progestrone insufficiency, corpus luteum insufficiency, menopausal symptoms, hyperprolactinemia. Vit. precise mechanism of action and its active constituents have not been established (Berger, 2000, Schellenberg, 2001, Tesch, 2003).

Characteristic constituents of the Vit. fruit is essential oils, irridoid glycosides, flavonoids, and also labdan diterpenoids, rotundifuran, vitexilactone, 6B, 7B-diacetoxy-13-hydroxy-labda-8-14-diene which have high binding affinity to dopamine receptors (Hoberg, 1999, 2000).

Material and Method

Plant material: Vit. fruits were collected from Ghom area (Tehran province, Iran). The plant was identified as *Vitex agnus castus* L. by Department of Pharmacognosy, Pharmacy Faculty, Tehran University of Medical Sciences. The fresh material was dried in air under shade then ground to a fine powder and using Percolation technique, the fruit hydroalcoholic extract was obtained. Then, the hydroalcoholic extract was filtered and the solvent was removed under reduced pressure at 30-40°C.

The resulting extract was dissolved in tween and added water, then was stored in refrigerator for biological studies.

Animals: Adult Balb/C male mice weighting 20-25gr were obtained from Pasteur institute of Iran. They were maintained in a room animals with a controlled temperature (23±2 °C) on a 12 L: 12D schedule and allowed free access to food and water.

Experimental groups: The LD50 of *Vit.* fruit extracts on the Balb/C male mice was obtained 1650mg/kg. To find this dose, we divided animals to many groups (n=10) and each group received a definite dosage, after 24 hours, number of dead animals was determined and then lethal dose (a dose that 50% of animals were killed) obtained.

Regarding to the LD50, these doses were used: 65, 165, 265, 365, and 465 mg/kg. All the injections were applied intraperitoneally for 30 days (n=6). After the last injection, under an anesthetic condition the blood samples of animals were collected from portal vein of liver and the serum was stored at -20 °C until assayed. Then, LH (Luteinizing Hormone), FSH (Follicle Stimulating Hormone) and Testosterone of the serums were measured by RIA.

Results

Vit. fruit extract decreases LH, FSH, and testosterone levels of the male mice serum.(Fig. 1, 2, 3) comparing to the control and sham groups. The optimum dose of the Vit. fruit extract was showed 365 mg/kg.

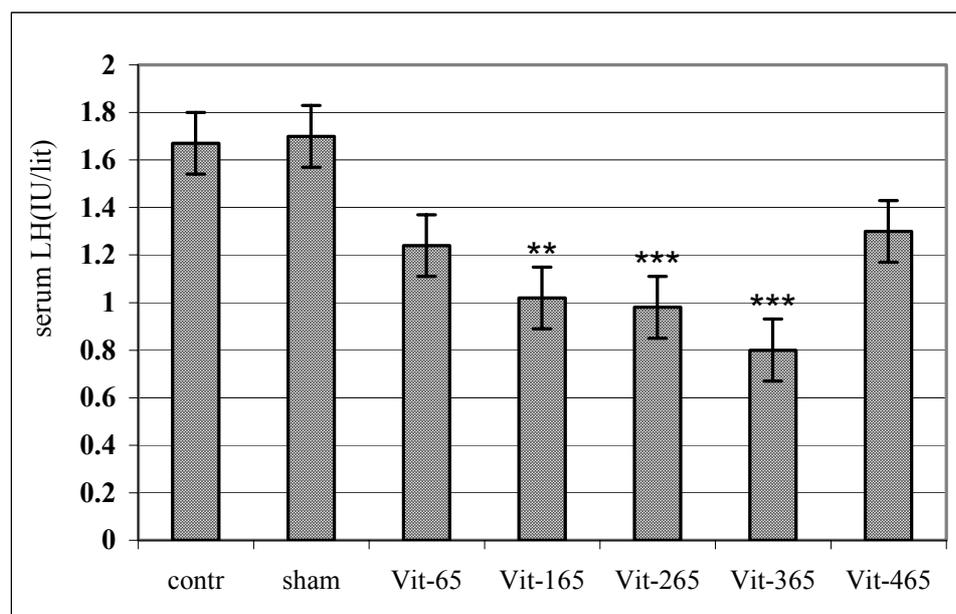


Figure 1- The effect of different doses of *Vitex agnus castus* L. extract on serum LH level. (** P<0.01 , *** P<0.001)

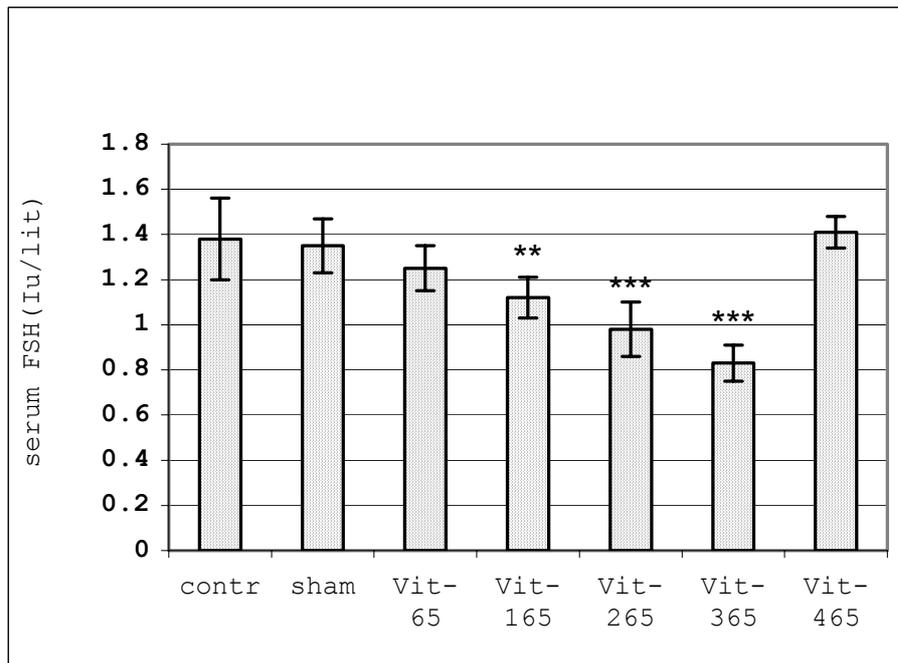


Figure 2 - The effect of different doses of *Vitex agnus castus* L. extract on serum FSH level. (** P<0.01, *** P<0.001)

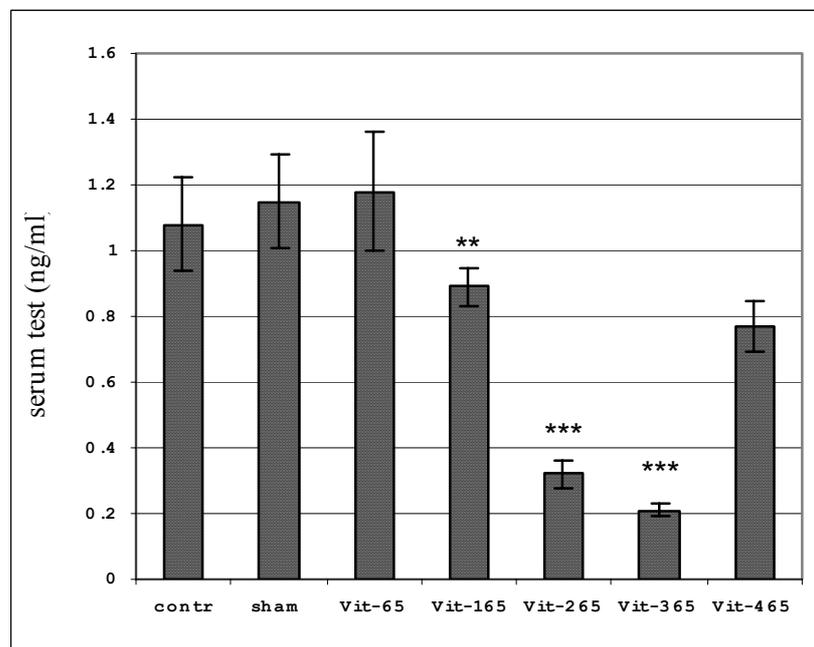


Figure 3- The effect of different doses of *Vitex agnus castus* L. extract on serum Testosterone level. (** P<0.01, *** P<0.001)

Discussion

The Premenstrual syndrome is due by the imbalance of estrogen and progesterone hormone levels. The imbalance of these two hormones can be regulated by Vit. fruit extract (Milewicz, 1996). This extract can increase progesterone effects and at the same time decrease estrogen effects in women and female guinea-pigs (Bone, 1994). The administration of this plant extract has a regulative effect on the level of LH and FSH secreted by adenohipophysis in women (Burch, 1999). Regarding to the mentioned findings, the effect site of this extract can be adenohipophysis in male as well as in the women and female animals. On the other hand, Vit. extract can inhibit the prolactine secretion from adenohipophysis, which is identically like the effect of dopamine agonist receptor named Lisuride. (Sliutz, 1993). By using dopamine receptor Binding assay, it was found that Vit. fruit extract has high binding affinity to the dopamine receptors (Jarry, 1994; Meier, 2000; Wuttke, 2003). In lactated and normal rat, Vit. fruit decreases the lactation (Christie, 1997).

Dopamine is known as a prolactine inhibitory factor, Which decreases the lactation. Vit. extract also decreases lactation. So, it can be suggested that probably Vit. acts through a dopaminergic pathway. On the other hand, dopamine is one of the neurotransmitter which has inhibitory effect on the Hypothalamic-Pituitary-Gonadal axis (Berne, 1998; Kacsoh, 2000). Regarding to the results of this study, it seems that the inhibitory effect of Vit. fruit extract on HPG axis is probably by activating the dopaminergic pathway.

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