

# Biological study of the extract of some species of vitex agnus-castus ( kafmurium ) grown in Egypt

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## Abstract

**Objective:** the study was designed to comparative the effect of alcoholic extract of vitex agnus –castus species and two types of hormone replacement therapy on female sex hormones and lipogram on overctomized female albino rats or during menopausal.

**Methods:** the plant samples were collected from the garden of faculty of science & garden of faculty of pharmacy of Zagazig University.The fresh plant ( aerial parts) macerated with 95% ethyl alcohol then evaporated by(labrota 4000-efficient).dose taken for 14das The selected dose is 16 gm /1000gm.

**Results:** the result were referred to group(3) were treated with (0.225gm of vitex agnus –castus"alba")showed significant increase in progesterone & estrogen and showed significant decrease in lipogram when compared with controlled group and other groups which treated with the other types of vitex plant and with double doses but group (7) were treated with klimadynon drug showed marked significant increase in progesterone, estrogen and lipogram.

**Conclusion:** the group was treated with (one dose daily for 14 days of vitex agnus- castus"varieties"Alba" given the best result increase female sex hormones and decrease lipogram .

## Introduction

### Chaste tree (vitex agnus-castus)

**Chaste tree Vitex** has been approved by German health authorities for PMS, breast tenderness, and irregularities in the menstrual cycle and is often recommended for women in early menopause experiencing irregular menstrual cycles (Stacie Geller, 2006). The progesterone like effect of Vitex has been verified by endometrial biopsy, analysis of blood hormone levels, and examination of vaginal secretions (Browen , 1997). The majority of research has been limited to PMS and breast tenderness and very little is known about the efficacy related to menopausal symptoms. The only study of Chaste tree alone in peri and postmenopausal women reported improvement in mood and hot flashes, although the study had no placebo or comparison group. (Lucks et al., 2002). Most often, when Chaste tree is used for menopause it is in combination with black cohosh and other herbs.

**Vitex agnus-castus**– chaste tree or monk’s pepper – has a long history as a medicinal plant in folk medicine for instance: ‘the seeds bring heat, help those bitten by animals and those retaining water and those that have chronic period troubles and inflammations about the womb’. From the 17th century, it was widely used as a common folk remedy for female troubles and imbalances and to stimulate the flow of milk (Hobbs, 1991; Meyer, 1993).

**Vitex agnus-castus L.** This plant has important medicinal properties and is especially used for treatment of premenstrual problems and hyperprolactinemia because of it is hormone-like effect (Milewicz et al., 1993; Odenthal, 1998; Lucks et al., 2002). In Anatolian folk medicine, Vitex agnus-castus is used as diuretic, digestive, antifungal and also against anxiety, early birth and stomachache (Baytop, 1984; Honda et al., 1996).

**Vitex agnus-castus**, a small shrub or tree up to 6 ft tall, is native to the Mediterranean and western Asia. It is now cultivated all over the World, including the southern part of the United States (Upton, 2001). Vitex agnus-castus has been used since ancient Greek times as a treatment for menstrual problems. In addition, it has been used to treat pain, swelling, inflammation, headaches, rheumatism, and sexual dysfunction (Upton, 2001). Treatment with vitex agnus-castus results in few unwanted side effects, and is therefore a popular alternative therapy for women who do not respond to or tolerate the hormonal or psychotropic drugs. The last decade has provided several successful clinical trials supporting the use of vitex agnus-castus for treatment of PMS (Lauritzen et al., 1997; Berger et al., 2000; Loch et al., 2000; Schellenberg, 2001; Atmaca et al., 2003).

The estrogenic compounds of this extract were identified as the flavonoids penduletin and apigenin (Jarry *et al.* 2006). A methanolic extract (not further characterized) showed significant competitive binding to estrogen receptor  $\alpha$  (IC<sub>50</sub> = 46  $\mu\text{g/ml}$ ) and estrogen receptor  $\beta$  (IC<sub>50</sub> = 64  $\mu\text{g/ml}$ ). Furthermore the extract stimulated the expression of the progesterone receptor but estrogen-dependent alkaline phosphatase activity was induced (Liu *et al.* 2001). Bioassay-guided isolation resulted in the isolation of linoleic acid as possible estrogenic component of the extract (Liu *et al.* 2004).

(*Mustafa, 2007*). *Vitex* has been used for hundreds of years to regulate the function of the reproductive organs in women (Christie & walker, 1998). It is also thought to exhibit a normalizing or balancing effect on hormone production, and to increase luteinizing hormone (LH) levels without affecting follicle stimulating hormone (FSH) in women (Schellenberg, 2001). 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; Tesch, 2003). 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 ; Hoberg *et al.*, 2000). In addition flavonoids may have antiviral and antioxidant effect and positive effect on the heart blood vessels (Brenda, 2005).

**The aim of this study** is to identify the prevalence and types of complementary and alternative medications (CAMs) used by women during menopause and explore potential associations between CAM use and menopausal symptoms. This is done through studying the effect of vitex on some lipogram and some male and female sex hormones. The use of supportive drugs like (hormonal replacement therapy) like cycloproygnova and klimadynon was also evaluated in virgin female albino rats in order to get best and increase fertility during menopause and enhance menopausal symptoms by increased level of estrogen hormone.

So, we want to pass through the light of different dose effect of two types of vitex extract with two concentration and two different types of hormone replacement therapy on enhancement menopausal symptoms by increase female sex hormones and increase fertility.

### Materials and methods

#### Plant material

Vitex plant were collected from the garden of the college of education (science & pharmacy) university of zagazig and authenticated at the department of drugs & zoology. The fresh materials aerial parts (leaves, stem and flowers) was cut into small pieces macerated in 95% ethyl alcohol till complete exhaustion then percolated after weak and freeze the crude viscous extract was obtained of this extract after removal of the solvent through vacuum pressure evaporation (labrota 4000-efficient) (1/2 liter of vitex agnus-castus-L) and (3/4 liter of vitex agnus-castus varieties "alba") , final viscous extract was stored in a refrigerator for biological studies. final viscous yield was suspended in gum acacia and dissolved in distilled water to prepare suspension in desired concentration just before use.

#### Experimental animals:

Sixty three clinically healthy virgin female albino rats were obtained from animal house of faculty of medicine - Zagazig University. Their weights ranged from (160- 200gm). The animals were housed in metal cages, bedded with wood shavings, kept under standard laboratory conditions of aeration and room temperature at about 25°C. The animals were allowed to free access of standard diet and water, were accommodated to the laboratory conditions for two weeks before being experimented.

#### Drugs

Used two types of hormone replacement therapy (cycloproygnova and klimadynon) these drugs purchased from local pharmacy.

#### Experimental groups

The animals were divided to nine groups. (7 rats in each group) eight groups of nine groups were removed the ovary (overctum) and leaved for 10 days to become healthy and the normal group. two groups treated with two different doses of vitex agnus-castus-L, two groups treated with two different doses of vitex agnus-castus varieties "alba", one group treated with mixture of two extract and two groups treated with two different types of hormone replacement therapy Another group served as the control and received 1ml distilled water.

#### Biochemical tests

Blood samples were collected after administration of the drugs and extracts for 14 days, individual blood was drawn by orbital puncture (from eye plexus) using heparinized micro hematocrit capillary tubes. Serum was harvested from blood without EDTA. Serum was separated by centrifuge at 3000 rpm for 10 minutes and subsequently used for the determination of cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL). also for determination of hormonal assay of progesterone, estrogen, prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

### Statistical analysis

Data were expressed as mean± S.E.M analyzed by one way ANOVA, using the SPSS software (version 14.0) in order to detect inter-group differences. P<0.05 was considered to be statically significant.

### Results

It was obvious that significant decrease with buffer group when compared with normal group in serum female sex hormones (progesterone and estrogen) and significant marked decrease in serum (cholesterol, triglycerides, HDL and LDL) with buffer group.

The results showed no effect of vitex agnus-castus on (prolactin, FSH and LH) all less than 0.1 µiu/ml.

Comparison between seven groups with each other and the effect of treatment of two types of alcoholic vitex extract with different concentration, also treatment of two types of drugs with buffer group and normal group and their effect on lipogram and female sex hormones. The results were obtained at table (1) and fig (1). Showed that group (5) virgin overctum female albino rats was treated with mixture of alcoholic extract (0.225gm of vitex agnus-castus- alba + 0.3375 gm of vitex agnus-castus –L) given significant decrease in serum progesterone hormone level.It was obvious group (5) given the lowest level of serum progesterone compare with all groups. Group (5) showed elevated significant increase serum cholesterol, HDL and LDL. The result showed that group (5) given the highest level of lipogram (cholesterol, HDL and LDL) when compared with all groups.

When compared all groups with each other the result afforded that group (4) was treated with double dose of white alcoholic extract (0.45gm) of vitex agnus-castus" alba " revealed significant increase with serum triglycerides concentration and given highest level of triglycerides more than all groups.also showed marked significant decrease in serum estrogen level when compared with all group given the lowest level of estrogen.

When compare all groups with each other showed that group (7) was treated with klimadynon drug (hormone replacement therapy) elevated significant increase in serum LDL concentration (low density lipid).also group (7) was treated with klimadynon drug showed significant in serum estrogen level and given the highest level of estrogen when compared with all groups. It was obvious that group (7) when compared with all groups given significant increase in progesterone and give highest level of progesterone after normal and more than all groups.

When compared all groups with each other showed that group (3) was treated with 0.225 gm of white alcoholic extract significant marked decrease in serum (cholesterol, triglycerides, HDL and LDL).also showed marked significant increase in serum progesterone level then group (2) was treated with double dose (0.675gm of vitex agnus-castus- L) when compared with groups were treated with vitex alcoholic extract.

But group (7) was treated with klimadynon give highest level of estrogen when compared with all groups then group (6) was treated with cycloprygnova drug.

### ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Progesterone	Between Groups	13.581	8	1.698	1481.970	.000
	Within Groups	.062	54	.001		
	Total	13.643	62			
Estrogen	Between Groups	34178.814	8	4272.352	4344.531	.000
	Within Groups	53.103	54	.983		
	Total	34231.917	62			
Cholesterol	Between Groups	27182.603	8	3397.825	111.202	.000
	Within Groups	1650.000	54	30.556		
	Total	28832.603	62			
triglycerides	Between Groups	3481.556	8	435.194	15.134	.000
	Within Groups	1552.857	54	28.757		
	Total	5034.413	62			
HDL	Between Groups	359.143	8	44.893	7.562	.000
	Within Groups	320.571	54	5.937		
	Total	679.714	62			
LDL	Between Groups	18866.413	8	2358.302	33.685	.000
	Within Groups	3780.571	54	70.011		
	Total	22646.984	62			

Table (1): Comparison between gp(1), gp(2), gp(3), gp(4), gp(5), gp(6),gp (7), gp8(control) and gp(9) normal gp and the effect of different doses and different types of vitex on female sex hormones (progesterone & estrogen) and lipogram (cholesterol, triglycerides, HDL &LDL).

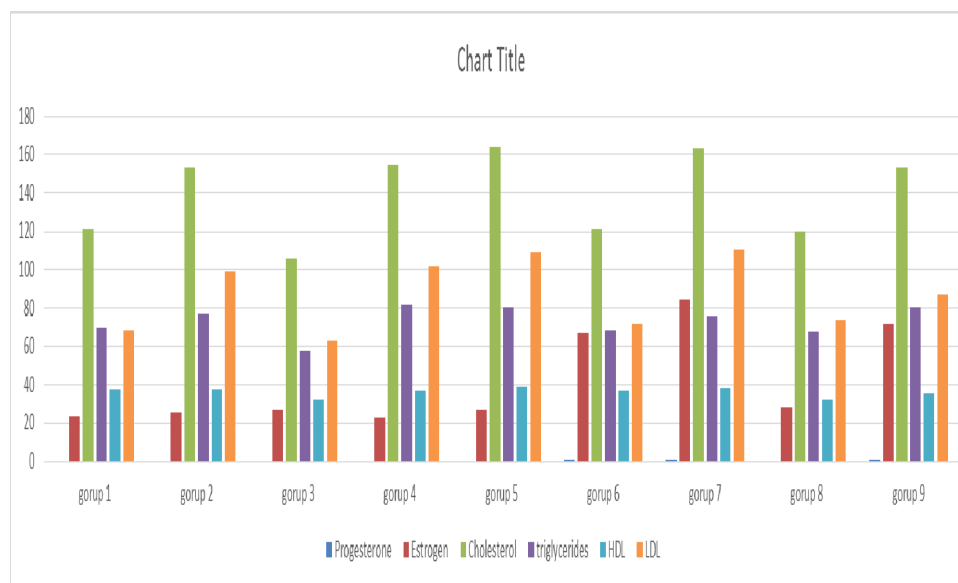


Fig (1): Comparison between gp(1), gp(2), gp(3), gp(4), gp(5), gp(6),gp (7), gp8(control) and gp(9) normal gp and the effect of different doses and different types of vitex on female sex hormones (progesterone & estrogen) and lipogram (cholesterol, triglycerides, HDL &LDL).

### Discussion

The result of this study showed that treatment for 14 days with ethanol extract of vitex agnus-castus

On serum progesterone level the obtained result showed significant increase in group (2) was treated with double dose of vitex agnus- castus-L (0.675gm of violet extract), group (3) was treated with 0.225gm of vitex agnus-castus "alba" , group (6) was treated with hormone replacement therapy (cyclopyrgnova) drug and group (7) was treated with (klimadynon drug) when compared with buffer group.

Our result in full agreement with (propping &katzorke., 1987). the progesterone levels were restored to normal or if there was a clear trend towards normal .

Our results in accordance with (StacieGeller, 2006). The progesterone like effect of Vitex has been verified by endometrial biopsy, analysis of blood hormone levels, and examination of vaginal secretions (Brownen , 1997). The majority of research has been limited to PMS and breast tenderness and very little is known about the efficacy related to menopausal symptoms. The only study of Chaste tree alone in peri and postmenopausal women reported improvement in mood and hot flashes, although the study had no placebo or comparison group. ( Lucks et al., 2002). Most often, when Chaste tree is used for menopause it is in combination with black cohosh and other herbs.

In our result showed significant decrease in serum progesterone with group(4) was treated with double dose of vitex agnus-castus "alba" and group (5) was treated with 0.3375gm of vitex agnus-castus-L+ 0.225gm of vitex agnus-castus "alba" when compared with buffer and normal group.

Studies show its significant effect on hypophysis, which is progesteronic-like and may decrease extra level of prolactin. (Azarnia et al., 2007) It is demonstrated that *Vitex* extract can operate as dopaminergic agonists which decrease the expressing prolactin fromhypophyseal cell cultures, *in vivo* (Jarry et al., 1991; Slitutz et al., 1993; Liu et al., 2004).Studies show that chasteberry may stimulate LH and vise-versa can suppress the FSH hormone hormone(Milewicz et al., 1993;Bhargava, 1989).*Vitex agnus-castus* potentially contains following chemicals: iridoid glycosides: eurostoside, agnoside, acubin(Gomaa et al., 1978; Hoberg et al., 1999).flavonoids: Casticin, kampferol, quercetageitin, vitexin, isorientin(Milewicz et al., 1993; Hirobe et al., 1997; Anonymous et al., 1998; snow, 1996). progestins: Progesterone, Hydroxyprogesterone (flowers and leavers), testosterone, epitestosterone (flowers), androstenedione (leaves). (Anonymous et al., 1998).alkaloids: viticin, volatile oil: 1, 8-cineol, limes, linalool, terpinyl acetate, pinenes and beta pinenes(Fleming, 1998; Du mee, 1993).essential fatty acids:palmitic acid, oleic acid, linoleic acid (Du mee, 1993).essential oil (Males et al., 1998).

(Azarnia et al., 2007).the *Vitex*(or maybe its components) crosses the placenta and enters fetal tissues. The increase in the fetus growth is striking. According to Weiss et al. report, *Vitex*acts on the diencephalohypophysial system *Vitex*increase LH production and mildly inhibits the release of FSH. The result is shift in the ratio of estrogen to progesterone, in favor of progesterone, (Sanderson et al., 1999). The studies show that progesterone is critical to ensuring bone health. It offers neuroprotection, contributes to cardiovascular health, assists normal brain development, and provides protection from some types of cancers,

(Boomsma&Paoletti, 2002). Therefore, *Vitex* which has progesteronic-like effect can have those benefits which are necessary for growth. It may submit the growth process.

According to Dr, Rudolf Fritz Weiss, vitex act on the diencephalohypophyseal system in other words, the hypothalamus and pituitary.

Vitex increases LH production and mildly inhibits the release of FSH the result is a shift in the ratio of estrogen to progesterone, in favor of progesterone. This is, in fact, a corpus luteum like hormone effect. (weiss ,1988) . The ability of vitex to raise or modulate progesterone levels in the body is therefore an indirect effect and not a direct hormonal action. (Amann , 1965.). This is in contrast to other phytomedicines . Like Black cohosh. Frequently used in gynecology because of their direct binding of estrogen receptors ("phyto-estrogens"). (Reichert ,1994.)

Another mechanism through which vitex agnus-castus may work is the opiate system, which consists of  $\mu$ ,  $\delta$ , and  $\kappa$  opiate receptors and endogenous opiate peptides such as  $\beta$ -endorphin. This peptide assists in regulating the menstrual cycle through inhibition of the hypothalamus–pituitary–adrenal axis (HPA), therefore decreasing the amount of gonadotropic releasing hormone (GnRH) acting on the pituitary, which decreases the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH, released from the pituitary, then enter into a complex feedback loop with progesterone and estrogen to regulate the menstrual cycle (Silberstein & Merriam, 2000). Levels of  $\beta$  endorphin decrease along with estrogen in the late luteal phase of the menstrual cycle, which correlates with the appearance of symptoms of PMS (Giannini et al., 1984, 1990; Chung & Coulam, 1988). Meier and co-workers reported that an ethanolic vitex agnus-castus extracts (Ze440) and several sub fractions of a methanolic extract had affinity for opiate receptors. The binding affinity (IC50) was reported to be at 20–70  $\mu$  g/ml. However, it remains unknown if vitex agnus-castus extracts produce agonistic or antagonistic effects. The information is important since an absence of agonistic activity would not support the use of vitex agnus-castus in PMS through opiate Mechanisms.

Our result revealed that significant marked decrease in serum high density lipoprotein (HDL) along the course of study when compared between buffer and normal group.

The decreased level of serum (HDL) obtained in our study as a result of treatment with. 0.225gm of white extract of Vitex agnus-castus "Alba" With group (3) when compared with buffer group and normal group

Whereas significant elevation in serum (HDL) was recorded after 14 day of treatment with Vitex agnus-castus – L and with double dose of Vitex agnus-castus –L double dose of white extract, mixture of (Vitex agnus-castus – L + Vitex agnus-castus- Alba) and with groups treated With Hormone replacement Therapy (Cyclopyrgnova) and (Klimadynon) When compared with buffer group and normal group.

Our result revealed that significant increase in serum (HDL) low density Lipoprotein with groups were treated with double dose of vitex as group(2) was treated with double dose of Vitex agnus-castus –L , group (4) was treated with Vitex agnus-castus" Alba" and group was treated with two extract 0.3375 gm of violet extract (Vitex agnus-castus –L ) and 0.225 mg as white extract (Vitex agnus-castus- Alba) and group (7) was treated with hormone replacement Therapy (Klimadynon) When compared with buffer group and normal group.

whereas groups were treated with one dose of vitex extract daily for 14 days as group (1) was treated with 0.3375 gm of Vitex agnus -castus –L, group (3) was treated 0.225 gm of white extract of vitex agnus-castus "alba" and group (6) was treated with cyclopyrgnova given significant decrease in serum (LDL) when compared with buffer and normal group.

On the other hand, flavonoids can decrease total serum protein and cholesterol. Anderson (Anderson, 1995). Pointed out that blood cholesterol and protein levels were reduced by using a plant extract containing flavonoids. James and Anderson (James & Anderson, 1994). found that flavonoids can dramatically lower blood levels of cholesterol and reduce the rate of formation of oxidized (LDL). The exact mechanism of flavonoid mediated lowering effect on cholesterol and proteins (Anderson, 1995; James & Anderson, 1994).are not yet established. Finally, it can be concluded that *vitex agnus-castus* extract can have a significant adverse effect on blood parameters and can lower total serum protein and cholesterol.

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