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The Effect of Vitex Agnus Castus Extract on the Blood Level of Prolactin, Sex Hormones Levels, and the Histological Effects on the Endometrial Tissue in Hyperprolactinemic Women

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Abstract

Objectives: Hyperprolactinemia leads to corpus luteum failure, sporadic ovulation, and ultimately, anovulation and amenorrhea. The purpose of this study was to investigate the effect of the vitex agnus-castus fruit extract (Vitagnus tablet) on the levels of blood prolactin and sex hormones and to compare its histological effect with that of bromocriptine and Dostinex on women with hyperprolactinemia.

Materials and Methods: To this end, of women at reproductive age with hyperprolactinemia who had referred to healthcare centers, 105 cases were selected and randomly assigned to three groups of bromocriptine, Dostinex, and Vitagnus. During the time of research (4 cycles), patients were treated with these drugs, and finally, the titer of prolactin, follicle-stimulating hormone (FSH), luteinizing hormone, and estrogen, and progesterone of the blood serum sample were measured accordingly. At the beginning of the follicular phase, the ultrasound scan was done to determine the endometrial thickness and if necessary, the histological study was conducted using the endometrial biopsy sample.

Results: Based on the results, the prolactin level in the bromocriptine group started to show a significant difference from previous cycles in the second cycle, and in the first cycle in the other two groups ($P<0.05$). In addition, the rate of the decrease in endometrial thickness in the Vitagnus group was significant compared to other groups ($P<0.05$). At the third stage and later, bromocriptine and Dostinex had a significant effect on the FSH level while the effect of Vitagnus was not significant at any of the stages. The results further revealed that the amounts of estradiol in the Vitagnus group had a significant increase compared to other groups ($P<0.05$). The effect of Vitagnus and Dostinex tablets on the HL level appeared from the 4th and 3rd cycles onward, respectively, while no significant effect of bromocriptine was found at any of the stages. Eventually, the effect of the Vitagnus tablet on progesterone was remarkable compared to the other two mediations in the 2nd and 3rd cycles.

Conclusions: Similar to other drugs, Vitagnus has a significant effect on the amount of prolactin and sex hormones and thus can be successfully used in treating hyperprolactinemia. Finally, reductions in endometrial thickness were significant in the Vitagnus group compared to the other two groups.

Keywords: Vitagnus, Sex Hormones, Bromocriptine, Dostinex, Hyperprolactinemia

Introduction

According to Tabak et al, the micromolar concentrations of dopamine inhibit calcium inflow and thus the secretion of the prolactin hormone by opening potassium channels and hyperpolarizing lactotrophs (1,2). During pregnancy, its level gradually rises to 600 ng/dL while it is yet inhibited by estrogen and progesterone. However, a combination of high prolactin levels and a sudden drop in estrogen and progesterone enables the body to produce milk in spite of the decreased prolactin level (200-400 ng/dL) after delivery (3).

In addition, Luangpirom et al reported that female mice treated with Hirsuta showed a significant increase in plasma prolactin while a decline in the follicle-stimulating hormone (FSH) level. They further found that male mice demonstrated decreased testicle weight, semen, and sperm count (4).

Hyperprolactinemia in women leads to menstrual and ovulation disorders and reduced post-ovulation progesterone levels, and if accompanied by galactorrhea, may be considered as the cause of amenorrhea in the absence of other causes. As prolactin rises, the state of a person changes from normal ovulation to corpus luteum failure, sporadic ovulation, and ultimately, anovulation and amenorrhea. It seems that the inhibition of gonadotropin-releasing hormone (GnRH) pulsatile secretions by prolactin is the cause of amenorrhea because the pituitary gland in these patients responds naturally to GnRH. Further, this inhibition might be due to an increase in opioids. Nevertheless, any therapy that reduces the blood prolactin level, whether the removal of the prolactin-secreting tumor or the suppression of

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prolactin secretions, will resume ovarian activity and menstrual function (5). GnRH is necessary for stimulating gonadotropin secretions (luteinizing hormone, LH, and FSH) and LH and FSH are not released if GnRh level is low and the production of the gamete and the synthesis of sex steroids will not be stimulated accordingly. Therefore, hyperprolactinemia is a case of infertility caused by hypogonadotropic hypogonadism (5).

In treating women with a combination of herbal extracts, Samuel et al reported a dosage-dependent reduction in the prolactin level while an increase in the levels of FSH, LH, and estrogen (6).

Considering the above-mentioned discussions, the present study aimed to evaluate the effect of vitex agnus-castus fruit extracts on the levels of sex hormones, and its histological effect on women with hyperprolactinemia in comparison with bromocriptine and Dostinex.

Materials and Methods

In general, 105 women out of those women at reproductive age with hyperprolactinemia referring to healthcare centers were selected and randomly classified into three groups of treatment with bromocriptine, Dostinex, and Vitagnus after signing the written consent form. The inclusion criteria included having hyperprolactinemia (the prolactin level of >530 mIU/mL), being in the reproductive age (18-45 years), and signing the written consent form while the exclusion criteria were pregnancy and the presence of an intolerable side effect.

Furthermore, the duration of the treatment was four menstrual cycles at most, and dependent variables were measured periodically during this time. Moreover, the FSH, LH, estrogen, and progesterone of the blood serum sample were used to determine the titer of prolactin. To examine follicular growth and development, the ultrasound scan was performed and then the follicular thickness and titer of serum estradiol and LH were measured in days 11-13 of the cycle, and finally, blood progesterone was estimated in the middle of the luteal phase to confirm the ovulation. At the beginning of the follicular phase, the ultrasound scan was done to determine the endometrial thickness and histological study was conducted using the endometrial biopsy sample if necessary.

Although Vitagnus is prescribed in an almost side-effect free dosage, patients were given the necessary information and the consent form regarding using Vitagnus as a herbal drug with potential effects on lowering the titer of serum prolactin.

The chi-square test and one-way ANOVA were used for qualitative and quantitative outcomes, respectively, and a 0.05 significance level was the baseline for statistical judgment of the hypotheses.

Results

Table 1 presents the mean age and weight of the participants divided by the groups.

In addition, the mean levels of prolactin are presented in Table 2.

Based on the statistical analysis, there was a statistically significant difference in the prolactin level in other groups at each stage in relation to the earlier stage except for the bromocriptine group. In other words, a significant difference from earlier stages was observed in the bromocriptine group, namely, two cycles were found after treatment initiation ($P<0.05$).

Table 3 summarizes mean endometrial thickness in days 1-3 of the menstrual cycle in participants divided by the groups.

The results showed that endometrial thickness significantly decreased in the Vitagnus group compared to the other two groups ($P<0.05$). Data on the mean FSH levels of the participants are provided in Table 4.

Based on the results, from stage three onward, bromocriptine and Dostinex tablets had a significant

Table 1. The Mean Age and Weight of the Participants

Type of Intervention	N	Age	Weight
		Mean ± SD	Mean ± SD
Bromocriptine	33	33.5 ± 66.83	67.7 ± 3636.61
Dostinex	35	32.4 ± 7143.99	65.6 ± 5714.20
Vitagnus	35	34.6 ± 857.02	67.9 ± 2286.71
Total	103	33.5 ± 4854.60	66.7 ± 7087.94

Note. SD: Standard deviation.

Table 2. Prolactin Levels of the Participants (mIU/mL)

Type of Intervention	One Week Before Treatment Initiation	One Cycle After Treatment Initiation	Two Cycles After Treatment Initiation	Three Cycles After Treatment Initiation	Four Cycles After Treatment Initiation
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Boromocriptine	832.198 ± 42.80	772.195 ± 42.83	386.103 ± 06.41	362.101 ± 48.95	369.111 ± 75.74
Dostinex	881.90 ± 14.77	839.169 ± 42.18	360.136 ± 85.30	360.136 ± 85.30	352.118 ± 91.35
Vitagnus	858.100 ± 67.86	755.179 ± 67.20	436.95 ± 20.65	412.98 ± 82.46	398.102 ± 84.25

Note. SD: Standard deviation.

effect on the FSH level, while the effect of Vitagnus was not significant at any of the stages. Table 5 lists the mean estradiol levels of the participants.

The results demonstrated that estradiol levels had a significant rise in the Vitagnus group in comparison to other groups ($P<0.05$). Table 6 presents the participants' mean LH levels.

Based on the obtained results, Vitagnus had a significant effect at stage 5 when compared to other stages ($P=0.024$), indicating that Vitagnus and Dostinex effects are manifested after the 4th and 3rd cycles, respectively. On the other hand, bromocriptine represented no significant effect at any of the stages. The mean progesterone levels of the three groups are presented in Table 7.

The findings indicated that Vitagnus has a greater effect on the blood progesterone level at stage 3 (2nd cycle) onward. Therefore, its effect in the 2nd and 3rd cycles was extremely greater compared to the other two drugs.

The results further showed that 21 (63.6%) women who received bromocriptine tablets reported nausea while no case of nausea was found with Vitagnus and Dostinex. Additionally, 48.5% of bromocriptine users reported dizziness whereas none of the Dostinex and Vitagnus users complained of this complication. Weakness was also reported by 6.1% of bromocriptine users while none of the users of Dostinex and Vitagnus indicated drug-associated weakness.

Discussion

Nearly all pituitary secretions are regulated by the hormonal or neural signals of the hypothalamus. More precisely, the secretion of different hormones (except for prolactin) decreases to extremely negligible amounts when the pituitary gland is surgically removed from its

Table 3. Mean (SD) of Endometrial Thickness in Days 1-3 of the Menstrual Cycle Divided by the Type of Intervention and Measurement Stage (mm)

Type of Intervention	One Week Before Treatment	One Cycle After Treatment Initiation	Two Cycles After Treatment Initiation	Three Cycles After Treatment Initiation	Four Cycles After Treatment Initiation
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Boromocriptine	5.3 ± 10.13	4.25 ± 83.05	3.2 ± 92.27	3.1 ± 65.65	4.1 ± 45.67
Dostinex	6.4 ± 22.32	6.3 ± 54.62	6.4 ± 34.40	4.1 ± 77.92	5.1 ± 05.78
Vitagnus	8.3 ± 40.82	8.3 ± 2.36	5.2 ± 94.95	4.1 ± 82.29	4.1 ± 05.49

Note. SD: Standard deviation.

Table 4. Mean (SD) of Blood FSH Levels in Days 9-12 of the Menstrual Cycle Divided by the Type of Intervention and Measurement Stage (mIU/L)

Type of Intervention	One Week Before Treatment	One Cycle After Treatment Initiation	Two Cycles After Treatment Initiation	Three Cycles After Treatment Initiation	Four Cycles After Treatment Initiation
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Boromocriptine	10.1 ± 27.48	10.1 ± 27.20	11.1 ± 81.33	11.1 ± 63.43	10.2 ± 36.07
Dostinex	9.3 ± 85.40	9.3 ± 85.40	9.2 ± 00.97	8.2 ± 71.75	10.2 ± 11.80
Vitagnus	11.2 ± 45.79	11.2 ± 48.74	10.1 ± 85.73	10.1 ± 80.64	10.2 ± 57.44

Note. SD: Standard deviation; FSH: Follicle-stimulating hormone.

Table 5. Mean (SD) of Serum Estradiol Levels in Days 9-12 of the Menstrual Cycle Divided by the Type of Intervention and Measurement Stage (pg/mL)

Type of Intervention	One Week Before Treatment	One Cycle After Treatment Initiation	Two Cycles After Treatment Initiation	Three Cycles After Treatment Initiation	Four Cycles After Treatment Initiation
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Boromocriptine	91.35 ± 81.50	91.35 ± 66.50	111.39 ± 96.99	108.40 ± 78.48	110.43 ± 90.29
Dostinex	80.25 ± 00.43	81.27 ± 42.34	102.47 ± 85.18	100.47 ± 0.09	105.43 ± 71.67
Vitagnus	70.28 ± 28.84	74.26 ± 0.03	127.33 ± 14.30	129.30 ± 14.61	136.30 ± 57.60

Note. SD: Standard deviation.

Table 6. Mean (SD) of Blood LH Levels in Days 12-14 of the Menstrual Cycle Divided by the Type of Intervention and Measurement Stage (mIU/mL)

Type of Intervention	One Week Before Treatment	One Cycle After Treatment Initiation	Two Cycles After Treatment Initiation	Three Cycles After Treatment Initiation	Four Cycles After Treatment Initiation
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Boromocriptine	12.3 ± 66.51	12.3 ± 66.51	13.2 ± 18.61	13.2 ± 18.61	12.2 ± 69.88
Dostinex	11.3 ± 85.57	11.3 ± 85.57	12.2 ± 85.99	13.2 ± 14.78	12.3 ± 71.82
Vitagnus	12.3 ± 91.53	12.3 ± 97.50	13.4 ± 80.54	13.4 ± 85.74	14.5 ± 42.24

Note. SD: Standard deviation.

Table 7. Mean (SD) of Blood Progesterone Levels in the Middle of the Luteal Phase Divided by the Type of Intervention and Measurement Stage (ng/mL)

Type of Intervention	One Week Before Treatment	One Cycle After Treatment Initiation	Two Cycles After Treatment Initiation	Three Cycles After Treatment Initiation	Four Cycles After Treatment Initiation
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Boromocriptine	3.3 ± 44.92	5.4 ± 63.93	5.5 ± 47.04	6.4 ± 2.95	3.3 ± 44.92
Dostinex	4.4 ± 26.96	6.5 ± 19.41	6.5 ± 30.32	7.4 ± 14.83	4.4 ± 26.96
Vitagnus	2.2 ± 87.42	8.4 ± 18.40	8.4 ± 94.62	10.4 ± 14.49	2.2 ± 87.42

Note. SD: Standard deviation.

natural position under the hypothalamus and implanted in another part of the body. Regarding anterior pituitary hormones, the releasing hormone is important while the inhibiting hormone is likely to have extensive control over prolactin. In addition, prolactin secretion from the anterior pituitary gland is completely or almost completely controlled by this inhibitory factor which is produced in the hypothalamus and transferred to the pituitary gland through the pituitary-hypothalamic system. Further, prolactin inhibiting hormone, which is catecholamine dopamine and produced by the arcuate nuclei of the hypothalamus, can 10 times reduce prolactin secretions (3). As mentioned earlier, the purpose of this study was to assess the effect of the vitex agnus-castus fruit extract on the blood prolactin level and compare its hormonal, metabolic, and histological effects with those of bromocriptine and Dostinex on women with hyperprolactinemia.

The results of the present study on the prolactin level showed that there was a slight difference among the interventions in reducing the prolactin. In other words, the effects of interventional methods are comparable. It was found that Vitagnus was far effective than the other drugs in reducing the prolactin level one cycle after starting the treatment although it was not noticeably different from Dostinex and bromocriptine.

Based on the results, Vitagnus reduced the endometrial thickness more effectively in days 1-3 of the menstrual cycle compared to the other two drugs. The analyses further suggested that Vitagnus was more effective in increasing estradiol levels.

On the other hand, the serum levels of FSH showed no statistically significant reduction in the Vitagnus group. In other words, Vitagnus did not have a significant effect on changing the serum FSH.

The results revealed no significant differences among the three therapeutic methods in influencing serum LH levels. However, the serum LH level in the Vitagnus group at stage 5 had a statistically significant difference from the other stages with a 0.14 effect size, indicating that the effect of Vitagnus is manifested after four cycles. On the other hand, Dostinex shows its effect in the 3rd cycle. Conversely, no significant effect was found for bromocriptine at any of the stages.

Based on the obtained results, the effect of Vitagnus on blood progesterone levels in the 2nd and 3rd cycles was extremely higher in comparison to the other two drugs.

During the time of the research, 21 (63.6%) women receiving bromocriptine reported nausea while no case of nausea was reported with Vitagnus and Dostinex users. Furthermore, 48.5% of bromocriptine users reported dizziness whereas none of Dostinex and Vitagnus users mentioned this complication. Weakness was reported by 6.1% of bromocriptine users as well. Contrarily, none of the users of Dostinex and Vitagnus reported drug-associated weakness. Based on the findings, 9.1% of Dostinex users complained about its high price while none of the participants in other groups had such complaints. Accordingly, there was a significant difference among the groups in terms of the side-effects of nausea, dizziness, and weakness, along with the price of the drug, but no significant difference was found in the occurrence of weakness.

In their study, Tabak et al concluded that the micromolar concentrations of dopamine inhibit calcium inflow and therefore the secretion of the prolactin hormone by opening potassium channels and hyperpolarizing lactotrophs. Moreover, 1000 lower concentrations of dopamine can stimulate prolactin secretions by creating a fast current of potassium (1) although no study has yet supported this finding. Prolactin secretion is stimulated by a variety of factors including oxytocin hormone, sleep, and maybe human placental lactogen hormone.

Based on the findings of Luangpirom et al, female mice treated with Hirsuta showed a significant increase in plasma prolactin while a decline in the FSH level and male mice demonstrated decreased testicle weight, semen, and sperm count (4).

Infertility causes mental complications and imposes high costs on families and society. Additionally, hyperprolactinemia in women leads to menstrual and ovulation disorders and reduces the post-ovulation progesterone level. Likewise, it may be considered as the cause of amenorrhea in the absence of other causes if accompanied by galactorrhea. In addition, the increased prolactin level causes a change in the state of a person from normal ovulation to corpus luteum failure, sporadic ovulation, and ultimately, anovulation and amenorrhea. It seems that the cause of amenorrhea is the inhibition of the gonadotropin-releasing hormone (GnRH) pulsatile secretion by prolactin because of the pituitary gland in these patients responds naturally to GnRH. Further,

this inhibition might be due to an increase in opioids. Nonetheless, any therapy that decreases the blood prolactin level (i.e., the removal of the prolactin-secreting tumor or the suppression of prolactin secretion) resumes ovarian activity and menstrual function (3,7). The serum prolactin level in normal men and women (i.e., non-pregnant and non-lactating) is 10 ng/dL at most, and clinical manifestations appear if it goes beyond 20 ng/dL. However, levels <100 ng/dL mostly have non-tumoral origins (8).

Drug-associated hyperprolactinemia (e.g., dopamine antagonists used for treating schizophrenia) may improve after stopping the drug, and if this is not an option, alternative treatment may be used for sex steroid hormones. The first-line treatment is the one with dopamine agonists. These drugs decrease the prolactin secretion and the size of the tumors. Bromocriptine and cabergoline (Dostinex) are among dopamine agonists that are usually used for treating hyperprolactinemia (8).

In their study, Samuel et al attempted to treat women with a combination of herbal extracts and reported a dosage-dependent reduction in the prolactin level while an increase in the levels of FSH, LH, and estrogen (6). The results of the present study also showed that Vitagnus slightly increased LH, progesterone, and estradiol, which is consistent with the findings of Shahnazi et al (9).

In 1986, American and Canadian researchers introduced vitex agnus-castus as a herbal compound to balance female hormones and improve menstrual and menopausal disorders (10). Vitex agnus-castus fruit extracts are used to produce different drugs in the form of drops, tablets, and capsules. Although drops have been more effective, they have a pungent and unpleasant taste that has not been eliminated yet. In 1995, this drug was developed in Iran under the name of Vitagnus as oral drops, and because of its unpleasant taste, it has been produced in the form of tablets and pills since 1997 with similar effects as oral drops. It should be noted that this drug has a delayed effect and is mostly ineffective in the first cycle. Moreover, the effects appear after one cycle or two cycles and maximize after 4-6 cycles (10). On the other hand, side-effects are rare and include rush, digestive disorders, headache, itching, and increased menstrual bleeding (11). The finding of the present study also supports Artz's findings with the effects of Vitagnus on various parameters appearing from the 3rd and 5th cycles (10).

Vitex agnus-castus has dopaminergic effects, and its important compounds, especially the essential oil, influence the hypothalamic-pituitary axis and lead to the reduced release of FSH and LH secretions, and consequently, increase the progesterone level (12,13). The results of the present study also demonstrated that Vitagnus increased progesterone and estradiol levels while it reduced the FSH level and made a small change in the LH level, which is in agreement with the findings of Shahnazi et al (9).

Different studies approved the effect of Vitagnus through balancing female hormones. For instance, Milewicz et al evaluated 52 women with hyperprolactinemia and found that this drug can revert the prolactin to its normal level. This effect also was proven in mice, suggesting that vitex agnus-castus inhibits the release of prolactin from pituitary cells probably because of the connection to dopamine receptors, and this effect is dosage-dependent (14). In another study, Ye et al reported that casticin with its anti-hyperprolactinemia activity is a flavonoid separated from vitex rotundifolia with a clear molecular mechanism (15). Its special chemical element, which is responsible for the clinical effects, is still unknown. Similarly, Hu et al found that flavonoids such as casticin in vitex rotundifolia are responsible for its effects. Casticin is a powerful painkiller and it seems to be an active element of Fructus Viticis, along with its anti-hyperprolactinemia effects (16). Additionally, this plant may be useful for hyperprolactinemia treatment (14). In addition, the in vitro examinations of animal specimens showed that high doses of vitex agnus-castus significantly reduced the amount of the produced milk while the findings of another study on animals indicated an increased amount of milk and breast size (17). In the first human studies, treatment with vitex agnus-castus resulted in an 80% increase in milk production and the amount of milk rose significantly following a 20-day treatment period (18). However, vitex agnus-castus extract can be used for the mild cases of hyperprolactinemia (19,20), and it has a long history in treating menstrual disorders, mastalgia, and premenstrual syndrome (PMS). Some in vitro studies regarding treating hyperprolactinemia found that vitex agnus-castus inhibits the prolactin secretion by connecting to dopamine receptors (19,21). It also has been shown to inhibit prolactin secretions in rats (22). In another study on women with latent hyperprolactinemia, vitex agnus-castus led to a significant reduction in the prolactin level and PMS symptoms, corrected luteal phase defects, and progesterone production (14). Likewise, Hamid et al reported that the extract of vitex agnus-castus was more effective than vitamin E in alleviating the anxiety of breast pain (23).

Given that pain and the increased prolactin level are the most common symptoms of PMS, Hu et al considered their impact on these symptoms as a criterion for anti-PMS effects and concluded that the casticin in vitex agnus-castus reduced the abnormal levels of serum prolactin by 50% and thus reported it as the most active element of vitex agnus-castus (16).

Conclusions

In general, the findings of this study confirmed that Vitagnus significantly reduced the prolactin level similar to bromocriptine and Dostinex.

Some recent studies reported a significant rise in progesterone and estrogen levels resulting from vitex

agnus-castus (9,24). The findings of the present study also demonstrated that Vitagnus significantly increased the estradiol level. Finally, Vitagnus increased the progesterone level initially, but it decreased its level in the 5th cycle, suggesting that in the short-term, the present finding is consistent with previous research.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

This study was approved by the ethical Committee of Tbariz University of Medical Sciences (Ethics No. IR.IAU.TABRIZ.REC.1395.1).

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