Qualitative Evaluation of Two Plant Extracts Vitexagnus and Dates Pollen to Some Sex Hormones Measurement in the Female Rats Induced by D-galactose

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Abstract

Vitexagnus-castus has long been used to treat female reproductive disorders and fertility in Europe for thousands of years. On the other hand, date palm pollen is a herbal combination in traditional medicines generally used to treat female and male fertility. Therefore, the present study aimed to evaluate these two plants qualitatively extracts VitexAgnus and dates pollen in female rats' hormonal measurement. After exposure to D-galactose, 60 Sprague Dawley rats (180±15 gram) weight and three months ages were randomly distributed into six groups, ten rats received normal saline (control group), ten rats received 70% of the ethanolic extract of Vitex (group 2), ten rats received d-galactose (3 groups), ten rats received date pollen extract (4 groups), ten rats received d-galactose, and Vitex (5 groups), and finally ten rats received d-galactose and dates pollen extract (six groups). Animals from each group underwent a blood hormone test LH, FSH, Progesterone, and Estrogen by using immunological assay enzyme_lnkedimmunosorbent assay kits.

The results found LH serum levels in the one group that received normal saline was significantly increased ($P< 0.01$) in the D-galactose groups, it significantly increased FSH serum levels in the control group that taken dates pollen ($P< 0.01$) rather than animals, which taken normal saline. Our results found a decrease in estrogen serum levels in the D-galactose groups rather than control groups. Finally, animals in the third group exposed to D-galactose at a dose rate on 500 mg/kg body weight for 30 days showed a significant decrease of Progestin level in serum of exposed rats. In conclusion: female hormonal measurement (LH) was increased, and (estrogen and progesterone) were dropped in the using date pollen. Otherwise, Vitexagnus-castus hasno significant differences in the LH and FSH serum levels.

Keywords: *Vitexagnus-castus, Dates palm pollen, D-galactose, sex hormone*

Introduction

A shrub native to Central and Mediterranean Asia is the Vitexagnus-castus tree. The shrub has long, finger-shaped leaves, dark purple berries, and blue-violet flowers. Vitexagnus-castus is used for conditions linked to the time, such as breast pain (breast pain), premenstrual syndrome (PM), and more serious symptoms of PMS. The fruit and seeds are used to produce medication (premenstrual dysphoric disorder or PMDD). It is often used for many other situations (1), although the bulk of these applications are not backed by sufficient scientific evidence. It grows from 2 to 4 meters in length and has many branches at the base. It has a silver-grey deciduous palm and has 5-7 leaves. The white flower is apically clustered. Its small black fruits with a diameter of half a cm. The shrub can withstand temperatures of 45 degrees Celsius, tolerate frost and exposure to direct sunlight(2). Some studies constrained-action of Vitexagnuscastus extracts and their composition on mouse pituitary cells

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under basal and stimulating conditions in primary cell culture. It was demonstrated that inhibition of prolactin is by using plant extracts. Both synthetic dopamine agonists and extracts of Vitexagnuscastus were significantly inhibited primary lactogenic hormone (PRL) secretion and TRH promoter of rat pituitary cells in outside living mousses. As a result, prolactin secretion can be inhibited by an antagonist of dopamine receptor addition (3). Therefore, due to the effect of dopamine, Agnuscastus can be considered an effective alternative drug for phytotherapy in the treatment of mild hyperprolactinemia[4].

Medicinal plants still exist as valuable therapeutic agents in traditional and modern medicine (5). Conventional Drugs are becoming more and more critical now. Search for a scientific basis in the research treat them (6). It comes from plants chemicals used to relieve impotence; they have the power to improve; these phytochemicals increase sexual desire and performance and sexual pleasure. Dates pollen grains are used to enhance fertility in women (7).

Date palm contained mainly cholesterol, a carotenoid routine of estrogen. It was known for the emergence of a gonadotropin function in the body of rats the date palm (phoeneixdactylifera l.) it is considered the most important source of food. This is good for both humans and animals (8). The date contains a high proportion of some sugars is as high as 88% miscellaneous (9). Dates are also rich in mineral salts and vitamins for digging dates, percentage non-reducing sugar content: 3.82% fructose 1.68 and 1.53, respectively, according to local medical practices, the date is considered tonic; some people think it is an aphrodisiac, and the plant flower is used as a laxative. The current study goal to qualitatively evaluate two plant extracts vitexAgnus and dates pollen in the sex hormonal measurement of female rats after exposure to d-galactose.

Materials and Methods

Experimental design

60 spray *Dawley rat* (180±15 grammes) weight and three months of age were randomly divided into six groups: control: subcutaneous injection of normal saline for 30 days and adjustable feeding of oral gavage twice daily for the last seven days; two groups; three-month-old mice's injection of saline under the skin for 30 days and 70% of the ethanol extract of Vitex at the same time. 600 mg/kg third group three-month-old mice were subcutaneously injected with d-galactose (Merck, Germany; 500 mg/kg/day for 30 days) and twice daily given saline solution by gavage for seven days in four groups: three-month-old mice were subcutaneously injected with date pollen extract three hundred mg/kg/day for 30 days and twice daily by gavage for seven days in saline solution. Five groups: 3-month-old mice's were subcutaneously injected with d-galactose (five hundred mg/kg/day for 30 days) and given Vitex (six hundred mg/kg/day for seven days by flexible oral feeding; six groups: 3-month-old mice's were subcutaneously injected with d-galactose (five hundred mg/kg/day for 30 days) and dated pollen extract (300 mg/ kg/day for seven days by flexible oral feeding.

Estrus cycle matching

In some respects, the correct matching of the rat estrus cycle is an essential experimental method. Initially, 100 mcg of estradiol valerate (synthetic estrogen) was being absorbed in 0.2 milliliters of olive oil and intramuscularly injected into the rat body. After forty-two hours, about fifty micrograms of progesterone was intramuscularly injected. All rats were stained and smeared after six h for the last four days of the experiment. The smear was fixed on a glass slide and stain with a 1% methylene blue aqueous solution, then perform microscopy[10].

Hormonal Measurement

The mice were anaesthetized with ketamine/xylazine after the experiment. Next, the heart extracted the blood samples and centrifuged them. Separate the serum and position it at -20 °C in the refrigerator before the hormone has been calculated. Enzyme-linked immunosorbent assay (ELISA) kits (biometric/USA) was used to test serum oestrogen and progesterone levels, and animal LH and FSH serum levels were assessed by ELISA assay kits (Biobase company, China). These kits have high specificity and sensitivity for LH and FSH rat detection.

Statistical Analysis

Mean, and the standard deviation was calculated and analyzed by Anova using software Past3 version 3.0, among six groups, it was essential to use *Tukey*pairwise to differentiate data between two groups as letters.

Results and Discussion

The effect of Vitex on blood sex hormone levels

Effect of Vitex and Dates pollen on the LH levels

In this study, compared, the LH levels of the d-galactose group increased significantly (P <0.01). Compared to the control animals, on the other hand, Vitex extract was reduced considerably the LH levels of animals treated with Vitex + d-galactose ($p < 0.05$) while, Compared to the dates pollen, the LH level of the Pollen + d-galactose group has no significantly ($p > 0.05$) decrease (Figure 1).

The efficacy of Vitexagnuscastus (Vitex; six hundred mg per kg) on the serum levels was illustrated as the mean ± the standard deviation. Based on one-way ANOVA and post hoc test of the least-significant difference between mean, and the result was compared to control group, the study found that LH serum levels were significantly increased (P< 0.01) in the d-galactose groups. A previous study was noted that d-galactose could increase LH levels in mice (11), the study also found a decrease levels of LH serum levels in rats blood in the d-galactose group by in the group was taken Vitexin extract this result agreement with (12) in Egypt who was found that Vitexn secretes actual amounts of androgen which converted to estrogen, it was found that no significant differences in the mean of LH levels between control and d-galactose groups, this results not agreement with (13) who was found that aqueous extract of DPP pollen can be used as a sex enhancer and seems to cure rats infertility.

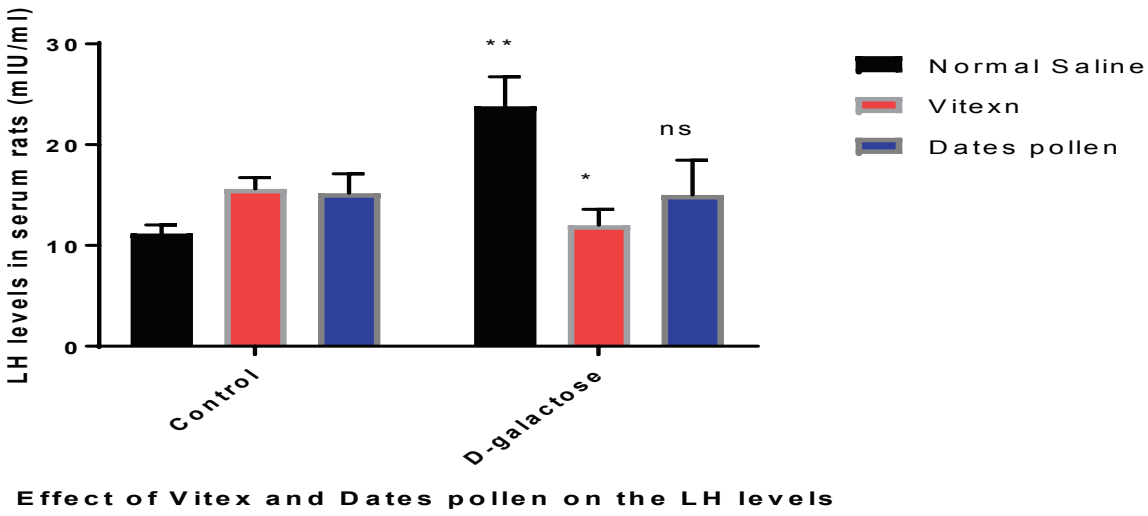

Figure1: Effect of Vitex and Dates pollen on the LH levels.

Values are appear as mean ± SD, N = 10 for each group.- C1: Rats given normal saline for 30 days and oral gavage 2daily for the last seven day .-C2: saline under the skin for 30 days, and at the same time, 70% of the an extract ethanolic of Vitex . six hundred mg / kg.-C3:d-galactosefive hundred mg / kg/ day for 30 daysgiven saline solution twice daily by gavage for 7 daysC4: date pollen extract three hundred mg / kg/ day for 30 days and were given saline solution twice daily by gavage for 7 daysC5:dgalactos five hundred mg / kg/ day for 30 dayand Vitex (600 mg / kg / by flexible feeding oral gavage 7 daysC6: d-galactose 500 mg / kg / day for 30 days anddate pollen extracts 300 mg / kg / by flexible feeding oral gavage 7 days * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, ns non-significant.

In this study, compared, the FSH levels of the d-galactose group decrease significantly (P <0.01). Among control animals, it was found increase levels of FSH in the dates pollen in the control group, on the other hand, Vitex extract and dates pollen was non significantly different in the FSH levels of animals treated with Vitex + d-galactose (p >0.05) and dates pollen +d-galactose (p >0.05 (Figure 2) (13). In an animal treated with a dose of 150 mg/ kg of Dates Pollen extract, he found the results revealed a significant (P< 0.05) increase in testosterone and FSH serum levels. Figure 2 presented that there was no significant difference between the control group, d-galactose group in the measurement of FSH serum levels, this result agreement with (12) who was found showed that the tested extract had no significant appearance on plasma serum of FSH in the normal group and female ovariectomized rats.

Our result found a significant increase in F.S.H. serum levels in the control group that took dates pollen (P< 0.01) rather than animals that took normal saline. The results were in agreement with (14) who investigated that F.S.H. levels were increased in his control groups 150 mg/K.B.W. from days 14 as (13.88 ± 0.26) to days 42 post-exposure levels (17.54 ± 0.6 ng/ml).

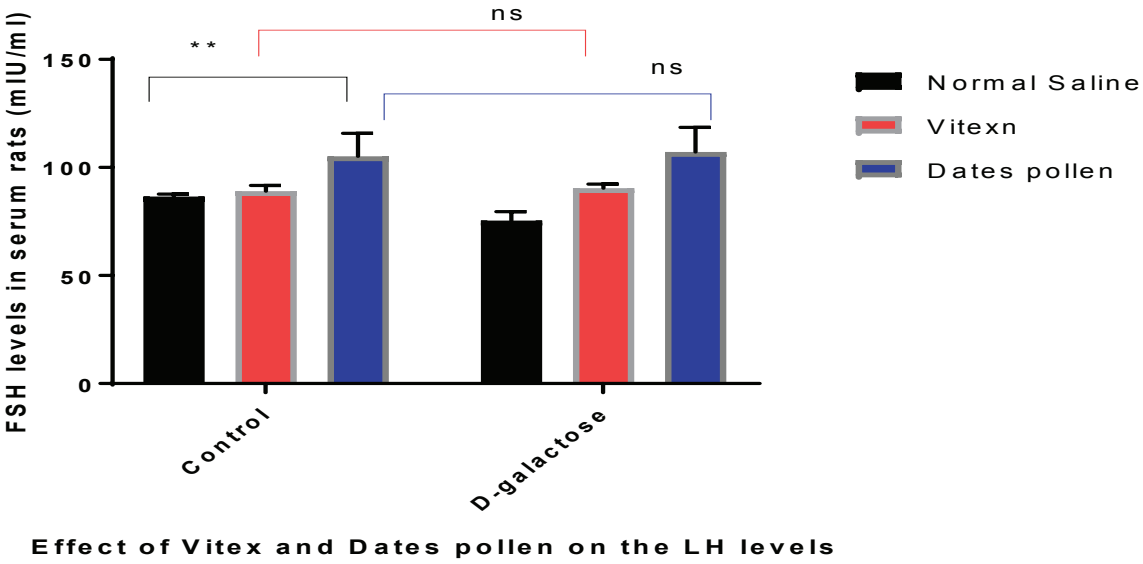

Figure2: Effect of Vitex and Dates pollen on the FSH levels

Values are appear as mean ± SD, N = 10 for each group.- C1: Rats given normal saline for 30 days and oral gavage 2 daily for the last seven day .-C2: saline under the skin for 30 days, and at the same time, 70% of the an extract ethanolic of Vitex . six hundred mg / kg.-C3: d-galactosfive hundred mg / kg/ day for 30 daysgiven saline solution twice daily by gavage for 7 daysC4: date pollen extract three hundred mg / kg/ day for 30 days and were given saline solution twice daily by gavage for 7 daysC5:d galactos five hundred mg / kg/ day for 30 dayand Vitex (600 mg / kg / by flexible feeding oral gavage 7 daysC6: d-galactose 500 mg / kg / day for 30 days anddate *Pollen extracts*300 mg / kg / by flexible feeding oral gavage 7 days* p < 0.05, ** p < 0. 01, and *** p < 0. 001 , ns non- significant

In this study, compared, the Estrogen levels of the control group decreased significantly (P <0.01) among control rats it was found reduced levels of estrogen in the fourth group after exposure to d-galactose, on the other hand, Vitex extract have was non significantly increase in the Estrogen levels of animals treated with Vitex + d-galactose (p <0.05) rather than control groups, while the study not found dates pollen +d-galactose have

different($p > 0.05$)with control groups (Figure 3).

Our results were not agreement with (15) who was found that a significant increase ($p<0.01$) at the level of progesterone and estrogen compared to the health group only in the experimental group type three (taking four hundred milligrams per kilogram palm pollen extract). Our results found a decrease of estrogen serum levels in the d-galactose groups due compare with control groups; this is because some studies are indicating apoptotic follicles increase and estrogen production reducing, the D-galactose toxicity form which plays a role in the development of primary ovarian insufficiency due to early depletion of ovarian follicles (16).

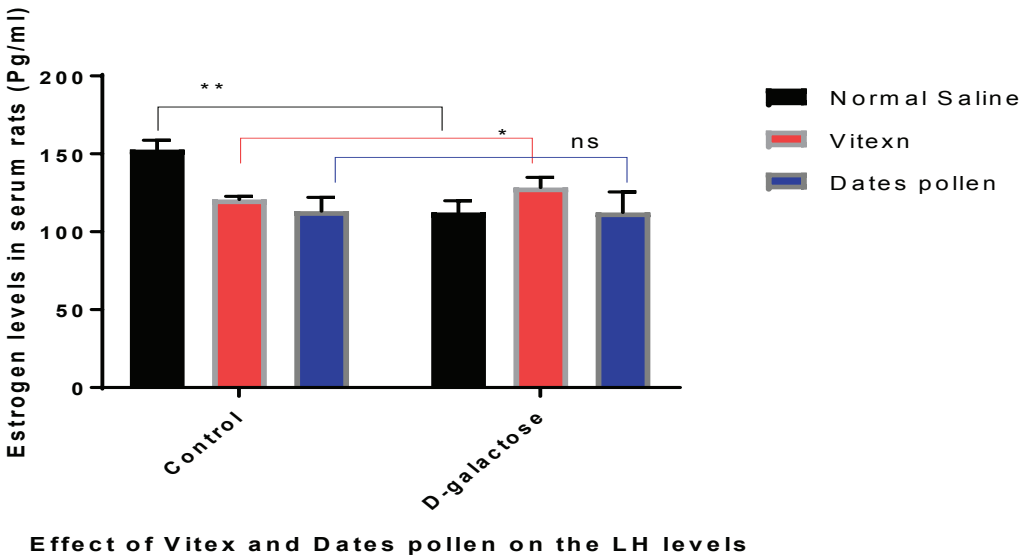

Figure 3: Effect of Vitex and Dates pollen on the Estrogen levels Values have appeared as mean ± SD, N = 10 for each group.- C1: Rats given normal saline for 30 days and oral gavage two days for the last seven days -C2: saline under the skin for 30 days, and at the same time, 70% of the extract ethanolic of Vitex. six hundred mg / kg -C3: galactose five hundred mg / kg / day for 30 days given saline solution twice daily by gavage for 7 days C4: date pollen extract three hundred mg / kg / day for 30 days and were given saline solution twice daily by gavage for 7 days C5:d-galactose five hundred mg / kg / day for 30 day and Vitex (600 mg / kg / by flexible feeding oral gavage 7 days C6: d-galactose 500 mg / kg / day for 30 days and date pollen extracts 300 mg / kg / by flexible feeding oral gavage 7 days * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, ns non-significant.Animals in the third group that exposed to d-galactose at a dose rate of 500 mg / k. B.W. for 30 days showed a significant decrease of progesterone level in serum of exposed rats, as well as sixth groups which received d-galactose+ date pollen as 500 mg/kg/day for 30 days and date pollen, extract 300 mg/kg, have a significant decrease in the progesterone serum levels than control groups. On the other hand, there were no significant differences ($P>0.05$) between the fourth and the second group in the taken of Vitexin; these results were in disagreement with (Ibrahim et al., 2008).

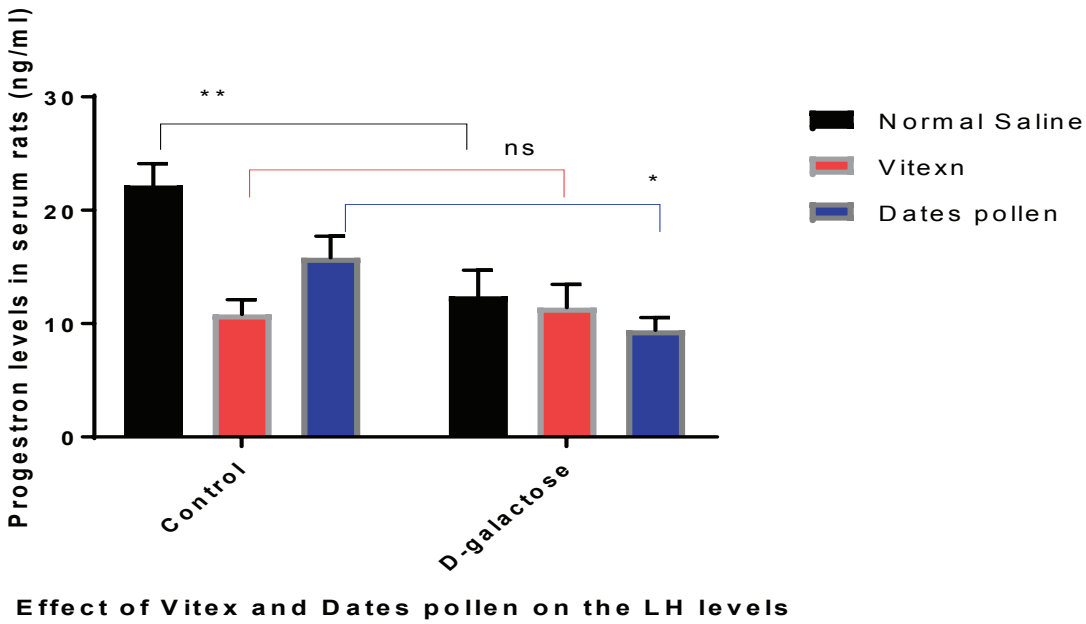

Figure 4: Effect of Vitex and Dates pollen on the Progesterone levels

Values appear as mean ± SD, N = 10 for each group.

- C1: Rats given normal saline for 30 days and oral gavage two days for the last seven day.-C2: saline under the skin for 30 days, and at the same time, 70% of the extract ethanolic of Vitex. six hundred mg / kg.-C3: galactose five hundred mg/kg/day for 30 days given saline solution twice daily by gavage for seven days C4: date pollen extract three hundred mg/kg/day for 30 days and were given saline solution twice daily by gavage for seven days C5:d-galactose five hundred mg/kg/day for 30 days and Vitex (600 mg/kg / by flexible feeding oral gavage seven days. C6: d-galactose 500 mg / kg / day for 30 days and date pollen extracts 300 mg / kg / by flexible feeding oral gavage 7 days. * $p< 0.05$, ** $p < 0.01$, and *** $p < 0.001$, ns non-significant.

The low progesterone levels in animals are unknown. However, Subclinical hyperprolactinemia has been suggested, or High levels of pre-menstruation or stress induced Prolactin also can inhibit the growth of the corpus luteum. Other Possible associations include those that are seen psychologically stressed (17), Excessive Exercise, or exposure to Endocrine Disruptive Chemicals (18).

In conclusion: female hormonal measurement (LH) was increased, and (estrogen and progesterone) were dropped in the using date pollen. Otherwise, Vitexagnus-castus have no significant differences in the LH and FSH serum levels.

Ethical Clearance- Taken from Basic Science, College of Dentistry, University of Al_Qadisiyah, Iraqcommittee

Source of Funding- This study is self-funded

Conflict of Interest - The authors declare no conflict of interest.

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