

# Effect of Vitex Agnus-Castus Ethanolic Extract on Sex Hormones in Streptozotocin-Induced Diabetic Rats

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## Abstract

**Objective:** Diabetes mellitus is recognized as one of the serious global health problems. There are evidences regarding the high prevalence of sexual dysfunction in diabetic patients. Experimental studies revealed a positive effect of Vitex agnus-castus (Vitex), on sexual function and behaviors. In this research, the effect of Vitex on sexual hormones in streptozotocin-(STZ) induced diabetic rats was investigated.

**Materials and methods:** A Thirty adult female Wistar rats were divided into five groups. 1-control group (non-diabetic), 2- diabetic group (received normal saline) and three induced diabetic groups treated with different doses (400, 200 and 100 mg/kg) of Vitex. Treatment groups received Vitex fruit extract by gavage for 7 days. The levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone and estrogen in serum were measured.

**Results:** Levels of LH, FSH, estrogen and progesterone and average body weight was lower in diabetic group compared to control group ( $p < 0.010$ ). Animals received high dose of Vitex fruit extract (400mg/kg) had significantly higher levels of serum LH, FSH, estrogen and progesterone compared to diabetic group ( $p < 0.010$ ). In animals receiving minimum dose (100mg/kg) of Vitex, no difference was observed compared to diabetic group ( $p > 0.010$ ).

**Conclusion:** It can be concluded that Vitex fruit extract probably has regulatory effect on diabetes-induced change in the levels of sex hormones in female rats. Vitex fruit extract can improve serum levels of sex hormones in an animal model of STZ-induced diabetes.

**Keywords:** Vitex Agnus-Castus; Diabetes; Streptozotocin; Sex Hormones

## Introduction

Diabetes is known as an important metabolic disease and a leading cause of disability and death worldwide. The disease causes wide range of implications in several organs including sexual glands in both men and women. The majority of diabetic patients experience sexual function deficiency such as loss of libido, erectile dysfunction and disorders of sexual desire, arousal, orgasm, and

pain (1, 2).

Regarding minimum toxicity and side effects, a wide range of medical plants are used to prevent adverse effects of diabetes mellitus. Vitex agnus-castus L. (VAC) is an important traditional herbal medicine plant belonging to the Verbenaceae family (3). The plant is used for treatment of several reproductive system conditions such as premenstrual syndrome (PMS), abnormal menstrual cycles, amenorrhea, mastodynia, hyperprolactinemia, premenstrual dysphoric disorder and lactation difficulties (4). Its main chemical ingredients include iridoid glycosides, flavonoids, diterpenes, and volatile oil. The current

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study was conducted to examine the effect of diabetes on the levels of sexual hormones and to investigate the effect of Vitex on diabetes-induced alterations of sex hormone in female rats.

### Materials and methods

**Hydro-alcoholic extract preparation:** Fresh fruits of Vitex were desiccated under shade. Then 500 gr of powdered fruit was macerated in 1L of 80% ethanol and maintained for 72 hours at room temperature. After filtering the combination with What-man No. 1 filter paper, the extract was dried using a rotary evaporator (Heidolph Laborota 4000, Germany). The obtained 80% ethanolic extract was stored at -20 °C until use.

**Experimental design:** Thirty adult female Wistar rats (*Rattus norvegicus*) (185 ± 20 g), purchased from the Animal House of the Pasteur Institute, were kept in standard condition with free access to food and water. Esterous cycle synchronization was performed based on method previously described (5). The rats made diabetics by single intraperitoneal injection of streptozotocin (STZ, Sigma, UK, 60 mg/kg; i.p.). After 4 days, animals having fasting blood glucose concentration ≥ 250 mg/dL were considered diabetic and used for the investigation.

**Animal grouping:** The animals were randomly divided into 5 groups of six: Group (C): non-diabetic rats received no treatment. Group (D): diabetic rats administered normal saline by gavage once daily for 7 days. Group (DL): diabetic rats received low dose of Vitex extract (100mg/kg). Group (DM): diabetic rats received medium dose of Vitex extract (200 mg/kg). Group (DH): diabetic rats received high dose of Vitex extract (400 mg/kg). The Vitex extract juice was administered once a day for 7 consecutive days using oral gastric gavages.

**Sample collection:** After one-week treatment, animals anesthetized with ether. Blood samples were obtained from the animals' hearts and the serum was separated by centrifugation (3000 rpm for 5 min).

The serums were kept in -20 °C until further analysis.

**Biochemical analysis:** LH and FSH levels were determined using commercial ELISA kit (CUSABIO China Inc) according to the manufacturer's protocol. Serum estrogen and progesterone levels were determined by radioimmunoassay using specific diagnostic kits (GmbH, IBL, Germany).

**Statistical analysis:** Data were presented as mean ± SEM. Normality of data were checked and approved using Kolmogorov–Smirnov test. Differences between the groups were assessed using the one-way analysis of variance (ANOVA) followed by TUKEY post- hoc test with SPSS version 19. The differences were considered significant at p < 0.01.

**Ethical consideration:** The study was conducted in accordance with protocols approved by the principles of the Declaration of Helsinki. The experimental method was confirmed by Hamedan University of Medical Science Animal Ethical Committee (NUM: IR.UMSHA.REC.1399.146).

### Results

**Effect of different doses of treatments on body weight:** At the end of the study, the body weight of diabetic and treatment groups (DL, DM and DH) showed significant (p = 0.008) reductions compared to control group. Significant increases in body weight were found in DM and DH groups compared to D group. No difference was observed in DL compared to D group (p > 0.010). There was significant increase in body weight in DH compared to DM group.

**Effect of different doses of treatments on LH serum concentration:** The results showed that LH serum level was significantly lower in the treatment groups compared to control group. The level of LH was significantly higher in DH compared to control group. There was no difference (p > 0.010) between group D and DL, however LH level was significantly higher (p < 0.001) in DM and DH compared to D group (Table 1).

**Table 1:** Serum Sexual hormone profile in studied groups

| Sexual hormones      | Group C      | Group D                  | Group DL                    | Group DM                   | Group DH                    |
|----------------------|--------------|--------------------------|-----------------------------|----------------------------|-----------------------------|
| FSH (ng/ml)          | 4.42 ± 23    | 1.04 ± 0.05 <sup>a</sup> | 1.58 ± 0.16 <sup>a</sup>    | 2.40 ± 0.15 <sup>acd</sup> | 4.95 ± 0.16 <sup>cd</sup>   |
| LH (ng/ml)           | 5.54 ± 0.19  | 2.16 ± 0.08 <sup>a</sup> | 2.62 ± 0.19 <sup>a</sup>    | 4.48 ± 0.16 <sup>bcd</sup> | 8.05 ± 0.23 <sup>bcd</sup>  |
| Estrogen (pg./ml)    | 48.30 ± 0.39 | 22 ± 0.71 <sup>a</sup>   | 27.76 ± 0.53 <sup>a c</sup> | 33.5 ± 0.50 <sup>acd</sup> | 45.80 ± 0.86 <sup>bcd</sup> |
| Progesterone (pg/ml) | 3.50 ± 0.18  | 1.14 ± 0.07 <sup>a</sup> | 1.56 ± 0.21 <sup>a</sup>    | 1.40 ± 0.18 <sup>a</sup>   | 5.01 ± 0.04 <sup>acd</sup>  |

Values are showed as mean ± SEM. a and b denote significant difference with control group at p < 0.001 and p < 0.01 respectively, c denotes significant difference with group D at p < 0.01, d denotes significant difference with group DL at p < 0.01. Differences between the groups were assessed using the one-way analysis of variance (ANOVA) followed by TUKEY post- hoc test.

**Effect of different doses of treatments on FSH serum concentration:** The results showed that FSH serum level was significantly lower in the D, DL and DM compared to control group. There was no difference between DH and control group. Likewise, there was no difference ( $p > 0.01$ ) between D and DL groups, however FSH level was significantly higher ( $p = 0.003$ ) in DM and DH compared to D group (Table 1).

**Effect of different doses of treatments on progesterone serum concentration:** The results showed that progesterone serum level was significantly ( $p = 0.008$ ) lower in the D, DL and DM group compared to control group. The progesterone level was significantly higher in DH compared to control group. There was no difference between D, DL and DM groups ( $p > 0.01$ ). The level of progesterone was significantly higher in DH compared to D and DL group (Table 1).

**Effect of different doses of treatments on estrogen serum concentration:** The results showed that estrogen serum level was significantly lower in the D, DL and DM group compared to control group. The estrogen level was significantly higher in DH compared to control group. The estrogen level was significantly higher in DL, DM and DH compared to D group. Furthermore, the estrogen level was significantly higher in DM and DH compared to DL group (Table 1).

## Discussion

The present study was conducted to investigate the effect of Vitex extract on sex hormones in diabetic rats. Diabetes mellitus was induced by STZ that impairs glucose oxidation, decreases insulin biosynthesis and secretion. Furthermore, nitric oxide molecule produced by STZ, destroys pancreatic islet cells (6). STZ-induced diabetes causes remarkable decline in body weight (7) which was also occurred in the current study.

Treatment with medium and high doses of Vitex extract significantly prevented the body weight loss; although, it was not restored to control levels. It is believed that Increase in muscle wasting and tissue protein degradation led to weight loss in diabetic patients (8). Findings of current study show that Vitex treatment has positive effect on maintaining body weights in diabetic rats. This protective effect on body weight of diabetic rats can be attributed to its ability to reduce hyperglycemia. A gradual increase in body weights of treated group with high dose of Vitex was close to that of control rats.

The current study showed that diabetes mellitus can change the levels of sex hormones. Furthermore, it

revealed that Vitex extract can attenuate diabetes induced alterations to some extent. Many studies reported disruption of normal functions in reproductive system of diabetic patients. Seethalakshmi et al. reported remarkable decreases in the levels of LH, FSH and testosterone in STZ-induced male diabetic rats (9). Furthermore, Bucholtz et al. found that in diabetic sheep with hypoinsulinemia the levels of LH decreases suggesting that insulin or insulin-dependent changes plays a role in regulating pulsatile GnRH secretion (10). Likewise, in STZ-induced diabetic rodents, reduced estradiol alongside increased testosterone levels in females is reported (11). In present study the decrease in estrogen, progesterone, LH and FSH levels was observed. In diabetic patients, it is believed that the levels of GnRH are decreased (12, 13) which lowers the levels of LH, FSH and sexual steroids. Our finding showed that Vitex could increase the levels of estrogen, progesterone, LH and FSH. Previous study showed that the Vitex extract can increase the progesterone level in animals with polycystic ovary syndrome (14) which is in agreement with our study. In ovariectomized rats, Vitex increases the level of the progesterone and estrogen and decreased LH without affecting FSH (15). It is believed that administration of this plant extract has a regulative effect on the level of sex hormones. Vitex secretes substantial amounts of androgen that is converted to estrogen (5). The imbalance between sexual hormones, is regulated through administration of Vitex extract in PMS patients (16).

Bergman et al. found that in women with oligomenorrhea and amenorrhea, Vitex cause significant increase in progesterone level during the luteal phase with minimum undesirable side effects (17). Furthermore, in women with luteal phase defects due to latent hyperprolactinemia, oral administration of Vitex reduced prolactin release, normalized luteal phases and eliminated deficits in the luteal progesterone synthesis (18). The precise mechanism of Vitex action is not fully discovered. It is believed that Vitex regulates the levels of sex hormones through pharmacological and physiological mechanisms. Apigenin is the most active phytoestrogen in which has the ability to bind to the estrogen receptor (19). In other hand, linoleic acid as an estrogenic compound in Vitex is able to induce certain estrogen-inducible genes by binding to estrogen receptors (20).

## Conclusion

In conclusion, the present study showed that, in the

female reproductive system, diabetes decreased serum levels of LH, FSH, estrogen and progesterone. The administration of Vitex could effectively improve these alterations and reduce the complication. Therefore, Vitex may be useful for the treatment of certain diabetes problems such as reproductive dysfunctions.

### Conflict of Interests

Authors have no conflict of interests.

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