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Original Article

# Effects of *Vitex agnus-castus* fruit on sex hormones and antioxidant indices in a D-galactose-induced aging female mouse model

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**Abstract**

*Background*: Aging is associated with the loss of endocrine function. In this study, *Vitex agnus-castus* (Vitex), which has antioxidant effects and high levels of phytoestrogen, was investigated with regard to the hypothalamic-pituitary-gonadal axis and antioxidant indices in natural aging and in a D-galactose induced aging model in female mice.

*Methods*: The mice were subcutaneously injected with D-galactose (500 mg/kg/d for 45 days). Extract of Vitex (600 mg/kg/bid for 7 days by gavage) was used to treat D-galactose-induced aging and natural aging in mice. Seventy-two female NMRI mice (48 3-month-old normal mice and 24 18-24-month-old mice), weighing 30–35 g were randomly divided into six groups: control, Vitex, D-galactose, Vitex + D-galactose, Aging, and Vitex + Aging. The antioxidant indices and sex hormone levels were subsequently measured by enzyme-linked immunosorbent assay kits.

*Results*: Body weight and the levels of malondialdehyde (MDA), follicle-stimulating hormone, and luteinizing hormone levels were significantly increased in the D-galactose aging and natural aging groups, whereas catalase and superoxide dismutase (SOD) activity and estrogen level were significantly decreased in these same groups. D-Galactose can also disrupt the estrous cycle and damage the uterus and ovarian tissues. Vitex could effectively attenuate these alterations.

*Conclusion*: Vitex improved some aging events in the reproductive system of female mice. Therefore, because of its apparent antiaging effects, Vitex can be suitable for some aging problems such as oxidative stress, female sex hormone deficiency, and an atrophic endometrium.



*Keywords:* aging; antioxidant index; D-galactose; estrogen; *Vitex agnus-castus*

## 1. Introduction

Aging is a biological process that leads to oxidative stress in cells and tissues. This process can enhance human vulnerability to cognitive dysfunction and impairment in physical, mental and social activities.[1,2] Continuous changes in neuroendocrine responses occur because of aging. These changes cause a "vicious cycle of endocrinosenescence and immuno-senescence".[3] In fact, aging is correlated with immune system dysregulation; a deficiency in sex hormones; an increase in oxidative stress and the level of inflammatory cytokines; and the development of chronic diseases such as cancer, type 2 diabetes mellitus, and neurological dysfunction.[4,5] Oxidative stress is generated by an imbalance between free radicals and antioxidants. Oxidative imbalance leads to aging because of the

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impairment of various biomolecular processes and the accumulation of damage over time.[6] Some reproductive diseases such as endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility are generated because of dysfunction in the antioxidant system caused by the production of reactive oxygen species (ROS).[7] D-Galactose is a reduced sugar that can be changed into glucose at normal concentrations and oxidized into aldehydes and hydrogen peroxide at high concentrations.[8] D-Galactose accelerates aging in rodents.[9] In addition, D-galactose induces oxidative stress by increasing the malondialdehyde (MDA) level and decreasing catalase and superoxide dismutase (SOD) activity.[10] D-Galactose results in the development of aging in the brain, kidney, liver, and blood cells, and it is associated with diabetes, arteriosclerosis, nephropathy, Alzheimer's disease, metabolic abnormalities, and the formation of extra ROS and neuronal damage.[10,11]

*Vitex agnus-castus* L. (Vitex) is a plant that belongs to the Verbenaceae family and is native to the middle Asian, southern European, and Mediterranean countries.[12] It is used as a treatment for premenstrual syndrome (PMS), abnormal menstrual cycles, amenorrhea, mastodynia, hyperprolactinemia, premenstrual dysphoric disorder, lactation difficulties, and low fertility.[13,14] This plant is composed of iridoid glycosides, flavonoids, diterpenes, and volatile oil.[14] Water extracts and ethanolic extracts of Vitex have antioxidant activity because of its flavonoid, diterpenoid, and ecdysteroid content.[15,16]

The number of aged individuals in societies is increasing and few research documents exist on aging models and on the effects of medicinal plants such as Vitex in aging models. This study therefore investigated the antiaging effects of Vitex on ovarian and uterine tissues, sex hormones, and antioxidant indices in female mice that underwent natural aging and D-galactose-induced aging.

## 2. Methods

### 2.1. Plant material

The fruits of Vitex were collected from Qom, Iran, and stored at the Department of Botany of Ahvaz Jundishapur University (Ahvaz, Iran) under voucher specimen number A14311001P. They were dried in the dark and then powdered by an electric mill. Two hundred fifty grams of the Vitex powder was mixed with 1 L of 70% ethanol, and then soaked for 72 hours in ethanol. The content was then filtered through a paper filter and funnel glass. The filtrate was transferred to a balloon and the solvent (purity of 10%) was removed in an environment with an ambient temperature under 70°C. The yield ratio of the extract was 9.8%. A dose of 600 mg/kg body weight was administered, based on the description of an earlier study by Ibrahim et al.[17]

### 2.2. Animals and experimental design

In this study, 72 female NMRI mice (48 young mice, 3 months old; 24 aged mice, 18–24 months old), weighing 30–35 g, were obtained from the Ahvaz Jundishapur University of Medical Sciences (AJUMS) Animal Facility (Ahvaz, Iran). All procedures involving animals were approved by the Animals Committee of AJUMS, and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. The mice were maintained in cages with free access to water and food with a 12/12 hour light-dark cycle and controlled temperature (22°C ± 2°C). The mice were randomly divided into six groups (12 animals per each group), as follows:

(1) Control group: Three-month-old mice were injected subcutaneously with normal saline for 45 days and concomitantly administered normal saline by gavage twice daily for the last 7 days.
(2) Vitex group: Three-month-old mice were injected subcutaneously with normal saline for 45 days and concomitantly administered 70% ethanolic extract of Vitex (600 mg/kg/bid; by gavage for the last 7 days).[17]
(3) D-Galactose group: Three-month-old mice were injected subcutaneously with D-galactose (Merck, Germany; 500 mg/kg/d for 45 days) and concomitantly administered normal saline by gavage twice daily for the last 7 days.[18]
(4) Vitex + D-galactose group: Three-month-old mice were injected subcutaneously with D-galactose (500 mg/kg/d for 45 days) and concomitantly administered Vitex (600 mg/kg/bid; by gavage for the last 7 days).
(5) Aging group: Mice 18–24 months old were injected subcutaneously with normal saline for 45 days and concomitantly administered normal saline by gavage twice daily for the last 7 days.
(6) Vitex + Aging group: Mice 18–24 months old were injected subcutaneously with normal saline for 45 days and concomitantly administered Vitex (600 mg/kg/bid; by gavage for the last 7 days).

### 2.3. Estrous cycle

In some respects, the process for correctly matching the estrous cycle in the mice was an experimental method. In the beginning, 100 μg of estradiol valerate was dissolved in 0.2 mL olive oil and injected intramuscularly. After 42 hours, 50 μg of progesterone was injected intramuscularly. All animals were smeared after 6 hours,[19] and the mice were also smeared in the last 4 days of the experiment. The smears were fixed on slides and stained with 1% aqueous methylene blue, after which they were examined microscopically.[17]

### 2.4. Analysis of tissue and serum

After the experiment, the mice were anesthetized with ketamine/xylazine. Thereafter, blood samples were collected from the heart and centrifuged. The serum was isolated and placed in a freezer at -20°C until hormonal assays were performed. Uterine and ovarian tissues were dissected and dried, and their collective weight relative to body weight was