

Pancreatic protective and hypoglycemic effects of *Vitex agnus-castus* L. fruit hydroalcoholic extract in D-galactose-induced aging mouse model

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Abstract

D-galactose induces pancreatic disorder along with aging mouse model. *Vitex agnus-castus* (VAC) has potential pancreatic protective effect. Hence, this study was designed to evaluate the hypoglycemic and pancreas protective effects of VAC hydroalcoholic extract in D-galactose-induced aging female mice. In the present experimental study, 72 adult female Naval Medical Research Institute (NMRI) mice (weighing 30–35 g) were divided into 6 groups of control, VAC hydroalcoholic extract, D-galactose, D-galactose + VAC hydroalcoholic extract, aged, aged + VAC hydroalcoholic extract. The aged model was prepared by subcutaneous injection of D-galactose for 45 days and, VAC hydroalcoholic extract was gavaged twice a day in the last 7 days. 24 h after the last drug and extract administrations, serum samples and pancreatic tissues were removed to evaluate experimental and histological determinations. Serum glucose level decreased in VAC, D-galactose and, aged-treated groups compared to the control ($P < 0.05$). Insulin level increased in VAC and decreased in D-galactose and aged VAC-treated mice compared to the control ($P < 0.05$). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) increased in D-galactose, aging, and VAC hydroalcoholic extract groups ($P < 0.05$) and, administration of VAC hydroalcoholic extract improved HOMA-IR in D-galactose and aging treated animals. Despite the size of pancreatic islets decreased in aged and D-galactose groups, VAC administration recovered it. Present data showed that VAC hydroalcoholic extract has hypoglycemic and pancreatic protective effects in natural aged and aging model mice.

Keywords: *Vitex agnus-castus*; Pancreas; D-galactose; Aged mouse

INTRODUCTION

Impairments of pancreatic physiological functions including alterations in growth, secretion, and motility have been occurred through the aging process (1). Age-associated diseases such as diabetes are more common in the elderly population. Further, animal model of aging play an important role in drug discovery for aging animal models. One of the important animal models of aging is D-galactose-induced aging mouse model used by different scientific groups (2). D-galactose is a reducing sugar,

which can be converted into aldose, hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-). These changes are similar to the normal aging process and demonstrate a reduction in antioxidant defense systems such as antioxidant enzyme activity (3). Glucose and lipid metabolism disorders have been considered as important factors contributing to the D-galactose induced aging mouse model (2).

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Also, a lot of evidence indicates that free radicals play an important role in the development of diabetes mellitus symptoms such as insulin resistance, impaired glucose tolerance and beta cell dysfunction (4).

Vitex agnus-castus (VAC) commonly known as chaste tree, belongs to the Lamiaceae family of plants and traditionally has been used as a remedy for menstrual problems including premenstrual symptoms and spasmodic dysmenorrhea, insufficient lactation and, acne. VAC has also been used as anticonvulsant, antiepileptic, sedative, and libido reducing plant in the Iranian traditional medicine. Phytochemical analysis of VAC revealed that the fruits, flowers and, leaves of this plant contain flavonoid, iridoids, volatile oils, and tannins (5). It is confirmed that VAC leave extracts has hypoglycemic activity in STZ-induced diabetic rats (6). Also, it has been revealed that D-galactose induces beta cell damage and reduces insulin secretion. Hence, this study was designed to evaluate the hypoglycemic and pancreas protective effects of the VAC hydroalcoholic extract in D-galactose induced aging mouse model.

MATERIALS AND METHODS

Plant extraction

In this experimental study, VAC desiccated fruits were obtained from green grocery of Ahvaz city, Iran and, scientifically authenticated by the Botany Department of Ahvaz Jundishapur University of Medical Science (AJUMS), Ahvaz, Iran. The fruits were powdered and, 250 g of its powder was soaked in 1 L of distilled water-ethanol mixture (70:30) and maintained for 48 h at room temperature. This mixture was filtered and centrifuged at 3000 rpm for 10 min. Then, the supernatant was taken away, dried at 37 °C and, the acquired semi-solid mass was stored at 4 °C until used (7).

Animal preparation

Since this plant has potential effects on aged and post-menopausal symptoms, it was decided to use 72 adult female Naval Medical Research Institute (NMRI) mice (weighing 30-35 g) in present study. The mice obtained

from the animal facility of AJUMS, treated according to AJUMS animal care guidelines with an ethics committee grantee ID number (IR. AJUMS. REC. 1394. 724) and kept at 20 ± 4 °C temperature with a 12-h light/ 12-h dark cycle. All animals had free access to tap water and commercial chow *ad libitum*.

Experimental design

Estrus cycle assessment

Since female steroid hormones can influence variations of serum insulin homeostasis and glucose metabolism through the estrus cycle (8), the same estrus cycle of hormonal synchronization was prepared in female mice. For this purpose, 42 h after injection of 100 µg estradiol valerate dissolved in 0.2 mL olive oil, 50 µg of progesterone was injected intramuscularly. Then, 6 h after the injection of progesterone, vaginal smears were prepared. All smears were fixed on slides in a warm condition, stained with methylene blue (1%) and examined by optical microscope (9,10).

Animals grouping

After induction of the same estrus cycle mice were divided into 6 groups of 12 each including 4 groups of young animals and 2 groups of old mice.

Young animals: control group, received a subcutaneous (s.c) injection of sodium chloride 0.9% as D-galactose solvent for 45 days and same solution was gavaged as VAC solvent twice a day in the last 7 days; VAC hydroalcoholic extract, received sodium chloride 0.9% s.c for 45 days and gavaged VAC extract (600 mg/kg) twice a day in the last 7 days (12); D-galactose, as aging model received D-galactose (500 mg/kg) s.c for 45 days and sodium chloride 0.9% was gavaged twice a day in the last 7 days (11); D-galactose + VAC hydroalcoholic extract, received D-galactose (500 mg/kg) s.c for 45 days and VAC extract (600 mg/kg) was gavaged twice a day in the last 7 days.

Aging animals consisted of 18- to 24-month old mice that received sodium chloride 0.9% s.c for 45 days and same solution as the gavage twice a day in the last 7 days, and aged + VAC hydroalcoholic extract including 18- to

24-month old mice that received sodium chloride 0.9% injection s.c for 45 days and were gavaged VAC extract (600 mg/kg) twice a day in the last 7 days.

Experimental measurement

Twenty four hours after the last D-galactose and extract administrations, animals were sacrificed under deep anesthesia by intraperitoneal injection of ketamine-xylazine mixture (70:10 mg/kg) and blood samples were obtained by cardiac puncture. Blood samples were centrifuged at 3000 rpm for 15 min to harvest their serum contents. Then, all samples maintained at -20°C until hormonal assessment (13). Glucose was measured by biochemical assay kits (Pars Azmoon, Iran) and serum insulin levels were determined by ELISA assays kits (Monobind, USA) (The sensitivity of hormone detection per assay tube was $0.182\ \mu\text{IU/mL}$). Further, homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated according to the following formula (14).

$\text{HOMA-IR} = \text{fasting insulin } (\mu\text{IU/dL}) \times \text{fasting glucose } (\text{mg/dL}) / 405.$

Histological assessment

Mouse pancreatic tissue was removed and fixed in 10% formalin solution. Then, tissues were dehydrated in graded alcohol concentrations and embedded in paraffin. Sections of 4-6 μm were prepared and stained

with hematoxylin & eosin (H&E). Six microscopic H&E stained slides per animal were examined for assessment of histological changes such as cellular swelling and diameters of islets which assessed by using Motic Images Plus 2.0 image analysis software. Finally, a blind method was used for slides reading (15,16).

Statistical assessment

All data are reported as mean \pm SEM and were analyzed using SPSS software with one-way ANOVA which was then followed by post hoc LSD tests. *P* values less than 0.05 was assumed statistically significant.

RESULTS

Hypoglycemic effects of *Vitex agnus-castus* extract

VAC hydroalcoholic extract decreased serum glucose levels in both VAC and D-galactose VAC-treated groups when compared to the controls ($P < 0.05$). Also, the same effect was observed in D-galactose VAC-treated mice in comparison with D-galactose group ($P < 0.05$). Moreover, administration of VAC extract reduced glucose serum levels in aged treated mice compared to aged and control groups ($P < 0.01$, Fig. 1). While serum insulin levels increased in VAC and aging groups, this hormone significantly decreased in aged VAC-treated mice when compared to the control animals ($P < 0.05$).

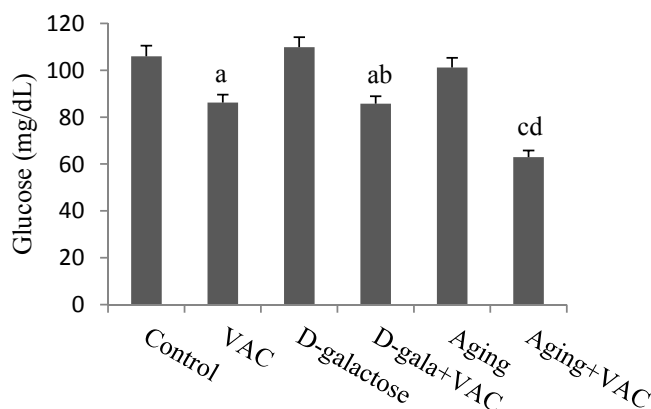


Fig. 1. Effect of *Vitex agnus-castus* (VAC) hydroalcoholic extract on serum glucose levels. ^aDenotes significant difference ($P < 0.05$) compared to the control, ^bdenotes significant difference ($P < 0.01$) compared to the D-galactose, ^cdenotes significant difference ($P < 0.01$) compared to the control, ^ddenotes significant difference ($P < 0.01$) compared to the aging animals.

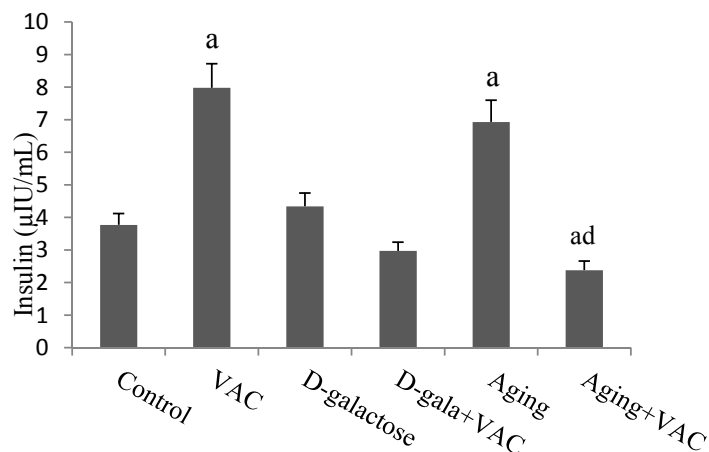


Fig. 2. Effect of *Vitex agnus-castus* (VAC) hydroalcoholic extract on serum insulin levels. ^aDenotes significant difference ($P < 0.05$) compared to the control, ^ddenotes significant difference ($P < 0.01$) compared to the aging mice.

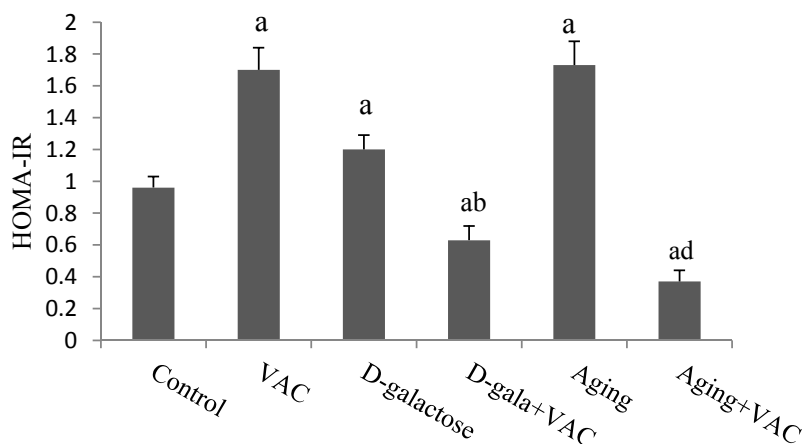


Fig. 3. Effect of *Vitex agnus-castus* (VAC) hydroalcoholic extract on homeostasis model assessment-estimated insulin resistance (HOMA-IR). ^aDenotes significant difference ($P < 0.05$) compared to the control, ^bdenotes significant difference ($P < 0.05$) compared to the D-galactose, ^ddenotes significant difference ($P < 0.01$) compared to the aging group.

Similarly, VAC extract administration reduced serum insulin levels in aged treated mice as appose to aged group ($P < 0.01$, Fig. 2). However, HOMA-IR, as an insulin resistance index, increased in D-galactose, aged and VAC groups; this parameter decreased in D-galactose and aged VAC-treated mice compared with control ($P < 0.05$). Further, the insulin resistance index showed a significant decrease in D-galactose VAC-treated mice as compared to D-galactose group ($P < 0.05$). Similar effects were observed in aged VAC-treated mice compared to aged group ($P < 0.01$, Fig. 3).

Histopathological and histomorphometric evaluation

All pancreatic sections revealed a normal appearance in control and VAC hydroalcoholic extract groups. In natural aged and D-galactose group, the diameter of islets were significantly decreased ($P < 0.05$). Cell swelling in pancreatic acini was significantly increased in these animals and these alterations were significantly attenuated by VAC extract. Corresponding data are shown in Fig. 4 and Table 1.

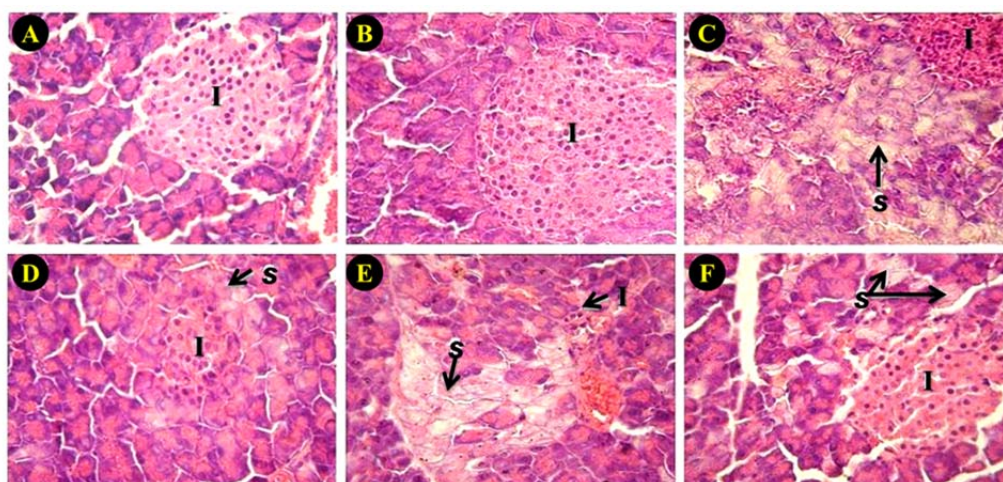


Fig. 4. Effect of *Vitex agnus-castus* (VAC) hydroalcoholic extract on pancreas histology. (A) control, (B) VAC hydro alcoholic extract, (C) D-galactose, (D) D-galactose + VAC hydro alcoholic extract, (E) aged, (F) aged + VAC hydro alcoholic extract (H&E stain, $\times 400$). (I) infiltration of inflammatory cells; (S) swelling cytoplasm.

Table 1. Effect of *Vitex agnus-castus* (VAC) extract on pancreatic histopathology of normal, D-galactose and aged mice.

| Groups | Histological features | |
|-------------------|-----------------------------|----------------------------------|
| | Cellular swelling (%) | Islet diameter (μm) |
| Control | 0 | 143.5 \pm 19.1 |
| VAC | 0 | 157.3 \pm 11.6 |
| D-galactose | 15.2 \pm 2.2 ^d | 97.7 \pm 8.9 ^d |
| D-galactose + VAC | 4.1 \pm 1.1 ^{dc} | 114.3 \pm 9.7 ^{dc} |
| Aged group | 9.3 \pm 1.7 ^d | 69 \pm 7.4 ^a |
| Aging + VAC | 2.6 \pm 0.9 ^{dc} | 107 \pm 9.5 ^c |

^aDenotes significant difference ($P < 0.05$) compared to the control, ^cdenotes significant difference ($P < 0.05$) compared to the aged, ^ddenotes significant difference ($P < 0.01$) compared to the control, ^{dc}denotes significant difference ($P < 0.01$) compared to the D-galactose. VAC; *Vitex agnus castus*.

DISCUSSION

The present study indicates that VAC induces hyperinsulinemia and hypoglycemic effects in the normal adult mice. Various studies showed that liver and exocrine tissues have the capacity of extra pancreatic insulin production and storage (17). Del Prato, *et al.* provided that the main cause of fasting hypoglycemia with hyperinsulinemia is the inhibition of hepatic glucose production concomitant with peripheral tissue insulin resistance (18). Also, Service, *et al.* demonstrated that beta cell hyper function can produce hypoglycemia through the excessive insulin secretion, which lead to insulin resistance (19). Hence, present results in normal mice indicate that VAC extract may affect through the beta cell hyper function to

increase insulin secretion but, there was not enough opportunity for insulin receptors to utilize this hormone and leads to increase HOMA-IR. Further, the glucose carrier isoform found in peripheral tissues is regulated by insulin. Insulin stimulation promotes the fusion of these carriers with the plasma membrane and accelerates glucose transport. On the other hand, because limited numbers of carrier molecules are present in the membrane, raising the solute concentration sufficiently will saturate all the carrier molecules so that no more glucose can be bound and this limits the maximal rate of transport (20). Hence, it could be suggested that despite of the increased serum insulin levels in normal VAC-treated mice, maximal transport of glucose has been occurred in them and this event leads to increase HOMA-IR levels. Gu, *et al.* reported

that although the fasting blood glucose (FBG) were within the normal range in aged animals the fasting insulin level increased in them. This finding suggested that old rats increased insulin secretion to maintain FBG in normal levels. Therefore, hyperinsulinemia was an indirect indication of insulin resistance. There is a lot of evidence that age and free radicals play an important role in the development of insulin resistance, impaired glucose tolerance and, beta cell dysfunction (21). Hence, compared to D-galactose-induced aging model, present data suggest that natural aged animals showed less beta cell dysfunction via increased more serum insulin levels. Regarding insulin resistance, several complications such as accumulation of lipid metabolism intermediates, increased muscle atrophy and decrease in glucose carrier isoform have been occurred through the aged process (22). Further, Stella, *et al.* study indicated that VAC extract decreases serum level of glucose in diabetic rats via modifying of glucose utilization, stimulates the regeneration of beta cells, and enhances glucose transport to the peripheral tissue (6). So, present results indicate that the hypoglycemic effects of VAC extract in D-galactose and aged treated animals may be occurred through the improvement of mentioned age-related criteria and it may be more effective on glucose transport carrier or insulin resistance, but future study is required to clarify the exact mechanism of this event.

In histological assessment we found that the diameter of islets was decreased by D-galactose which may relate to a decrease in insulin secretion. The interstitial fibrosis progression has a main factor in the distortion of exocrine and endocrine pancreas histoarchitecture that is related to vascular, ductal insular alterations. Further, the progressive enhancement of the pancreatic adipose tissue was noted in the aging rats frequently which is associated with the natural degenerative event of aging (23). Cell swelling was observed in pancreatic acini. It is well known that cell swelling can lead to cell death. Treatment of animals with the VAC hydroalcoholic extract showed an increase in diameter of pancreatic islets and a decrease in

acinar cell damages. Thus, VAC may suppress apoptosis in both D-galactose and aging animals. Anti apoptotic effect of VAC has been shown in previous studies (24,25).

CONCLUSION

Present data revealed that VAC hydroalcoholic extract has a hypoglycemic and pancreatic protective effect in natural aged and aging model treated animals. Extract treatment improved hyperglycemia, insulin resistance and beta cell reduction induced by aging. Further studies are required to determine the exact mechanism of VAC on age-related changes in the pancreas.

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