Short Paper

Effects of hydro-alcoholic extract of *Vitex agnus-castus* fruit on kidney of D-galactose-induced aging model in female mice

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Summary

The aim of the present study was to evaluate the effect of a hydro-alcoholic extract of *Vitex agnus-castus* (VAC) fruit on blood urea nitrogen (BUN), creatinine (Cr) and, kidney histology of a female mouse model of D-galactose induced aging. In this experimental study, 72 NMRI mice were divided into 6 groups: control, VAC, D-galactose, D-galactose+VAC, aging, and aging+VAC. D-galactose was injected for 45 days and, VAC extract administered in the last 7 days, twice a day. Serum BUN and Cr levels were not significantly changed in the D-galactose and natural aged animals in comparison to control group. Histological changes such as nuclear pyknosis, proximal cell swelling, infiltration of inflammatory cells, tubular dilatation and, vasodilatation were observed in both D-galactose and natural aged mice. Further, glomerules diameter was decreased in them. Administration of VAC could attenuate the histological alterations. These results indicate that VAC may have beneficial effects on aging and aging related kidney disease.

Key words: Aging, D-galactose, Kidney, *Vitex agnus-castus*

Introduction

The kidney is the most susceptible organ to the development of functional and structural age-related damage (Gomes *et al*., 2009). Many morphological changes are observed in the kidneys with aging such as glomerular and tubular destructure. In addition, the number and volume of glumeroli and kidney tubules decrease in the aging process (Silva, 2005).

Vitex agnus-castus (VAC) is a small tree that belongs to the *Verbenaceae* family and grows in the whole Mediterranean, tropical and, subtropical regions of the world. The main compounds of VAC are glycosides, flavonoids, diterpenoids, steroids, and essential oils such as linoleic acid (Nabih Rashed, 2013). *Vitex agnus-castus* was used to reduce the symptoms of menopause such as hot flashes, depression, and sleep disturbances (Sakhavar *et al*., 2013). Hence, we used female animals to induce D-galactose aging model. Because the growth of the older population causes an increase in aging-related disorders, including kidney disease and their economic burden, research on anti-aging agents are necessary to improve the health of the elderly population. Thus, in present study, anti-aging effect of VAC on the kidney of mice was investigated.

Materials and Methods

Plant extraction

Desiccated VAC fruits were obtained from green grocery of Qom, Iran. The powder was macerated for 48 h using 70% ethanol and 30% water (Ahangarpour *et al*., 2013).

Experimental design

In this experimental study, 48 (3-month-old) and 24 (18–24-month-old) adult female NMRI mice (30-35 g) were used. The mice were treated according to Ahvaz Jundishapur University of Medical Sciences (AJUMS) animal care guidelines and kept at 20 ± 4°C temperature with a 12:12 h light-dark cycle. They had free access to tap water and commercial chow *ad libitum*.

After conformation of estrous cycle synchronization, the mice were randomly divided into six groups (12 mice per group) as follow:

1) Control group received saline daily for 45 days by SC and concomitant gavage of saline for 7 days twice a day.

2) VAC group received saline for 45 days and concomitant gavage of 600 mg/kg/bid VAC for 7 days (Ibrahim *et al*., 2008).

3) D-galactose group received D-galactose 500 mg/kg

daily for 45 days and concomitant gavage of saline for 7 days twice a day (Ho *et al*., 2003).
4) D-galactose+VAC group received D-galactose 500 mg/kg/daily for 45 and concomitant gavage of 600 mg/kg/bid VAC for 7 days.
5) Aging group received saline daily for 45 days by SC and concomitant gavage of saline for 7 days twice a day.
6) Aging+VAC group received saline daily for 45 days by SC and concomitant gavage of 600 mg/kg/bid VAC for 7 days.

One day after last administration, the mice were sacrificed. Serum samples were obtained from heart for evaluation of BUN and creatinine (Cr) (Ahangarpour *et al*., 2014).

Histopathological and histomorphometric evaluation

The kidneys were removed and fixed in 10% formalin solution. Six hematoxylin and eosin (H&E) slides per animal were assessment for histological changes such as cellular swelling, nuclear pyknosis, infiltration of inflammatory cells, brush border loss, tubular dilatation and vasodilatation. For each treatment, the average percentage of each criteria was determined as previously described (Mirhoseini *et al*., 2012). Infiltration of inflammatory cells was classified into 4 categories:
Normal (0), weak (1), moderate (2), intense (3) and the averages were considered. Diameter of tubules, glomerules and dilated vessels were assessed by using Motic Images Plus 2.0 image analysis software. Slides were read in a "blind" fashion (Khorsandi *et al*., 2013).

Statistical analysis

The data were analyzed using one-way ANOVA followed by post hoc LSD test and were presented as the mean±SD. $P<0.05$ was considered significant.

Results

Organ weight

Body weight in D-galactose group was significantly higher than control group ($P<0.05$). Kidney weight was significantly decreased in D-galactose group. *Vitex agnus-castus* could significantly attenuate these alterations (Table 1).

Effect of VAC on serum BUN and Cr levels

There were no significant differences in serum BUN, and Cr levels between groups. Administration of VAC did not significantly change them (Figs. 1 and 2).

Histology assay

All kidney sections revealed a normal appearance in control and VAC groups. D-galactose significantly increased vasodilation, brush border loss, tubular dilation and cell swelling in proximal tubules, while the diameter of glomerules was significantly decreased ($P<0.01$). Kidneys of natural aged animals showed similar histology to the D-galactose group. Administration of the

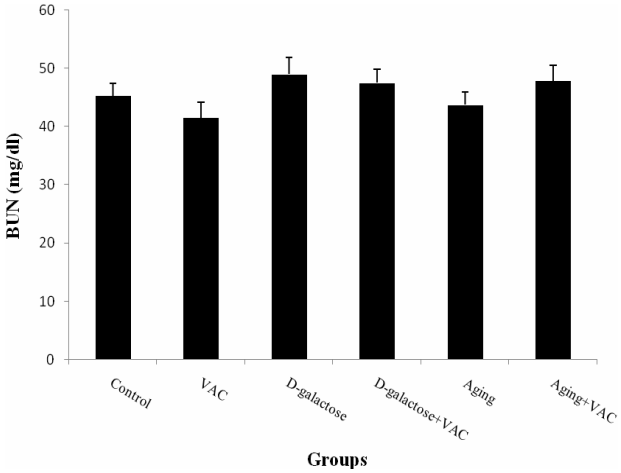

Fig. 1: Effect of VAC on BUN level. Results presented as mean±SEM

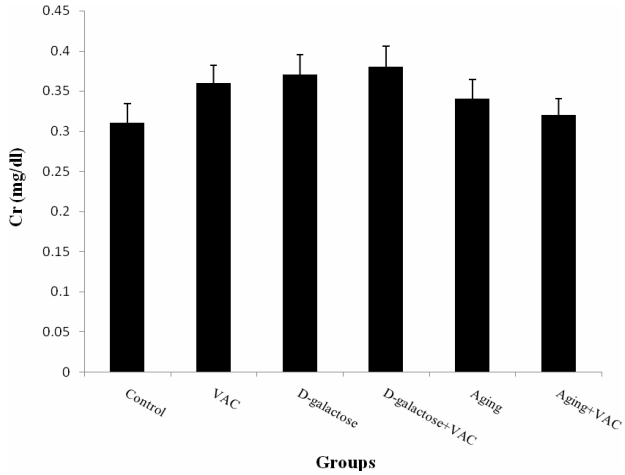

Fig. 2: Effect of VAC on serum Cr level. Results presented as mean±SEM

Table 1: Effect of VAC on body weight, kidney weight and kidney/body weight ratio in various groups (mean±SD)

Groups	Body weight (g)	Kidney weight (g)	Kidney/body weight ratio (%)
Control	29.95 ± 0.19	0.182 ± 0.01	0.607 ± 0.05
VAC	29.82 ± 1.1	0.190 ± 0.01	0.637 ± 0.05
D-galactose	32.98 ± 0.4*	0.105 ± 0.01	0.318 ± 0.03*
VAC+D-galactose	29.58 ± 0.4†	0.168 ± 0.01	0.567 ± 0.03
Aging	32.78 ± 0.42*	0.155 ± 0.01	0.472 ± 0.02*
VAC+Aging	29.82 ± 0.95$	0.178 ± 0.02	0.596 ± 0.04

* $P<0.05$, † $P<0.05$, and $ $P<0.05$ indicate comparison control, D-galactose, and aging groups, respectively

Table 2: Effect of VAC on histological criteria in various groups (mean±SD)

Histological criteria	groups					
	Control	VAC	D-galactose	D-galactose+VAC	Aging	Aging+VAC
Normal (%)	98.3	98.7	57.4±8.2**	79.3±11.5**†	59.4±9.3**	63.7±10.1$^{**\$}$
Cellular swelling (%)	0±0	0±0	21.2±5.2**	10.1±2.6**††	19.3±3.7**	17.6±4.3$^{**\$}$
Brush border loss (%)	0.9±0.1	1±0.3	7.6±2.8**	4.7±1.5**†	8.57±2.1**	7.8±1.9$^{**\$}$
Tubular diameter (µm)	27±3.4	25.3±6.1	48.4±5.1**	38.2±4.9**†	45±3.6**	25±4.2**
Vessels diameter (µm)	12.5±1.5	12.3±2	90.4±6.5**	25.3±4.3**††	89.3±7.2**	19.3±3.4$^{*\$\$}$
Glomerul diameter (µm)	99.7±9.6	102.3±11.1	56.7±6.9**	91±7.9**††	69±8.1*	84±5.5$^{\$}$
Pyknosis (%)	1.1±0.09	0.9±0.08	38.4±5.6**	11.3±4.5$^{*\$\$}$	19.3±7.1	6.4±1.1
Infiltration of inflammation cells	0	0	2.5**	0.4**††	1.8**	0.3$^{**\dagger\dagger\$\$}$

(* P<0.01, ** P<0.001), († P<0.01, †† P<0.001), and ($^{\$}$ P<0.01, $^{\$\$}$ P<0.001) indicate comparison control, D-galactose, and aging groups, respectively

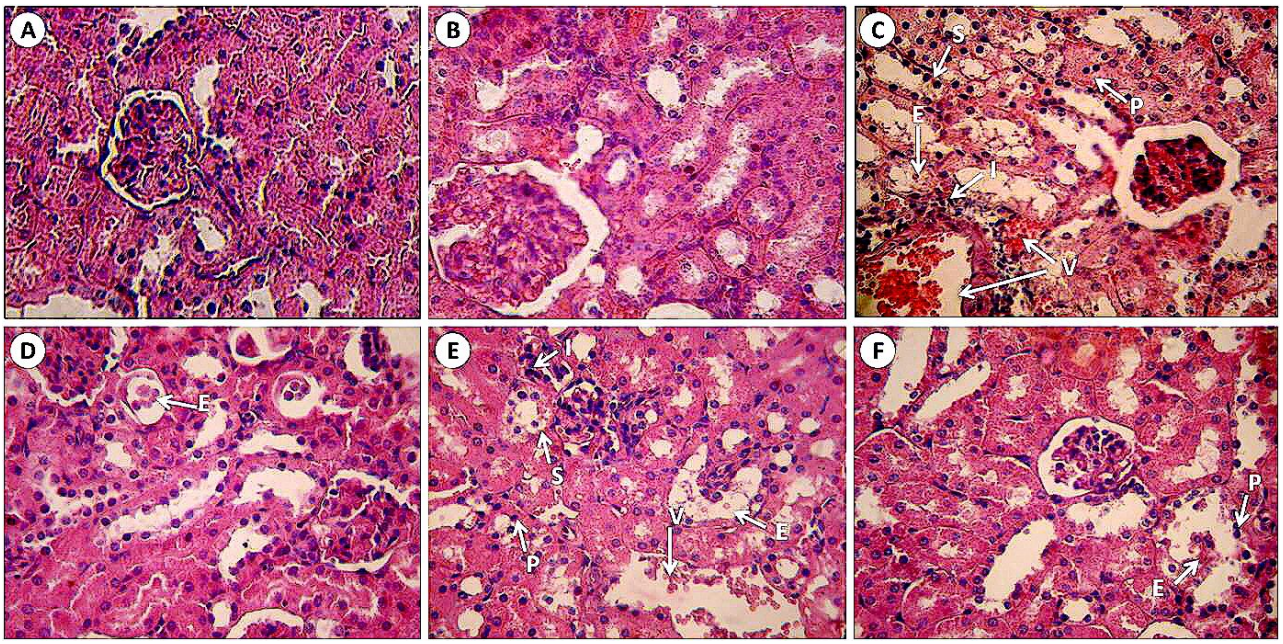

Fig. 3: Effect of VAC on kidney histology. A: Control, B: VAC, C: D-galactose, D: D-galactose+VAC, E: Aging, and F: Aging+VAC. I: Infiltration of inflammatory cells, S: Swelling cytoplasm, V: Vasodilation, P: Pyknosis, and E: Exfoliated cells, (H&E, ×400)

VAC could effectively improve (P<0.01) these changes (Table 2 and Figs. 3A-F).

Discussion

Present study demonstrates that VAC can effectively attenuate D-galactose induced aging in the kidneys of mice. As shown in the results, kidney weights were decreased in D-galactose groups. D-galactose increased proximal cell swelling, pyknosis and tubular dilation while the diameter of glomerules was decreased. Bitzer and Wiggins (2016) have revealed that the total number of glomeruli in the kidney reduces with age. As shown in the results, in aged animals, nuclear pyknosis was increased. Pyknosis is the irreversible nuclear chromatin condition of a cell necrosis or apoptosis (Kroemer *et al*., 2009). Thus reduction in kidney weight may be related to cell death in kidney tissue.

D-galactose could induce infiltration of inflammatory cells in kidney tissue. Park *et al*. (2014) and Li *et al*. (2015) have also revealed that, the D-galactose injection induces kidney inflammation. While the number of glomerules and renal tubules decrease in the aging process, the kidney vasculature appears to remain in a constant state of vasodilation to compensate for underlying sclerotic damage with aging. Renal function is then maintained despite a decrease in kidney functional reserve (Brenner *et al*., 2011). As reported in the results Cr levels and BUN were not changed in all experimental and aging groups. As a person ages, the musculoskeletal system atrophies and is replaced by fat. Hence, less Cr is produced, less Cr is excreted, and the aging kidney is able to maintain homeostasis (Radke, 1994).

Vitex agnus-castus could effectively attenuate body and organ weight as well as histological changes of kidneys in both D-galactose model and aging mice. The exact mechanism of VAC has not been clarified in this study. As mentioned in the introduction, VAC contains flavonoids identified as important antioxidants. Antioxidants are able to scavenge free radicals, which have destructive effects on many tissues (Sarıbaz *et al*.,

2007).

Vitex agnus-castus could suppress inflammation in kidney tissue. Antiinflammatory effects of VAC components were previously reported (Ku *et al*., 2014). The appearance of inflammatory cells in kidney tissue suggests that the D-galactose can interact with proteins and enzymes in the interstitial tissue of the kidney, interfering with the antioxidant defense mechanism and leading to generation of reactive oxygen species, which in turn may induce an inflammatory response (Orazizadeh *et al*., 2014). Thus the reduction in infiltration of inflammatory cells may relate to antioxidant activity of VAC. It is well known that apoptosis in aging process can reduce numbers of glomeruli and apoptosis in kidney tubules. We have also shown that the diameter of glomerulus increases by VAC in aged animals. Thus, VAC may suppress apoptosis in both D-galactose and aged mice and subsequently increase kidney weight. The antiapoptitic effect of VAC has been shown in previous studies (Wang *et al*., 2015).

Until now, use of VAC has been limited to improvement in menopausal signs such as hot flash. Present study demonstrates that VAC can reduce aging alterations in kidneys besides female reproductive system. This information provides a stimulus for true clinical investigations.

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Conflicts of interest

The authors have no conflicts of interest to report with respect to this paper.

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