The effect of *Marrubium vulgare* on contractile reactivity of aorta in diabetic rats

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Abstract

BACKGROUND: The incidence of atherosclerosis and cardiovascular diseases increases in diabetes mellitus patients. Therefore, the effects of a two-month oral administration of *Marrubium vulgare* (MV) on contractile reactivity of isolated aorta in an experimental model of diabetic rats were evaluated in the present study.

METHODS: Male Wistar rats (n = 44) were randomly divided into control, MV-treated control, diabetic, and MV-treated diabetic groups. For induction of diabetes, streptozotocin (STZ) was intraperitoneally administered (60 mg/kg). MV-treated groups received MV mixed with standard pelleted food at a weight ratio of 1/15. After 2 months, contractile reactivity of aortic rings to potassium chloride (KCl) and noradrenaline was determined using isolated tissue setup.

RESULTS: Serum glucose levels showed significant increases in the diabetic group at 4th and 8th weeks ($P < 0.001$), while this increase was not observed in MV-treated diabetic group at the 8th week. In addition, the latter group showed a lower contraction to KCl ($P < 0.05$) and noradrenaline ($P < 0.05$) as compared to the diabetic group. Meanwhile, there was no significant difference between the control and MV-treated control groups regarding contractile reactivity.

CONCLUSION: It can be concluded that oral administration of MV for 2 months could attenuate the contractile responsiveness of the vascular system which may prevent the development of hypertension in diabetic rats.

Keywords: *Marrubium vulgare*, Vascular System, Diabetes Mellitus, Contractile Response, Rat.

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Introduction

Diabetes mellitus is one of the most common endocrine disorders with an increasing trend in humans.[1] Elimination or relative reduction of insulin in this disease is accompanied with acute and chronic metabolic disorders and other complications such as retinopathy, vascular involvement, neuropathy, skin lesions, and cardiovascular disorders.[2] Several factors like increased free oxygen radicals due to elevated blood sugar level, and intensified lipid peroxidation increase the incidence of atherosclerosis and cardiovascular disorders. The main objective of diabetes mellitus treatment is to cause normoglycemia, and to prevent or postpone diabetes complications. Heterogeneity of the disease calls for finding effective compounds with the least complications to treat diabetes mellitus and its consequent disorders.[3] Although herbs and their derivatives have always been discussed as treatments of diabetes mellitus and its complications, they have not been reliably proved to be efficient.[4] However, the beneficial effects of Marrubium vulgare (MV) have been documented. It is evident that the administration of this herb causes clear hypoglycemia, reduces serum cholesterol and triglyceride, and has a beneficial effect on carbohydrate and lipid metabolism.[4] Furthermore, MV and similar herbs have high concentrations of protective and antioxidant compounds, like flavonoids,[5] which are effective in attenuating oxidative stress caused by oxygen free radicals that occur in diabetes.[6] Sahpaz et al. found that phenylpropanoid esters derived from MV have anti-inflammatory properties via inhibition of cyclooxygenase.[7] Moreover, its hypotensive effect in experimental hypertension model, its appetizing and antitussive effects, and its application in acute bronchitis and maldigestion have been confirmed.[8] Therefore, the main objective of the present study was to investigate the effects of long term, oral administration of MV (for 2 months) on contractile responses of isolated aorta in experimental diabetic male rats.

Materials and Methods

This experimental study used 44 male Wistar rats (Pasteur Institute, Tehran) weighing 230 to 280 g. All

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animals were housed in groups of 3-4 in each cage at 20-22°C. They had access to tap water ad libitum. They consumed pellets with or without certain amount of MV for two months. In order for the rats to adapt with the new environment, all tests were conducted at least two weeks after their settlement.

MV enriched feed pellets were prepared by picking the aerial part of the plant in August. Physical health of the plant was approved and then the plant was ground. The powder was mixed at a weight ratio of 1:15 with powdered feed to make pelleted feed again.[9] In the present study, only male rats with non-fasting blood sugar less than 250 mg/dl were used. They were randomly allocated into 4 groups of control, control using MV, diabetic, and diabetic using MV. To make rats diabetic, a single dose of streptozotocin (STZ) (60 mg/kg) dissolved in cold normal saline solution was infused intraperitoneally.[10] The volume of the infused solution for each rat was 0.5 ml. Serum glucose was measured using glucose oxidase kit before starting the project, in the fourth and eighth weeks. Two months later, rats were anesthetized using ether. After dissecting their chest, thoracic aorta was removed and put in Krebs solution (which was continuously perfused with carbogen). Krebs solution used in all tests comprised of NaCl (118.5 mmol), KCl (4.74 mmol), $CaCl_2$ (5.2 mmol), $MgSO_4$ (1.18 mmol), $NaHCO_3$ (24.9 mmol) $KHPO_4$ (1.18 mmol), and Glucose (10 mmol).[10]

While aorta was in cold Krebs solution, it was cleaned off of connective tissue, and then cut into 4 mm rings. To make sure of endothelium health, after causing contraction using 10^{-6} M noradrenaline, acetylcholine (10^{-5} M) was added to the tissue bath. Observing more than 30% relaxation in aorta rings was the criterion for detecting healthy endothelium.[10] To record responses of aorta rings, they were put parallel to one another using L shaped platinum wires. They were connected to a glass hook on one side and to an isometric transducer (F-60) on the other. After applying a resting tension of 2 g to aorta rings, the tissue was left for 60-90 minutes to be stable. The Krebs solution was changed every 30 minutes. After reaching equilibrium, the tissue was exposed to increasing concentrations of KCl (5-10 mM), and noradrenaline (10^{-9} to 10^{-4} M). To record and analyze data, Physiograph I software (Behineh Arman Co., Tehran, Iran) was used. Contractile response was recorded as g/mm^2 in all tests. To measure cross sectional area (CSA), the conventional method described by Abebe et al. was used.[10] To compare results of weight, and serum glucose parameters in each group before and after the study, repeated measures analysis of variance (ANOVA) was used. To compare groups with regard to the results of vascular contractions, one-way ANOVA, and Tukey's post-hoc test were used. The significance level was considered as $P < 0.05$.

Results

In the present study, the weight of rats was determined in the week before the intervention, and in the fourth and eighth weeks after the intervention (Table 1). There was no significant difference among the groups before the intervention in this regard. In addition, untreated diabetic rats had a significant weight reduction in the fourth ($P < 0.01$) and the eighth ($P < 0.005$) weeks as compared to the week before the intervention. The diabetic group using MV had a significant weight reduction in the eighth week as compared with the week before intervention, and to a lesser extent than the untreated diabetic group ($P < 0.01$). In addition, the difference between the treated and untreated groups was significant only in the fourth week ($P < 0.05$).

In this study, serum glucose levels were determined in the week before the intervention, and in the fourth and eighth weeks after the intervention (Table 1). No significant differences were found between the groups before the intervention. However, in untreated diabetic rats, there was a significant increase in glucose level in weeks 4 and 8 ($P < 0.001$) as compared with the week before the intervention. Furthermore, treating rats with MV did not change glucose level in the control group in the fourth and eighth weeks after the intervention as compared with the week before the intervention. Moreover, treating diabetic rats with MV did not make any significant changes in serum glucose levels in weeks

Table 1. The effect of oral administration of MV on weight and serum glucose level of control and diabetic rats in week 0 (before the intervention), week 4, and week 8

	Body weight			Serum glucose (mg/dl)		
	Week 0	Week 4	Week 8	Week 0	Week 4	Week 8
Control	294 ± 4.9	310.5 ± 6.3	325.6 ± 6.1	132.8 ± 11.5	119.8 ± 11.2	133.8 ± 10.2
Control + MV	305.8 ± 6.2	313.9 ± 8.1	318.9 ± 8.2	147.1 ± 5.2	151.2 ± 8.1	142.8 ± 6.8
Diabetic	294.6 ± 6.8	224.1 ± 11.4*	198.1 ± 8.7†	124.6 ± 6.8	418.6 ± 12.5$^{¥}$	405.4 ± 13.1$^{¥}$
Diabetic + MV	301.9 ± 7.9	263.1 ± 9.5	227.9 ± 9.7*	135.6 ± 9.5	369.2 ± 13.7$^{¥}$	351.8 ± 13.9$^{¥}$

* $P < 0.01$, † $P < 0.005$, $^{¥}$ $P < 0.001$ (in comparison with the week before the intervention)

4 and 8 as compared to the untreated diabetic group.

Contractile response to KCl and noradrenaline on aortic rings with endothelium after 2 months followed a concentration dependent model (Figure 1). Maximum contractile response for KCl (50 mM) and noradrenaline (10^{-4} M) did not show significant differences between the two groups of control and control using MV. Furthermore, diabetes increased aortic ring response to KCl and noradrenaline. In addition, treating diabetic rats with MV significantly decreased maximum contractile response to KCl and noradrenaline as compared with the untreated diabetic group. Maximum contractile responses to KCl and noradrenaline in the treated diabetic group were respectively 20.3% ($P < 0.005$) and 19.1% ($P < 0.05$) less than that in the untreated diabetic group.

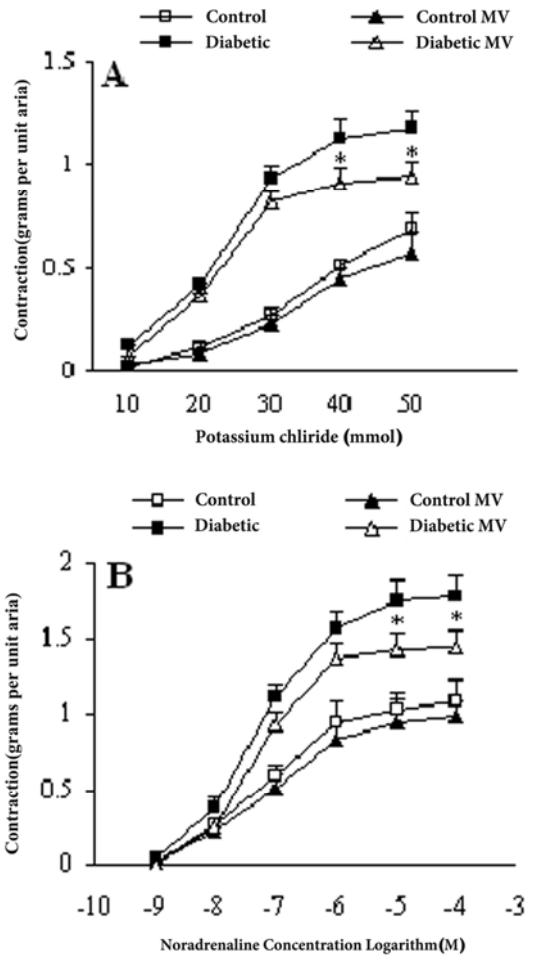

Figure 1. Contractile response to KCl (A) and noradrenaline (B) in different groups after 2 months.
MV: Marrubium vulgare
*$P < 0.05$ (as compared with the diabetic group)

Discussion

The findings of the present study showed that serum glucose level in the diabetic group treated with MV did not significantly reduce as compared with that in the diabetic group. However, treatment with aerial part of MV significantly reduced maximum contractile response to nonspecific agonists of KCl, and specific agonists of noradrenaline, as compared with the untreated diabetic group.

Various mechanisms are involved in disturbing the structure and function of blood vessels in diabetes mellitus. For instance, the capacity of the endothelium in synthesis of vasodilators such as prostacyclin and nitric oxide is reduced and vasoconstrictors such as endothelin are produced profusely. Although there is no definite evidence about the role of chronic hyperglycemia in causing macrovascular complications of diabetes, some believe hyperglycemia per se and its consequent intensified oxidative stress cause such complications.[11] Recent studies have shown that in diabetes mellitus, glucose metabolism and protein glycosylation produce free oxygen radicals. Increased free radicals and reduced antioxidant defense have an important role in causing atherosclerosis and increased permeability and sclerosis of blood vessels. In addition, in diabetic patients, production of free radicals through autoxidation of glucose, activation of cyclooxygenase pathway, and production of active oxygen by carbohydrates and lipids are increased.[12] Our results showed that contractile response of aortic rings with endothelium to noradrenaline and KCl in diabetic male rats significantly increased as compared to healthy animals, which is in line with the results of other studies.

The present study revealed that oral administration of MV for two months can reduce maximum contractile response in diabetic rats after adding KCl and noradrenaline to samples with endothelium. Although the chemical nature of active ingredients of MV with antidiabetic and vascular protection properties is not completely known, its beneficial effects can be attributed to active ingredients especially flavonoids with antioxidant properties.[5] It is evident that diabetes is caused by increased glycosylation of various substrates in target regions, and by intermediating final and complicated products of glycosylation. Increasing oxidative stress caused by free radicals, active oxygen, intensified lipid peroxidation and proteolysis creates serious complications in diabetic people and animals.[11-13] Therefore, it is probable that plants similar to MV can decrease diabetes complications because of containing flavonoid

compositions with antioxidant properties.[14,15] It has been proved that polyphenyls and phenylpropanoids in the aerial parts of the plant reduce lipid peroxidation at cell level that causes unfavorable functional and structural changes in cardiovascular system in diseases like diabetes mellitus.[16]

Conclusion

In conclusion, long-term oral administration of aerial part of MV is effective in reducing contractile response of vascular system and probably prevents hypertension in diabetic rats. However, it is recommended to do more extensive researches to determine the mechanism of reducing contractile response of vascular system

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