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Effect of apigenin on endothelium-dependent relaxation of aorta in streptozotocin-induced diabetic rats

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Abstract

Objective: The current study aimed at evaluating the effect of flavonoid apigenin on contractile response and relaxation of aorta in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods: Thirty-two rats were randomly divided into four groups of control, apigenin, diabetic (DM), and DM + apigenin. DM was induced in rats using intraperitoneal (i.p.) injection of STZ at 60 mg/kg. The two treatment groups received apigenin at a dose of 10 mg/kg i.p. every other day for four weeks. At the end of the study, the contractile responses of aorta to potassium chloride (KCL), phenylephrine (PE), and the relaxation response of the aorta to acetylcholine (ACh) were measured.

Results: Treatment with apigenin significantly reduced plasma glucose in diabetic rats in the 2nd and 4th weeks as compared to those of the DM group (P = 0.01). The DM+apigenin group had less contractile response as compared to that of the DM group, although the difference was not statistically significant (P >0.05). Treatment of diabetic rats with apigenin could significantly reduce the maximum contractile response as compared to that of the DM group (P = 0.05). The contractile response to ACh was significantly higher in the DM + apigenin group as compared to that of the DM group (P = 0.05).

Conclusion: The results of the current study indicated the hypoglycemic effects of apigenin in diabetic rats. In addition, it was observed that the administration of apigenin to diabetic and healthy rats could reduce contractile response of aorta to PE and increase the relaxation response to ACh.

Keywords: Apigenin, Diabetes mellitus, Aorta, Streptozotocin, Endothelium

1. Introduction

Diabetes mellitus (DM) is one of the most important non-communicable diseases. The increasing prevalence and various complications of the disease made it a great dilemma of the health system of different communities. The prevalence of DM in adults in 2017 was estimated more than 450 million people and expected to reach about 700 million by 2045. In addition, about 5 million people died of DM in 2017 and the direct medical costs were US$ 850 billion (1). Vascular complications caused by DM, e g, endothelial dysfunction, are among the commonest causes of mortality and morbidity in patients with DM (2). These vascular complications are associated with exacerbation of lipid peroxidation and oxidative stress caused by hyperglycemia in patients with DM. Therefore, the improvement of antioxidant defense is a therapeutic goal in DM (3). Owing to the increase in human knowledge about the variation of DM, finding compounds with minimal side effects to treat diabetes

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and its related disorders is of particular importance. Since ancient time, medicinal plants and their derivatives, including polyphenols and flavonoids, have a special place in medical sciences to treat DM and also cardiovascular diseases due to their antioxidant properties and lower side-effects (3, 4). Apigenin is a flavone and an aglycone derived from glycosides found in many fruits. Results of previous studies indicate hypoglycemic effects of this component (5-9). Also, its beneficial effects on cardiovascular system and protective effects on endothelium of aorta against oxidative stress and vascular relaxation are confirmed (10, 11). Due to the importance of finding alternative therapies in DM and their complications, the current study aimed at evaluating the effect of apigenin on contractile response and relaxation of the aorta in diabetic rats.

2. Materials and Methods

2.1. Animals

The current study was performed on 32 Albino Wistar male rats (Pasteur Institute of Iran, Tehran) weighing 220-310 g. Conditions for animals were provided according to protocols and standards recommended by the National Institutes of Health of the United States (NIH) for the maintenance and use of laboratory animals and existing facilities. Animals were kept in cages, four rats per cage, at 22-24°C with free access to drinking water and rodent chow (Pars Feed Co., Iran).

2.2. Methodology

Rats used in the current study were maintained under normal conditions, without fasting, with serum glucose levels of <250 mg/dL. Serum glucose level was measured taking retro-orbital blood samples, based on glucose oxidase technique (ZiestChem Diagnostics Co., Iran). Then, the rats were randomly assigned into four groups of control, apigenin, DM, and DM + apigenin.

To induce diabetes in the two decided groups, rats were injected intraperitoneally (i.p.) with a single dose of 60 mg/kg of streptozotocin (STZ) (Sigma-Aldrich, USA) dissolved in cold saline. Ten days after STZ injection, diabetic rats were identified by urine test strips based on urine glucose level. The two treatment groups received apigenin (Sigma-Aldrich, USA) 10 mg/kg i.p. every other day for four weeks. Rats were re-examined for serum glucose and weight at the end of the 2nd and 4th weeks.

2.3. Measurement of contractile response and relaxation of aorta

At the end of the procedure, the rats were anesthetized, and after opening the chest, the aorta was removed and placed in cold Krebs-Henseleit solution (pH 7.4); then the aorta was separated from the surrounding graft tissue and cut into 4-mm rings. In the current study, acetylcholine (Ach) was used to induce relaxation and phenylephrine (PE) to induce contractile response. To measure aortal contractile response, aortal rings were connected to a metal hook by parallel L-shaped platinum wires, from one side, and to the F-60 isomeric transducer (Narco Biosystem, USA) on the other side. Hence, the amount of stretch was transferred to the amplifier and data were recorded and analyzed. In the current study, the initial tension applied to aortic rings was 1.5 g. After applying the initial stretch, the tissue was left for 60-90 minutes to get back to normal state. Then, the increasing concentrations of potassium chloride (KCl) (10-50 mM) was used to ensure the health and integrity of the tissue, and tissue sections with a weak contractile response (<0.2 g at 50 mM KCL) were excluded from the study. Then, the rings were exposed to increasing concentrations of PE (10-9 to 10-5 M), and as soon as endothelium-dependent contraction of aorta reached its minimum, Ach was added to the tissue bath cumulatively at 10-9 to 10-4 M concentrations. The log concentration-response curve for ACh was drawn. In all of the vascular contractile and relaxation responses, the contraction level was reported as gram per unit area of tissue and the degree of relaxation as a percentage of the maximum contraction at the presence of PE (12,13).

2.4. Statistical analysis

In the current study, the results were expressed as mean ± standard error. One-way ANOVA and Tukey post hoc test were employed for intergroup comparisons at different intervals and concentrations and $P<0.05$ was considered as the level of significance.

3. Results

3.1. Animals' weight

Evaluation of the body weight of rats had no significant difference between the groups in a week prior to the study onset, while in the 4th week, the body weight of rats in the DM groups significantly decreased, compared to baseline ($P=0.01$). The difference between DM and DM + apigenin groups in the 2nd and 4th weeks was not significant (Fig. 1).

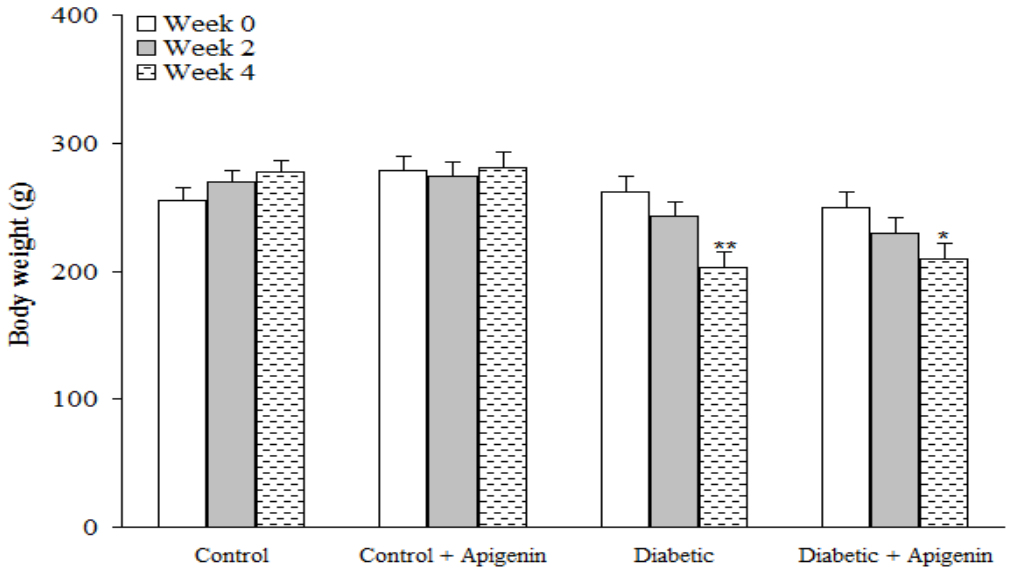

Figure 1. Body weight changes prior to and throughout the study in all four study groups, *P<0.05 **P <0.01 as compared to baseline of the same group (Repeated measures ANOVA).

3.2. Serum glucose level

There was no significant difference in serum glucose level among the groups in a week prior to the study onset. In DM groups, a significant increase was observed in serum glucose level in the 2nd and 4th weeks. Treatment with apigenin led to a significant decrease in serum glucose level of the DM group in the 2nd and 4th weeks (P = 0.01) as compared with those of the DM group in the same weeks (Fig. 2).

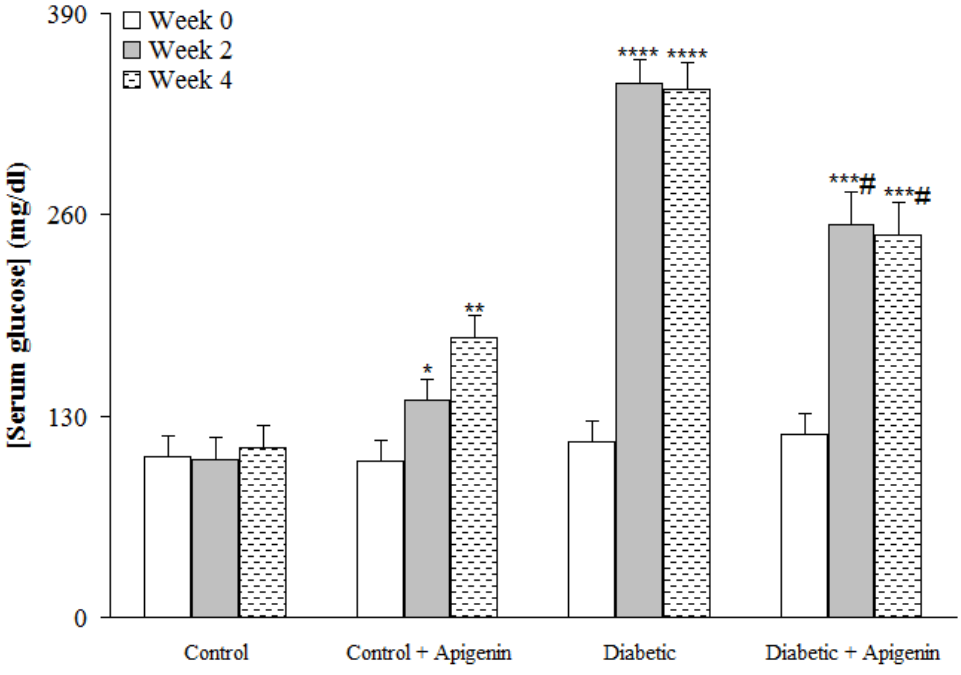

Figure 2. Serum glucose changes prior to and throughout the study in all four study groups, *P<0.05 **P<0.01 ***P<0.005 ****P<0.0005 compared to baseline of the same group, #P <0.01 compared to DM group of the same week (Repeated measures ANOVA).

3.3. Contractile response of aorta to KCL

As shown in Figure 3, the contractile response of aorta to KCL in the study groups had a concentration-dependent manner. For this purpose, the increasing KCL concentrations from 10 to 50 mM were used to induce contractile response. According to the study results, the highest contractile response belonged to the DM group, however, compared to that of the control group the difference was statistically insignificant. The DM + apigenin group had a lower contractile response compared to that of the DM group, although the difference was statistically insignificant. The DM + apigenin group also showed a lower contractile response compared to that of the control group, although the difference was not statistically significant (Fig. 3).

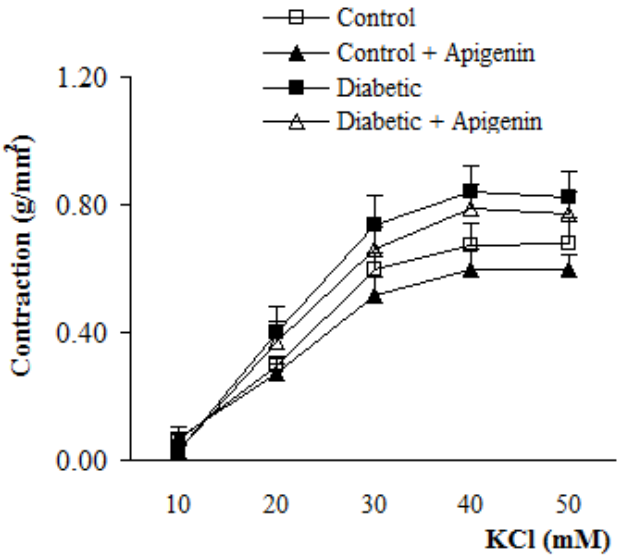

Figure 3. Contractile response of aorta to KCL in all four study groups

3.4. Contractile response of aorta to PE

As shown in Figure 4, the contractile response of aorta to PE in different groups had a concentration-dependent manner (10^{-9} to 10^{-5} M), and DM significantly increased the responsiveness of aortic rings to PE at $\geq 10^{-7}$ M (P = 0.01). Treatment of diabetic rats with apigenin could significantly reduce the maximum contractile response of aorta compared to that of the DM group (P = 0.05). In the case of the DM + apigenin group, a decreasing trend was observed at $\geq 10^{-7}$ M in comparison with that of the control group; however, the difference was statistically significant only at the dose of 10^{-5}M (P = 0.05) (Fig. 4).

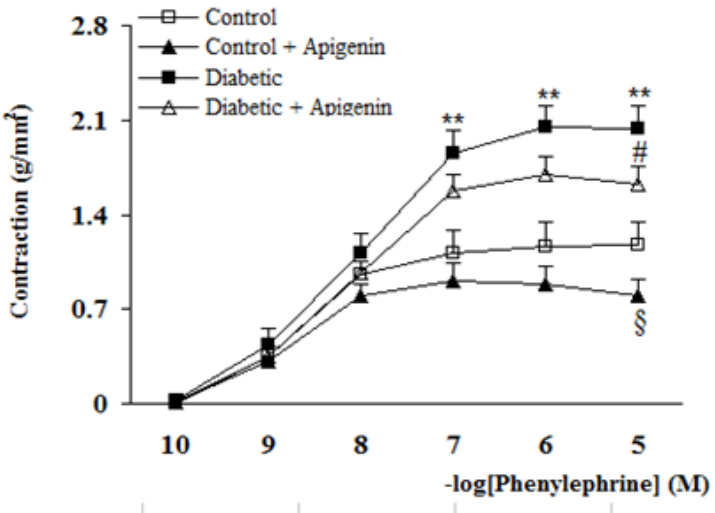

Figure 4. Contractile response of aorta to PE in all four study groups, **P<0.01 compared to Control group, #P<0.05 compared to DM group, §P<0.05 compared to Control group (One-way ANOVA).

3.5. Relaxation response of aorta to ACh

The relaxation response of aorta to Ach had a concentration-dependent manner; hence, the response to relaxation increased with increasing Ach concentration (Fig 5). Ach, at 10^{-9} to 10^{-4} M concentrations, was used to induce a relaxation response. The results indicated that DM can significantly reduce the contractile response of aorta at $\geq 10^{-6}$ M and more compared with that of the control group (P = 0.05) and (P = 0.01) in the DM + apigenin group compared with the DM group. In the DM + apigenin group, the relaxation response to Ach was significantly higher at $\geq 10^{-6}$ M and more compared to that of the DM group (P = 0.05 and 0.01, respectively). In the DM + apigenin group, the relaxation response at $\geq 10^{-6}$ M was higher than that of the control group (P <0.05); the difference was statistically significant at 10^{-5} M (P = 0.05) (Fig. 5).

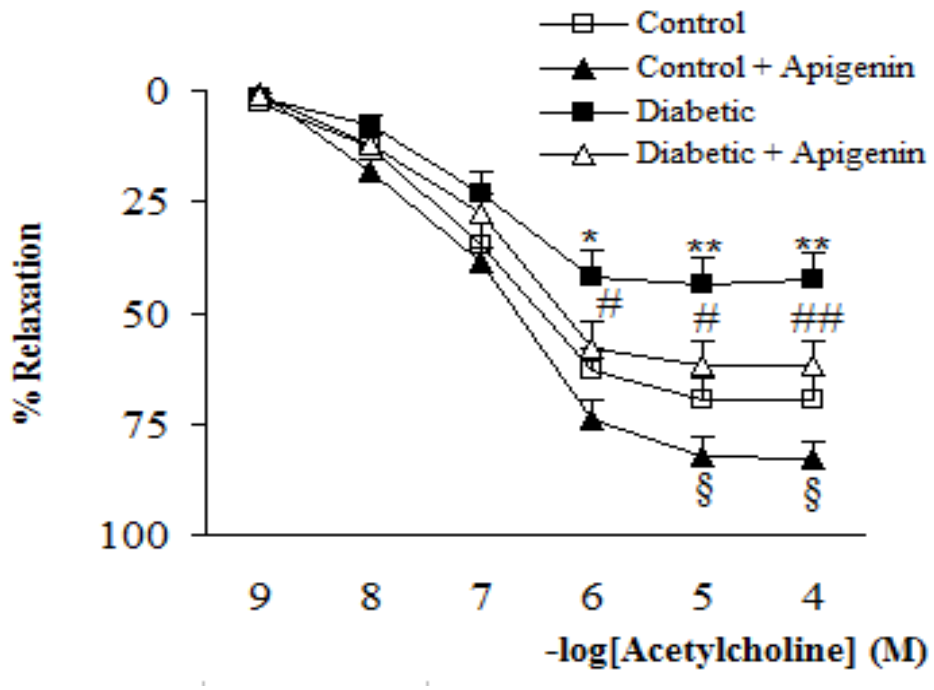

Figure 5. Contractile response of aorta to ACh in all four study groups, *P<0.05 **P<0.01 compared to Control group, #P<0.05 ##P<0.01 compared to DM group, §P<0.05 compared to Control group (One-way ANOVA).

4. Discussion

The findings of the current study on the effect of apigenin in STZ-induced diabetic rats revealed that administration of this flavonoid at the dose of 10 mg / kg for four weeks in STZ-diabetic rats caused a significant decrease in serum glucose level in the DM + apigenin group, but had no effect on their weight loss. In studies by Liu et al., (9) and Qin et al., (7) apigenin also reduced serum glucose levels in STZ-induced diabetic rats. The results of these studies were consistent with those of the current study. In this regard, it was shown that flavonoid and polyphenolic compounds can reduce their blood glucose by hypoglycemic effects (3). Apigenin is also one of the flavonoid-rich compounds (6); therefore, its effects on reducing serum glucose levels can be justified. In addition, similar to the results of the current study, in the studies by Malik et al., (5) and Mahajan et al., (8) chronic administration of apigenin to STZ-induced diabetic rats did not have an effect on the weight of rats, which seems that the hypoglycemic effect of apigenin is independent of its impact on weight.

In the current study, the contractile response of endothelial aortic rings to PE in diabetic rats significantly increased, compared to those of the control group; consistent with the findings of Mahdavi et al. (13). This finding can be justified due to the pathological effects of DM, since DM and hyperglycemia disrupt the structure and function of the vessels through various mechanisms and increase the contractile response. In DM, the production of vasoconstrictors such as endothelin and some prostaglandins increase and on the other hand, the production of endothelium-derived vasodilators such as nitric oxide (NO) and prostacyclin decreases. In addition, the increased production of free radicals and oxidative stress, increased intracellular diacylglycerol, followed by increased intracellular calcium and the activation of protein kinase C, followed by alteration of genes transfer into endothelial cells, glucose metabolism disorders, and non-enzymatic

glycosylation of proteins and nitric oxide synthase are other mechanisms that cause disruption in the function and structure of endothelium and even more complications (12,14).

In the current study, the endothelium-dependent contractile response of aortic rings was significantly lower in diabetic rats than the control ones. Since the relaxation response of aorta to Ach exerts via the production of NO by endothelium, and in DM, due to oxidative stress and production of active oxygen species, reduced activity of NO synthetase and increased amount of asymmetric dimethyl arginine, result in the reduced production of NO (15), however, it seems that in the diabetic animals of the current study, NO production also reduced, and accordingly, the contractile response was lower.

In the current study, it was found that apigenin reduced the maximum contractile response of aortic rings to PE and increased the relaxation degree of aortic rings contracted in response to Ach. To explain the beneficial effects of apigenin on vascular responses, evidence from previous studies on the antioxidant effects of this compound are noteworthy. Findings of the study by Suh et al., indicated that apigenin can effectively prevent damage to pancreatic beta cells, dose- and time-dependently, which can be attributed to its antioxidant and oxidative stress decreasing effects (16). In addition, Wang et al., suggested that apigenin can protect the pancreatic beta cells from tissue damage caused by oxidative stress; therefore, this substance can be useful to treat DM and its complications (17). The anti-oxidative-stress effect of apigenin may have also occurred in the current study, resulting in decreased vascular dysfunction. Similar to the results of the present study, Jin et al., showed that apigenin can improve impairments in aortic relaxation response under oxidative stress conditions by affecting NO (10). Therefore, some of the beneficial effects of apigenin on vascular responses in the current study can be attributed to the NO mediation system. Also, Sui et al., in a study on the effect of apigenin endothelium-dependent relaxation of aorta in rats showed that apigenin can dose-dependently cause relaxation in the PE-contracted aorta via intensifying the activity of NO synthetase, increasing NO synthesis, activation of potassium inhibitory channels, and inhibition of calcium channels (11). Therefore, it seems that increased relaxation response and decreased contractile response in the presence of apigenin in the current study were partly due to the mediation of NO system and the ion channels of the membrane, which of course requires further investigation. In addition, in a study by Yamagata et al., it was found that apigenin, by reducing the harmful effects of high level of serum glucose and inhibiting the effects of TNFα, reduced endothelial cell dysfunction and the risk of cardiovascular disease, and since DM is associated with cardiovascular complications, can also have beneficial effects in this regard (18).

Conclusion

The results of the current study showed that administration of apigenin cannot significantly reduce the weight of DM animals, but has hypoglycemic effects on them. In addition, it was observed that administering apigenin in diabetic and healthy rats reduced contractile response of aorta to PE and increased the relaxation response of aorta to ACh.

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Conflict of interest

The authors declared no conflict of interest.

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