



## Salvigenin has Potential to Ameliorate Streptozotocin-induced Diabetes Mellitus and Heart Complications in Rats

Hamid Mohammad Sadeghi<sup>1</sup>, Amirhossein Mansourabadi<sup>2\*</sup>,  
Mohammad Ebrahim Rezvani<sup>1</sup>, Mojtaba Ghobadi<sup>1</sup>, Nastaran Razavi<sup>3</sup>  
and Mahmood Bagheri<sup>4</sup>

<sup>1</sup>Department of Physiology, Shahid Saddughi University of Medical Science, Yazd, Iran.

<sup>2</sup>Department of Immunology, Shahid Saddughi University of Medical Science, Yazd, Iran.

<sup>3</sup>Shahrekord University of Medical Science, Shahrekord, Iran.

<sup>4</sup>Department of Medical Physics, Shahid Saddughi University of Medical Science, Yazd, Iran.

### Authors' contributions

This work was carried out in collaboration between all authors. Author HMS designed the study, wrote the protocol and wrote the first draft of the manuscript. Author AM managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process. Author MER identified the species of plant. All authors read and approved the final manuscript.

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### ABSTRACT

**Objectives:** Flavonoids are the active ingredients, found in herbal remedies for amelioration the variety of disorders. Salvigenin is a plant flavenoid, which is found in *Salvia officinalis*. Salvigenin has an antioxidant, anti-inflammatory, anti-tumor and vascular relaxant activities. This study was conducted to evaluate the possible antidiabetic and cardioprotective effects of salvigenin.

**Materials and Methods:** 32 wistar rats were made diabetic using streptozotocin (60 mg/kg, i.p.). Rats were divided into four groups that treated with salvigenin at doses of 5, 10 or 25 mg/kg. All the treatments were administered orally for 4 weeks. At the end of the experiment, the blood samples

\*Corresponding author: E-mail: [a.mansourabadi.67@gmail.com](mailto:a.mansourabadi.67@gmail.com);

were collected for determining the FBS, HbA1C, insulin, triglyceride, cholesterol and HDL. After 72 hrs, animals were anesthetized; hearts were removed quickly and mounted on Langendorff apparatus. The max pressure, heart rate, max dP/dt, contractility index and coronary flow were measured.

**Results:** Administration of salvigenin exhibited a significant reduction in fasting serum glucose, triglycerides, total cholesterol, HbA1c and increased level of plasma insulin and HDL in diabetic rats ( $P<0/05$ ). Salvigenin could significantly increased hemodynamic indices such as max pressure, max dP/dt, contractility and coronary flow ( $P<0/05$ ).

**Conclusion:** Salvigenin improved diabetes through decreasing blood glucose, lipid profile, HbA1c. Increased insulin secretion can be a mechanism for antidiabetic effect of salvigenin. Regarding the antidiabetic and cardioprotective effects of salvigenin, it can be concluded that this flavonoid compound can be potentially used to reduce diabetes and it's cardiovascular complications.

**Keywords:** Type 1 diabetes; Salvigenin; isolated hearts system (Langendorff); HbA1c; rats.

## 1. INTRODUCTION

Diabetes is an increasing metabolic disorder resulting from a defect in increased insulin secretion, insulin action or both. Defects in insulin secretion or signaling leads to chronic hyperglycemia associated with disturbances in the metabolism of carbohydrates, fats and proteins [1]. Hyperglycemia and glycation and the subsequent neuronal dysfunction, dysfunction of kidney and other organs are the long-term consequences of blood-sugar during diabetes [2,3]. Diabetes causes secondary patho-physiologic changes in multiple organs of the body, and cardiovascular diseases are one of the consequences of the disease. Increased incidence of congestive heart failure, myocardial infarction, reduced muscle strength and arrhythmias have been observed in people with diabetes [4]. This disorder is due to coronary artery disease and cardiomyocyte dysfunction [5].

Now, the main treatment for diabetes is the effective use of drugs such as insulin and amino analogues, sulfonylureas and Biguanides. In recent years, in order to control, prevent and delay the development of diabetes outcomes, the use of herbs and drugs derived from them is increasing [6]. Many active compounds are found in plants, such as guanidine in Galga and myricetin in okra, that a synthetic form of them has been identified and even used to treat diabetes [5]. One of the plants, that has anti-diabetic effects and there is considerable evidence for proving that in traditional medicine [6] and also in scientific papers [7-11], is *Salvia officinalis*, which naturally grows in Asia and the Mediterranean [12,13].

*Salvia*, the largest genus of the Lamiaceae family, includes about 900 species, spread throughout the world. Many species of *Salvia*, including *Salvia officinalis* L. (sage), have been used as traditional herbal medicine against a variety of diseases [7]. In its leaves and root, there are compounds such as caffeic acid, gallic acid and flavonoids such as Salvigenin, terpenes and polyphenol such as tannins [14,15]. Salvigenin (5-hydroxy 6, 7, 4 tri methoxy flavones) is an active flavonoid in the leaves and aerial part of this plant and other plants that has anti-diabetic effect [11,15]. So far, direct evidence for anti-diabetic effects of this flavonoid hasn't been reported yet. Eidi et al. [16] have studied the effect of oral administration of wild garlic, which contains flavonoid, on blood-glucose level and blood lipids of rats with type 2 diabetes. This study showed that oral administration of wild garlic in the diet of diabetic rats significantly reduced the blood glucose, cholesterol and triglycerides. In another study, Raafatian et al. in [17], have studied on salvigenin protective effect on SH-SY5Y cells against hydrogen peroxide treatment and found out that Salvigenin protects cells against the oxidative stress induced by hydrogen peroxide by increasing Otto phage and by weakening apoptosis. Anti-tumor effects [18], neuron-protection [17] and compounding with DNA [18] of this flavonoid have been reported in previous studies. On the other hand, in many studies, the effect of flavonoids in food, on increased insulin sensitivity and on increased insulin secretion have been expressed. In this study, the effect of salvigenin extracted from *Salvia officinalis*, on type 1 diabetes and cardiovascular dysfunction have been evaluated.

## 2. MATERIALS AND METHODS

### 2.1 Extraction of Methanolic Plant Material

Sage leaves were collected from Mashhad city, IRAN, in summer and identified in Ferdowsi Department of Mashhad University (Voucher number: 043722, deposited in: Farabi Herbarium, Director: Dr. B. Hamidi). The plant was cleaned, shed dried at 25°C, and the dried leaves of the plant were ground with a blender, and the powder was kept in nylon bags in a deep freezer until the time of experiments. About 60 g of powder were submitted to extraction with 300 ml methanol (80%) in a Soxhlet apparatus for 72 h. After extraction, the solvent was filtered and then evaporated under reduced pressure by Rotavapor. The H-NMR spectrum of the extract showed that the extract contains flavonoids.

### 2.2 Extract Purification

For isolation and purification of compounds in the plant extracts, column chromatography (CC) was used. For this purpose, a column of silica gel (230-400 mesh ASTM) (100 cm × 2 cm with gel 60 and particle size from 0.040 to 0.063 mm, Merck product), mixed with hexane were used.

### 2.3 Animals

Thirty two Wistar rats weighing 300±25 that bred in the animal house of Shahid Sadoughi Medical School, Yazd, Iran were selected. Animals were housed at controlled temperature (22± experiments reported 2°C) with a 12 h-light/dark cycle and with standard lab chow and tap water *ad libitum* were kept in special cages. This study was carried out in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals [19].

### 2.4 Induction of Diabetes

The overnight fasted adult wistar rats were induced diabetic with streptozotocin (STZ, ENZO, Argentina) 65 mg/kg, i.p (The STZ was dissolved in the citrate buffer 0.01 M, PH:4.5). Diabetes was proved by measuring the fasting blood-glucose concentration 72 h after injecting STZ. The wistar rats with the blood-glucose level above 300 mg/dl were considered to be diabetic.

### 2.5 Treatment Groups

Diabetic rats were randomly divided into four groups of eight rats: control group administrated

normal saline daily and treatment group received orally 5, 10 and 25 Salvigenin mg/kg daily for four weeks. Four weeks after gavage, cardiovascular parameters were measured in all groups.

### 2.6 Blood Sampling

After 4 weeks of treatment, blood samples were drawn from the retro-orbital sinus. Fasting blood sugar (FBS) were determined by enzymatic method of glucose oxidase. The serum insulin levels were estimated by using the radioimmunoassay kit (Diasorin, Italy). Hemoglobin A1C, lipid profile and body weight were measured [20,21]. Triglycerides [22], serum cholesterol [23], high-density lipoprotein [24] and glycosylated hemoglobin A1C were measured too.

### 2.7 Langendorff Apparatus

The Langendorff apparatus was used for perfusion of isolated rat heart [25,26]. Briefly, treated rats were anesthetized with sodium thiopental (75 mg/kg, i.p.) and heparinized with heparin (1000 IU, i.p.). Then, the hearts were removed immediately and aortas were cannulated and perfused retrograde with Krebs-Ringer solution containing (mM): NaCl 118.0, KCl 4.7, CaCl<sub>2</sub> 1.25, KH<sub>2</sub>PO<sub>4</sub> 1.2, MgSO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25.0, and glucose 11.0 that equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at about 37°C. The PH of perfusing solution was controlled 7.35-7.45. The constant pressure of perfusion was 100 cm H<sub>2</sub>O. Ventricular pressure was recorded continuously by an adjustable water filled probe that inserted into the left ventricle via left atria and linked to pressuring transducer (AD instruments, Australia). Electrocardiogram (EKG) was recorded permanently through two electrodes placing on apex and one placing on the base of ventricles (AD instruments, Australia). The temperature of cardiac jacket was maintained about 37°C using warm water recycle. Coronary flow was collected manually at various times (at baseline and at 5, 15, 30, 60, 90 minutes of reperfusion).

### 2.8 Statistical Analysis

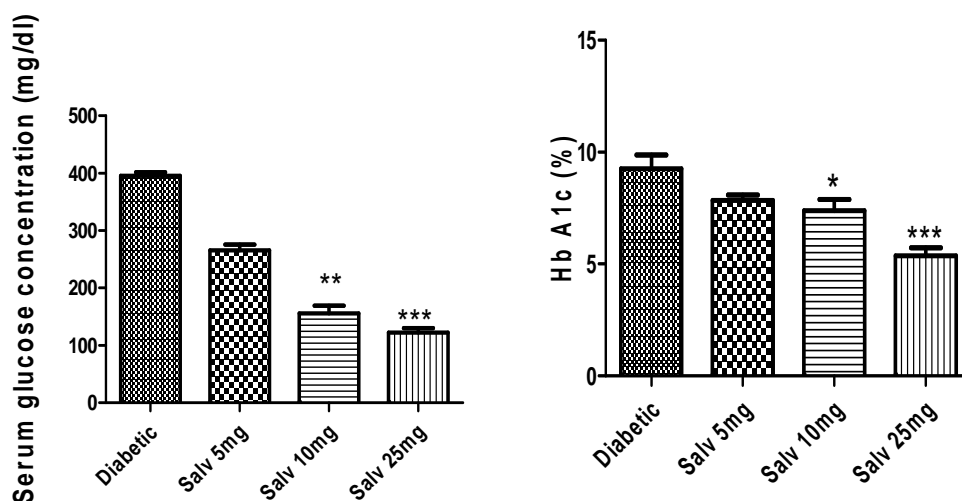
Data were expressed as mean ± SEM and were analyzed by statistical software (version 5.0, Statview, St. Louis, MO) using one-way analysis of variance (ANOVA) followed by Tukey's test and cardiac index by two-way ANOVA (Two-Way ANOVA). p < 0.05 were considered significant.

### 3. RESULTS

As shown in Fig. 1, Salvigenin administered at doses of 5, 10 and 25 milligrams per kilogram, as a significant and dose-dependent, decreased fasting glucose in diabetic animals ( $p < 0.05$ ). Also Salvigenin at all doses have no significant dose-dependent effect on glycosylated hemoglobin (Hb A1c) ( $p < 0.05$ ). Insulin in the group receiving 5 mg ( $p < 0.05$ ) and 10 mg Salvigenin significantly increased ( $p < 0.01$ ) (Fig. 2).

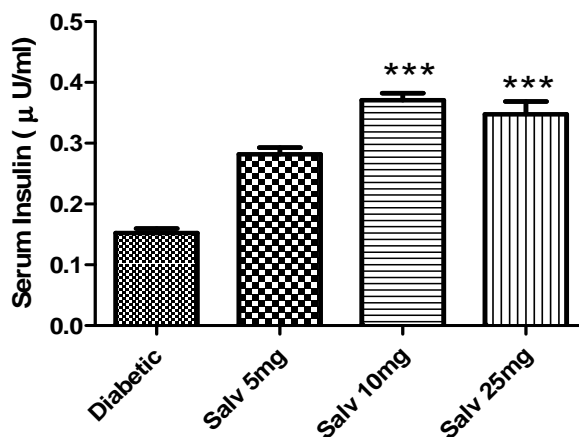
Fig. 3 shows that long-term administration of Salvigenin at doses of 10 and 25 mg per kg were significantly reduced body weight of diabetic animals ( $p < 0.05$ ).

Furthermore, Salvigenin effect on blood lipid profile is shown in Fig. 4. The levels of triglycerides, total cholesterol and LDL cholesterol were significantly reduced at all doses ( $p < 0.01$ ). On the other hand, only at a dose of 25 mg per kg could significantly raise HDL cholesterol levels in the blood ( $p < 0.01$ ).



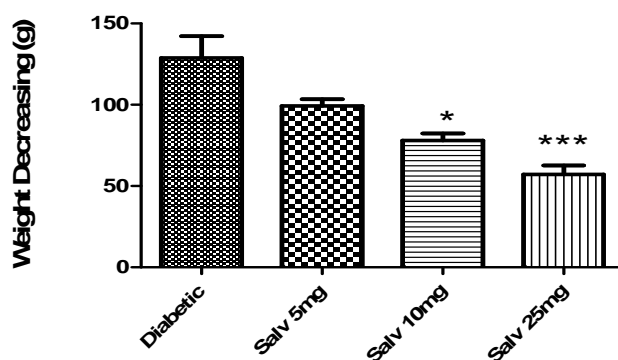
**Fig. 1. Salvigenin effect on fasting blood glucose and glycosylated hemoglobin in streptozotocin-induced diabetic rats. As is clear Salvigenin has different dose levels of fasting blood glucose and glycosylated hemoglobins were significantly reduced)**

\*\*\*: significant difference from the control group ( $p < 0.001$ ). \*\*: Significantly different from the control group ( $p < 0.01$ ). \*: significant difference from the control group ( $p < 0.05$ )

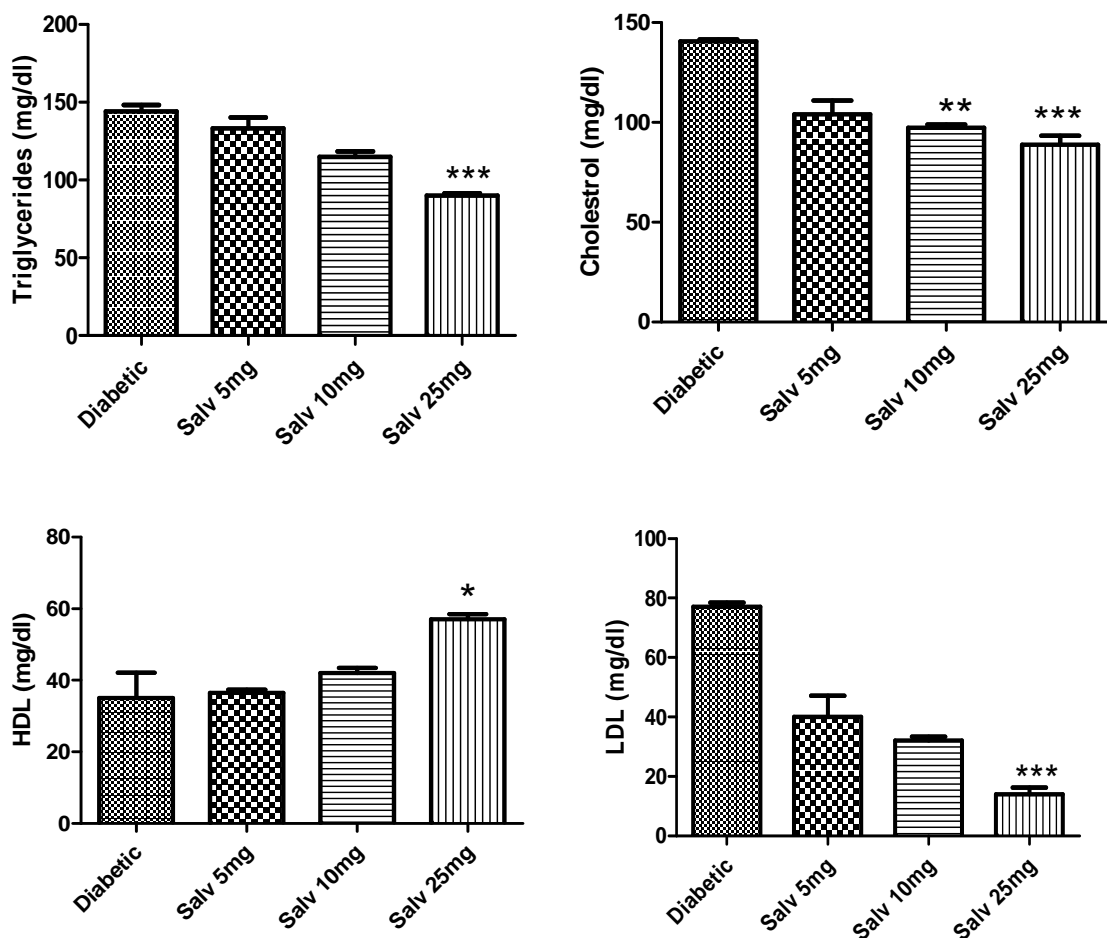


**Fig. 2. Salvigenin effect on plasma insulin in streptozotocin-induced diabetic rats. As it is evident at doses of 5 and 10 mg Salvigenin able kg significantly increased plasma insulin levels**

\* And \*\*, respectively, a significant difference with  $p < 0.05$  and  $p < 0.01$  in the control group



**Fig. 3. Salvigenin effect on body weight in streptozotocin-induced diabetic rats. As it is evident at doses of 25 and 10 has Salvigenin mg per kg of body weight loss was significantly inhibited**  
 \* And \*\*, respectively, a significant difference with  $p < 0.05$  and  $p < 0.01$  in the control group



**Fig. 4. Salvigenin effect on serum lipid levels in streptozotocin-induced diabetic rats. Various forms above shows that Salvigenin has different dose levels of triglycerides, and total cholesterol was significantly reduced. Salvigenin whereas the HDL level was effective only at high doses, it significantly raises**

\*\*\*: significant difference from the control group ( $p < 0.001$ ). \*\*: Significantly different from the control group ( $p < 0.01$ ). \*: significant difference from the control group ( $p < 0.05$ )

### 3.1 Effect of Salvigenin on Cardio Metabolic Risk Factors in Diabetic Rats

Below the maximum pressure, at times before ischemia, immediately after ischemia, 15 and 30 minutes after ischemia, Different doses of Salvigenin has a maximum pressure in the left ventricle after ischemia times, but this increase was not significant compared with the control group ( $p < 0.001$ ) (Table 1).

The heart beat Rate before ischemia, 15 and 30 minutes after ischemia were measured. heart rate increased after administer of doses 10 and 25 mg Salvigenin after ischemia but this was not significant compared to control group ( $p < 0.05$ ) (Table 2).

The maximum rate of pressure change in different periods, before ischemia, immediately after ischemia, 15 and 30 minutes after ischemia is shown in Table 3. at doses of 10 and 25 mg,

Salvigenin has a maximum rate of pressure change at different times. This increases of maximum rate of pressure change at doses of 25 mg before ischemia ,and 10 mg at 30 minutes after ischemia was significant compared to controls ( $p < 0.01$ ) (Table 3).

Ventricular contraction time before ischemia, immediately after ischemia, 15 and 30 minutes after ischemia is shown in Table 4. Salvigenin at doses of 10 and 25 mg can increase contraction of the left ventricle at different times significantly compare to the control group ( $p < 0.001$ ) (Table 4).

The results show that the discounted rate on coronary flow in all groups after ischemia and ischemia in all groups, the more time passes the coronary flow rate decreases. Salvigenin coronary flow rate for the group that received a dose of 10 mg kg at all times to find a significantly increased compared to controls ( $p < 0.001$ ) (Fig. 5).

**Table 1. Effect of different doses Salvigenin the maximum pressure in the left ventricle before and after ischemia**

Time	Control	5 mg Salvigenin	10 mg Salvigenin	25 mg Salvigenin
Basal time	69.12	77.63	69.50	66.56
ischemia	0	0	0	0
0 min after ischemia	70.07	77.18	114.52***	107.54***
15 min after ischemia	80.34	84.47	100.32**	110.62***
30 min after ischemia	80.86	84.93	102.99*	105.83**

\*\*\*: Significant difference from the control group ( $p < 0.001$ ) \*\*: Significantly different from the control group ( $p < 0.01$ ). \*: Significant difference from the control group ( $p < 0.05$ )

**Table 2. Effect of different doses of Salvigenin on heart rate before and after ischemia**

Time	Control	5 mg Salvigenin	10 mg salvigenin	25 mg salvigenin
Basal time	210.62	205.87	215.93	225.87
ischemia	0	0	0	0
0 min after ischemia	190.512	156.61	211.37	198.79
15 min after ischemia	210.53	209.20	186.91	185.97
30 min after ischemia	193.45	209.49	200.37	205.54

**Table 3. Effect of different doses of Salvigenin on maximum rate of pressure change per unit before and after ischemia**

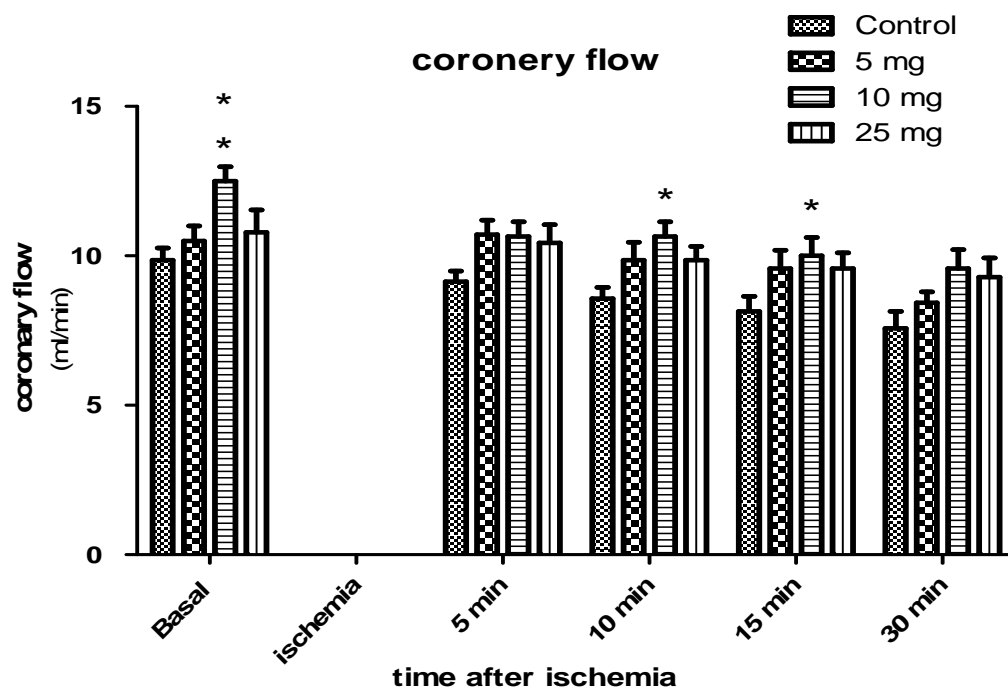
Time	Control	5 mg salvigenin	10 mg salvigenin	25 mg salvigenin
Basal time	2447.11	2360.17	2766.3	2531.06
ischemia	0	0	0	0
0 min after ischemia	3020.85	3005.2	3067.4	3191.25
15 min after ischemia	2417.62	2545	2874.5	3208.86***
30 min after ischemia	2368.28	2537.61	2922	3213.7**

\*\*\*: Significant difference from the control group ( $p < 0.001$ ). \*\*: Significantly different from the control group ( $p < 0.01$ )

**Table 4. Effect of different doses of Salvigenin on the ability of left ventricular contractility of before and after ischemia**

Time	Groups	Control	5 mg salvigenin	10 mg salvigenin	25 mg salvigenin
Basal time		44.43	45.79	60.19**	65.15***
ischemia		0	0	0	0
0 min after ischemia		39.87	43.31	57.39**	61.27***
15 min after ischemia		42.83	42.56	62.08***	64.83***
30 min after ischemia		38.49	40.48	51.20*	57.26***

\*\*\*: Significant difference from the control group ( $p < 0.001$ ). \*\*: Significantly different from the control group ( $p < 0.01$ ). \*: Significant difference from the control group ( $p < 0.05$ )

**Fig. 5. Effect of different doses of Salvigenin on coronary flow after ischemia**

\*\* : Significant difference from the control group ( $p < 0.01$ )

#### 4. DISCUSSION

The results show that Salvigenin, flavonoids extracted from *Salvia officinalis*, reduced blood sugar and glycosylated hemoglobin in rats with type 1 diabetes. This flavonoid increases plasma insulin level in diabetic rats. Consumption of salvigenin can reduce the level of triglyceride, cholesterol and level of LDL in plasma. Consumption of salvigenin can increase level of HDL in plasma in animals with type 1 diabetes. In addition, salvigenin protects rats with type 1 diabetes from severe weight loss.

However, there have been studies in scientific resources on anti-diabetic and metabolic effects of *Salvia officinalis*, from which salvigenin has

been extracted, but no studies have been reported on salvigenin metabolic effects on diabetic and non-diabetic animals. Medium- and long-term administration of hydro-alcoholic extract of leaves of *Salvia officinalis*, reduced fasting glucose in diabetic animals. Another study showed that oral administration of infused *Salvia officinalis* will increase liver cells respond to insulin and can inhibit new glucose production of these cells and will reduce blood glucose of health animals [11]. Eidi et al showed that *Salvia officinalis* leaf extract increases plasma insulin levels and have shown hypoglycaemic activity on rats with type 2 diabetes [27].

Anti-diabetic properties of this plant can be attributed to flavonoid compounds. Flavonoids,

like Salvigenin, are compounds that can help to improve diabetes with different mechanisms.

The results of this study suggest that weight loss in type 1 diabetes is controlled by Salvigenin. Other studies have shown that weight loss in type 1 diabetes are due to protein and fat catabolism and dehydration caused by polyuria [28]. Also, salvigenin has an important role in preventing protein and fat degradation and dehydration by decreasing fasting blood sugar and glycosylated hemoglobin [29,30].

Effects of flavonoids on reducing glycosylated hemoglobin levels have been reported in many studies [31,32]. Glycosylated hemoglobin level shows the average amount of glucose in the past 2 or 3 months [33,34] and its great importance in the prevention of diabetes complications and early glycation products [34]. Increased level of glycosylated products stimulates specific receptors of these products on the surface of macrophages and increases the production of inflammatory mediators such as IL-6 and TNF $\alpha$  [35]. In this study, salvigenin could control the progress of this process by reducing glycosylated hemoglobin level and, therefore, control diabetes. Studies have shown that most of the flavonoids control inflammation by controlling the activity and production of enzymes that produce eicosanoids [36]. As salvigenin is not a flavonoid, its anti-diabetic effect can be due to induced inflammation caused by streptozotocin and high-fat food.

evidence indicates that oxidative stress plays an important role in the pathogenesis of chronic diseases such as diabetes. Free radicals that are the base of oxidative stress, are produced in the case of diabetes, because of the oxidation of glucose, non-enzymatic glycosylation of proteins and oxidative decomposition of this glycosylated products [37]. Flavonoids, due to the phenolic groups, have a higher anti-oxidant effect than other compounds present in herbs [38]. Since it is widely accepted that the antioxidant compounds have beneficial effects on chronic diseases such as diabetes [31,39] and the antioxidant effect of salvigenin has been proved [40], it is possible that part of its anti-diabetic effects are caused by its anti-oxidant effect.

Furthermore, in previous studies and the present study show that flavonoids increase insulin secretion, insulin response and the number of beta cells in diabetic animals [41]. It is likely that increases of blood insulin level, maybe related to

flavonoid effects on increases of insulin secretion, cell response to insulin and increased number of beta cells. The numbers of beta cells and cell sensitivity to flavonoids have not been studied in this study but insulin level have been evaluated.

Studies have shown that polyphenolic compounds reduce cardiovascular complications of diabetes by eliminating free radicals and controlling diabetes parameters [42-47]. Free radicals are produced by different mechanisms. Increased activity of xanthine oxidase in reperfusion injury phase produces superoxide ions that are damaging free radicals. Flavonoids, such as salvigenin, with their antioxidant effect [48], can reduce oxidative damage of ischemia-reperfusion injury by inhibiting the activity of xanthine oxidase [49,50].

Polyphenolic compounds increase insulin resistance by increasing bioavailability of nitric oxide in the endothelium and by reducing the formation of reactive oxygen and nitrogen [51]. Several studies suggest a relationship between insulin resistance and endothelial dysfunction [52]. A number of flavonoids improve endothelial function [53]. Antioxidants improve the function of endothelial cells in patients with diabetes, expressing that oxidative stress plays an important role in the dysfunction of endothelial cells [54,55].

Flavonoid intake may reduce the risk of death from coronary disease [56-58,42]. Daily consumption of flavonoid-rich plants such as tea may reduce the risk of atherosclerosis and coronary disease, as well as a preventive effect against stroke [59-61].

The protective effects of polyphenols against cardiovascular disease in general, is attributed to the ability of these compounds to modulate endothelial function, antioxidant and anti-inflammatory properties, induced nitric oxide production and expansion of blood vessels, inhibiting the hyperactivity of platelets, inhibiting proliferation and angiogenesis [62-64]. The epidemiological evidence and clinical studies on this subject in recent years has emphasized that Polyphenols have a significant impact on prevention of cardiovascular disease, especially coronary heart disease and myocardial infarction [65-67].

Aides Dugan et al. have studied on the relaxant effect of salvigenin flavonoid on rat aorta. Their



results indicated that salvigenin is a flavonoid affecting vessel relaxant and this action is mediated by nitric oxide and endothelium-derived prostacyclin. Vessel relaxant effect of this flavonoid is like apigenin and both of them do it by NO. Flavones effectively overcome the contraction of the entry of calcium into cells or released intracellular calcium. In addition, other mechanisms, such as inhibition of intracellular protein kinase C, or nucleotide phosphodiesterase or activated potassium channels may attend by vascular relaxant effect of these flavonoids. Recent studies show a relationship between the protective effects due to their antioxidant activity that causes the production of endothelial NO. As a consequence of this vessel relaxant effect, salvigenin that is mediated by endothelium, can prevent Oxidative damage and cardiovascular consequences of diabetes [68].

## 5. CONCLUSION

In general it can be concluded that salvigenin as a flavonoid can do its anti-diabetic effects through reduction of blood glucose and by decreasing glycosylated hemoglobin, increasing insulin secretion and improving lipid profiles. To know more about its anti-diabetic mechanisms and its use in treatment, further studies are necessary. According to previous studies and positive effects of salvigenin flavonoid on hemodynamic parameters in diabetic hearts, it can be concluded that this flavonoid compound can be used to control oxidative stress and cardiovascular complications of diabetes.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

As this study performed on animals, human ethical approval is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care*. 1996;19(3): 257-67.
2. Gumieniczek A, Hopkala H, Wójtowicz Z, Nikolajuk J. Changes in antioxidant status of heart muscle tissue in experimental diabetes in rabbits. *Acta Biochimica Polonica-English Edition*. 2002;49(2):529-36.
3. Oberley LW. Free radicals and diabetes. *Free Radical Biology and Medicine*. 1988;5(2):113-24.
4. Haryson. *Ossol teb dakheli*. 2008;1.
5. Dhalla NS, Liu X, Panagia V, Takeda N. Subcellular remodeling and heart dysfunction in chronic diabetes. *Cardiovasc Res*. 1998;40(2):239-47.
6. Galer BS, Gianas A, Jensen MP. Painful diabetic polyneuropathy: Epidemiology, pain description, and quality of life. *Diabetes Research and Clinical Practice*. 2000;47(2):123-8.
7. Khattab HA, Mohamed RA, Hashemi JM. Evaluation of hypoglycemic activity of *Salvia officinalis* L. (Sage) infusion on streptozotocin-induced diabetic rats. *Journal of American Science*. 2012;8(11).
8. Lima CF, Azevedo MF, Araujo R, Fernandes-Ferreira M, Pereira-Wilson C. Metformin-like effect of *Salvia officinalis* (common sage): Is it useful in diabetes prevention? *British Journal of Nutrition*. 2006;96(02):326-33.
9. Eidi M, Eidi A, Zamanizadeh H. Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*. 2005; 100(3):310-3.
10. Eidi A, Eidi M, Shahmohammadi P, Mozaffarian V, Rustaiyan A, Mazooji A. Antidiabetic effect of *Salvia verticillata* L. aerial parts in normal and streptozotocin-induced diabetic rats. *Int J Pharmacol*. 2011;7:66-73.
11. Eidi A, Eidi M. Antidiabetic effects of sage (*Salvia officinalis* L.) leaves in normal and streptozotocin-induced diabetic rats. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. 2009;3(1):40-4.
12. Maksimović, Milka, Danijela Vidic, Mladen Miloš, Marija Edita Šolić, Sabaheta Abadžić, and Sonja Siljak-Yakovlev. Effect

- of the environmental conditions on essential oil profile in two Dinaric *Salvia* species: *S. brachyodon* Vandas and *S. officinalis* L. *Biochemical Systematics and Ecology*. 2007;35(8):473-478.
13. Sookto Tularat, Theerathavaj Srithavaj, Sroisiri Thaweboon, Boonyanit Thaweboon, Binit Shrestha. *In vitro* effects of *Salvia officinalis* L. essential oil on *Candida albicans*. *Asian Pacific Journal of Tropical Biomedicine*. 2013;3(5):376-380.
  14. Newall CA, Anderson LA, Phillipson JD. *Herbal medicines. A guide for health-care professionals: The Pharmaceutical Press*; 1996.
  15. Brieskorn CH, Kapadia R. XXIII. 5-Methoxy salvigenin in leaves of *Salvia officinalis* Pl. *Med*. 1979;35:376-8.
  16. Eidi A, Eidi M, Esmaeili E. Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. *Phytomedicine*. 2006;13(9):624-9.
  17. Rafatian G, Khodaghali F, Farimani MM, Abraki SB, Gardaneh M. Increase of autophagy and attenuation of apoptosis by Salvigenin promote survival of SH-SY5Y cells following treatment with H<sub>2</sub>O<sub>2</sub>. *Molecular and Cellular Biochemistry*. 2012;371(1-2):9-22.
  18. Habibi Z, Hassan ZM, Noori S, Mozafari V, Yoosefi-Mohammadi M-M, Hassani L. Interaction of salvigenin with DNA. *Daneshvar*. 2011;18(92):69-76.
  19. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*. 1983;16(2):109-10.
  20. Hultman E. Rapid specific method for determination of aldosesaccharides in body fluids; 1959.
  21. Hyvärinen A, Nikkilä EA. Specific determination of blood glucose with o-toluidine. *Clinica Chimica Acta*. 1962;7(1):140-3.
  22. Fossati P, Principe L. Estimation of the concentration of triglyceride in plasma and liver. *Clinical Chemistry*. 1982;28:2077-81.
  23. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clinical Chemistry*. 1974;20(4):470-5.
  24. Warnick G, Nguyen T, Albers A. Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. *Clinical Chemistry*. 1985;31(2):217-22.
  25. Lépicier P, Bouchard JF, Lagneux C, Lamontagne D. Endocannabinoids protect the rat isolated heart against ischaemia. *British Journal of Pharmacology*. 2003;139(4):805-15.
  26. Skrzypiec-Spring M, Grotthus B, Szeląg A, Schulz R. Isolated heart perfusion according to Langendorff— Still viable in the new millennium. *Journal of Pharmacological and Toxicological Methods*. 2007;55(2):113-26.
  27. Hakim ZS, Patel BK, Goyal RK. Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. *Indian Journal of Physiology and Pharmacology*. 1997;41:353-60.
  28. Prince PSM, Menon VP, Pari L. Hypoglycaemic activity of *Syzygium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology*. 1998;61(1):1-7.
  29. Kamalakkannan N, Prince PSM. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. *Basic & Clinical Pharmacology & Toxicology*. 2006;98(1):97-103.
  30. Esmaeili MA, Zohari F, Sadeghi H. Antioxidant and protective effects of major flavonoids from *Teucrium polium* on  $\beta$ -cell destruction in a model of streptozotocin-induced diabetes. *Planta medica*. 2009;75(13):1418-20.
  31. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. *The American Journal of Clinical Nutrition*. 2002;76(3):560-8.
  32. Asgary S, Naderi G-A, Movahedian Attar A, Sajjadian A, Kafil F, Fatehi Z. Inhibitory effects of *Crataegus curvisepala*, *Salvia hydrangea*, and *Betula pendula* on in-vitro protein glycosylation. *Arya Atheroscler*. 2010;1(4).
  33. Larsen ML, Hørder M, Mogensen EF. Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. *New England Journal of Medicine*. 1990;323(15):1021-5.
  34. Rahbar S, Blumenfeld O, Ranney HM. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochemical and Biophysical Research Communications*. 1969;36(5):838-43.
  35. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of

- type 2 diabetes. *Diabetes Care*. 2004; 27(3):813-23.
36. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of Pharmacological Sciences*. 2004;96(3): 229-45.
  37. Mehta JL, Rasouli N, Sinha AK, Molavi B. Oxidative stress in diabetes: A mechanistic overview of its effects on atherogenesis and myocardial dysfunction. *The International Journal of Biochemistry & Cell Biology*. 2006;38(5):794-803.
  38. Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine & Pharmacotherapy*. 2005;59(7):365-73.
  39. Lukačínová A, Mojfiš J, Beňačka R, Rácz O, Ništiar F. Structure-activity relationships of preventive effects of flavonoids in alloxan-induced diabetes mellitus in rats. *Journal of Animal and Feed Sciences*. 2008;17(3):411-21.
  40. Eidi M, Eidi A, Zamanizadeh H. Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*. 2005; 100(3):310-3.
  41. Pinent M, Castell A, Baiges I, Montagut G, Arola L, Ardévol A. Bioactivity of flavonoids on insulin-secreting cells. *Comprehensive Reviews in Food Science and Food Safety*. 2008;7(4):299-308.
  42. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Archives of Internal Medicine*. 1995;155(4):381-6.
  43. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004;109(21 suppl 1):II-2-II-10.
  44. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Annals of Internal Medicine*. 1996;125(5):384-9.
  45. Hollman P, Katan MB. Health effects and bioavailability of dietary flavonols. *Free Radical Research*. 1999;31:S75-80.
  46. Hirvonen T, Pietinen P, Virtanen M, Ovaskainen M-L, Häkkinen S, Albanes D, et al. Intake of flavonols and flavones and risk of coronary heart disease in male smokers. *Epidemiology*. 2001;12(1):62-7.
  47. Sesso HD, Gaziano JM, Liu S, Buring JE. Flavonoid intake and the risk of cardiovascular disease in women. *The American Journal of Clinical Nutrition*. 2003;77(6):1400-8.
  48. Miura K, Kikuzaki H, Nakatani N. Antioxidant activity of chemical components from sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) measured by the oil stability index method. *Journal of Agricultural and Food Chemistry*. 2002;50(7):1845-51.
  49. Chang W-S, Lee Y-J, Lu F, Chiang H-C. Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Research*. 1992; 13(6A):2165-70.
  50. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, et al. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *Journal of Natural Products*. 1998;61(1):71-6.
  51. McCarty MF. Potential utility of natural polyphenols for reversing fat-induced insulin resistance. *Medical Hypotheses*. 2005;64(3):628-35.
  52. Kim J-a, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction molecular and pathophysiological mechanisms. *Circulation*. 2006;113(15):1888-904.
  53. Baron AD, Brechtel-Hook G, Johnson A, Cronin J, Leaming R, Steinberg H. Effect of perfusion rate on the time course of insulin-mediated skeletal muscle glucose uptake. *American Journal of Physiology-Endocrinology and Metabolism*. 1996; 271(6):E1067-E72.
  54. Dubrey SW, Reaveley DR, Seed M, Lane DA, Ireland H, O'Donnell M, et al. Risk factors for cardiovascular disease in IDDM. A study of identical twins. *Diabetes*. 1994;43(6):831-5.
  55. Pietta P, Minoggio M, Bramati L. Plant polyphenols: Structure, occurrence and bioactivity. *Studies in Natural Products Chemistry*. 2003;28:257-312.
  56. Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: A cohort study. *BMJ*. 1996;312(7029):478-81.

57. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. *American Journal of Epidemiology*. 1999;149(10):943-9.
58. Hertog MG, Feskens EJ, Kromhout D, Hollman P, Katan M. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. *The Lancet*. 1993;342(8878):1007-11.
59. Keli SO, Hertog MG, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: The Zutphen study. *Archives of Internal medicine*. 1996;156(6):637-42.
60. Yao LH, Jiang Y, Shi J, Tomas-Barberan F, Datta N, Singanusong R, et al. Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition*. 2004;59(3):113-22.
61. Hodgson JM. Tea flavonoids and cardiovascular disease. *Asia Pac J Clin Nutr*. 2008;17(Suppl 1):288-90.
62. Vita JA. Polyphenols and cardiovascular disease: Effects on endothelial and platelet function. *The American Journal of Clinical Nutrition*. 2005;81(1):292S-7S.
63. Stoclet J-C, Chataigneau T, Ndiaye M, Oak M-H, El Bedoui J, Chataigneau M, et al. Vascular protection by dietary polyphenols. *European Journal of Pharmacology*. 2004;500(1):299-313.
64. Mulvihill EE, Huff MW. Antiatherogenic properties of flavonoids: Implications for cardiovascular health. *Canadian Journal of Cardiology*. 2010;26:17A-21A.
65. Michalska M, Gluba A, Mikhailidis DP, Nowak P, Bielecka-Dabrowa A, Rysz J, et al. The role of polyphenols in cardiovascular disease. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2010;16(5):RA110-9.
66. Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, et al. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition*. 2008;88(1):38-50.
67. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*. 2005;45(4):287-306.
68. Uydeş-Doğan BS, Takır S, Özdemir O, Kolak U, Topçu G, Ulubelen A. The comparison of the relaxant effects of two methoxylated flavones in rat aortic rings. *Vascular Pharmacology*. 2005;43(4):220-6.

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