Ethyl acetate fraction of *Allium hirtifolium* improves functional parameters of isolated hearts of diabetic rats

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Abstract

Objective: *Allium hirtifolium* (Persian shallot) has a hypoglycemic effect on diabetic animals. The aim of this study was to assess the effect of the ethyl acetate fraction of *Allium hirtifolium* on the function of isolated hearts of diabetic rats.

Methods: The control and diabetic animals were randomly divided into four groups: saline- or extract-treated controls (n=10 and n=6, respectively) and saline- or extract-treated diabetic rats (n=8 and n=9, respectively), which received normal saline or extract for four weeks by daily gavage. The hearts were perfused according to the Langendorff method. Cardiac function parameters, including left ventricular developed pressure (LVDP), heart rate (HR), rate pressure product (RPP; LVDP×HR), and dp/dt were measured.

Results: The findings of this study showed that in the extract-treated diabetic rats, LVDP (94.5±9.1 mm Hg, mean±SEM), HR (249±15 beats/min), RPP (22732±1246) and +dp/dt (2598±230) at the baseline were significantly higher than those in the saline-treated diabetic animals, (71.5±4.0), (189±6), (13923±984), and (1701±124), respectively. Furthermore, RPP and HR were also significantly higher than the corresponding values obtained in the saline-treated diabetic rats after ischemia.

Conclusion: Besides blood glucose lowering action, oral administration of the ethyl acetate fraction of *Allium hirtifolium* significantly improved the baseline and post-ischemic cardiac function parameters in streptozotocin-induced diabetic rats. *(Anatol J Cardiol 2017; 17: 452-9)*

Keywords: diabetes, *Allium hirtifolium*, isolated heart, cardiac function

Introduction

Diabetes mellitus is a common disorder and an important risk factor for cardiovascular diseases (1). Diabetic patients are more prone to heart diseases, including myocardial infarction and severe ventricular arrhythmias (2). Owing to the effectiveness of herbal drugs and their low cost, they are widely prescribed, even when their biologically active constituents are not fully identified (3). *Allium hirtifolium* (Persian shallot) is a nutritive plant that belongs to the Alliaceae family (4). Based on available pharmaceutical investigations, the antioxidant and hepatoprotective effects of *Allium hirtifolium* have been demonstrated. In addition, *Allium hirtifolium* extract has antioxidant properties comparable to or slightly higher than garlic extract (5). The commonly known phytochemical compounds identified in *Allium hirtifolium* are saponins, sapogenins, and flavonoids, including shallomin, quercetin, and kaempferol (5).

Certain flavonoids in the diet can have significant cardiovascular benefits (6). *Allium hirtifolium* with a high content of flavonoids and phenolic components changes the postprandial serum lipid profile, endothelial markers, and thrombogenic factors and might be beneficial in patients with cardiovascular diseases (7). The Persian shallot significantly reduces blood glucose levels in diabetic rats (8), and aqueous shallot extract has a hypoglycemic effect on fructose-induced insulin resistance animals (9). No data is available regarding the cardiovascular effects of *Allium hirtifolium*. Given that the ethyl acetate fraction of shallot has the highest flavonoid content (38% of total flavonoid) compared with other fractions (10, 11), this study was conducted to assess the effect of the ethyl acetate fraction of *Allium hirtifolium* on the pre- and post-ischemic function of isolated hearts of diabetic rats.

Methods

This experimental animal study was designed to evaluate possible cardiac protective effects of the ethyl acetate fraction of *Allium hirtifolium* on streptozotocin (STZ)-induced diabetes in rats.

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Drugs and chemicals

Ethanol, methanol, ethyl acetate, butanol, hexane and STZ were purchased from Sigma Chemicals (St. Louis, Mo., USA). All other chemicals were purchased from Merck (Darmstadt, Germany).

Animals

All experiments were approved by the Ethics Committee of the Kermanshah University of Medical Sciences. The study was supported by Medical Biology Research Center, Kermanshah, Iran. Male Wistar rats weighing 200–250 g were housed three per cage at the constant temperature of 22°C–24°C, with a 12-hour light/dark cycle and relative air humidity of 40%–60%. The rats fed on standard pellet chow and water ad libitum. All the animals used in the present study received humane care in compliance with the institutional animal care guidelines.

Experimental protocols and animal grouping

Initially, the animals were randomly divided into two groups of diabetic and control rats. Diabetes was induced via a single intraperitoneal injection of STZ (60 mg kg^{-1} body weight) dissolved in 0.1 M cold citrate buffer (pH 4.5) (12). The control rats were just injected with citrate buffer. Five days after STZ or citrate buffer injection, blood samples were taken from the retro-orbital plexus of the median canthus under ether anesthesia (13, 14). Serum glucose level was measured using a glucose oxidase method (glucose oxidase kit, Zist Chimi, Tehran, Iran). The rats with fasting serum glucose concentration higher than 200 mg dL^{-1} were considered diabetic. Afterwards, the diabetic rats were randomly allocated into two groups: 1) the saline-treated diabetic rats (n=8) and 2) the extract-treated diabetic rats (n=9) that received normal saline or ethyl acetate fraction of shallot extract (5 mg kg^{-1} body weight), respectively, for four weeks via daily gavage.

The control rats were also randomly allocated into two groups: 1) the saline-treated control rats (n=10) and 2) the extract-treated control rats (n=6) that received normal saline or the ethyl acetate fraction of the shallot extract (5 mg kg^{-1} body weight), respectively, for four weeks via daily gavage.

The body weight and serum glucose levels were measured three times throughout this four-week period: 1) when the rats were allocated into the groups (the initial day of the experiment), 2) after two weeks of the treatment with the ethyl acetate fraction of shallot extract or normal saline, and 3) after four weeks of the treatment period (at the time of heart isolation).

Isolated rat heart preparation

Four weeks after treatment with the ethyl acetate fraction of shallot extract or normal saline through gavage, the animals were anesthetized by intraperitoneal administration of pentobarbital sodium (60 mg kg^{-1}, Sigma). The hearts were quickly removed and soaked in Krebs solution at 4°C. The aortic root was cannulated and retrogradely perfused in accordance with the Langendorff method using Krebs solution (pH 7.4) containing the following: NaCl (118 mM), $NaHCO_3$ (25 mM), KCl (4.8 mM), KH_2PO_4 (1.2 mM), $MgSO_4$ (1.2 mM), glucose (11 mM), and $CaCl_2$ (1.2 mM). The buffer was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 at 37°C. Perfusion was performed under a constant hydrostatic pressure of 85 cm H_2O. A latex balloon was placed in the left ventricle through the mitral valve. This balloon was filled with distilled water and connected to a pressure transducer (MLT 844; AD Instruments, New South Wales, Australia) through a rigid polyethylene tube. For continuous monitoring of cardiac function parameters, the pressure transducer was connected to a computer via a power lab (model ML825; AD Instruments). At the beginning of the experiment, the balloon volume was adjusted to produce an end diastolic pressure of 5–10 mm Hg. This volume was then kept constant during the experiment. The indices of myocardial function included left ventricular developed pressure (LVDP), which was defined as peak systolic pressure minus end diastolic pressure, heart rate (HR; beats/minute), and rate pressure product (RPP=LVDP×HR). Coronary flow (CF) was measured through volumetrical collection of the coronary effluent per minute. Cardiac parameters were measured after the 20 min stabilization period. After stabilization, global normothermic ischemia was induced by halting perfusion and immersing the heart in Krebs buffer at 37°C for 40 minutes. Following ischemia, the hearts were reperfused for 45 minutes (15).

Plant material and extraction

Allium hirtifolium (Persian Shallot) bulbs were purchased from a local vegetable market in Kermanshah, Iran. The bulbs were cleaned and shed dried at 25°C, and the dried material was ground in a blender. The powder was kept at –20°C until the time of experiments.

Preparation of ethyl acetate fraction was performed by successive fractionation (10, 11). Briefly, the homogenate of Persian shallot powder (100 g) was extracted with 50% (v/v) ethanol, stirring for 24 h. The extract was filtered, then centrifuged at 12000 g for 20 min at 4°C and evaporated under reduced pressure to dryness. For solvent fractionation, the extract was resuspended in distilled water and then partitioned successively with n-hexane (Hex), ethyl acetate (EA), and n-butanol (BuOH), leaving a residual aqueous fraction (Aq). Each fraction was evaporated under reduced pressure to yield Hex (0.35%), EA (0.45%), BuOH (8.7%), and Aq fractions (90.5%), respectively. Ethyl acetate fraction was used to treat the animals.

Statistics

All data are expressed as mean±SEM (standard error of mean). To test normality of distributions, the Kolmogorov–Smirnov test was applied. Regarding the normal distribution of the data, independent samples t-test was used to evaluate the significant difference between the two sets of data obtained from the independent groups. The plasma glucose concentration and body weight were analyzed using repeated measures ANOVA followed by Bonferroni as a post-test. One-way ANOVA

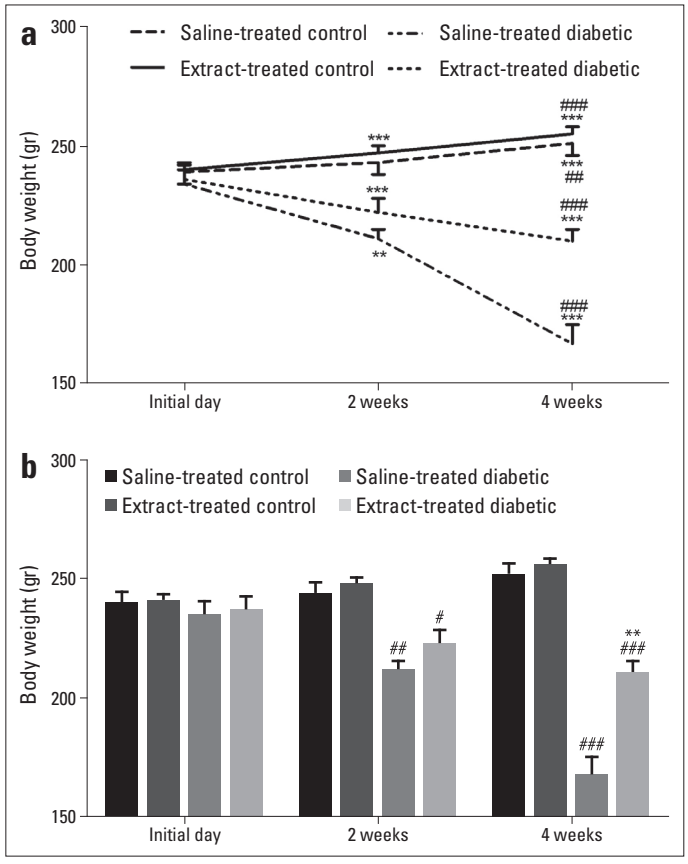

Figure 1. The effect of oral administration of the ethyl acetate fraction of *Allium hirtifolium* extract on the body weight of the animals during four weeks of the experiment. The values are expressed as mean±SEM for 6–10 rats in each group. (a) The trend of the body weight of the animals during the experiment period. Repeated measures ANOVA is followed by Bonferroni post-test. ## or ** $P<0.01$, ### or *** $P<0.001$. Symbol *, significant difference in comparison to the initial day. Symbol #, significant difference compared to week two. (b) One-way ANOVA is followed by Tukey's post-test. # $P<0.05$, ## or ** $P<0.01$, ### $P<0.001$. Symbol #, significant difference versus the saline-treated control group. Symbol *, significant difference versus the saline-treated diabetic group

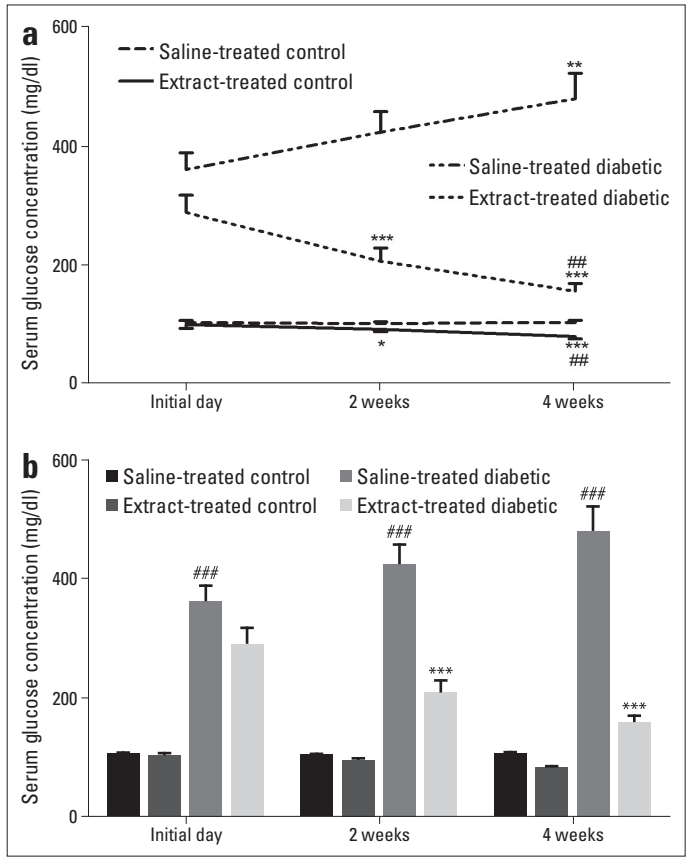

Figure 2. The effect of oral administration of the ethyl acetate fraction of *Allium hirtifolium* extract on the serum glucose levels of the animals during four weeks of the experiment. The values are expressed as mean±SEM, for 6–10 rats in each group. (a) The trend of the serum glucose concentrations of the animals during the experiment period. Repeated measures ANOVA is followed by Bonferroni's post-test. #$P<0.05$, ## or ** $P<0.01$, ### or *** $P<0.001$. Symbol *, significant difference versus the initial day. Symbol #, significant difference versus the week two. (b) One-way ANOVA is followed by Tukey's post-test. ## $P<0.01$, and ### or *** $P<0.001$. Symbol #, significant difference versus the saline-treated control group. Symbol *, significant difference versus the saline-treated diabetic group

was applied to analyze a set of independent groups and Tukey posthoc test was used for multiple comparisons as offered by GraphPad InStat version 3.0 (GraphPad Software Inc., La Jolla, CA, USA). Differences with a p value of <0.05 were considered statistically significant.

Results

Body weight

The weights of the animals during four weeks of the treatment with the ethyl acetate fraction of shallot extract or normal saline are illustrated in Figure 1a, b. Repeated measures ANOVA on the data in each group revealed that the body weight in the saline and extract-treated control rats gradually increased throughout the experiment period, whereas in the saline- and extract-treated diabetic rats this parameter significantly changed in the opposite direction (Fig. 1a).

One-way ANOVA analysis on the data showed that body weights of the different groups were the same in the initial day of the experiment while at the end of the second and fourth weeks this parameter was significantly lower in the saline-treated diabetic rats compared to the saline-treated control rats ($p<0.01$ and $p<0.001$, respectively). The results also indicated that four weeks extract administration in the diabetic rats, significantly improved weight gain in comparison to the values in the saline-treated diabetic rats ($p<0.01$) although their average body weights were still lower than those of the saline- and extract-treated controls (Fig. 1b).

Serum glucose concentration

Serum glucose concentrations of the animals in the initial day of the experiment period and two and four weeks later are demonstrated in Figure 2a, b. The analysis of serum glucose concentrations with repeated measures ANOVA revealed that in the saline-

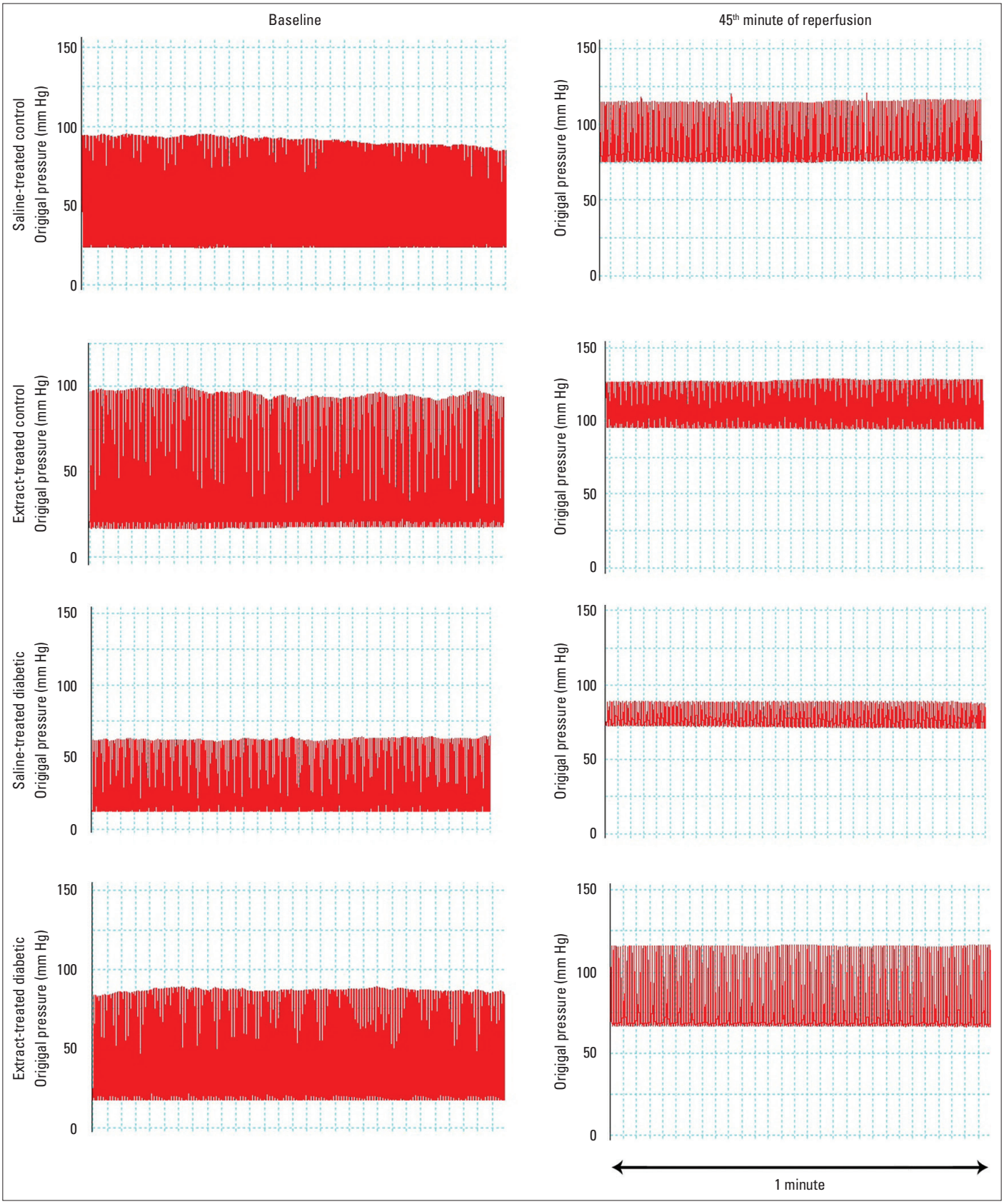

Figure 3. Typical traces of PowerLab-recorded left ventricular developed pressure before and after exposure to 40 minute global ischemia in the four experimental groups

Table 1. The effect of oral administration of ethyl acetate fraction of *Allium hirtifolium* extract on cardiac function parameters of isolated hearts of the control and diabetic groups

		Experimental groups			
Periods	**Cardiac parameters**	**Saline-treated control (n=10)**	**Extract-treated control (n=6)**	**Saline-treated diabetic (n=8)**	**Extract-treated diabetic (n=9)**
Baseline	HR	291.0±9.3	268.4±11.4	189.0±6.1$^{\#\#\#}$	249.0±15.4**
	LVDP	78.6±4.7	83.6±4.3	71.4±4.0	94.5±9.1
	RPP	22644±1210	21838±1642	13923±984$^{\#\#\#}$	22732±1246***
	CF	10.6±0.3	11.08±0.7	7.93±0.4$^{\#\#}$	8.83±0.5$^{\#}$
	+dp/dt	2221±173	2388±171	1701±124	2598±230**
	–dp/dt	-1632±93	-1912±144	-1388±82	-1819±40
45th minute of reperfusion	HR	240.8±6.1	248.3±9.6	181.9±14.3$^{\#\#\#}$	267.3±6.3***
	LVDP	34.2±2.5	37.0±5.4	21.1±1.4	32.15±5.1
	RPP	8322±473	9835±1180	3911.82±476$^{\#\#}$	8382±1174**
	CF	5.1±0.2	5.5±0.4	4.1±0.3	5.3±0.3
	+dp/dt	1049±141	728±151	411±77$^{\#}$	724±163
	–dp/dt	-825±125	-493±110	-300±52$^{\#\#}$	-492±101

CF - coronary flow, mL/minute; HR - heart rate, beats/minute; LVDP - left ventricular developed pressure, mm Hg; LVDP×HR, RPP - rate pressure product. Values has been presented as mean±SEM. # or *P<0.05, ## or ** P<0.01, ### or *** P<0.001. Significant differences versus the saline-treated control and the saline-treated diabetic groups are shown by symbols # and *, respectively. For more statistical analysis see the text

treated control rats this parameter was constant throughout the experiment period. On the other hand, after four weeks of diabetes induction, serum glucose levels in the saline-treated diabetic rats significantly increased in comparison to the initial day. On the contrary, it significantly decreased throughout the experiment period in the extract-treated diabetic and control rats (Fig. 2a).

One-way ANOVA analysis of the data showed that diabetes in the saline-treated diabetic group resulted in a significant elevation of serum glucose concentration compared to the saline- and extract-treated controls. Treatment with shallot extract in the extract-treated diabetic rats decreased the serum glucose levels after two and four weeks of the experiment compared to those in the saline-treated diabetic rats ($p<0.001$). However, serum glucose concentrations were still significantly higher than those in the saline- and extract-treated control rats (Fig. 2b).

Cardiac function parameters

The typical traces of left ventricular developed pressure recorded in this study are illustrated in Figure 3. The values of cardiac function parameters, including RPP, LVDP, HR, dp/dt, and CF, at the 20th minute of baseline and 45th minute of the reperfusion period are shown in Table 1.

Baseline

The results of a one-way ANOVA test in the isolated heart experiments showed that the baseline values of cardiac function parameters, including RPP, HR, and CF, in the saline-treated diabetic rats were significantly lower than the corresponding values in the saline- and extract-treated controls. Furthermore, according to independent samples t-test, +dp/dt in the saline-treated diabetic group was significantly lower than that in the saline-treated control group ($p<0.05$). On the other hand, the baseline values of all cardiac function parameters except CF in the extract-treated diabetic rats did not differ significantly compared to the corresponding values in the extract- and saline-treated controls. In other words, there were no significant differences between the baseline values of these three groups before ischemia. As it is shown in Table 1, the values of RPP, HR, and +dp/dt in extract-treated diabetic rats are significantly higher than the corresponding values in the saline-treated diabetic rats. In addition, the results of independent samples t-test revealed that LVDP in these two groups was significantly different ($p<0.05$).

Reperfusion

As illustrated in Table 1, the values of RPP and HR in the saline-treated diabetic rats at the 45th minute of the reperfusion period are significantly lower than the corresponding values in the saline- and extract-treated controls, whereas the values of RPP, LVDP, and HR in the extract-treated diabetic rats did not significantly differ from the corresponding values in the saline- and extract-treated controls. The RPP and HR values of the isolated hearts of the extract-treated diabetic rats are significantly higher than the corresponding values in the saline-treated diabetic animals (one-way ANOVA).

Discussion

The results of this study demonstrated that, in addition to lowering the blood glucose level, oral administration of the ethyl acetate fraction of *Allium hirtifolium* significantly improved the

baseline and post-ischemic cardiac function parameters deteriorated in the STZ-induced diabetic rats.

Considering the fact that diabetes mellitus can result in cardiac function disturbances, we aimed to assess the effect of the ethyl acetate fraction of *Allium hirtifolium* on the function of isolated hearts of diabetic rats in the normal and post-ischemia conditions.

Mahmoodi et al. (8) have shown that oral administration of hydroalcoholic extract of *Allium hirtifolium* markedly enhances the GCK mRNA expression in the rat liver and consequently decreases blood glucose. In our study, we used the ethyl acetate fraction of *Allium hirtifolium*, and a prominent hypoglycemic effect was reached through using much lower doses (5 mg/kg) compared to Mahmoodi's study (100 and 200 mg/kg), indicating higher purification of the active ingredients and hence higher potency.

It is worth noting that, while the serum glucose level gradually increased in the saline-treated diabetic group, it changed in the opposite direction in the extract-treated control and diabetic animals. In other words, the longer the animals received the extract, the lower serum glucose levels they presented.

During the four-week experimental period, body weights were reduced in the saline- and extract-treated diabetic rats; however, the extent of reduction was lower in extract-treated diabetic rats so that their weights were significantly higher than those of the saline-treated diabetic animals at the end of the fourth week. The failure of STZ-induced diabetic rats to gain weight has already been reported (16–18). Administration of the extract restored these levels toward a normal range. The ability of the extract to partially restore body weight seems to be because of its ability to reduce hyperglycemia (16, 19). This may also be due to the protective effect of the extract in controlling muscle wasting, i.e., reversal of gluconeogenesis (20). As oxidative stress has been shown to play a key role in the pathogenesis of diabetes, antioxidants may play a role in the alleviation of diabetes (21). It is possible that treatment with the extract might lead to a better utilization of nutrients in the diet and thus a gain in weight.

The results of the current study provide evidence for the marked decrease in cardiac function parameters as a result of diabetes before and after ischemia. These findings are consistent with the results presented by Ravingerova et al. (22) and Nemeth et al. (23), who showed that 4- and 8-week diabetes significantly decreases baseline heart rate and coronary flow. Although the actual mechanisms are not completely understood, some possible factors that might be involved in the progression of cardiovascular malfunction in diabetic rats include decreased hepatic glucokinase and hexokinase activity, increased oxidative stress due to excessive production of oxygen free radicals, reduced antioxidant defense systems (8, 24, 25), decreased endothelial nitric oxide (NO) release or production, decreased reactivity of vascular smooth muscle to NO in diabetic animals, and increased production of superoxide anions (26).

In addition to the partial improvement of blood glucose concentrations, the results of our study are indicative of improvement of cardiac functional parameters of the diabetic rats in the baseline and post-ischemic periods following extract administration. Therefore, some part of the improvement can be explained by the efficacy of the extract in improving the glucose metabolism, but some of it may also result from the antioxidant and other therapeutic properties of the ingredients.

It has been reported that the hypoglycemic effect of shallot extract may be induced by its flavonoid compounds, especially quercetin, which is a highly potent antioxidant flavonoid (4, 5). Studies have shown that quercetin is capable of increasing both glucose tolerance and hepatic glucokinase activity in STZ-induced diabetic rats (27–29). Diets with a high content of quercetin have been associated with decreased risk of cardiovascular diseases (30–32). Consistent with this idea, in vivo treatment with shallot extract reduces the end-products of lipid peroxidation (33, 34). Furthermore, the lipid-peroxidation-lowering effect of shallot extract may be induced via its direct superoxide scavenging properties or indirectly increased NO synthesis (35). Moreover, Persian shallot is full of organosulfur compounds, especially allicin. These compounds may be responsible for some beneficial properties of this plant (36, 37). In agreement with our findings, several studies have shown the hypoglycemic effect of garlic, which is attributed mainly to allicin-type compounds (38, 39). Therefore, the hypoglycemic and the beneficial effects of ethyl acetate fraction of shallot on the cardiac function of diabetic rats can be explained by its antioxidant potency, which needs to be elucidated in the future studies.

The vessels of diabetic patients show marked abnormalities in endothelial function, characterized by reduced NO bioactivity and increased superoxide production (40). Impaired NOS activity and reduced NO bioavailability are common initiators of cardiovascular dysfunction (41). Some medicinal plants may improve cardiovascular function through nitric oxide modulation (42). In our study, the protective effect of the extract might have been caused by increased NO bioactivity, resulting in improvement of cardiac function parameters.

Study limitations

Lack of quantitative analysis of nitric oxide pathway and other biochemical parameters can be considered as a limitation of this study.

Conclusion

Besides lowering blood glucose level, oral administration of ethyl acetate fraction of *Allium hirtifolium* significantly improved the baseline and post-ischemic cardiac function parameters in the STZ-induced diabetic rats. Therefore by extension it can be used in the management of diabetic and cardiac dysfunction. Further studies are recommended to investigate the underlying mechanisms.

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