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Original Article

Enriched-asafoetida diet attenuates hyperglycemia, oxidative stress and endothelial dysfunction in type 2 diabetic rats

Mansour Esmailidehaj[1*], Mahboobe Kahtenaroon[1], Mohammad Ebrahim Rezvani[1], Hossein Azizian[1], Alimohammad Ranjbar[2]

1. Department of Physiology, School of Medicine, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran
2. Department of Pharmacognosy, School of Pharmacy Shahid Sadoughi University of Medical Sciences, Yazd, Iran

ABSTRACT

Introduction: It has been documented that oxidative stress and inflammation are the main causes of diabetic-induced disorders. Several studies have been reported the antioxidant and antidiabetic effects of prepared asafoetida extracts, from Ferula assa-foetida L. species in the Apiaceae family. The aim of the present study was to evaluate the effects of enriched-asafoetida diet (EAD) 0.5% and 2% on plasma level of glucose, hemoglobin A1C (HbA1C), insulin, lipid profile and hepatic enzymes, and vascular endothelial dysfunction induced by type 2 diabetes (T2D).
Methods: Thirty-two male Wistar rats were divided into four groups: 1) control group, 2) diabetic group, 3 and 4) diabetic groups received EAD0.5% and EAD2% for 4 weeks, respectively. T2D was induced by intraperitoneal injection of nicotinamide and streptozotocin. At the end of the experiment, the plasma level of glucose, lipid profile, insulin, oxidative stress, hepatic enzymes and vascular dysfunction were evaluated.
Results: Fasting blood sugar, HbA1C, oxidative stress and hepatic enzymes significantly decreased and plasma level of insulin markedly increased in the EAD0.5 compared to the diabetic group. The plasma lipid profile was improved in the EAD0.5 group. The response of thoracic aorta rings to vasodilators and vasoconstrictor substances was considerably improved in EAD0.5 than in the diabetic group. The EAD2 did not have a significant effect on diabetic-induced disorders.
Conclusion: The results of the present study suggest that the effects of EAD on biological disorders caused by T2D are dependent on the percentage of asafoetida in the diet.

Keywords:
Type 2 diabetes
Asafoetida
Hemoglobin A1C
Vascular dysfunction
Lipid profile

Introduction

Diabetes mellitus is a chronic endocrine disorder that adversely affects the body metabolism of all substances especially carbohydrates and fats due to insulin deficiency or insulin resistance. Arterial atherosclerosis, dyslipidemia, retinopathy, nephropathy and hypertension are the most common chronic side effects of diabetes. Chronic elevation of blood glucose causes vascular dysfunction through oxidative stress altering the expression of nitric oxide synthase and protein kinases (Hink et al.,

* Corresponding author: Mansour Esmailidehaj, med1354@ssu.ac.ir
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2001; Kolluru et al., 2012; Patel et al., 2012). Short-term and long-term antioxidant therapy could improve vascular dysfunction from diabetes (Sotnikova et al., 2013; Nishikawa et al., 2000; Esmaeili et al., 2017). Also, with increasing the plasma concentration of glucose, hepatic enzymes especially aminotransferases are leaked from hepatocytes into the circulation and serve as an indicative of hepatocyte injury (Harris, 2005).

There are many medicinal plants from various parts of the world with antidiabetic effects (Chukwuma et al., 2019). Daily consumption of these herbs is increasing worldwide, because most people are believed that they are with a fewer side effects (Kasaian et al., 2016). *Ferula asafoetida* is a perennial and aromatic herb contains an exudate known as asafetida that is obtained by incision of its roots and stems in the summer (Mahendra and Bisht, 2012; Kasaian et al., 2016; Amalraj and Gopi, 2017). Asafetida contains carbohydrates, proteins, fats, moisture and minerals which are divided into three main parts: resin, gum and volatile oil (Mahendra and Bisht, 2012; Amalraj and Gopi, 2017). A number of studies have been reported that various prepared asafoetida extracts has antinociceptive, hepatoprotective, anti-inflammatory, antioxidant, antidiabetic and anti-obesity effects (Azizian et al., 2012; Amalraj and Gopi, 2017; Chandran et al., 2017). In addition, some studies indicated that asafoetida could relax the smooth muscle of intestine (Fatehi et al., 2004; Hejazian et al., 2014), trachea (Kiyanmehr et al., 2016), veins (Mahendra and Bisht, 2012) and uterus (Hejazian et al., 2014). In previous study, we pretreated rats with asafoetida at the dose of 25, 50 and 100 mg/kg once a day, for 4 weeks. The results using isolated hearts shown that asafoetida has cardioprotective effects at low-dose and cardiotoxic effects at higher dose (Esmailidehaj et al., 2014).

To the best of our knowledge, it has not been investigated the effect of enriched-asafoetida diet on diabetes. So, the main goal of the present study was to find out the effect of enriched-asafoetida diet (EAD) 0.5% and 2% on plasma lipid profile, fasting blood sugar (FBS), insulin secretion, water and food intake, the plasma level of oxidative stress, hepatic function and especially vascular endothelial dysfunction in type 2 diabetic rats.

Materials and methods

Animals

Thirty-two male Wistar rats weighing 250-30 g were kept in the institutional animal resource facility of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, under 12/12-hour dark/light cycle with temperature of 22±2°C, humidity of 55% and free access to water and food. The experimental protocol was performed according to the International Guidelines for the Care and Use of Animal Laboratory and confirmed by the Ethic Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (IR.SSU.MEDICINE.REC.1397.046).

Preparation of enriched-asafoetida diet

Asafoetida collection was reported previously (Esmaeili et al., 2017). To make an enriched-asafoetida diet 0.5% and 2%, 0.5 and 2g asafoetida were added to 100g chew, respectively. All rats had free access to their foods throughout the experiment.

Induction of type 2 diabetes (T2D)

To induce T2D (Husna et al., 2018), rats were kept fasting for 12h with *ad libitum* access to water. Nicotine amide (NA) was then injected intraperitoneally at the dose of 100mg/kg. After 15min, streptozotocin (STZ) was injected intraperitoneally at a dose of 50mg/kg. NA was dissolved in distilled water and STZ in cold citrate buffer (pH 4-4.5). Diabetes was verified 72h later by evaluating the FBS level using glucose-oxidase reagent strips (Easy Gluco). Animals with FBS ranged from 200 to 400mg/dl were considered to be diabetic.

Experimental design

To perform this study, rats were assigned in one of four groups (n=8): 1) Ctrl, control group; 2) ND, diabetic rats given normal diet; 3) EAD0.5, Diabetic rats given 0.5% enriched-asafoetida diet; 4) EAD2, Diabetic rats given 2% enriched-asafoetida diet. Rats in groups 3 and 4 were received EAD containing 0.5% and 2% asafoetida for four weeks after induction of T2D. The dose of asafoetida was selected according to the previous work (Mallikarjuna et al., 2003).

Measurement of food and water intake and body weight

Body weight was measured prior to induction of diabetes and 4 weeks later. Then, the percentage of changes in body weight compared to the time before induction of diabetes was determined. Food and water intake were

evaluated at the end of the study.

Biochemical blood analysis

To analyze the biochemical blood parameters, rats fasted overnight for 12h. Then they were anesthetized by ketamine (70mg/kg, IP) and xylazine (10mg/kg, IP) and blood sample was collected from their hearts. The glycosylated hemoglobin from whole blood and plasma lipid profile were determined by a commercial kit (Pishtazteb, Iran) according to the manufacturer's protocols. To assay the plasma concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and plasma lipid profile including total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglyceride, serum was separated from the blood by centrifugation at 3000rpm for 10min. Then, their concentrations were measured by commercial assay kits (Pars Azmoon, Iran) according to the manufacturer's protocols.

FBS was determined using a commercial kit (Pars Azmoon, Iran) and plasma insulin level was assayed by ELISA kit (Hangzhou, Eastbiopharm, China) according to the manufacturer's guidelines.

Measurement of plasma level of malondialdehyde

To determine the level of oxidative stress in plasma, malondialdehyde (MDA) level, as a marker of lipid peroxidation (Castedo et al., 2005), was assayed using a commercially available ELISA kit (ZellBio, Germany) according to the manufacturer's protocol.

Measurement of vascular tension

Briefly, after anesthetizing the animals with ketamine (70mg/kg, IP) and xylazine (10mg/kg, IP), abdominal muscles were cut and the chest opened. The thoracic aorta was removed and transferred to a plate containing prepared Krebs solution saturated with 95% O2 and 5% CO2. After removing the connective and adipose tissues, the aorta was cut into 2–3mm rings. To record the tension, the aortic rings were placed in an organ bath with one side of the rings fixed to the bottom of the organ bath and their opposite side attached to an isometric transducer (F60, NARCO, UK) connected to a power lab data acquisition system (AD Instrument, Australia). In order to relieve the stress-induced relaxation, the rings were stabilized with a tension of 1 gram for one hour. Then endothelial-dependent relaxation and endothelium-independent relaxation mechanisms were assessed in the presence of acetylcholine (Ach, 40μM) and sodium nitroprusside (Snp, 10nM), respectively. The influx of calcium through cell membranes of smooth muscle cells was evaluated by phenylephrine (Phe, 0.1M) and calcium chloride (1.2M). The release of calcium from the sarcoplasmic reticulum was evaluated by phenylephrine (0.1M) in a free-calcium medium. The contractile response of the rings was recorded by Power Lab data acquisition apparatus using Lab Chart Pro 7 software (AD Instruments, Australia).

Statistical analysis

Data were analyzed using GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA). The Shapiro Wilks W test was used to check the normality of the data. Data on vascular tensions were evaluated using a two-way analysis of variance (two-way ANOVA). Other data were analyzed using the one-way ANOVA followed by the post hoc Tukey test. The data are shown as mean±SEM. Finally, $P<0.05$ was considered statistically significant.

Results

Assessment of body weight and water and food intake

Figure 1 addresses the effect of EAD on the body weight, food intake and water intake in type-2 diabetic rats four weeks after induction of diabetes. As shown in Figure 1A, body weight was significantly decreased in all diabetic groups compared to the control group ($P<0.001$). There was not any significant difference between ND group and EDA0.5 and EAD2 groups. Figure 1B shows that food intake was significantly increased in all diabetic groups than the control group ($P<0.001$ for ND group, $P=0.01$ for EAD0.5 and $P<0.001$ for EAD2 groups). Although, food intake was lower in EAD groups especially in EAD0.5 group, there had no any significant difference between the EAD groups compared to the ND group. However, Figure 1C indicates that water intake was significantly increased in all diabetic groups ($P<0.001$) groups compared to the control group. It was lower in EAD groups compared to the ND group that was statistically significant in EAD0.5 group compared to the ND group ($P<0.001$).

Assessment of fasting blood sugar and insulin

Figure 2A shows that FBS significantly increased

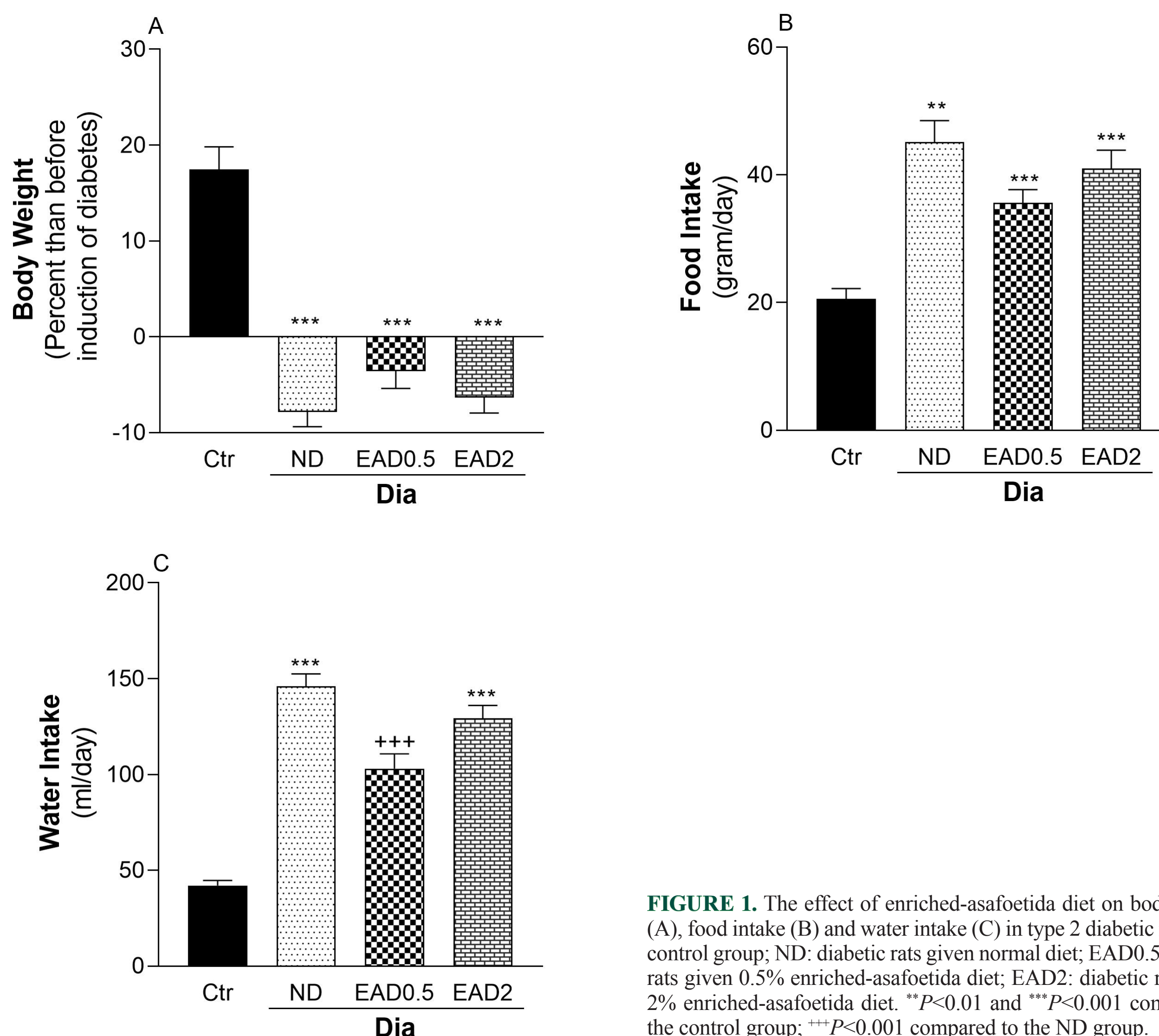


FIGURE 1. The effect of enriched-asafoetida diet on body weight (A), food intake (B) and water intake (C) in type 2 diabetic rats. Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: diabetic rats given 0.5% enriched-asafoetida diet; EAD2: diabetic rats given 2% enriched-asafoetida diet. $^{**}P$<0.01 and $^{***}P$<0.001 compared to the control group; ^{+++}P<0.001 compared to the ND group.

in all diabetic groups compared to the control group (*P*<0.001). It was significantly lower in the EAD0.5 group (*P*=0.038) compared to the ND group. Although, the plasma level of insulin, four weeks after induction of diabetes, was increased in all diabetic groups (Figure 2B), there was only a marked difference between the control group and EAD0.5 group (*P*<0.01).

Assessment of plasma level of hemoglobin and hemoglobin A1C

Figure 3A indicates that hemoglobin concentration was significantly decreased in ND group (*P*=0.01) and EAD group (*P*<0.01) compared to the control group. Although, it was higher in EAD0.5 group than the ND group, it was not significant, statistically. The plasma level of HbA1C is an important predictive marker in diabetic patient (Taylor et al., 1988). It was significantly decreased in ND group (*P*<0.01) compared to the control group (Figure 3B). Although, the HbA1C was lower in EAD groups, there was only a significant between EAD0.5 group and ND group (*P*<0.05).

Assessment of plasma lipid profile

Figure 4 shows that the effect of EAD on plasma lipid profile in type 2 diabetic rats. The plasma level of triglyceride (Figure 4A) was markedly increased in ND and EAD2 groups (*P*<0.001) than the control group. The plasma level of triglyceride was considerably lower in EAD0.5 group than the ND group (*P*<0.05). Like the plasma level of triglyceride, the plasma level of total cholesterol (Figure 4B) was considerably decreased in ND and EAD groups (*P*<0.001) than the control group. It was markedly lower in EAD0.5 group (*P*<0.05).

As shown in Figure 4C, the plasma level of LDL was

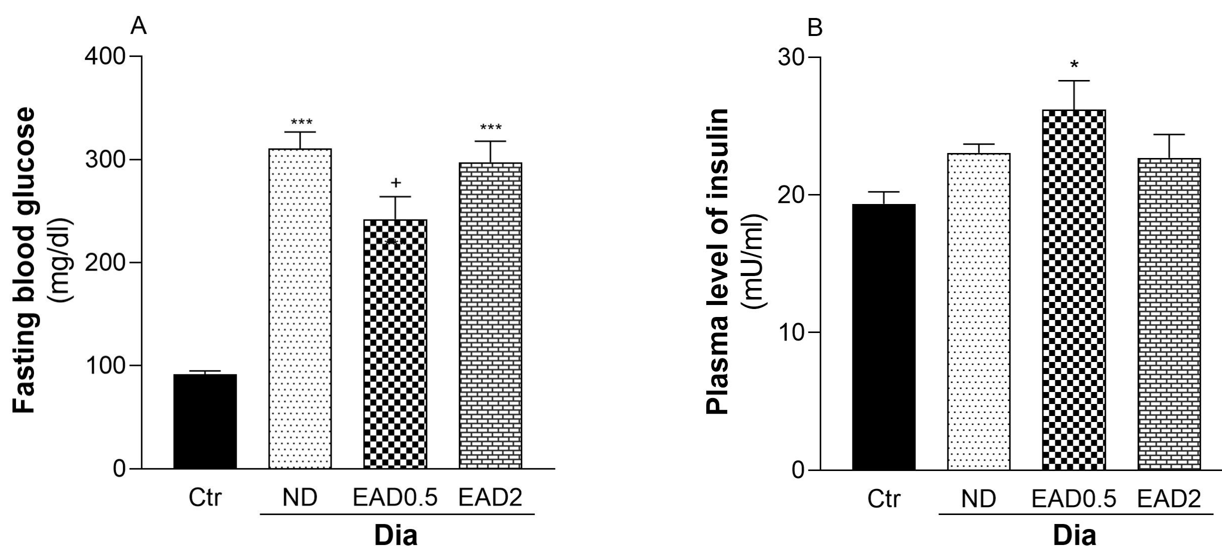


FIGURE 2. The effect of enriched-asafoetida diet on fasting blood sugar (A) and plasma level of insulin (B) in type 2 diabetic rats. Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: diabetic rats given 0.5% enriched-asafoetida diet; EAD2: diabetic rats given 2% enriched-asafoetida diet. $^{*}P$<0.05 and $^{***}P$<0.001 compared to the control group; ^{+}P<0.5 compared to the ND group.

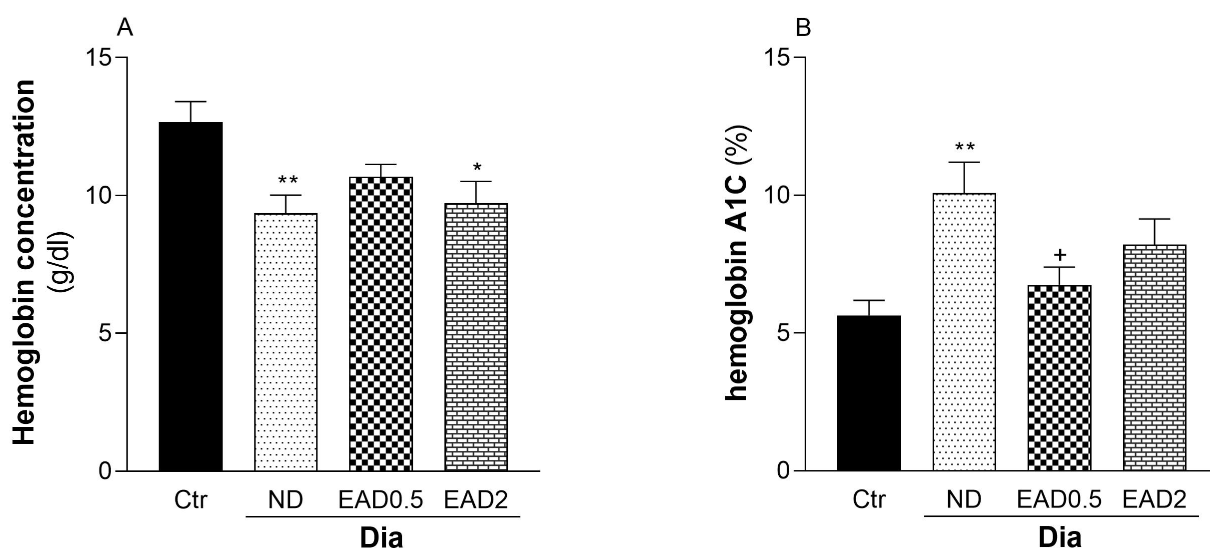


FIGURE 3. The effect of enriched-asafoetida diet on the plasma level of hemoglobin (A) and hemoglobin A1C (B) in type 2 diabetic rats. Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: diabetic rats given 0.5% enriched-asafoetida diet; EAD2: diabetic rats given 2% enriched-asafoetida diet. $^{*}P$<0.05 and $^{**}P$<0.01 compared to the control group; ^{+}P<0.5 compared to the ND group.

markedly increased in all diabetic groups compared to the control group (*P*<0.001 for ND and EAD0.5 and *P*<0.01 for EAD2). It was lower in EAD0.5 group than the ND group (*P*<0.01). The plasma level of HDL (Figure 4D) was significantly decreased in ND group (*P*<0.001) and EAD (*P*<0.001) groups than the control group. Although it was higher in EAD0.5 group compared to the ND group, it was not statistically significant (*P*>0.2).

Assessment of plasma level of malondialdehyde and hepatic enzyme

As shown in Figure 5A, STZ-NA-induced T2D increased the plasma level of MDA compared to the control group (*P*<0.001). EDA0.5 significantly decreased the plasma level of MDA compared to the ND group (*P*<0.05). In contrast, the plasma level of MDA was markedly higher in the EDA2 group compared to the control group (*P*<0.05), but there was not a significant

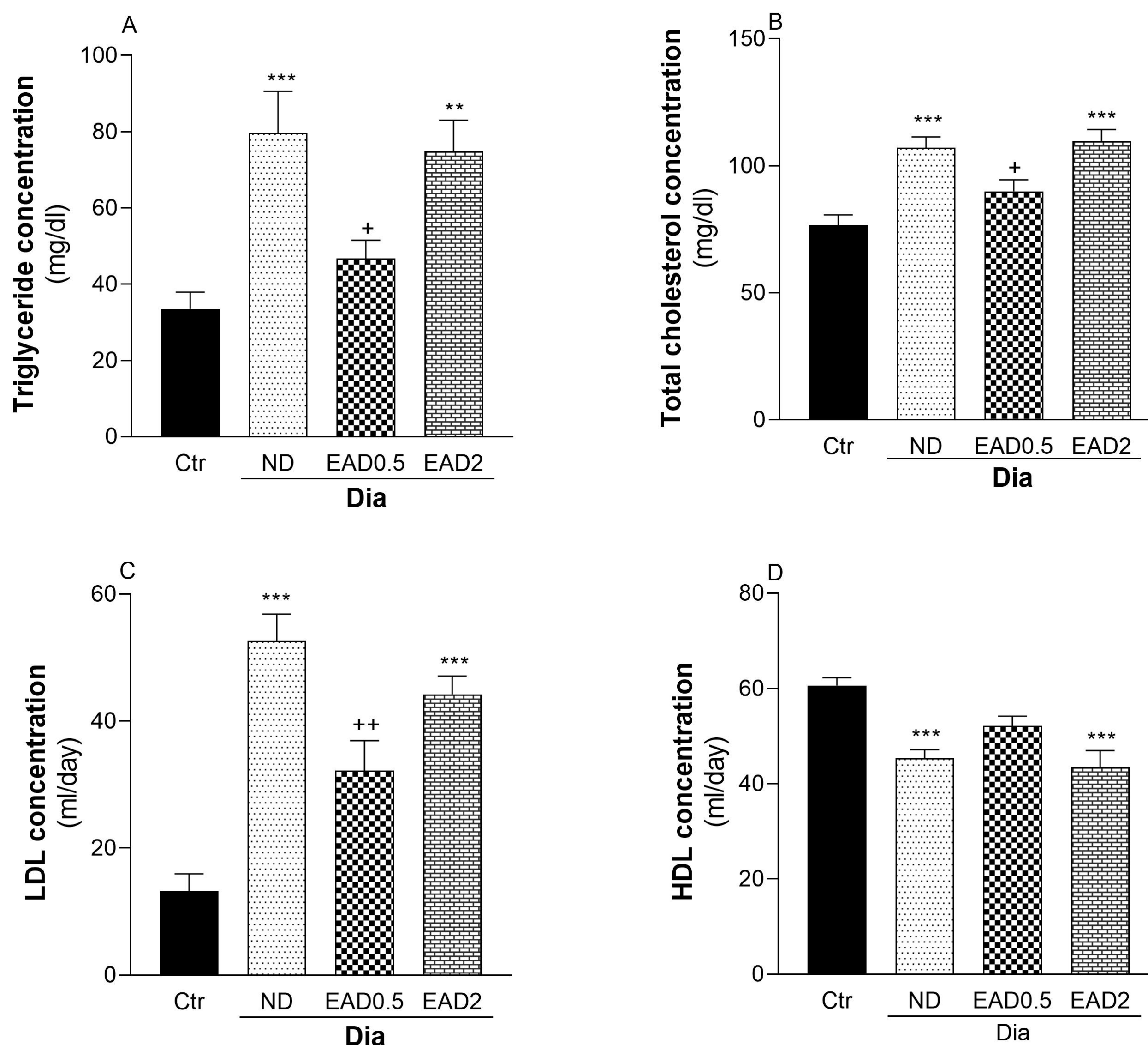


FIGURE 4. The effect of enriched-asafoetida diet on the plasma lipid profile in type 2 diabetic rats. LDL: low density lipoprotein; HDL: High density lipoprotein; Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: diabetic rats given 0.5% enriched-asafoetida diet; EAD2: diabetic rats given 2% enriched-asafoetida diet. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to the control group; $^{+}P<0.05$ and $^{++}P<0.01$ compared to the ND group.

difference between EAD0.5 group and EAD2 group. Figures 5B and 5C shows that diabetes induced by STZ-NA significantly led to an increase in plasma level of liver enzymes AST ($P<0.05$) and ALT ($P=0.01$) compared to the control animals. Although, the plasma level of AST and ALT was lower in EAD0.5 compared to ND group, it was not statistically significant ($P=0.06$ and $P=0.25$, respectively).

Assessment of the vascular endothelium-dependent and independent pathways

Figure 6A shows the effect of EAD on the endothelial-dependent relaxation. After a steady-state with a tension of 1g for one hour, the contractile force of aortic rings was significantly increased in all groups by adding 80mM KCL to the ring's media. After 15min, there was no significant difference between groups. Then, Ach was added to the ring's media in the presence of KCL. In response to Ach, the tension of the rings was significantly lower in the control group and EAD0.5 group compared to the ND group ($P<0.05$).

Figure 6B indicates the effect of EAD on the endothelial-independent relaxation. After a steady-state with a tension of 1g for one hour, the contractile force of aortic rings was significantly increased in all groups by adding KCL to the ring's media. After 15min, there was no sig-

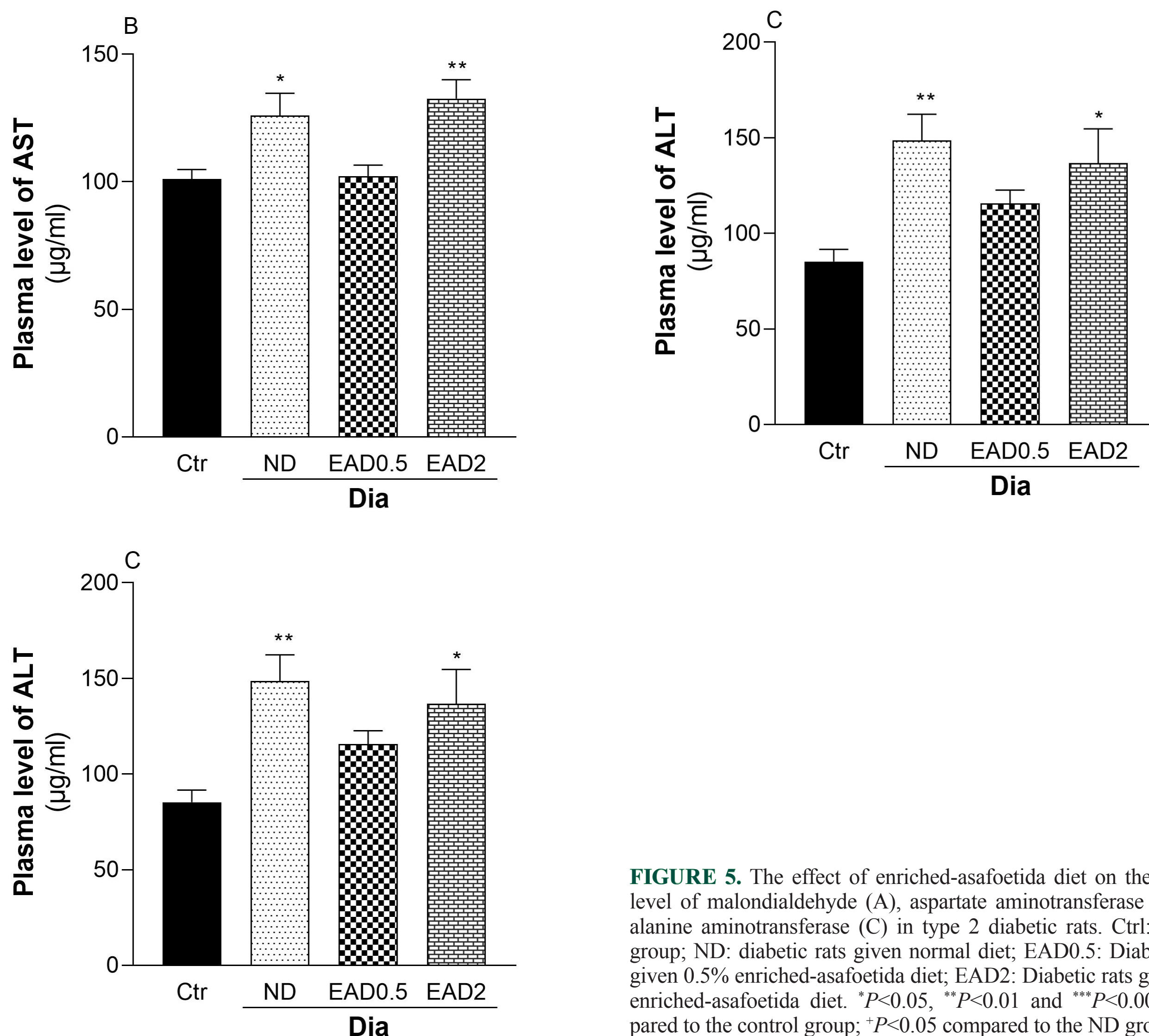


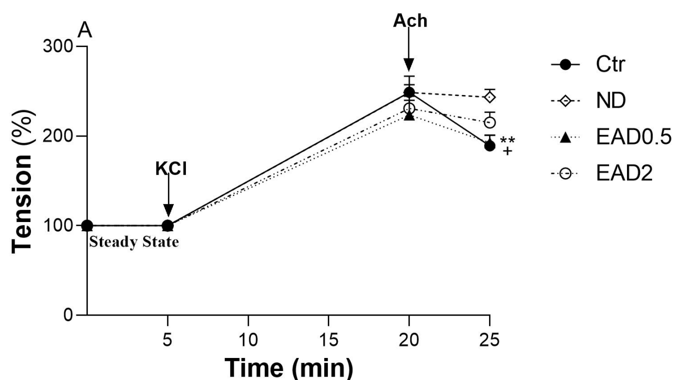
FIGURE 5. The effect of enriched-asafoetida diet on the plasma level of malondialdehyde (A), aspartate aminotransferase (B) and alanine aminotransferase (C) in type 2 diabetic rats. Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: Diabetic rats given 0.5% enriched-asafoetida diet; EAD2: Diabetic rats given 2% enriched-asafoetida diet. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to the control group; $^{+}P<0.05$ compared to the ND group.

nificant difference between groups. Then, Snp was added to the medium in the presence of KCL. In response to Snp, the tension of the rings only was significantly lower in the control group and EAD0.5 group compared to the ND groups ($P<0.05$).

Assessment of calcium influx and release in vascular smooth muscle

Figure 7A indicates the effect of EAD on the influx of calcium in response to phenylephrine. After a steady-state with a tension of 1g for one hour, the contractile force of aortic rings was considered as 100%. Following adding Phe to ring's medium, the contractile response significantly increased in all groups. The tension was significantly higher in ND group compared to the control group ($P<0.05$). On the other hand, it was considerably lower in EAD0.5 group than the ND group ($P<0.05$).

In order to evaluate the effect of EAD on the intracellular calcium release, the free-calcium medium was used. After a steady-state with a tension of 1g for one hour, the contractile force of aortic rings, in the calcium-free medium, was considered as 100%. Adding Phe to this medium led to the release of calcium from intracellular organelles especially sarcoplasmic reticulum. Thereby, Phe causes a significant increase in contractile response of all experimental groups. There was no significant difference among all groups. Then, calcium was added to the rings medium, in the form of CaCl2, to assess the effect of EDA on the influx of calcium from extracellular. As shown on the right side of the figure, the tension was significantly lower in the control and EAD0.5 groups compared to the ND groups ($P<0.05$).



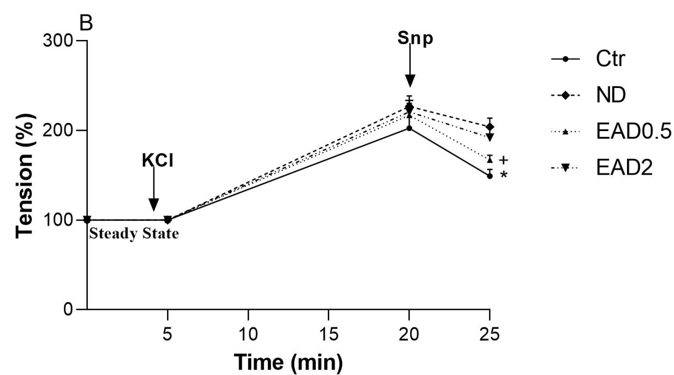
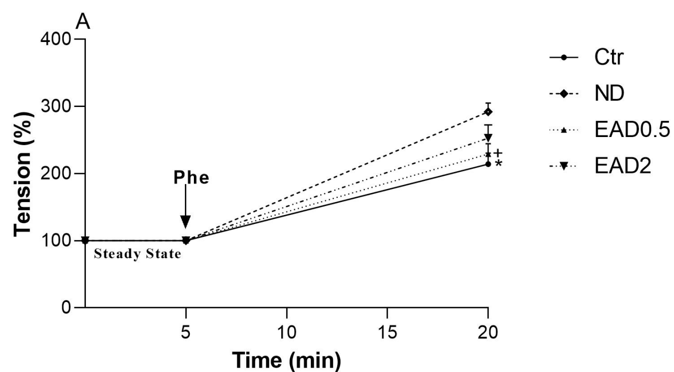


FIGURE 6. The effect of enriched-asafoetida diet (EAD) on endothelium-dependent (A) and independent (B) relaxation in type 2 diabetic rats. Ach: acetylcholine; Snp: sodium nitroprusside; KCL: potassium chloride; Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: diabetic rats given 0.5% enriched-asafoetida diet; EAD2: diabetic rats given 2% enriched-asafoetida diet. $^{*}P<0.05$ and $^{**}P<0.01$ mean the difference between the control and ND groups; $^{+}P<0.05$ mean the difference between the EAD0.5 and ND groups.



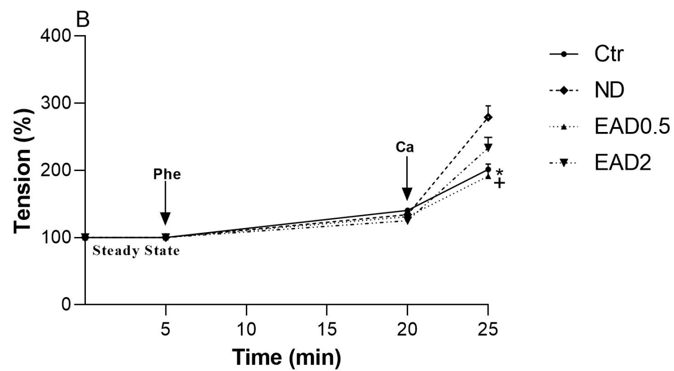


FIGURE7. The effect of enriched-asafoetida diet (EAD) on the calcium influx (A) and the intracellular calcium release (B) in type 2 diabetic rats. Ach: acetylcholine; Snp, sodium nitroprusside; KCL: potassium chloride; Ctrl: control group; ND: diabetic rats given normal diet; EAD0.5: diabetic rats given 0.5% enriched-asafoetida diet; EAD2: diabetic rats given 2% enriched-asafoetida diet. $^{*}P<0.05$ and $^{**}P<0.01$ mean the difference between the control and ND groups; $^{+}P<0.05$ mean the difference between the EAD0.5 and ND groups.

Discussion

The results of the present study indicate that EAD0.5 but not EAD2 had a marked antihyperglycemic effect in STZ-NA-induced diabetic rats which was evident by a decrease in blood glucose and an increase in the blood level of insulin. These effects were associated with decreased oxidative stress which in turn improved the hepatic function and the responsiveness of the aorta to

vasodilators and vasoconstrictors.

Asafoetida is known to have a hypoglycemic effect in diabetic rats (Abu-Zaiton, 2010; Latifi et al., 2019). STZ-NA-induced diabetes makes an experimental diabetic model that imitates some features of type 2 diabetes such as partial responsiveness to glucose and partial sensitivity to insulinotropic agents without induction of obesity, primarily thorough reduced pancreatic insulin reservoirs and insulin secretion deficiency (Fararh et al., 2002). The glucose moiety of STZ causes its uptake by pancreatic beta cells through glucose transporter 2. After entering to cells, STZ damages DNA strands that induce poly-ADP-ribose synthetase which in turn can give rise to NAD depletion. Also, the metabolism of STZ in beta cells produces free radicals that contribute to the additional DNA damage. Nicotinamide, a poly-ADP-ribose synthetase inhibitor, protects the beta-pancreatic cells via the decrease in the level of NAD and proinsulin (Palsamy and Subramanian, 2008). According to the above information, STZ in the presence of nicotineamid can give rise to induction of type 2 diabetes. Accordingly, this experimental diabetic model was used as a suitable model to investigate the antidiabetic effects of EAD.

Our data showed that EAD0.5 resulted in a significant increase in body weight and a significant decrease in food and water intake as well as a significant decrease in plasma level of glucose and a significant increase in the blood level of insulin. The decrease in body weight of diabetic rats might be the result of defective carbohydrate metabolism for ATP production which leads to cells use lipids and proteins as the source of energy. This change causes tissue protein wasting especially in skeletal muscles which in turn can give rise to body weight lost (Pepato et al., 1996; Brodsky, 1998; Palsamy and Subramanian, 2008; Kasetti et al., 2010; Husna et al., 2018). It appears that EAD0.5 activates the pancreatic β cells to increase insulin secretion (Mahendra and Bisht, 2012) which needs more research in the future to demonstrate the effect of EAD on insulin release from the pancreas and its relation to plasma level of glucose. These findings are consistent with others (Abu-Zaiton, 2010). They reported that asafoetida extract significantly reduced blood glucose and increased serum insulin in diabetic rats by alloxan. On the other hand, it seems that EAD2 had a cytotoxic effect on pancreatic β cells that are according to our previous reports on the heart (Esmailidehaj et al., 2014).

As previously noticed, STZ induces diabetic oxidative stress by overexpression of free radicals that more aggravate the function of the beta cells to secret a sufficient amount of insulin (Giugliano et al., 2008). In our study, the oxidative stress of STZ was evident by higher plasma level of MDA and blood level of HbA1c compared to the control group. It was significantly decreased in the EAO0.5 group but not in the EAD2 group. We suggested that EAD0.5 inserted its effect through stimulation of beta cells to secret more amount of insulin. It needs more research in the future to know why EAD2 could have not improved the diabetic-induced oxidative stress. It is likely the lack of the effect of EAD2 is attributable to the high dose of cytotoxic substances (Lee et al., 2009).

Chronic hyperglycemia causes an increase in the glycation of Hb (Nishikawa et al., 2000; Giugliano et al., 2008; Pari and Sankaranarayanan, 2009). HbA1c is considered a more accurate and reliable measure for the management of patients with diabetes than FBS (Taylor et al., 1988; Kristová et al., 2008). Also, it has been considered as a predictor of diabetic complications such as nephropathy, retinopathy, nephropathy and neuropathy (Howlett and Ashwell, 2008). The increase in the level of HbA1C implies the oxidation of sugars (Huebschmann et al., 2006). EAD0.5 significantly decreased the level of HbA1C as well as MDA a marker of oxidative stress. The antioxidant effect of asafoetida is attributable to the phenolic, flavonoid and sesquiterpenoid compounds (Dehpour et al., 2009). The decrease in the blood level of Hb in diabetic rats may be due to an increase in the blood level of HbA1C that was increased in the EAD0.5 group.

Dyslipidemia in diabetes has a strong relationship with cardiovascular diseases (Wold et al., 2005). Elevated plasma levels of TC and LDL, and decreased plasma levels of HDL increase the risk of coronary heart diseases and atherosclerosis (Kasetti et al., 2010). Treating diabetic rats with EAD0.5, but not EAD2, could improve the lipid profile. Al-Awadi and Shoukry (1988) reported that asafoetida lowers the plasma level of TC level in diabetic rats. The reason for this effect is due to the presence of compounds such as flavonoids and saponins in the asafoetida which exert their hypolipidemic effect by suppressing cholesterol absorption and increasing its secretion through bile excretion (Sharma et al., 2008). Given that EAD0.5 has been able to lower liver enzyme

levels, this effect could be a reason for increased TC clearance and decreased TC level (Krishna Kundu et al., 2012).

Plasma concentration ALT and AST, the markers of hepatocyte injury, is increased in diabetic patients (Harris, 2005). ALT and AST are glycogenosis enzymes that their gene expression suppresses by insulin (O'Brien and Granner, 1996). Thus, the increased concentration of ALT and AST in EAD0.5 might be the result of insulin deficiency as well as the effect on a high level of oxidative stress in hepatocytes. In addition, STZ had a hepatotoxic effect (Palsamy and Subramanian, 2008). The hepatoprotective effect of EDA0.5 may be related to its antioxidant (Shivashankara et al., 2012), anti-inflammatory (Iranshahy and Iranshahi, 2011; Mahendra and Bisht, 2012) and inhibition of liver oxidase (Soni et al., 1993; Mahendra and Bisht, 2012) properties. The current results are consistent with the results of others (Ayoubi et al., 2013; Esmailidehaj et al., 2013).

It has been documented that diabetic-induced vascular complications are resulting from an increase in oxidative stress (Palsamy and Subramanian, 2008). Augmentation of endogenous antioxidant capacity could preserve the ability of vascular endothelium to mediate vascular dilation (Palsamy and Subramanian, 2008). In the present study, the vascular response of diabetic rats partly preserved in the EAD0.5 group that might be due to the antioxidant activity of asafoetida.

Vascular relaxation occurs through two pathways: 1) endothelial-dependent pathway through synthesis of nitric oxide and 2) endothelial-independent pathway (Taylor et al., 1988; Mitchell et al., 2008). Diabetes-induced hyperglycemia disrupts blood vessels through various mechanisms, such as increased oxidative stress, increased secretion of inflammatory cytokines, altered gene expression and vascular endothelial cell signaling (Kristová et al., 2008). It has been documented that antioxidant therapy improves vascular dysfunction in patients with diabetes (Sotnikova et al., 2013). Previous studies have been shown that asafoetida has antioxidant and anti-inflammatory effects as well as anti-diabetic effects (Iranshahy and Iranshahi, 2011; Shivashankara et al., 2012; Amalraj and Gopi, 2017; Latifi et al., 2019). Also, we have reported that asafoetida essential oil has a vasodilatory effect on thoracic aorta rings (Esmaeili et al., 2017). The aim of this study was to determine the effect of EAD on vascular impairment induced in type 2 diabetics' rats. We evaluated the effect of EAD on both endothelium-dependent and endothelium-independent pathways using Ach and Snp, respectively. As the results showed, EAD0.5 but not EAD2, significantly increased the relaxation response of the rings to Ach and Snp. Also, EAD0.5 but not EAD2, could significantly reduce the contractile response of aortic rings to epinephrine. EAD did not have any significant effect on the release of calcium from intracellular resources. It is consistent with the previous studies on the smooth muscles of the ileum and trachea (Kiyanmehr et al., 2016). It appears EAD0.5 changes the response of aortic rings to vasodilators and vasoconstrictors through its antioxidant effect to attenuate oxidative stress in the aortic tissue. Also, it appears that the effect of EAD0.5 on the response of the aorta to Ach and Snp might partly result from the change in the sensitivity of vascular smooth muscle. We have previously reported the vasodilatory effect of asafoetida essential oil (Esmaeili et al., 2017). We did not really know why EAD2 did not improve the vascular response in diabetic rats. We postulated that it may be mediated through the higher concentration of toxic substances in the EAD2 group.

After addition of KCL to organ bath medium, the contractile force of the aortic rings significantly increased in all groups compared to the baseline level. KCL-induced contraction is caused by an increase in potassium and depolarization of the smooth muscle fibers that lead to the opening of L-type calcium channels (Fatehi et al., 2004). In this study, treatment with a diet containing asafoetida did not significantly change the contractile response of the aortic rings to KCL compared to the ND group. In our previous study (Esmaeili et al., 2017) the essential oil of asafoetida significantly reduced the aortic contractions caused by KCl. This difference might be related to the differences in composition of asafoetida and its essential oil and the experimental models used.

Conclusion

The main findings of the present study showed that EAD0.5 improved complications caused by T2D, including increased FBS, plasma total cholesterol, blood glycated hemoglobin, plasma oxidative stress, vascular contractile disorder and hepatic dysfunction. It is possible that these effects of EAD were mediated by the stimulatory effect on pancreatic beta cells to increase insulin secretion and by its antioxidant effect. Then, it

is needed to investigate more on its cellular mechanisms in the future.

Conflict of interest

The authors declare that there is no conflict of interest.

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