Pretreatment with Assafoetida exerts dose-dependent dual effects on rat hearts

Mansour Esmailidehaj, Mohadeseh Kakoo, Mohammad Ebrahim Rezvani, Mohammad Hossein Mosaddeghmehrjardi[1]

Departments of Physiology and [1]Pharmacology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Submitted: 05-12-2012 Revised: 19-01-2013 Published: 17-04-2014

ABSTRACT

Context: Although many studies displayed the favorable effects of Assafoetida, some of them reported that high doses of Assafoetida could lead to harmful effects. **Aims:** In this study, the effect of pretreatment with Assafoetida investigated on ischemic–reperfusion injury in isolated rat heart model. **Materials and Methods:** Thirty two male Wistar rats were divided into 4 groups of eight. Group 1 as the control (Con) group and three other groups as the treatment groups that given Assafoetida by gavage at levels of 25, 50 and 100 mg/kg, once a day for four weeks (T25, T50 and T100 groups). Then their hearts were subjected to 30 min global ischemia and 90 min reperfusion under langendorff apparatus. **Results:** The data shown that hemodynamic parameters including left ventricular developed pressure (LVDP) and maximum and minimum of pressure changes (±dp/dt) were increased in T25 and decreased in T50 and T100 groups during reperfusion in comparison with Con group. There was not any significant difference in the incidence of irreversible ventricular fibrillation between T25 and Con group, while it was increased in T50 and T100 groups significantly. There was not any significant difference in infarct size between all groups. **Conclusion:** These data indicate that pretreatment of rats with Assafoetida have cardioprotective effects in low doses and cardiotoxic effects in higher doses. Therefore, it needs more investigation in the future.

Key words: Assafoetida, arrhythmia, cardiac function, infarction, rat

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.131026

Quick Response Code:

INTRODUCTION

Since plants are rich in pharmaceutical substances and many people prefer to consume herbal medicine instead of synthetic drugs, there is a great attention to find out the useful therapeutic compounds of herbs.[1-3] One of these herbal constituents is Oleo-gum-resin or Assafoetida (Anghoze in Iran) that earned from *Apiacea Ferula Alliacea*.[4-6]

Apiacea Ferula Alliacea is a wild and native plant in Iran[6] that grows up to the height of 2 meters.[2,7,8] It is a stout and hollow plant with succulent stems. It has leaves of 30-40 cm with tripinnate.[1] Its usage part is Assafoetida that is acquired by incision of its root or stem.[4,5] Among the most important compounds of the Assafoetida could point to coumarin derivatives, assafeotidnol A and B, sesquiterpene, arabinose, rhamnose and ferulic acid.[1-4,6,9]

Address for correspondence:
Dr. Mansour Esmailidehaj, Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
E-mail: ned1382@gmail.com

Assafoetida has been used traditionally for treatment of epilepsy, bronchitis, whooping cough and amenorrhea.[10] Experimental and clinical studies have shown that it has anticarcinogenic, hypotensive, antiviral, antidiabetic, antioxidant, contraceptive, antifungal, antithepatotoxicity and anticoagulant properties.[1-5,7,9] On the other hand, some studies reported paradoxical or no biological effects of Assafoetida.[6,10] For example, some researchers have reported that administration of atherogenic diet containing 1.5% assafoetida or gastric intubation of the dried assafoetida did not elicited hypolipidemic effect in rats.[10-12]

On the other hand, despite many therapeutic methods to relief the ischemic myocardium, but cardiovascular diseases are still the most common cause of death around the world.[13-16] Therefore, improved myocardial resistance against ischemic-reperfusion injury or decreased consumption of substances that might lead to increased susceptibility of the heart to ischemia would have a great relevance.

In this study, we wanted to known whether pretreatment of rats with Assafoetida at the levels of 25, 50 and 100 mg/kg

for four weeks has any effect on ischemic-reperfusion injury in isolated rat heart or not?

MATERIALS AND METHODS

Animals

Thirty two Male Wistar rats weighing 250-300 g were used to perform this study. They were kept under standard conditions in the laboratory animal house of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, with 12 h dark/light cycle, humidity of about 55%, environmental temperature of 22 ± 2°C and free access to water and food. The care and the use of animals were conducted according to the Ethical Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Assafoetida preparation

Assafoetida was collected from Dorbid area of Yazd, Iran, in spring season (Eastern length of 54°C, 31', 55" and Northern wide of 22°, 07', 07" and 2010 meters height from sea surface). A voucher specimen (A2343) is deposited at the Herbarium of the Herbal Medicine Research Center of Shahid Sadoughi University of Medical Sciences of Yazd, Iran.

10 g Assafoetida was soaked in 100 ml normal saline overnight. Thereafter, the prepared solution was filtered through a paper filter. Finally, its volume was increased to 100 ml to adjust to concentration of 100 mg/ml of Assafoetida. The solution was stirred before giving to rats.

Experimental groups

In this study, 32 Male Wistar rats were divided into four groups of eight as follow:

Group 1 as the control group (Con group) was given 1ml of normal saline by gavage as a vehicle, once a day for four weeks. Groups 2-4 as the treatment groups (T25, T50 and T100 groups) were given Assafoetida by gavage at levels of 25, 50 and 100 mg/kg in 1ml of normal saline, once a day, for four weeks, respectively. The selected doses adopted according to the previous studies.[6]

Acute toxicity test

For toxicological study, five groups of rats (n = 6) were given a single dose of 10, 50, 250, 1250 and 2500 mg/kg Assafoetida, respectively. After 48 h, the motor behavior, lethality and other signs and symptoms of toxicity were determined.

Isolation of the heart

After four weeks, all animals were anesthetized with sodium thiopental (100 mg/kg, i.p) and heparinized intra-peritoneally with 1000 IU heparin. Then, the thorax was opened, the heart removed and placed in cold Krebs solution. Aorta was cannulated retrogradly to perfuse coronary arteries under Langendorff apparatus with Krebs solution. The buffer was composed of (mmol/L) NaCl 118.0, KCl 4.7, $CaCl_2$ 1.25, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.0 that equilibrated with 95% O_2 and 5% CO_2 at about 37°C. PH was maintained at about 7.35-7.45. The perfusion pressure was kept at about 70-80 mmHg. To record intra-ventricular pressures, a water filled balloon connected to a pressure transducer (Narco, UK) inserted into the left ventricle. The volume of the balloon was then increased gradually to get a left ventricular end diastolic pressure (LVEDP) of 4-8 mmHg. Two electrodes were placed on the base of the aorta and the apex of the heart to record electrocardiogram (ECG) permanently. A water filled jacket with internal temperature of about 37°C was placed around the heart to maintain its surrounding temperature approximately near the normal range. Coronary flow was collected manually at various times (at baseline and at 5, 15, 30, 60, 90 min of reperfusion).

Ischemic-reperfusion injury

Following 20 min stabilization, all hearts were subjected to 30 min global ischemia and 90 min reperfusion. LVEDP, left ventricular end systolic pressure (LVESP), left ventricular development pressure (LVDP; means LVESP - LVEDP), maximum and minimum of pressure changes (±dp/dt), coronary flow (CF) and heart rate (HR) were recorded before and during ischemic-reperfusion procedure on different times (at baseline and at 5, 15, 30, 60, 90 min of reperfusion) Using power lab data acquisition apparatus (lab chart 7, ADI, Australia).

Infarct size measurement

Following 90 min reperfusion, the hearts were frozen and then cut into 2 mm slices. Next, the slices were stained by 1% tetrazolium chloride stain at 37°C for 20 min. Thereafter, the slices were placed in 10% formalin to increase their contrasts. Finally, we photographed from both sides of the slices by a digital camera (Sony cyber shot dsc-hx1, Resolution 9.1 mega pixel). Infarct size (white color area) was expressed as the percentage of the whole area of myocardial tissue using C8 Photoshop software.

Creatin kinase activity assay

Five min after reperfusion, CK activity was measured in coronary efflux using a Commercial kit from ZiestChemie Diagnostics (Tehran, Iran).

Statistics

Data are shown as Mean ± SEM and percentage of the incidence. Data of the heart running and iVF were analyzed by Fisher exact test. Hemodynamic data including LVEDP,

LVESP, LVDP and ±dp/dt and coronary flow were analyzed by two-way ANOVA repeated measure test that the time was considered as one variable and the treatment as another one. The data related to the infarct size that had normal distribution were analyzed by one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Acute toxicity test

In all doses used in this experiment no signs and symptoms of toxicity and mortality were observed. Furthermore, we could not calculate the LD50.

Basal running of the heart

As we know, when the heart is removed from the body, cannulated and perfused under langendorff apparatus, it almost always starts to beat regularly. However, in our study, all the hearts in the Con and T25 groups started to beat regularly and none of them had any problem in the running or beating. But, in group T50, four of eight (50%) and in group T100, two of eight (25%) of the hearts run hardly [Figure 1a]. However, following 20 min stabilization, expect two hearts in T50 group, all the hearts in T50 and T100 groups run regularly [Figure 1b].

Hemodynamic parameters

LVESP

As Figure 2a shown, there was not any significant difference in LVESP between groups at the end of stabilization period (before ischemia). However, LVESP was higher in T25 and T100 groups and lower in T50 group than the Con group during reperfusion. There was only statistical significant difference between T25 and T100 groups than the Con group at times of R30 and R60 of reperfusion. The other important point of this figure is the LVESP in T25 and T100 groups was higher at times R30 and R60 than the basal time.

LVEDP

Figure 2b shown the LVEDP maintained between 4-8 mmHg before global ischemia. It was increased during ischemia and the first 5 minof reperfusion. Then, it was decreased gradually in all groups. It was higher in T25 and T100 groups in comparison with the Con group that was only significant at times of R30 and R90.

LVDP

Figure 2c indicates there was not any considerable difference in LVDP at the basal time among all groups. Following reperfusion, it was highly decreased immediately in all groups, but there was not any significant difference between the groups. Then, it was increased gradually. LVDP was higher in T25 group and lower in T50 and T100 groups than the Con group that was significant between T25 and Con groups at time of R60.

Heart rate

As Figure 2d shown, Like LVESP, LVEDP and LVDP, heart rate (HR) had not any statistical difference at the basal time. It was highly decreased at the time of R5 in all groups. Since only one of the hearts in T50 group run (or beat) at time of R5, there was only one sample during this time. In comparison with the Con group, HR was reduced significantly in T50 group during reperfusion times. It was also reduced in T100 group, but increased at the end of reperfusion. There was not any considerable difference between the T25 and Con groups.

± dp/dt

At the basal time, +dp/dt had not any significant

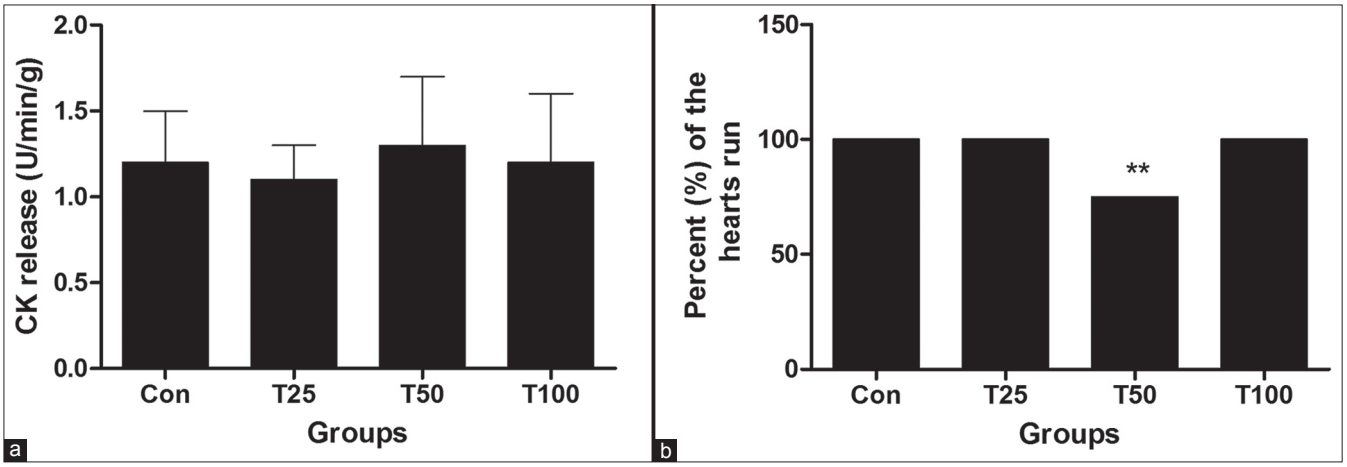

Figure 1: The effect of pretreatment of rats with Assafoetida on the running (beating) of the heart under langendorff apparatus before global ischemia. Con, Control group; T25, T50 and T100 mean pretreatment of rats with oral doses of 25, 50 and 100 mg/kg of Assafoetida for four weeks before isolation of the heart. $^{**}P < 0.01$ vs. Con group

difference [Figure 3a], although it was higher in T100 group. +dp/dt was decreased in the beginning of reperfusion and increased gradually during the rest of the reperfusion in all groups, but it did not reach to the basal value. Following

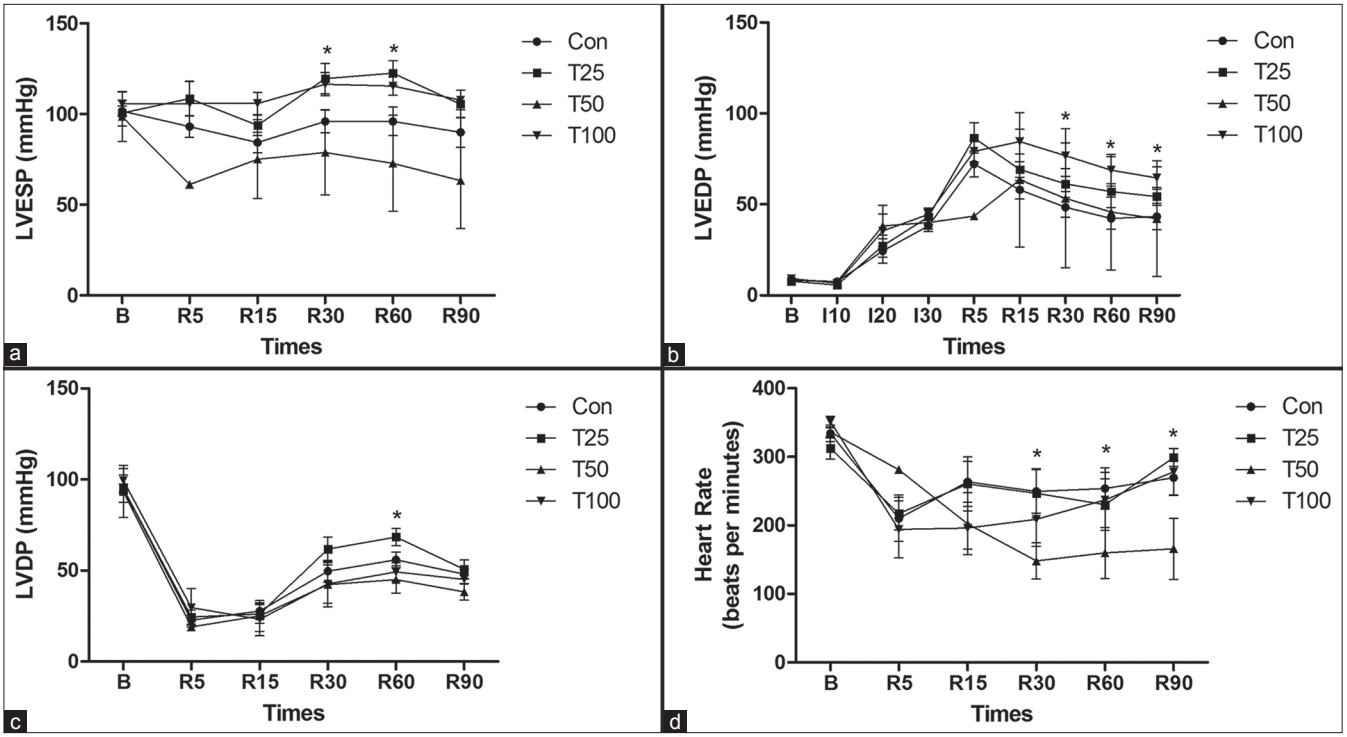

Figure 2: The effect of pretreatment of rats with Assafoetida on the LVESP (a), LVEDP (b), LVDP (c) and HR (d) during 30 min global ischemia and 90 min reperfusion in isolated rat hearts. Con, Control group; T25, T50 and T100 mean pretreatment of rats with oral doses of 25, 50 and 100 mg/kg of Assafoetida for four weeks before isolation of the heart. B, baseline time, I, ischemic time, R5, R15, R30, R60 and R90 mean 5, 15, 30, 60 and 90 min after reperfusion. *$P < 0.05$ compared to the Con group

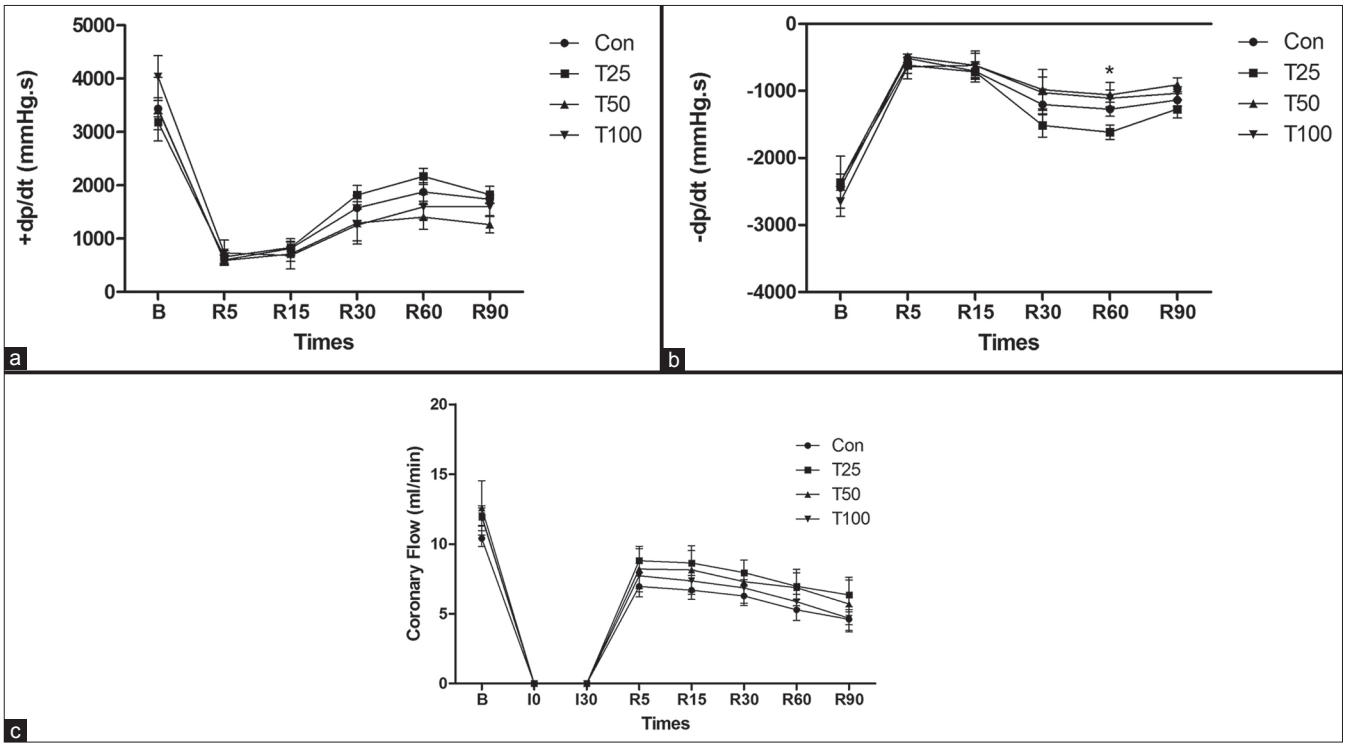

Figure 3: The effect of pretreatment of rats with Assafoetida on the +dp/dt (a), –dp/dt (b) and CF (c) during 30 min global ischemia and 90 min reperfusion in isolated rat hearts. Con, Control group; T25, T50 and T100 mean pretreatment of rats with oral doses of 25, 50 and 100 mg/kg of Assafoetida for four weeks before isolation of the heart. B, baseline time, I, ischemic time, R5, R15, R30, R60 and R90 mean 5, 15, 30, 60 and 90 min after reperfusion. *$P < 0.05$ compared to the Con group

15 min reperfusion, +dp/dt was higher in T25 group and lower in T50 and T100 groups compared with the Con group without statistical significance.

Figure 3b shows that, like +dp/dt, −dp/dt had not any marked difference at the basal time between group, although it was more negative in the T100 group. It was reduced (less negative) dramatically at the initiation of reperfusion and increased (more negative) gradually throughout the reperfusion in all groups. Following 15 min reperfusion, −dp/dt was lower in the T50 and T100 groups and higher in the T25 group compared to the Con group. There was only a significant difference between the T25 and the Con groups at time R60.

Coronary flow

Coronary flow (CF) was reduced to zero during global ischemia in all groups [Figure 3c]. Following reperfusion, CF was highly increased at first and reduced gradually at the rest of the reperfusion in all groups. CF was higher in T25, T50 and T100 groups compared to the Con group at both the basal and reperfusion times without any statistical significancy. Among treatment groups (T25, T50, T100), it was highest in the T25 group and lowest in the T100 group.

Irreversible ventricular fibrillation

The incidence of irreversible ventricular fibrillation (iVF) during reperfusion time was significantly higher in T50 and T100 groups compared to the Con group. It was 12.5% (1 of 8) in the Con group, 12.5% (1 of 8) in the T25 group, 33.3% (2 of 6) in the T50 group and 25% (2 of 8) in the T100 group [Figure 4a].

Infarct size

There was not any significant difference in the infarct size between groups [Figure 4b]. It was 51 ± 4, 55 ± 4.2, 52 ± 9.2 and 63 ± 3.8% in the Con, T25, T50 and T100 groups, respectively.

Creatin kinase activity assay

There was no any significant difference in CK activity of coronary efflux between Assafoetida pretreatment groups and control group. It was 1.2 ± 0.3, 1.1 ± 0.2, 1.3 ± 0.4,

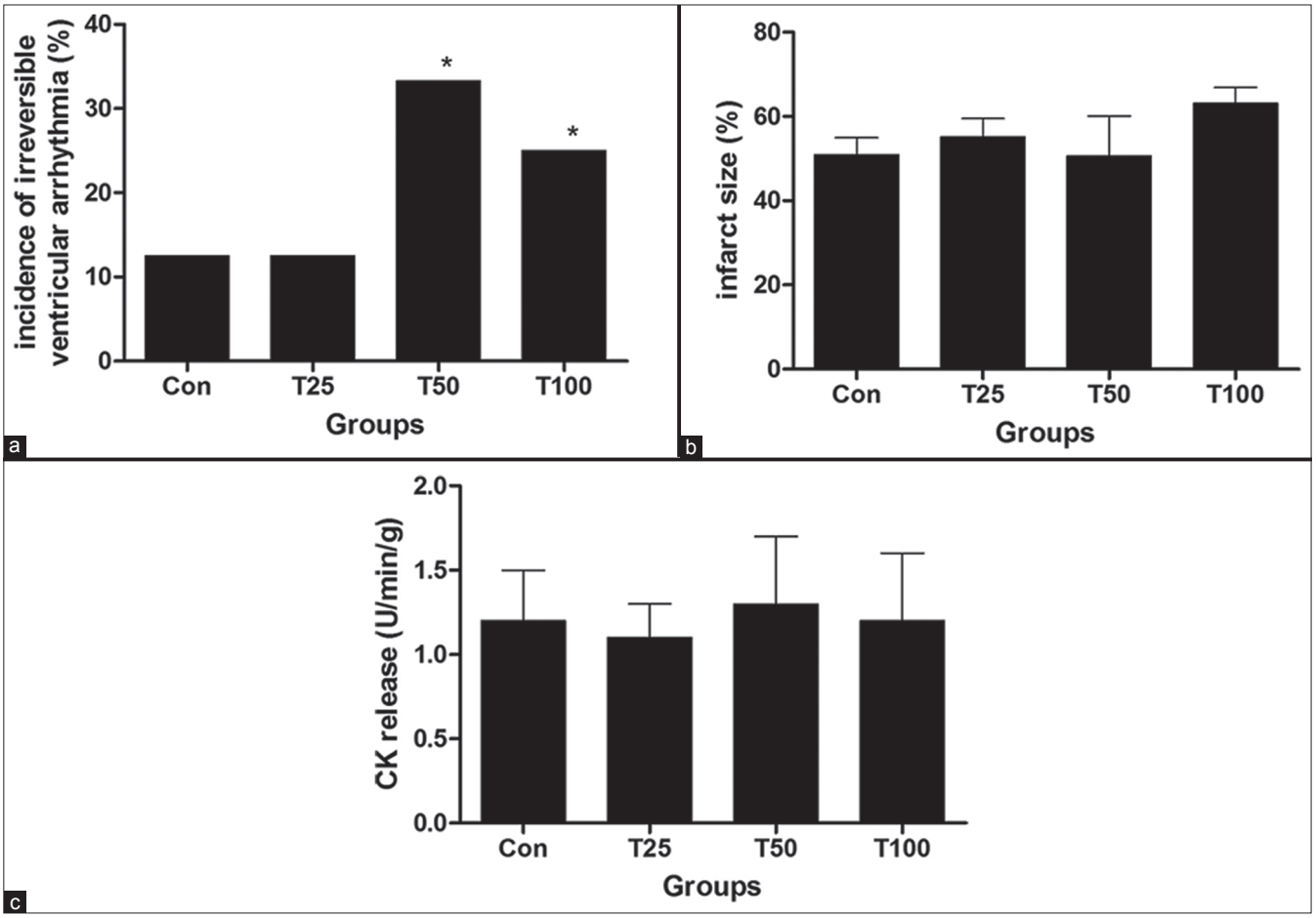

Figure 4: The effect of pretreatment of rats with Assafoetida on the incidence of irreversible ventricular fibrillation (a), infarct size (b) and creatine kinase (c) following 30 min global ischemia and 90 min reperfusion. Con, Control group; T25, T50 and T100 mean pretreatment of rats with oral doses of 25, 50 and 100 mg/kg of Assafoetida for four weeks before isolation of the heart. *$P < 0.05$ compared to the Con group

and 1.2 ± 0.4 in the Con, T25, T50 and T100 groups, respectively [Figure 4c].

DISCUSSION

The striking findings of this study shows that pretreatment of rats with Assafoetida has dose - dependent effects. Due to the low dose (25 mg/kg) has elicited anti-stunning and anti-arrhythmic effects and its high doses (50 and 100 mg/kg) have cardiotoxic effects. These are obvious with improved cardiac functions and decreased incidence of arrhythmia in lower dose of assafoetida, and decreased cardiac functions, increased incidence of arrhythmia and bad running of the hearts under langendorff apparatus in higher doses. On the other hand, it appears that the consumption of Assafoetida has not any effect on the infarct size.

Assafoetida is gained from the plant named *Apiacea Ferula Alliacea* that grows in Iran,[2,5,6,17] Afghanistan,[7,9] Kashmir[5,7,9,17] and Pakistan.[17] Indeed, *Apiacea Ferula Alliacea* is native to Mediterranean area and central part of Asia.[1]

In the present study, when the rats were pre-treated with 25 mg/kg Assafoetida (orally, once a day for four weeks) and then their hearts excised and placed under langendorff apparatus, they start to beating the same as the control hearts that received only vehicle [Figure 1]. But, when the rats were pre-treated with higher doses of Assafoetida (50 and 100 mg/kg), their hearts start to beating hardly to stable before subjected to global ischemia and even some of them did not stable and went to irreversible ventricular fibrillation (iVF). We do not have any explanation for this problem by high doses of Assafoetida. Therefore, it needs further investigation in the future.

Although, lower dose of Assafoetida could induce better cardiac functions during reperfusion times, especially at times of 30 and 60 min of reperfusion (increased LVDP, ±dp/dt and CF), it appears that it had not inserted these effects through inhibition of calcium channels directly or indirectly. Because, LVEDP has increased during reperfusion in all pre-treated groups compared with the control groups [Figure 2b]. It was only statistically significant in group T100 that points out Assafoetida has lead to calcium overload. We do not know whether Assafoetida influence calcium channels directly or indirectly that need more research in the future. Fatehi and co-workers reported that intravenous administration of a single dose of Assafoetida (0.3-2.2 mg/100 g body weight) had hypotensive effects and the more was the dose, the greater was its hypotensive effect. They have not pointed to its mechanisms in their study.[3]

One of the most interesting points in the our study is that LVESP increased in T25 and T100 groups and decreased in T50 group in comparison with the control group [Figure 2a], but LVDP was higher in T25 group and lower in T50 and T100 groups in comparison with the control group [Figure 2c]. We again could not explain the cellular mechanism of Assafoetida on intra-cellular calcium homeostasis. Also, as shown in the Figure 3c, CF is higher in all pre-treated groups in comparison with the control group at the baseline and reperfusion times, although it was not statistically significant. These data has shown that Assafoetida has lead to coronary vasorelaxation at all doses, especially at the lower doses. These data are consistent with the hypotensive effect of Fatehi's study who reported the hypotensive effect of Assafoetida in anesthetized rats (intravenous administration) and its antispasmodic using isolated illume of guinea pig.[3]

Previous studies have reported the antioxidant effect of Assafoetida that maybe related to its ferulic acid and coumarin derivatives.[3,6,18] It is likely the low doses of Assafoetida have antioxidant and its higher doses have pro-oxidant effect in the heart. So, it underlined the lower doses of Assafoetida are investigated in the same as the method of the present study to know whether our hypothesis is correct or not? If so, the protective effects of Assafoetida may be related to its antioxidant.

Despite of antiarrhythmic and antistunning effects of Assafoetida, pretreatment of rats with Assafoetida had not anti-infarct effects [Figure 4b and c]. Some studies reported that it has not hypolipidemic property in rats given high cholesterol diet.[10,19,20] But, it may induce anti-infarct effect at lower doses that recommend for the future studies.

Finally, since ischemic heart diseases and life threatening of arrhythmia are the common causes of cardiac sudden death around the world,[21,22] then, these patients have to warn in consuming Assafoetida.

CONCLUSIONS

The results of this study, point out that pretreatment of rats with Assafoetida has antistunning and antiarrhythmic effects at lower doses and cardiotoxic effects at higher doses. In addition, we stressed that other similar studies have to done on the lower doses of Assafoetida in other animal species and methods. Also, it has to consider an especial care in prescribing different doses of Assafoetida for patients with cardiovascular diseases.

REFERENCES

1. Bamoniri A, Mazoochi A. Determination of bioactive and fragrant molecules from leaves and fruits of Ferula assa-foetida L.

growing in central Iran by nano scale injection. Dig J Nanomater Biostruct 2009;4:323-8.

2. Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition. Grasas y Aceites (Sevilla) 2009;60:405-12.
3. Fatehi M, Farifteh F, Fatehi-Hassanabad Z. Antispasmodic and hypotensive effects of Ferula asafoetida gum extract. J Ethnopharmacol 2004;91:321-4.
4. Eigner D, Scholz D. Ferula asa-foetida and Curcuma longa in traditional medical treatment and diet in Nepal. J Ethnopharmacol 1999;67:1-6.
5. Hassani SB, Saboora A, Radjabian T, Husseini HF. Effects of temperature, GA3 and cytokinins on breaking seed dormancy of ferula assa-foetidal. Iran J Sci Technol 2009;33:75-85.
6. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin) A review. J Ethnopharmacol 2011;134:1-10.
7. Khajeh M, Yamini YA, Bahramifar N, Sefidkon F, Pirmoradei MR. Comparison of essential oils compositions of ferula assa-foetida obtained by supercritical carbon dioxide extraction and hydrodistillation methods. Food Chem 2010;90:636-44.
8. Otroshy M, Zamani A, Khodambashi M, Ebrahimi M, Struik PC. Effect of exogenous hormones and chilling on dormancy breaking of seeds of Asafoetida (Ferula assafoetida L.). Res J Seed Sci 2009;2:9-15.
9. bd El-Razek MH, Ohta S, Ahmed AA, Hirata T. Sesquiterpene coumarins from the roots of Ferula assa-foetida. Phytochemistry 2001;58:1289-95.
10. Ross IA. Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses, 3rd ed. United States: Humana Press Inc; 2005.
11. Al-Awadi FM, Gumaa KA. Studies on the activity of individual plants of an antidiabetic plant mixture. Acta Diabetol Lat 1987;24:37-41.
12. Mansurov MM. Effect of Ferula asafetida on the blood coagulability. Med Zh Uzb 1967;6:46-9.
13. Kaminski KA, Bonda TA, Korecki J, Musial WJ. Oxidative stress and neutrophil activation of the two keystones of ischemia/reperfusion injury. Int J Cardiol 2002;86:41-59.
14. Williams RS, Benjamin IJ. Protective responses in the ischemic myocardium. J Clin Investig 2000;106:813-8.
15. Wu ZK, Iivainen T, Pehkonen E, Laurikka J, Tarkka MR. Ischemic preconditioning suppresses ventricular tachyarrhythmias after myocardial revascularization. Circulation 2002;106:3091-6.
16. Xiao J, Liang D, Zhang H, Liu Y, Li F, Chen YH. 4'-Chlorodiazepam, a translocator protein (18 kDa) antagonist, improves cardiac functional recovery during postischemia reperfusion in rats. Exp Biol Med (Maywood) 2010;235:478-86.
17. Helal EG, Mostafa AM, MhMood AF, Kahwash AA. Hypoglycemic And Hyperinsulinemic Effects Of Ferula Assafoetida On Diabetic Male Albino Rats. Egypt J Hosp Med 2005;21:95-108.
18. Alqasoumi S, Al-Dosari M, Alhowiriny T, Al-Yahya M, Al-Mofleh I, Rafatullah S. Gastric antiulcer activity of a pungent spice ferula assafoetidal. in rats. Farmacia 2011;59:750-59
19. Joshi P. Herbal drugs used in Guinea worm disease by the tribals of southern Rajasthan (India). Pharm Biol 1991;29:33-8.
20. Kamanna VS, Chandrasekhara N. Effect of garlic (Allium sativum Linn) on serum lipoproteins and lipoprotein cholesterol levels in albino rats rendered hypercholesteremic by feeding cholesterol. Lipids 1982;17:483-8.
21. Canyon SJ, Dobson GP. Protection against ventricular arrhythmias and cardiac death using adenosine and lidocaine during regional ischemia in the *in vivo* rat. Am J Physiol Heart Circ Physiol 2004;287:H1286-95.
22. Wu ZK, Iivainen T, Pehkonen E, Laurikka J, Tarkka MR. Perioperative and postoperative arrhythmia in three-vessel coronary artery disease patients and antiarrhythmic effects of ischemic preconditioning. Eur J Cardiothorac Surg 2003;23:578-84.

Cite this article as: Esmailidehaj M, Kakoo M, Rezvani ME, Mosaddeghmehrjardi MH. Pretreatment with Assafoetida exerts dose-dependent dual effects on rat hearts. Phcog Mag 2014;10:147-53.

Source of Support: Nil **Conflict of Interest:** None declared.