Original Article

Extract from *Teucrium polium L.* Protects Rat Heart against Oxidative Stress Induced by Ischemic–reperfusion Injury

Maryam Mahmoudabady[1,2], Milad Haghshenas[3], Saeed Niazmand[1]

From the [1]Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, [2]Neurogenic Inflammation Research Centre, Mashhad University of Medical Sciences, [3]Pharmacological Research Center of Medicinal Plants, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Background: The deleterious effect of oxidative stress on myocardial ischemia–reperfusion (I/R) has already been shown in previous studies. Since *Teucrium polium* has anti-oxidative and cardio-protective properties, the aim of this study was to investigate the effects of this plant on I/R injuries in the isolated rat heart. **Materials and Methods:** The myocardial I/R injury of rat was created by Langendorff retrograde perfusion technology. The heart was preperfused with Krebs–Henseleit (K-H) solution containing *T. polium* extract for 20 min before 20 min global ischemia, and then the reperfusion with K-H bicarbonate buffer was conducted for 40 min. The left ventricular developed pressure and the maximum up/down rate of the left ventricular pressure ($\pm dp/dt_{max}$) were recorded by physiological recorder as the myocardial function. Lactate dehydrogenase (LDH) and creatine kinase (CK) activities in the effluent were measured to determine the myocardial injury degree. Thiobarbituric acid reactive substances (TBARS), total thiol groups (−SH), superoxide anion dismutase (SOD), and catalase (CAT) in myocardial tissue were detected to determine the oxidative stress degree. **Results:** The results showed that the pretreatment with *T. polium* significantly enhanced cardiac parameters and the coronary artery flow, decreased the LDH, CK activities, and TBARS level, whereas it increased − SH groups, SOD and CAT activities. **Conclusions:** Our findings indicated that *T. polium* could provide protection for heart against the I/R injury which may be related to the improvement of myocardial oxidative stress states.

Keywords: *Cardiac function, ischemia–reperfusion, oxidative stress, rat, Teucrium polium*

Introduction

Myocardial disability and tissue damage following ischemia/reperfusion (I/R) is a common disadvantage in patients with some heart diseases. I/R happens in various situations, including thrombolysis, percutaneous coronary angioplasty, organ transplantation, and coronary bypass. I/R injury describes the additional damage occurs by reperfusion in tissues endured ischemia.[1] In the process of reperfusion, tissue cells produce excessive reactive oxygen species (ROS), and hence oxidative stress is an important way involved in I/R injury. Studies have shown that increased expression of antioxidant enzymes will protect against postischemic injury which can lead to apoptosis.[2] Former reports revealed that antioxidant therapy after I/R could improve myocardium after ROS-induced damage.[3,4] Antioxidant enzymes such as superoxide dismutase and catalase (CAT) are capable to protect cardiac tissue against the detrimental effects of ROS.[5,6] Previously, it is shown that ROS scavenger antioxidants could act as a treatment for I/R-mediated cardiac injury. Thus, it is necessary to find out and identify suitable antioxidant interventions to rectify myocardial tissue damage and dysfunction induced by I/R.[7] The presence antioxidant agents in the plants species or in natural resources have been previously highlighted by many studies.[8-10] The medicinal use of plants dates back to ancient times. *Teucrium polium* L. (*Lamiaceae*) is a perennial shrub, 20–50 cm high, distributed widely in the dry and stony places of the hills and deserts of almost all Mediterranean countries, South Western Asia, Europe and North Africa. Sessile, oblong or linear leaves have a length of about 3 cm. *T. polium* that locally called kalpooreh is widely found in Iran has small cluster of pink to white flowers with an aromatic odor originated from its bruised foliage.[11] Phytochemical investigations have shown

How to cite this article: Mahmoudabady M, Haghshenas M, Niazmand S. Extract from *Teucrium polium L.* Protects Rat Heart against Oxidative Stress Induced by Ischemic–reperfusion Injury. Adv Biomed Res 2018;7:15.

Received: September, 2016. Accepted: February, 2017.

Address for correspondence:
Dr. Saeed Niazmand, Department of Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. E-mail: niazmands@mums.ac.ir

Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_218_16

Quick Response Code:

that *T. polium* contains various compounds such as terpenoids, flavonoids, and iridoids.[12] *T. polium* is a plant that has been used for over 2000 years in traditional medicine due to its diuretic, diaphoretic, tonic, antipyretic, antispasmodic, and cholagogic properties.[13,14] In addition, the plant possesses hypoglycemic, insulinotropic, and anti-inflammatory activities[15] and has antinociceptive and antioxidant properties.[16-18] There is increasing evidence of cardiovascular effects of *T. polium* such as positive inotropic and chronotropic,[19] reducing of blood pressure,[20,21] and lowering blood lipid.[22] Thus, regarding to above-mentioned properties of this plant, we decided to evaluate the effects of *T. polium* on cardiac functional parameters and oxidative stress injury in a model of I/R injury in the isolated rat heart.

Materials and Methods

Plant material and preparation of the extract

The aerial part of *T. polium* was collected in autumn from the Khorasan Province, Ferdows, Iran, and identified in the Ferdowsi University Herbarium (voucher No. 152-2016-4) and then dried at room temperature. The aerial parts (300 g) of the plant were soaked in ethanol (50%) for 48 h and filter paper was used to filter the solute after mixing. The solution was then dried using a 40°C oven for 72 h. The dried extract was dissolved in the distilled water to make different concentrations of 0.5, 1, and 2 mg/ml.[21,23]

Animals and experimental design

In this study, fifty male Wistar rats weighing 250–280 g (obtained from animal house of Mashhad University of Medical Sciences [MUMS]) were divided into five groups, $n = 10$ for each. The following groups were involved in the study; groups of Control, Control-ischemia (CI), Ext 0.5, Ext 1 and Ext 2.

Animals were maintained under 12 h light and 12 h of darkness, unrestricted access to food and water at 22 ± 4°C. All experiments were conducted in accordance with the Animal Experimentation Ethics Committee of MUMS (approval no. 910762). The animals were heparinized (375 units/200 g) and anesthetized by intraperitoneal injection of ketamine (60 mg/kg), after tracheotomy and opening the chest, the hearts were excised quickly and cooled in ice-cold saline until contractions stopped. Hearts were then mounted on a Langendorff apparatus and perfused isovolumically at a constant pressure of 70 mmHg with Krebs–Henseleit bicarbonate (K-H) buffer of the following composition (in mmol/L): NaCl, 120; $HNaCO_3$, 25; KCl, 4.8; $MgSO_4$, 1.33; KH_2PO_4, 1.2; $CaCl_2$, 1.6; Na_2 EDTA, 0.02; and glucose, 10. The perfusate was gassed with 95% O_2 and 5% CO_2 (pH 7.4) and kept at a constant temperature of 37°C. Hearts in control group were uninterruptedly perfused with K-H buffer purely for the 80 min. CI group hearts were perfused first for 20 min, and then we suspended the infusion for 20 min and reperfusion for 40 min. Hearts in pretreated groups were perfused first for 20 min instead of K-H buffer with different concentrations of *T. polium* extract (0.5, 1 and 2 mg/ml) and then, we suspended the infusion for 20 min, and reperfusion was continued for 40 min with K-H buffer.[24]

Measurement of heart cardiodynamic parameters

For measurement of left ventricular pressures (LVPs), the left atrium was exposed, and a latex balloon connected to pressure transducer was inserted into the left ventricle through the mitral valve. The volume of the balloon was adjusted to obtain left ventricular end-diastolic pressure of 10 mmHg. The indices of myocardial function were the left ventricular developed pressure (LVDP), which was defined as peak systolic pressure minus end diastolic pressure, heart rate (HR; cardiac spontaneous rhythm was counted per min), peak rate of contraction ($+dp/dt_{max}$), peak rate of relaxation ($-dp/dt_{min}$), and the rate pressure product (RPP = LVDP × HR) which were obtained using a digital data acquisition system (AD Instrument, Australia).[25] Coronary flow (CF) was measured by timed collections of the coronary effluent during all phases of the experiment.

Enzymes activities assays

To determine lactate dehydrogenase (LDH) and creatine kinase (CK) activity, as myocardial damage markers in the perfusate, samples were collected from the coronary effluent before 2 min of ischemia and during the first 8 min of reperfusion. LDH and CK were assayed spectrophotometrically using commercial kits (Pars Azmoon, Iran).

Assay of oxidative stress

When the perfusions finished, the hearts were frozen under the condition of −70°C to prepare for further testing. The frozen hearts were crushed to powder by liquid nitrogen-chilled tissue pulverizer. For tissue analyses, weighed amounts of the frozen tissues were homogenized in appropriate buffer using microcentrifuge tube homogenizer.

Assessment of lipid peroxidation

Malondialdehyde (MDA) level is an index of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a TBA-reactive substance (TBARS) and produces a red complex. Briefly, 1 mL of homogenates was added to 2 mL of a complex solution containing TBA/trichloroacetic acid/hydrochloric acid, and it was then boiled in a water bath for 40 min. After reaching to the room temperature, the solution was centrifuged at 1000 g for 10 min. The absorbance was read at 535 nm[26] and was expressed as TBARS in nmol/g tissue weight, using an extinction coefficient of 1.56×10^5 cm^{-1}/M^{-1}.

Determination of thiol concentration

2, 2'-dinitro-5, 5'-dithiodibenzoic acid (DTNB) reagent, which reacts with the SH group, was used to determine the total thiol groups. The produced yellow complex has a peak absorbance at 412 nm. Briefly, 50 μL of tissue homogenates was added to 1 ml tris-ethylenediaminetetraacetic acid (EDTA) buffer (pH = 8.6), and the absorbance was read at 412 nm against tris-EDTA buffer alone (A1). Then, 20 μL of 10 mM solution of DTNB was mixed with the solution, and it was stored in room temperature for 15 min, and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as blank (B).[27] The thiol levels were determined by a spectrophotometric method based on the use of Ellman's reagent (DTNB solution), and the results were expressed per gram of tissue.

Total thiol concentration (mM) = (A2 − A1 − B) ×1.07 (/0.05 × 14.150).

Determination of superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured by the procedure of Madesh and Balasubramanian. A colorimetric assay involving generation of superoxide by pyrogallol autooxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) to its Formosan by the SOD was measured at 570 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition in the MTT reduction rate.[28]

Determination of catalase activity

CAT activity was determined by the method of Aebi with hydrogen peroxide (30 mM) as the substrate.[29] By measuring the decrease in absorbance at 240 nm/min, the rate constant of the enzyme was determined (EC 1.11.1.16). One unit of CAT activity is determined as the micromoles of the hydrogen peroxide consumed per 100 g sample. One unit (U) is defined as the amount of enzyme which decomposes 1 mol of H_2O_2 per min at 25°C and pH 7.0.

Analysis of results and statistical analysis

Data were expressed as mean ± standard error mean. Comparisons between groups in cardiodynamic and cardiac enzyme values were made using repeated measures ANOVA (with Tukey–Kramer multiple posttest for the most effective dose). In biochemical assays, comparison between groups was performed using ANOVA with Tukey–Kramer multiple posttest. Significance was set at $P < 0.05$. All statistical analyses were made using GraphPad Instat version 3.00 (GraphPad Software, San Diego, CA, USA).

Results

Since the changes in all cardiac parameters were almost similar in all of the time duration before ischemia and as well as those of after reperfusion, we mentioned only the average changes of 2 min before ischemia and the first 10 min of reperfusion period.

The effects of *Teucrium polium* on cardiac function and coronary flow

Two minutes after the reperfusion, HR in all of the extract groups showed a significant increase compared to the CI group ($P < 0.01$ to $P < 0.001$), as well as this increasing trend continued by the end of the reperfusion period [Figure 1].

In the first 10 min after the establishment of reperfusion, LVDP in Ext 0.5 group and RPP in both groups of Ext 0.5 and Ext 1 increased compared to the CI group ($P < 0.05$ to $P < 0.01$) [Figure 1].

$+dp/dt_{max}$ also showed increasing trend during the first 10 min of reperfusion in a group of Ext 0.5 compared to CI group ($P < 0.05$). On the other hand, $-dp/dt_{min}$ in all groups of extract decreased compared to the CI group ($P < 0.05$ to $P < 0.01$) [Figure 2].

CF in all three groups of the extract increased compared to the CI group during the first 10 min of reperfusion, however, this increase was significant only in the group of Ex 0.5 ($P < 0.05$) [Figure 3].

The effects of *Teucrium polium* on cardiac lactate dehydrogenase and creatine kinase release

The extent of reperfusion injury in all groups of hearts was determined from the release of a marker intracellular enzyme into the effluent. As shown in Figure 3, after 20 min of ischemia, in the first moments, the leakage of LDH and CK markedly increased in CI group compared to other pretreated groups. The *T. polium* pretreatment by dose of 0.5 mg/ml significantly reduced the I/R-induced increase in LDH and CK release in rat heart ($P < 0.05$ to $P < 0.01$) [Figure 3].

The effects of *Teucrium polium* on oxidative stress state induced by ischemia–reperfusion Injury

To identify the possible mechanisms of *T. polium* on cardioprotection, the TBARS and thiol levels as well as SOD and CAT activities were determined in myocardial tissue. As shown in Figure 4, the TBARS level was significantly decreased in all groups of extract compared to CI group ($P < 0.05$ to $P < 0.01$) and the total thiol concentration that had been decreased in CI group showed significant increase in all groups of extract compared to CI group ($P < 0.05$ to $P < 0.01$).

The SOD activity significantly increased in EX 0.5 group after reperfusion compared to CI group ($P < 0.05$) and CAT activity showed augmentation in extract pretreated groups ($P < 0.01$ to $P < 0.001$), whereas these values showed significant reductions in CI group compared to control group ($P < 0.01$ to $P < 0.001$) [Figure 5]. The effects of the two higher concentrations of *T. polium* on

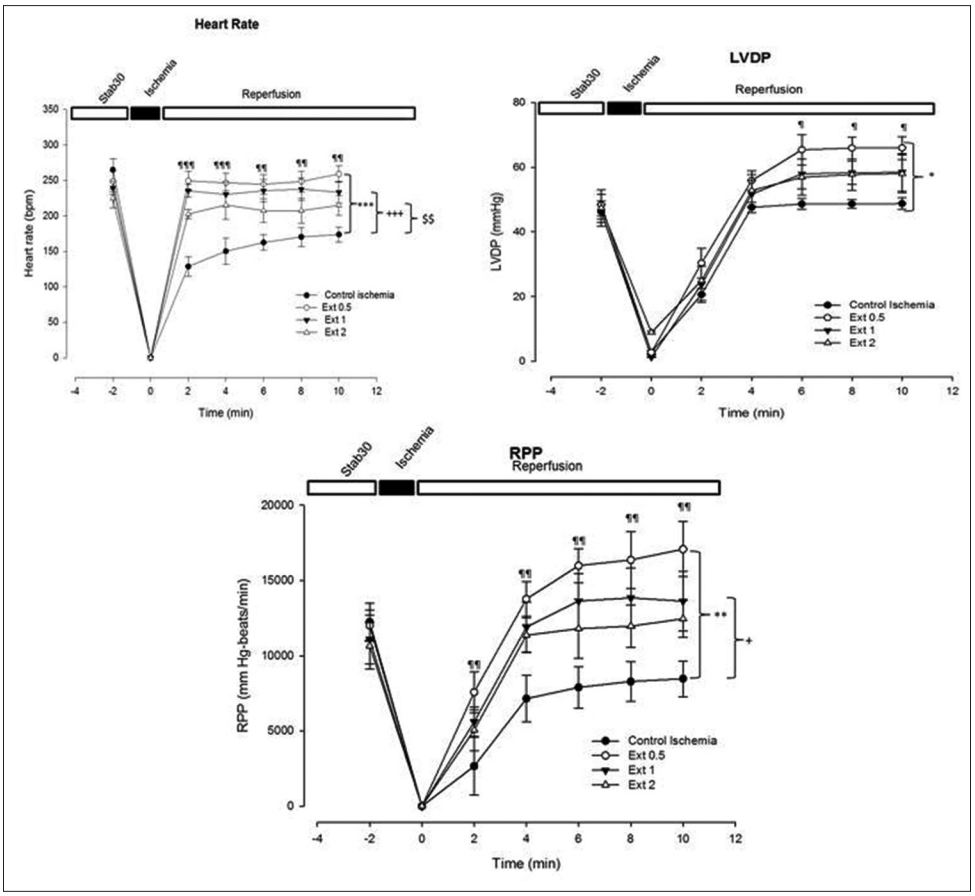

Figure 1: Time course of change in heart rate, left ventricular developed pressure and rate pressure product in control ischemia and different concentrations of *Teucrium polium* extract (0.5, 1 and 2 mg/ml; Ext 0.5, Ext 1, Ext 2) pretreated rat groups during ischemia–reperfusion. The data provided are mean ± standard error mean. For repeated measures ANOVA; **P* < 0.05, ***P* < 0.01 and ****P* < 0.001, ^{+}P < 0.05 and ^{+++}P < 0.001, $^{\$\$}P$ < 0.01, for Tukey–Kramer multiple posttests; $^{¶}P$ < 0.05, $^{¶¶}P$ < 0.01 and $^{¶¶¶}P$ < 0.001, all compared to control ischemia group

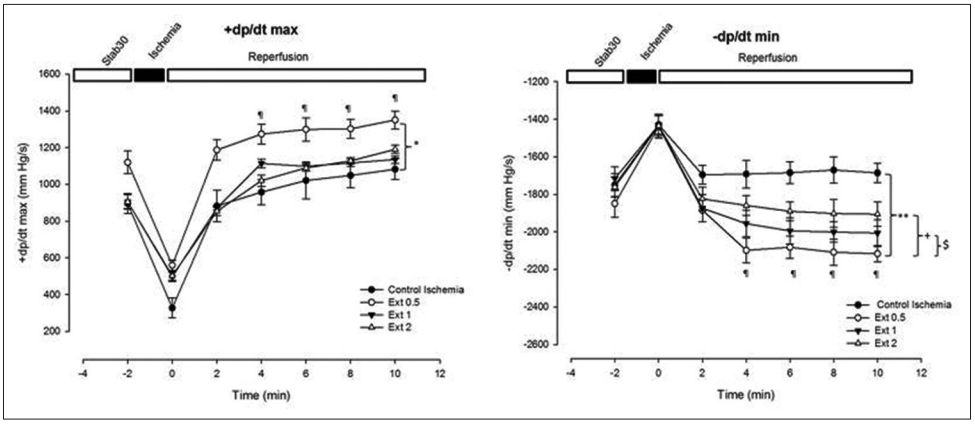

Figure 2: Time course of change in the rate of ventricular pressure development (+dp/dt) and the rate of ventricular pressure decline (−dp/dt) in control ischemia and different concentrations of *Teucrium polium* extract (0.5, 1, and 2 mg/ml; Ext 0.5, Ext 1, Ext 2) pretreated rat groups during ischemia–reperfusion. The data provided are mean ± standard error mean. For repeated measures ANOVA; **P* < 0.05 and ***P* < 0.01, ^{+}P < 0.05, $^{\$}P$ < 0. 05, for Tukey–Kramer multiple posttests; $^{¶}P$ < 0.05, all compared to control ischemia group

oxidative stress parameters were lower than the effect of its low concentration ($P < 0.05$ to $P < 0.001$).

Discussion

It has been a general consensus about the role of oxidative stress, which is associated with increased formation of ROS, in the pathogenesis of I/R injury.[30] A clinical consequence after therapeutic procedures such as thrombolysis, angioplasty, and coronary bypass surgery could be I/R-induced myocardial damages. These impairments include cardiac contractile dysfunction, arrhythmias as well as irreversible myocyte damage. The

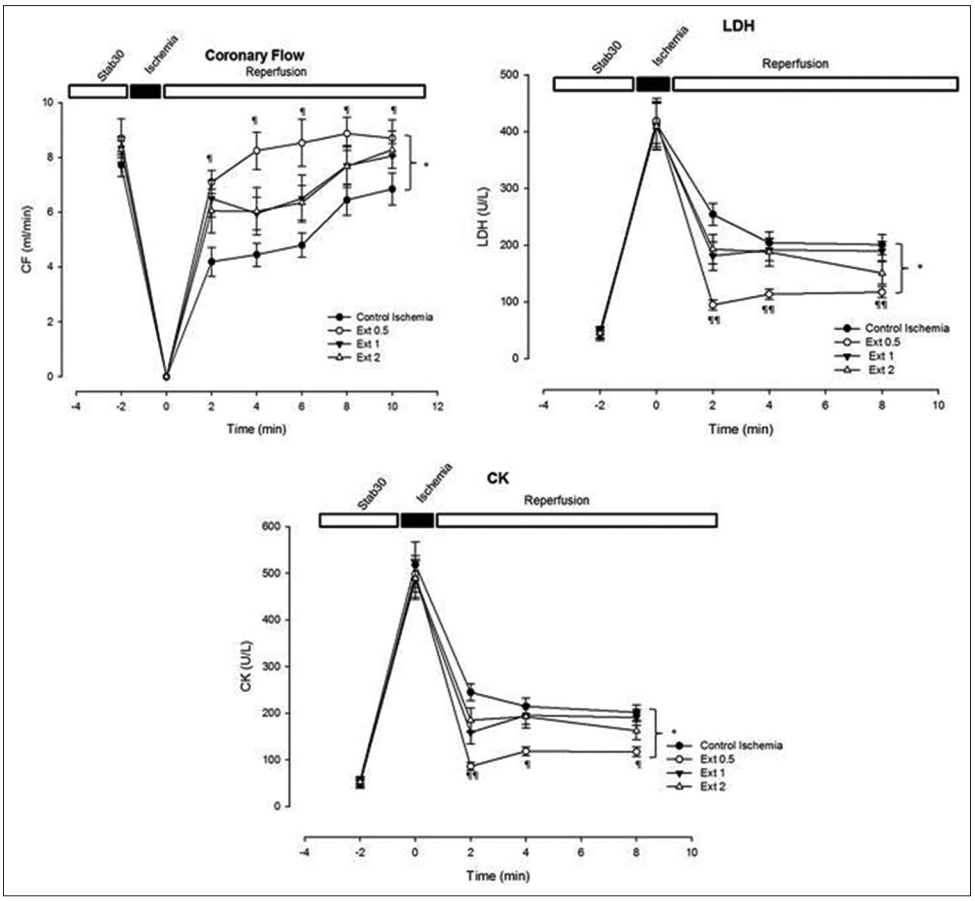

Figure 3: Time course of change in rate of coronary flow, outflow of lactate dehydrogenase and creatine kinase enzymes in control ischemia and different concentrations of *Teucrium polium* extract (0.5, 1 and 2 mg/ml; Ext 0.5, Ext 1, Ext 2) pretreated rat groups during ischemia–reperfusion. The data provided are mean ± standard error mean. For repeated measures ANOVA; **P* < 0.05 and ***P* < 0.01, for Tukey–Kramer multiple posttests; ¶*P* < 0.05 and ¶¶*P* < 0.01, all compared to control ischemia group

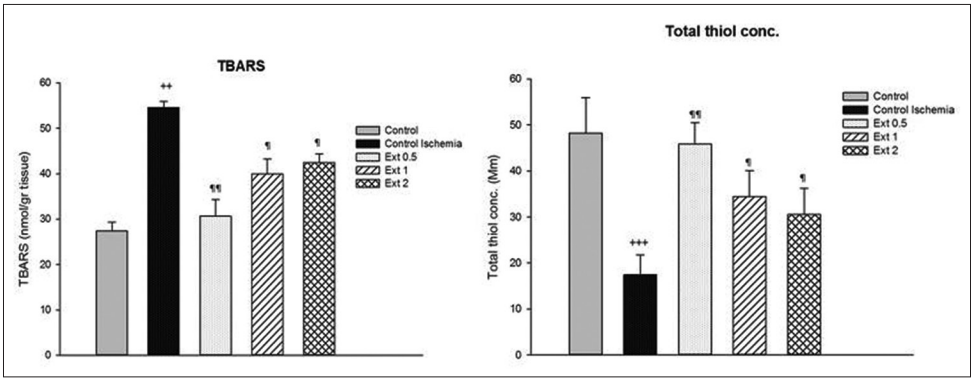

Figure 4: The level of formation of thiobarbituric acid reactive substances, expressed as nmol malondialdehyde/g heart weight and total thiol concentration in the heart tissues of control, control ischemia and different concentrations of *Teucrium polium* extract (0.5, 1 and 2 mg/ml; Ext 0.5, Ext 1, Ext 2) pretreated rat groups during ischemia–reperfusion. The data provided are mean ± standard error mean ++*P* < 0.01 and +++*P* < 0.001 compared to control group, ¶*P* < 0.05 and ¶¶*P* < 0.01 compared to control ischemia group

imbalance between the formation of oxidants and the availability of endogenous antioxidants in the heart is thought to be one of the reasons for these shortcomings.[31] In the present study, this imbalance was obvious after ischemia, administration of hydroalcoholic extract of *T. polium* caused significant augmentation of endogenous myocardial antioxidants (SOD, CAT, thiol) and reduced basal lipid peroxidation (TBARS) after reperfusion of ischemic hearts.

The recovery of cardiac function and myocardial enzyme release has been considered as our goals to evaluate the effects of *T. polium* on I/R injury. Pretreatment with *T. polium* however resulted in decreased I/R injury and excess ROS generation; this led to an improved cardiac

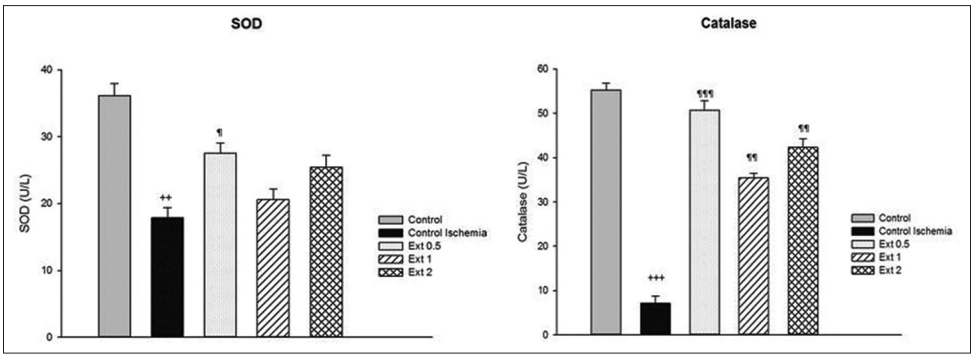

Figure 5: Superoxide dismutase and catalase enzyme activity values in the heart tissues of control, control ischemia and different concentrations of *Teucrium polium* extract (0.5, 1 and 2 mg/ml; Ext 0.5, Ext 1, Ext 2) pretreated rat groups during ischemia–reperfusion. The data provided are mean ± standard error mean $^{++}P < 0.01$ and $^{+++}P < 0.001$ compared to control group, $^{¶}P < 0.05$, $^{¶¶}P < 0.01$ and $^{¶¶¶}P < 0.001$ compared to control ischemia group

function. In our work, this was reflected by the reducing necrotic damage (LDH and CK release). In the absence of this protection, additional cell damages including necrosis and apoptosis which lead to more release of myocardiac enzymes will be predictable. Cardiodynamic parameters, including HR, alongside with CF measurement are considered as improvement factors of left ventricular function in isolated rat hearts.[24] From this study, HR, LVDP, RPP, the recovery rate of ± dp/dt_{max} and CF values during the reperfusion period were increased significantly by *T. polium* pretreatment. These data suggested that *T. polium* could ameliorate cardiac function after I/R injury.

There is a wide range of studies on the antioxidant and free radical scavenging properties of *T. polium* using *in vitro* and *in vivo* models.[11] Regarding the highest total flavonoids (0.20% of air-dried aerial parts) content in the *T. polium* extract, investigators concluded that the antioxidant effects of the plant could be due to the flavonoids, but could also be a result of the activity of other secondary biomolecules present in the extract.[24] Flavonoids are known as a good protector of myocardial tissue against I/R injury due to their diverse properties, such as anti-inflammatory, antioxidant, vasodilatory, and antiplatelet aggregation.[32] On the other hand, previous studies have been shown *T. polium* has inotropic and chronotropic effects[19,23] so improvement of cardiodynamic parameters in pretreated groups after reperfusion could be attributed to these properties of plant.

Hypotensive[21] and vasodilatory effects of *T. polium* has been observed previously in our lab (some parts of data not published), thus the augmentation of CF in extract pretreated groups could be due to this vasorelaxation which was mainly mediated by inhibition of calcium influx in vascular smooth muscle cells, moreover, vasodilation in lower concentrations mediated by nitric oxide and prostacyclin. Although considering to the good effects of *T. polium* on LVDP in this study, it seems the reduction of diastolic LVP ameliorates CF.

Our data demonstrated that the lower concentration of extract had more effectivity on cardiodynamics and oxidative balance, former studies showed cytotoxicity of *T. polium* in different types of cell lines,[33,34] and also hepatotoxicity of this plant was reported by some investigators.[35,36] On the other hand, since our results rely on *in vitro* data and regarding to the toxicity of *T. polium*, to better evaluation the efficiency of this plant, it is recommended much lower doses of its extract considered in further studies.

Conclusions

This study demonstrated a cardioprotective effect of *T. polium* on isolated heart against oxidative stress during ischemia reperfusion injury; however, further studies are needed to determine the related signaling pathways of specific *T. polium* compounds and/or their respective metabolites as well as to confirm that *T. polium* could be a potentially therapeutic substance.

Acknowledgment

The authors would like to thank research affairs of Mashhad University of Medical Sciences for their financial support.

Financial support and sponsorship

Research Affairs of Mashhad University of Medical Sciences (grant No. 910762).

Conflicts of interest

There are no conflicts of interest.

References

1. Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, Tang CS. Apelin protects heart against ischemia/reperfusion injury in rat. Peptides 2009;30:1144-52.
2. Wang D, Guo X, Zhou M, Han J, Han B, Sun X. Cardioprotective effect of the aqueous extract of lavender flower against myocardial ischemia/reperfusion injury. Journal of Chemistry 2014.
3. Chen EP, Bittner HB, Davis RD, Folz RJ, Van Trigt P. Extracellular superoxide dismutase transgene overexpression preserves postischemic myocardial function in isolated murine hearts. Circulation 1996;94 9 Suppl:II412-7.
4. Du Y, Guo H, Lou H. Grape seed polyphenols protect cardiac

cells from apoptosis via induction of endogenous antioxidant enzymes. J Agric Food Chem 2007;55:1695-701.
5. Ambrosio G, Zweier JL, Duilio C, Kuppusamy P, Santoro G, Elia PP, *et al.* Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. J Biol Chem 1993;268:18532-41.
6. Maulik N, Sharma HS, Das DK. Induction of the haem oxygenase gene expression during the reperfusion of ischemic rat myocardium. J Mol Cell Cardiol 1996;28:1261-70.
7. Swaminathan JK, Khan M, Mohan IK, Selvendiran K, Niranjali Devaraj S, Rivera BK, *et al.* Cardioprotective properties of *Crataegus oxycantha* extract against ischemia-reperfusion injury. Phytomedicine 2010;17:744-52.
8. Devi R, Banerjee SK, Sood S, Dinda AK, Maulik SK. Extract from *Clerodendron colebrookianum* Walp protects rat heart against oxidative stress induced by ischemic-reperfusion injury (IRI). Life Sci 2005;77:2999-3009.
9. Augusti KT, Arathy SL, Asha R, Ramakrishanan J, Zaira J, Lekha V, *et al.* A comparative study on the beneficial effects of garlic (*Allium sativum* Linn), amla (*Emblica Officinalis* Gaertn) and onion (*Allium cepa* Linn) on the hyperlipidemia induced by butter fat and beef fat in rats. Indian J Exp Biol 2001;39:760-6.
10. Helen A, Krishnakumar K, Vijayammal PL, Augusti KT. Antioxidant effect of onion oil (*Allium cepa*. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol Lett 2000;116:61-8.
11. Bahramikia S, Yazdanparast R. Phytochemistry and medicinal properties of *Teucrium polium* L. (*Lamiaceae*). Phytother Res 2012;26:1581-93.
12. Piozzi F, Bruno M, Rosselli S, Maggio A. Advances on the chemistry of furano-diterpenoids from *Teucrium* genus. Heterocycles 2005;65:1221-34.
13. Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. J Ethnopharmacol 2002;83:251-65.
14. Zargari A. Vol. 4. Tehran: Tehran University Press; 1997. Medicinal Plants; p. 103.
15. Esmaeili MA, Yazdanparast R. Hypoglycaemic effect of *Teucrium polium*: Studies with rat pancreatic islets. J Ethnopharmacol 2004;95:27-30.
16. Ljubuncic P, Dakwar S, Portnaya I, Cogan U, Azaizeh H, Bomzon A. Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. Evid Based Complement Alternat Med 2006;3:329-38.
17. Abdollahi M, Karimpour H, Monsef-Esfehani HR. Antinociceptive effects of *Teucrium polium* L total extract and essential oil in mouse writhing test. Pharmacol Res 2003;48:31-5.
18. Couladis M, Tzakou O, Verykokidou E, Harvala C. Screening of some Greek aromatic plants for antioxidant activity. Phytother Res 2003;17:194-5.
19. Niazmand S, Erfanian Ahmadpoor M, Moosavian M, Derakhshan M. The positive inotropic and chronotropic effects of *Teucrium polium* L. extract on guinea pig isolated heart. Pharmacologyonline 2008;2:588-94.
20. Bello R, Calatayud S, Moreno L, Beltran B, Primo Yufera E, Esplugues J. Effects on arterial blood pressure of the methanol extracts from different *Teucrium* species. Phytother Res 1997;11:330-1.
21. Mahmoudabady M, Shafei MN, Niazmand S, Khodaee E. The effects of hydroalchoholic extract of *Teucrium polium* L. on hypertension induced by angiotensin II in rats. Int J Prev Med 2014;5:1255-60.
22. Rasekh HR, Khoshnood-Mansourkhani MJ, Kamalinejad M. Hypolipidemic effects of *Teucrium polium* in rats. Fitoterapia 2001;72:937-9.
23. Niazmand S, Esparham M, Hassannia T, Derakhshan M. Cardiovascular effects of *Teucrium polium* L. extract in rabbit. Pharmacogn Mag 2011;7:260-4.
24. Yu J, Wang L, Akinyi M, Li Y, Duan Z, Zhu Y, *et al.* Danshensu protects isolated heart against ischemia reperfusion injury through activation of Akt/ERK1/2/Nrf2 signaling. Int J Clin Exp Med 2015;8:14793-804.
25. Niazmand S, Fereidouni E, Mahmoudabady M, Mousavi SM. Endothelium-independent vasorelaxant effects of hydroalcoholic extract from *Nigella sativa* seed in rat aorta: The roles of Ca2+ and K+ channels. Biomed Res Int 2014;2014:247054.
26. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 1990;9:515-40.
27. Sharma JB, Sharma A, Bahadur A, Vimala N, Satyam A, Mittal S. Oxidative stress markers and antioxidant levels in normal pregnancy and pre-eclampsia. Int J Gynaecol Obstet 2006;94:23-7.
28. Madesh M, Balasubramanian KA. Microtiter plate assay for superoxide dismutase using MTT reduction by superoxide. Indian J Biochem Biophys 1998;35:184-8.
29. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
30. Fan H, Yang L, Fu F, Xu H, Meng Q, Zhu H, *et al.* Cardioprotective effects of salvianolic acid a on myocardial ischemia-reperfusion injury *in vivo* and *in vitro*. Evid Based Complement Alternat Med 2012;2012:508938.
31. Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovasc Res 2000;47:446-56.
32. Akhlaghi M, Bandy B. Mechanisms of flavonoid protection against myocardial ischemia-reperfusion injury. J Mol Cell Cardiol 2009;46:309-17.
33. Eskandary H, Rajabalian S, Yazdi T, Eskandari M, Fatehi K, Ganjooei NA. Evaluation of cytotoxic effect of *Teucrium polium* on a new glioblastoma multiforme cell line (REYF-1) using MTT and soft agar clonogenic assays. Int J Pharmacol 2007;3:435-7.
34. Nematollahi-Mahani SN, Rezazadeh-Kermani M, Mehrabani M, Nakhaee N. Cytotoxic effects of *Teucrium polium* on some established cell lines. Pharm Biol 2007;45:295-8.
35. Mazokopakis E, Lazaridou S, Tzardi M, Mixaki J, Diamantis I, Ganotakis E. Acute cholestatic hepatitis caused by *Teucrium polium* L. Phytomedicine 2004;11:83-4.
36. Savvidou S, Goulis J, Giavazis I, Patsiaoura K, Hytiroglou P, Arvanitakis C. Herb-induced hepatitis by *Teucrium polium* L.: Report of two cases and review of the literature. Eur J Gastroenterol Hepatol 2007;19:507-11.