Effects of hydroalcoholic extract of *Cynodon dactylon* (L.) pers. on ischemia/reperfusion-induced arrhythmias

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ABSTRACT

Background and purpose of the study: Probable antiarrhythmic effects of *Cynodon dactylon* (L.) pers. (family Poaceae) against ischemia/reperfusion (I/R)-induced arrhythmias were investigated in isolated rat heart.

Methods: The hearts were subjected to 30min regional ischemia followed by 30min reperfusion and perfused with hydroalcoholic extract of rhizome of *C. dactylon* (25, 50, 100 and 200µg/ml).

Results: During ischemia, the extract produced marked reduction in the number, duration and incidences of ventricular tachycardia (VT) at 25 and 50µg/ml ($p<0.001$ and $p<0.01$, respectively). Total number of ischemic ventricular ectopic beats (VEBs) were lowered by 25-100µg/ml ($p<0.001$, $p<0.001$ and $p<0.05$, respectively). At the reperfusion phase, *C. dactylon* (25 and 50µg/ml) decreased incidence of VT from 100% (control) to 13 and 33% ($p<0.001$ and $p<0.05$) respectively. Duration and number of VT and total VF incidence were also reduced at the same concentration ($p<0.05$ for all). Perfusion of the extract (25-100µg/ml) was markedly lowered reversible VF duration from 218±99sec to 0 sec, 0 sec and 10±5sec ($p<0.01$, $p<0.01$ and $p<0.05$) respectively. Moreover, *C. dactylon* (25 and 50µg/ml) decreased number of total VEBs from 349±73 to 35±17 ($p<0.001$) and 66±26 ($p<0.01$). In this study, it was also shown that perfusion of the extract produced a marked and concentration-dependent positive inotropic effect.

Conclusion: The findings of this study indicate that *C. dactylon* produce protective effects against I/R-induced arrhythmias in isolated rat hearts probably by increase in the myocardial contractility and as a result by improvement of hemodynamic factors.

Keywords: *Cynodon dactylon* (L.) pers., ischemia/reperfusion, arrhythmias, isolated heart; rat

INTRODUCTION

Cynodon species are members of the *Cynodonteae* tribe and the *Chloridoideae* sub-family (1). *Cynodon dactylon* (*C. dactylon*, Bermuda grass) is a resilient, perennial grass distributed all over the world and especially it is native to the warm temperate and tropical regions (1-2). Leaf, root and rhizome of the plant have been used in folk medicine of different countries (3, 4), as anti-inflammatory (3, 5), anticystitis (3), antihypertensive (3, 6), antihysteria, antipsychotic (2) antigonorrheal infection (4), antiviral, as well as hypolipidemic, hypoglycemic agent (6). In India, the plant is reputed for the treatment of melena, thirst, anorexia, burning sensations of the body, pruritis, miscarriage and erysipelas (6-7), and its leaf juice with a pinch of common salt has been used orally in stomachache (5). Decoction of whole plant has been given orally to cure menstrual problem (8).

In Iranian folk medicine, the root and rhizome of the plan have been used in the treatment of depression, naused, cough, epilepsy and hemo-rrhage (9). In some provinces of Iran (such as Azerbaijan and Kurdistan), *C. dactylon* which is locally named as *Chayer*, has been traditionally used for cardiovascular diseases. Many people of these regions believe that the extract of the rhizome has curative effects in coronary artery diseases and in heart failure. In addition, uncontrolled studies by some Iranian cardiologists have shown cardioprotective effects in the patients who used the plant traditionally. To date, there is no report on protective effects of

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the plant on cardiovascular diseases and in the present study the effects of hydroalcoholic extract of *C. dactylon* rhizomes on ischemia/ reperfusion (I/R)-induced arrhythmias in isolated rat heart was investigated.

MATERIALS AND METHODS

Plant materials

Rhizomes of *C. dactylon* were freshly collected from the field (Maragheh- East Azerbaijan, Iran) in November, 2005 and a voucher specimen was deposited at herbarium of School of Pharmacy, Tabriz University of Medical Sciences. The collected rhizomes were washed and dried at room temperature in shade.

Preparation of the plant extracts

Two hundred grams of the dried and powdered rhizomes of *C. dactylon* were extracted three times by maceration with 1 L of a mixture of methanol-water (70:30) each time for 12 h. The combined extracts were filtered and evaporated under reduced pressure and temperature (40 °C) to dryness. Dried residue (15 g) was kept at 4 °C until used.

Chemical tests

The crude extract of *C. dactylon* was screened for the presence of different classes of compounds by some modifications in standard methods (10-12). Thin Layer Chromatography procedures using precoated silicagel plates (Merck; GF_{254}, 0.25 mm) were performed to confirm the results of screenings (14). The following spray reagents were used for detection of respective classes of compounds: NEU (for polyphenoles/flavonoids), Antimony trichloride in chloroform (for steroidal saponins/sterols), Kedd reagent (for cardiac glycosides), Dragendorrf's reagent (for alkaloids), 5% Ethanolic sodium hydroxide (for anthraquinones) (13-14). Since the dried extract contained mainly flavonoid glycosides, it was standardized in its flavonoid glycosides content (4.6%) by the reported method (15).

Free radical scavenging activity (DPPH assay)

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula of C18H12N5O6, was obtained from Fluka Chemie AG, Bucks. Quercetin (a well-known natural antioxidant) was obtained from Merck Company. The antioxidant activity of the extract was determined by a reported method (16,17).

Quantitative assay

The extract was dissolved in MeOH to obtain a concentration of 0.5 mg/mL. Dilutions were made to obtain concentrations of $5x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$, $5x10^{-5}$, $5x10^{-6}$, $5x10^{-7}$, $5x10^{-8}$, $5x10^{-9}$, $5x10^{-10}$ mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for completion of reactions. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicates and the average absorption was recorded for each concentration. The same procedure was followed for quercetin in MeOH as positive controls.

Animals and surgical procedure

The hearts of male Sprague-Dawley rats (280-320g) were rapidly excised and mounted via the aorta on a standard Langendorff perfusion apparatus with a perfusion pressure of 100 cm H_2O. Modified Krebs-Henseleit buffer solution containing (mM): NaCl (118.5), $NaHCO_3$ (25.0), KCl (4.8), $MgSO_4$ (1.2), KH_2PO_4 (1.2), D-glucose (12.0) and $CaCl_2$ (1.7) of pH of 7.4 as the perfusion medium was gassed with 95% O_2 5% CO_2, pH 7.4 at 37°C throughout the experiment (18-21). An epicardial ECG was recorded by a polygraph during the experiment using two silver electrodes attached directly to the heart. A fluid filled balloon was introduced into the left ventricle and inflated to give a pre-load of 8–10 mmHg (22). Hemodynamic factors including heart contractility force, left ventricular developed pressure (LVDP), rate pressure product (RPP) and coronary flow rate (CFR) were measured. CFR was measured by a time collection of the coronary perfusate that dripped from the heart. RPP was calculated by multiplying LVDP by HR.

A 4/0 braided silk suture was placed around the left anterior descending coronary artery. Following 20min stabilization, coronary occlusion (30min) was achieved by threading the loose ends of the ligature through a polyethylene occluder and clamping in place. Release of the clamp allowed reperfusion of the previously ischemic tissue (30min). Based on the Lambeth conventions, the ECGs were analyzed to determine the total number of ventricular ectopic beats (VEBs), the number of beats occurring as ventricular tachycardia (VT), and the incidence and duration of VT and ventricular fibrillation (VF) during both ischemia and reperfusion phases (23). The isolated hearts were allocated randomly to one of the following 5 groups (n=8-12 in each group): (1) control; (2-5) in which the hearts were perfused with rhizome hydroalcoholic extract of *C. dactylon* (25, 50, 100 and 200µg/ml, respectively). Selection of concentrations for the experiment was based on the results of a previous pilot study and for the preparation of the required concentrations, different amounts of the dried extract was completely dissolved in fresh Krebs-

Table 1 Effects of total extract of rhizome of *C. dactylon* (25-200µg/ml) on cardiac arrhythmias during 30min ischemia and 30min reperfusion in isolated rat hearts.

Groups	Ischemia time			Reperfusion time		
	VT Counts	VT Duration (sec)	Reversible VF Duration (sec)	VT Counts	VT Duration (sec)	Reversible VF Duration (sec)
Control	280±89	58±19	6±4	154±29	23±5	218±99
C. dactylon (25µg/ml)	10±4 ***	2±1 ***	0±0	17±10 ***	3±2 **	0±0 **
C. dactylon (50µg/ml)	22±18 **	7±4 **	0±0	42±20 *	7±4 *	0±0 **
C. dactylon (100µg/ml)	71±35	14±6	3±3	85±31	17±7	10±5 *
C. dactylon (200µg/ml)	154±40	20±7	5±4	161±46	29±8	43±15

Total VEBs is sum of arrhythmias occurring as single, salvos and VT. *** p<0.001, ** p<0.01, * p<0.05 compared to the control value. n= 8-12 rats in each group.

Henseleit solution then filtered and the hearts were perfused with enriched solution for the whole period of ischemia and reperfusion. To determine the isolated hearts' contractility force, LVDP as an index of contractility force was measured. Krebs buffer solution containing different concentrations of the extract was perfused for 1min at 20min intervals and maximum responses were recorded and compared to pre-perfusion values. The experiments were carried out in accordance with regulations of Tabriz University of Medical Sciences guideline for the care of laboratory animals.

Statistical analyses

Except for the incidences of VT and VF, all results are expressed as mean±SEM. One-way ANOVA with LSD post hoc test was carried out to test any differences between the mean values of hemodynamic factors and heart contractility force. To compare the number of VEBs and duration of VT and VF between groups, the Mann-Whitney non-parametric U test was employed. For the analysis of the incidences of VT and VF Fisher's exact test with Yates correction was used. Differences between groups were considered significant at a level of $p<0.05$.

RESULTS

Phytochemistry results

Phytochemical screenings showed that the rhizomes total extract of *C. dactylon* have significant amounts of sugars, flavonoids, sterols, steroidal saponins and trace amount of alkaloids.

DPPH assay

RC_{50} values (the concentration of the extract that reduces 50% of DPPH) for quercetin (standard) and the extract were 2.88×10^{-5} and 0.346 mg/ml, respectively.

Antiarrhythmic effects

Effects of *C. dactylon* on numbers, duration and incidences of arrhythmias during 30min ischemia followed by 30min reperfusion are summarized in Table 1 and Figures 1 and 2. In the ischemic time, perfusion of the hearts with 25 and 50µg/ml hydroalcoholic extract of *C. dactylon* produced significant reduction in the number and duration of VT ($p<0.001$ and $p<0.01$, respectively). The total number of ischemic VEBs were lowered from 667±148 in the control to 38±17 and to 56±22 in the treated group by the same concentrations ($p<0.001$ for both). The incidence of ischemic VT was also significantly decreased from 100% in the control group to 20 and 17% by *C. dactylon* (25 and 50µg/ml, respectively). Perfusion of the hearts at the dose of 100µg/ml produced significant reduction in the number of ischemic VEBs ($p<0.05$). Similar to the ischemic phase, *C. dactylon* at the concentration of 25 and 50µg/ml, significantly reduced the number and duration of reperfusion induced VT (Table 1). During reperfusion, incidences of VT was also lowered from 100% in the control group to 13 and 33% by the same concentrations ($p<0.001$ and $p<0.05$, respectively). At the same time, total VF incidences was decreased by 25 and 50µg/ml ($p<0.05$ for both). *C. dactylon* (25 and 50µg/ml) decreased the total number of VEBs from 349±73 (control) to 35 ± 17 ($p<0.001$) and 66 ± 26 ($p<0.01$). Perfusion of the extract (25-100µg/ml) markedly lowered reversible VF duration from 218±99sec in the control group to 0 sec, 0 sec and 10±5sec ($p<0.01$, $p<0.01$ and $p<0.05$, respectively).

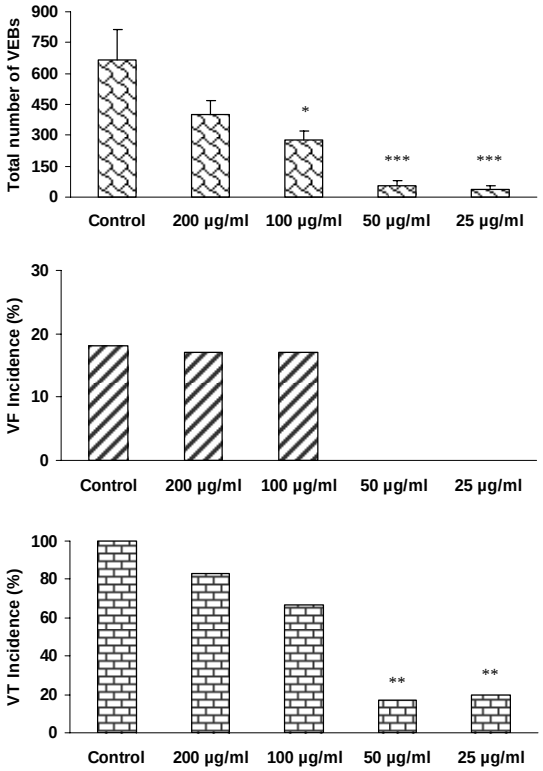

Figure 1. Effects of hydroalcoholic extract of rhizomes of *C. dactylon* (25-200µg/ml) on ischemic phase arrhythmias in isolated rat hearts. Total VEBs is sum of arrhythmias occurring as single, salvos and ventricular tachycardia (VT). *** p<0.001, ** p<0.01 and * p<0.05 compared to the control, respectively. n= 8-12 in each group.

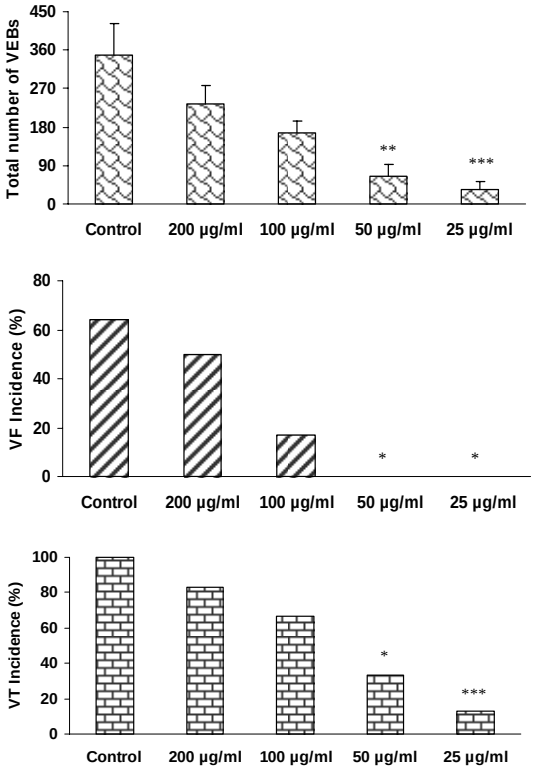

Figure 2. Effects of hydroalcoholic extract of rhizomes of *C. dactylon* (25-200µg/ml) on reperfusion phase arrhythmias in isolated rat hearts. Total VEBs is sum of arrhythmias occurring as single, salvos and ventricular tachycardia (VT). Total ventricular reversible and irreversible fibrillation (VF) incidence was recorded. *** p<0.001, ** p<0.01 and * p<0.05 compared to the control, respectively. n= 8-12 in each group.

Effects on cardiac hemodynamic functions

During ischemia, LVDP was significantly increased in the groups who received 25 and 50µg/ml of the extract (p<0.05 for both) compared to the control (Table 2). However, the effect was not significant at higher concentrations of the extract. Similarly, RPP was significantly increased only in concentrations of 25 and 50µg/ml in comparison with the control group. CFR was not significantly changed throughout the ischemia. At the reperfusion phase, *C. dactylon* (25 and 50µg/ml) elevated LVDP phase (p<0.05 for both) but at higher concentrations failed to increase this parameter. While RPP in comparison to the control was not changed by *C. dactylon*, perfusion of the hearts by 25 and 50µg/ml of *C. dactylon* produced significant improvement in RPP versus other concentrations. At the same time, CFR was lowered by all concentrations of *C. dactylon* (Table 2).

Effects of the extract on contractility force

Perfusion of the hearts with hydroalcoholic extract of rhizomes of *C. dactylon* (25, 50, 100 and 200µg/ml) produced potent and clear positive inotropic effects in isolated rat hearts. Compared to the control value (100), contractility force was increased by the above concentrations to 124±3% (p<0.05), 131±8% (p<0.01), 163±14% (p<0.001) and 143±15% (p<0.001), respectively (Figure 3).

DISCUSSION

Results of present study clearly show that hydroalcoholic extract of the rhizome of *C. dactylon* produces antiarrhythmic effects against I/R-induced arrhythmias when it was used during 30min ischemia and 30min reperfusion.

Perfusion of low concentrations of the extract produced significant reduction in the number, duration and incidences of VT and the number of VEBs during I/R. In addition, *C. dactylon* produced marked reduction in reversible VF duration and incidence of total VF at reperfusion time. During both ischemia and reperfusion phases, antiarrhythmic effects of *C. dactylon* were reversely dependent on the extract concentration, where lower concentrations showed greater effects. The discrepancies between the inhibitory

effects upon low concentrations and ineffectiveness at higher concentrations of the extract might be explained by this hypothesis that some of the active constituent(s) of *C. dactylon* at high concentration may probably exhibit pro-arrhythmic properties. It is also likely that the total extract may have components with both anti- and pro-arrhythmic effects.

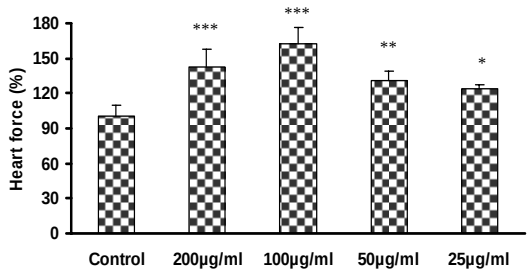

Figure 3. Effects of hydroalcoholic extract of rhizomes of *C. dactylon* (25-200µg/ml) on heart force in isolated rat hearts. *** $p<0.001$, ** $p<0.01$ and * $p<0.05$ compared to control value. n= 8-12 in each group.

In addition, the low concentrations of the total extract of *C. dactylon* produced significant improvement in LVDP and RPP (as a marker for heart performance). Similar to the antiarrhythmic effects, changes in hemodynamic factors was reversely dependent on the concentration of *C. dactylon.* Consistent with the above results, our unpublished data have shown that infusion of low dose infusion of the extract improved hemodynamic factors in isolated rat hearts while higher doses produced marked but sudden elevation in the heart force followed by severe arrhythmias and heart death. Although antiarrhythmic effects of *C. dactylon* extract has not been reported, but application of similar methods for effects of other medicinal plants against I/R-induced arrhythmias are not uncommon. In another study infusion of a hydroalcoholic extract of the flowering tops of Crataegus meyeri in anaesthetized male rats, resulted in a significant decrease in the total number of VEBs, mainly by reduction in the number of beats occurring as VT. Also a significant reduction in the time for VF without significant changes in the heart rate and blood pressure during the infusion of hydroalcohil and ethyl acetate extracts have been reported (24). However it has been reported that long-term application of Crataegus oxyacantha on I/R-induced arrhythmias did not show any cardioprotective effects neither in the heart in situ nor in the Langendorff preparations. (25). In agreement with results of this study, Nasa et al. demonstrated that Crataegus extract (0.05%) had cardioprotective effects on the ischemic-reperfused heart (26).

Results of this study showed marked positive inotropic effects of the extract at all concentrations. Phytochemical analysis have shown that the extract contain flavonoids, sterols and steroidal saponin (27,28). It has been reported that some plant's saponine improve cardiac function in the early stage after myocardial infraction in rats (29). Although it has been shown that different classes of flavonoids scavenge oxygen free radicals (30), since free radical scavenging activity of the extract was not significant in comparison with the standard it is unlikely that the antiarrhythmic action of the extract is directly related to its antioxidant effect.

From the results of this study, it seems that total extract of the rhizome of *C. dactylon* has some important effects on hemodynamic factors such as heart contractility, LVDP and could RPP could recover ischemic-reperfused isolated rat hearts and consequently has antiarrhythmic activity. It is also likely that *C. dactylon* has direct antiarrhythmic effects against I/R-induced arrhythmias. Future studies are required to determine the exact cardioprotective mechanism(s) of action of the extract.

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