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Efficacy of *Melissa officinalis* in Suppressing Ventricular Arrhythmias following Ischemia-Reperfusion of the Heart: A Comparison with Amiodarone

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Key Words

Melissa officinalis (lemon balm) · Heart · Ischemia-reperfusion · Ventricular arrhythmias

Abstract

Objective: We aimed to assess the influence of *Melissa officinalis* (lemon balm), a well-known herbal drug with numerous applications in traditional and modern medicine, on cardiac conduction and susceptibility to lethal ventricular arrhythmia. ***Materials and Methods:*** Forty-two male Wistar rats were divided into a control group (CTL), an *M. officinalis* group that received the aqueous extract of *M. officinalis* L. intraperitoneally (i.p.) at dosages of 50, 100, 200 and 400 mg/ml/kg, respectively, and an amiodarone group (Amio group) that received 30 mg/ml/kg i.p. of amiodarone. Heart ischemia/reperfusion was induced by the ligation and release of the left anterior descending branch of the left coronary artery. ***Results:*** There were no statistical differences between the groups in the basal heart rate and blood pressure. PR, corrected QT (QTc) and QRS intervals increased in the *M. officinalis* and Amio groups. PR and QTc were statistically significant only in the Amio group and QRS was significant only in the group receiving 400 mg of *M. officinalis* (M400 group) in comparison with the CTL group. During the reperfusion period, the decrease in ventricular fibrillations was statistically significant in all groups (except the M400 group) when compared with the CTL group. The score of arrhythmia severity also decreased, but was statistically significant only in the Amio group ($p < 0.05$ vs. CTL group). ***Conclusions:*** Our findings suggest that *M. officinalis* extract has a mild protective effect against reperfusion-induced lethal ventricular arrhythmias in rats.

Introduction

Melissa officinalis (lemon balm) belongs to the genus *Melissa* (family Lamiaceae) and is native to Europe, central Asia and Iran, but is now also common around the world and a well-known herbal drug in both Eastern and Western societies [1]. Traditionally, lemon balm is used to treat depression and melancholy [1], bronchitis and asthma [2], menstrual problems, hypertension, migraines, vertigo, fever [1, 3], snake bite [3], eczema and gout [1]. The herb can lift the spirits [1] and comfort the heart, and is useful for the mind, liver, spleen, digestion and fainting [1, 4]. Experimental research conducted in recent years has demonstrated the effectiveness of *M. of-*

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ficinalis in the prevention and treatment of Alzheimer's disease [5]. It also possesses antiviral, antibacterial, antifungal and antioxidant properties [6–8], has antihyperlipidemic and hepatoprotective effects [9] and displays antitumoral activity [10, 11]. Hassanzadeh et al. [12] and López et al. [13] report the neuroprotective properties of *M. officinalis*. Its positive effect on anxiety and sleep disturbances has also been reported [14]. Consumption of *M. officinalis* for the treatment of sleep disturbances and functional gastrointestinal complaints is approved by the German Commission E [15]. Internal use of this drug is recommended by the European Scientific Cooperative on Phytotherapy for the treatment of several neurological and gastrointestinal disorders [15].

Traditional medicine texts report in favor of the cardiovascular effects of *M. officinalis*, but less attention has been paid to such properties in modern scientific research. In an empirical study, Gazola et al. [16] indicated that *M. officinalis* aqueous extract significantly reduced the cardiac rate of isolated rat heart but had no effect on the heart contractile force. *M. officinalis* is in common use, but there is a lack of information about its influence on the electrical properties of the heart. This study was conducted to elucidate the effect of this herb on electrocardiogram (ECG) parameters and susceptibility to lethal ventricular arrhythmias in rats.

Material and Methods

Sodium thiopental and amiodarone were purchased from Sandoz (Austria) and Amin (Iran), respectively.

M. officinalis Extract

M. officinalis was collected in the spring of 2012 from the Sirjan area of the Kerman Province, Iran, as identified and confirmed by Dr. A.A. Maghsoudi Moud of the Department of Botany, Bahonar University of Kerman, Iran. A voucher specimen (KF1429-1) of the plant was deposited in the Herbarium Center, Faculty of Pharmacy, Kerman University of Medical Sciences. The dried aerial parts of the plants were ground and powdered to a particle size of about 0.5 mm. Boiling distilled water (10 ml) was added to the powder for 10 min to brew the *M. officinalis*. The mixture was then filtered and the liquid evaporated under vacuum at 45–50°C. The resulting material was then dried completely at 70°C as reported previously [16]. Finally, the prepared extract was stored in glass vials at –20°C prior to use. On the day of the experiment (half an hour before use), the required amounts of extract were weighed, dissolved in saline and then injected intraperitoneally (i.p.).

Animals

This experiment was performed on 42 male Wistar rats aged 3 months and weighing 250–350 g. The animals were divided into the control group (CTL group), 4 groups receiving different doses of *M. officinalis*, i.e. 50, 100, 200 and 400 mg/ml/kg (M50, M100, M200 and M400 groups), respectively, and the group that received amiodarone (Amio group), a classic antiarrhythmic drug. There were 6–8 animals in each group, housed in a temperature-controlled (23 ± 1°C) room with a 12-hour light-dark cycle. CTL rats received 1 ml/kg of saline (i.p.) as the vehicle of extract and the Amio group received 30 mg/ml/kg amiodarone 30 min before the basal blood pressure (BP) and ECG recording. The M50, M100, M200 and M400 rats received i.p. doses of the aqueous extract of *M. officinalis* L. This study was conducted according to the national guidelines for animal studies (Ethic Committee Permission No. 90/29P, Kerman University of Medical Sciences).

BP and ECG Recording and Heart Ischemia-Reperfusion Induction

Full details of the procedure have been described previously [17]. Briefly, under anesthesia (sodium thiopental 50 mg/kg i.p.), the right common carotid artery was cannulated with a catheter filled with saline plus heparin which connected to a pressure transducer of a PowerLab system to record the arterial BP throughout the experiment. During chest surgery and ischemia-reperfusion, the animals were artificially ventilated with room air at 50 strokes/min (a stroke volume of 0.8 ml/100 g of body weight). After the left thoracotomy and separation of the 5th and the 6th ribs by a small retractor, the pericardium was opened. A 6-0 silk suture was placed under the left anterior descending coronary artery at the level of the left atrial appendage. Basal ECG (limb lead II) and BP were recorded following recovery time from surgery (15 min). Exclusion criteria were the presence of cardiac arrhythmia or a sustained drop in mean arterial BP to <70 mm Hg. For ischemia induction, two ends of the suture were passed through a small plastic tube and then the tube and suture were pulled so that they were clamped and pressed together against the myocardium for a period of 10 min. Ischemia was confirmed by ST segment (the duration of time from the end of the S wave to the beginning of the T wave) elevation on ECG and myocardial color change. Reperfusion was initiated by releasing the clamp and removing the tube and tension on the suture. Recording of BP and ECG were continued during the reperfusion period for 15 min after the ischemia period was over [17].

Measured and Calculated Parameters

Mean arterial pressure (MAP) was calculated with the formula: MAP = Pd + (Ps – Pd)/3, where Pd is the diastolic arterial pressure and Ps is the systolic arterial pressure.

Basal PR (i.e. the time from the beginning of the P wave and the beginning of the QRS wave) and QT (the time from the beginning of the Q wave to the end of the T wave) intervals were calculated by means of ECG-recorded strips of 2 min each. To obviate the dependence of QT interval on heart rate (HR), corrected QT (QTc) intervals were calculated using Bazett's formula, normalized as $QTcn\text{-}B = QT/(RR/f)^{1/2}$, where RR is the R–R interval (i.e. the time between two successive R waves) and f = 150 ms [17, 18].

During the reperfusion period, ventricular arrhythmias were analyzed according to the Lambeth Conventions and defined as premature ventricular beats (PVBs) or premature ventricular contraction (PVC), discrete and identifiable premature QRS complexes (salvo), two or three consecutive PVBs (ventricular tachycardia, VT), a run of four or more consecutive PVBs (ventricular fibrillation, VF) and a signal where individual QRS deflections could not easily be distinguished from each other and where rate could no

Table 1. RR interval and QRS interval, HR and BP in all groups during the periods of the experiment

Group	RR interval, ms	QRS interval, ms	HR, beats/min	Basal MAP, mm Hg	End ischemia MAP, mm Hg	End reperfusion MAP, mm Hg
CTL	153±3	15.9±0.3	379±6	115±4	59±10‡	60±10‡
M50	167±6	15±0.8	362±14	105±8	46±11‡	56±6‡
M100	172±5	15.5±0.4	352±11	108±8	82±8‡	69±8‡
M200	153±4	18.1±0.8	380±7	112±5	80±14†	93±7†,#
M400	171±7	18.4±1.1*	355±14	104±4	78±6‡	77±4‡
Amio	158±5	16.6±0.5	386±12	109±7	53±13‡	61±8‡

Values are mean ± SEM.
* $p < 0.05$ versus the CTL, M50 and M100 groups. † $p < 0.05$ and ‡ $p < 0.001$ versus its basal value. # $p < 0.05$ versus the CTL, M50 and Amio groups.

longer be measured [19]. Episodes of PVC, VT and VF were counted and the duration of VT plus VF was measured in seconds.

The severity of arrhythmias was presented quantitatively by a previous scoring system with a slight modification [18], defined as: 0: <10 PVCs, 1: ≥10 PVCs, 2: 1–5 episodes of VT, 3: >5 episodes of VT or 1 episode of VF, 4: 2–5 episodes of VF, 5: >5 episodes of VF and 6: VT or VF or both with a duration of >300 s.

Statistical Analysis

One-way ANOVA and the post hoc Tukey test were used to compare HR, BP and basal ECG parameters and the number and duration of arrhythmias in the different groups. HR and BP differences within each group during the various stages of the experiment were determined using repeated-measures ANOVA and the Bonferroni test. The arrhythmia scores in each group were compared using the nonparametric Kruskal-Wallis and Mann-Whitney U tests. $p < 0.05$ was considered statistically significant. The results are presented as mean ± SEM.

Results

Hemodynamics

Just before the induction of ischemia, the values of basal MAP and HR did not show any significant differences between groups (table 1).The ischemia period was associated with a significant reduction in MAP in all groups versus their related basal values ($p < 0.05$ for M200 and $p < 0.01$ for the other groups; table 1). During reperfusion, MAP showed an ascending trend in the CTL, M50, M200 and Amio groups; however, at the end of this period (i.e. similar to the ischemia period), BP was significantly lower in all groups when compared to their baseline values. At the end of reperfusion, BP was higher in the M200 group than in the CTL, M50 and Amio groups ($p < 0.05$; table 1).

Basal ECG

There were some differences in the basal ECG of the groups. PR interval and QTcn-B tended to increase in all the treatment groups, but were significant only in the Amio group when compared with the CTL group ($p < 0.05$ for PR interval and $p < 0.01$ for QTcn-B; fig. 1). This pattern was also observed in the case of the QRS interval, but was significant only in the M400 group ($p < 0.05$ vs. the CTL, M50 and M100 groups; table 1).

Susceptibility to Arrhythmia during Reperfusion

There were no significant differences between groups in the incidence of PVCs, VT/VF episodes and VT/VF duration (fig. 2a–c). However, the number of VFs decreased significantly in all the treatment groups, except the M400 group, when compared to the CTL group ($p < 0.05$; fig. 2d). The score of arrhythmia severity decreased in all groups, but was significant only in the Amio group ($p < 0.05$ vs. CTL group; fig. 3).

Discussion

This study indicated the slowed heart's electrical conduction and the mild antiarrhythmic properties of *M. officinalis* in an in vivo experimental model; these manifested as a partial PR and QTc prolongation, a reduction in the incidence of VF and, to some extent, an attenuation of arrhythmia severity during the reperfusion period. The pattern of the *M. officinalis* effects on the electrical properties of the heart was similar to but weaker than the effects of amiodarone. On the other hand, pretreatment with *M. officinalis* at dosages of 100–400 mg/kg (especially 200 mg/kg) improved the BP level during heart

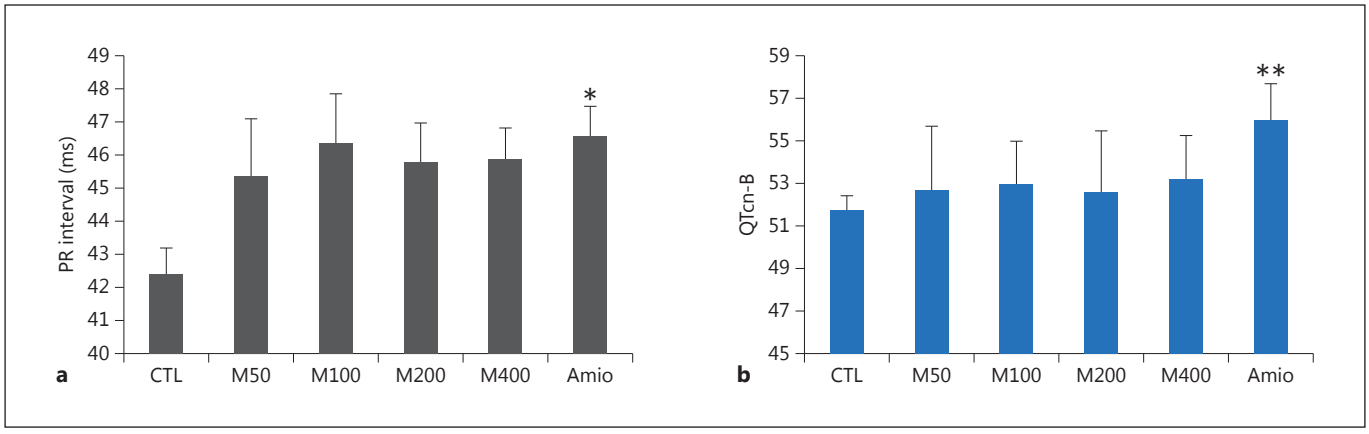

Fig. 1. Basal PR interval and QTcn-B in each group of animals (n = 6–8). Values are mean ± SEM. Amiodarone significantly increased the PR (**a**) and QTc (**b**) intervals. All doses of *M. officinalis* prolonged the PR and QTc intervals (not statistically significant). * $p < 0.05$, ** $p < 0.01$ vs. CTL group.

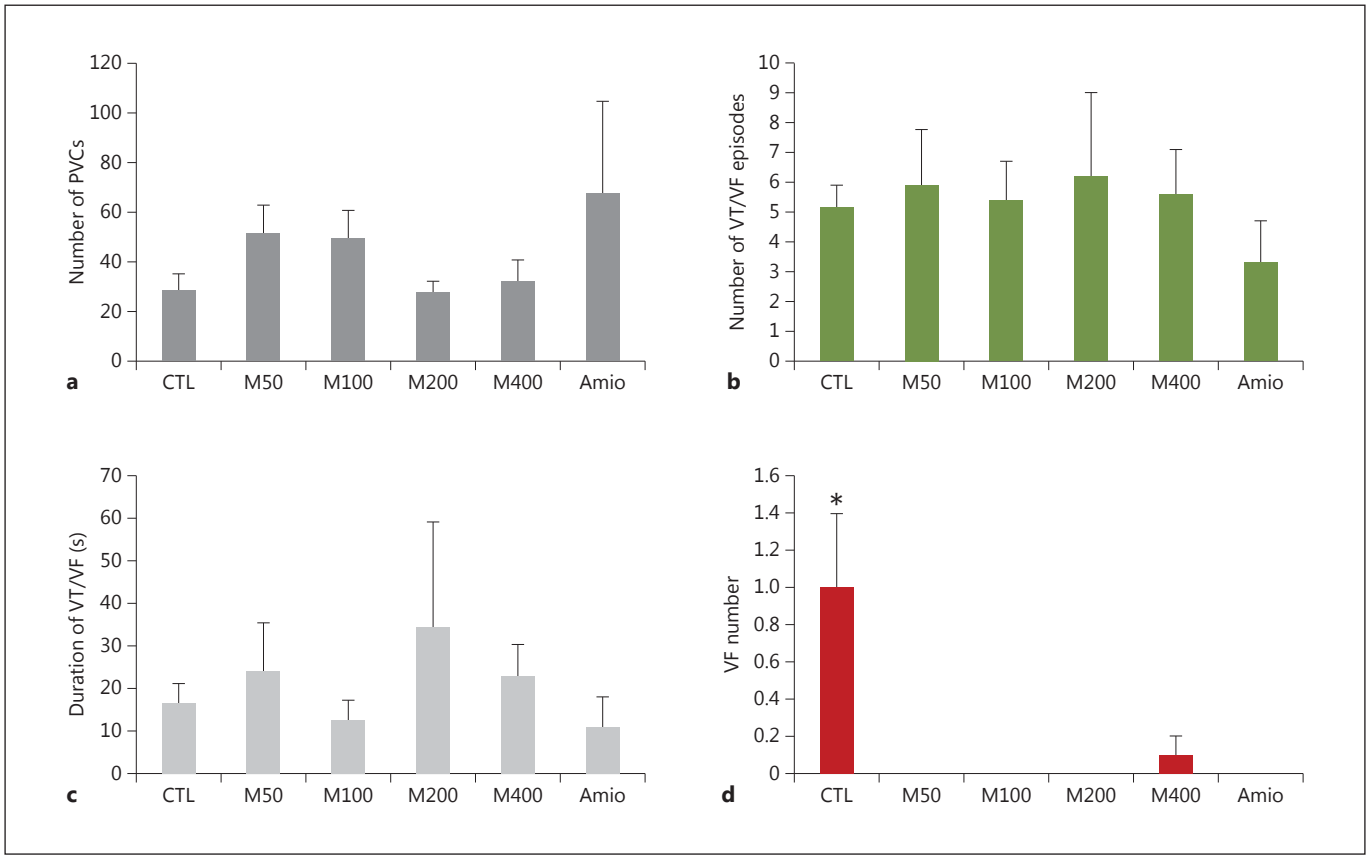

Fig. 2. Effects of different doses of *M. officinalis* on reperfusion-induced ventricular arrhythmias. Data are mean ± SEM. *M. officinalis* extract did not have a significant effect on the number of PVCs (**a**) and VT/VF episodes (**b**) or VT/VF duration (**c**), but its lower doses, i.e. 50, 100 and 200 mg/kg, reduced the number of VFs (**d**) in a manner equivalent to that with amiodarone. * $p < 0.05$ vs. CTL group.

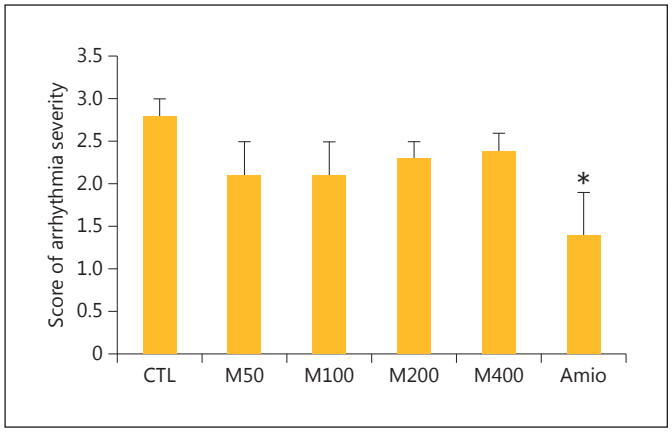

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Fig. 3. Despite the reduction of arrhythmia score in all test groups, this variable was only significant in the Amio group. Scores were defined as: 0: <10 PVCs, 1: ≥10 PVCs, 2: 1–5 episodes of VT, 3: >5 episodes of VT or 1 episode of VF, 4: 2–5 episodes of VF, 5: >5 episodes of VF, 6: VT or VF or both with a duration of >300 s. * $p < 0.05$ vs. CTL group.

ischemia-reperfusion. Amiodarone is important among the class 3 antiarrhythmic drugs; it blocks potassium, sodium and calcium channels and exerts a noncompetitive adrenergic blocking effect [20]. The electrophysiological manifestations of amiodarone include the prolongation of PR, QRS and QT intervals, which is a consequence of decreasing the heart conduction velocity and increasing the action potential duration and effective refractory periods via the inhibition of the sodium, potassium and calcium channels [20–23]. Consistent with previous reports [17, 20–23], in our study, amiodarone induced the prolongation of the PR and QTc intervals, thus confirming its antiarrhythmic effect.

Therefore, with the existing information, it is difficult to explain our findings concerning the mechanisms involved in the electrophysiological and antiarrhythmic properties of this agent, but there is some speculation. According to the similar pattern of ECG alteration after administering *M. officinalis* and amiodarone, the effects of lemon balm may be partly via the same pathways involved in the action of amiodarone; *M. officinalis* contains alkaloid compounds such as saponins and glycosides which could cause inhibition of cation channels [24]. Gazola et al. [16] reported that the extract of *M. officinalis* significantly reduced the cardiac rate of rat isolated heart, and suggested that this effect may provoke muscarinic receptor stimulation by the activity of its alkaloid compounds [25]. In our study, we did not observe the negative chronotropic effect of *M. officinalis* extract. The discrepancy here could have been due to the two different methods of conduction used, i.e. in vivo and isolated heart perfusion, with the result that the influence of some endogenic factors such as autonomic nervous system feedback were not easily observed in the in vitro method [16]. However, the stimulatory effect of *M. officinalis* on heart muscarinic receptors (acetylcholine receptors), which leads to the opening of acetylcholine-dependent potassium channels, may be involved in slow atrioventricular conduction and the PR interval prolongation. Lemon balm also contains flavonoids, phenolic acids, terpenes, rosmarinic acid and caffeic acids, all of which can have antioxidant effects [1, 26]. There is some evidence that the production of oxygen free radicals increases in ischemic tissue during heart reperfusion [26, 27], and reactive oxygen species scavengers are useful in reperfusion-induced arrhythmias [28, 29]. Therefore, some of the antiarrhythmic effects of *M. officinalis* may result from its antioxidant features which stabilize the redox balances of the heart.

Conclusion

Our findings show that *M. officinalis* extract provided a mild protective effect against ischemia-reperfusion-induced lethal ventricular arrhythmias in rats. This effect was revealed as a significant reduction in the incidence of VF, the most dangerous of the cardiac arrhythmias. According to the information available on the properties of lemon balm, this promising effect may be mediated via muscarinic receptor stimulation, increasing the effective refractory period of cardiac cells and the prevention of redox imbalance. However, further studies are needed to determine the mechanisms involved as well as the exact fraction of *M. officinalis* extract that is responsible for its electrophysiological and antiarrhythmic effects.

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