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Negative Chronotropic and Antidysrhythmic Effects of Hydroalcoholic Extract of Lemon Balm (*Melissa Officinalis* L.) on $CaCl_2$-Induced Arrhythmias in Rats

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Abstract

Background: In many cases, myocardial infarction leads to arrhythmia. Since antioxidant agents have an important protective role in heart disease, these compounds in medicinal plants are used in traditional medicine. Lemon balm extract, compared to other plants of the lamiaceae family, has been proven to have significant amounts of antioxidant compounds. The aim of this study was to assess the effect of the hydroalcoholic extract of lemon balm (Melissa officinalis L.) on CaCl2-induced arrhythmias in rats.

Methods: This research is an experimental study; male adult Sprague Dawley rats that weighed 200-250 g were divided randomly into three groups, i.e., 1) control (normal saline, 1 ml/kg/day), 2) extract (100 mg/kg), and 3) extract (200 mg/kg). The normal saline and the extracts were gavaged for 14 consecutive days. After anesthesia, lead II electrocardiograms were recorded for calculating the rats' heart rates (HRs). Arrhythmia was induced by intravenous injection of CaCl2 solution (140 mg/kg), and the percentages of incidence of ventricular tachycardia (VT), ventricular fibrillation (VF), and ventricular premature beats (VPB) were recorded. The results were analyzed by using Fisher's exact test and one-way ANOVA. P-values less than 0.05 were considered as significant level.

Results: Heart rates and percentages of incidence of VPB, VT, and VF were reduced significantly in extract groups (with the highest activity at 200 mg/kg) in comparison with the control group.

Conclusion: Melissa officinalis was considered to be an antiarrhythmic agent because it reduced the percentage of incidence of VPB, VT, and VF in the groups that received it. The results indicated that Melissa officinalis had a protective effect on the heart.

Keywords: arrhythmia, Melissa officinalis, chronotropic effect, rats

1. Introduction

Cardiac death is the most common cause of mortality in industrial countries. Ventricular tachycardia and ventricular fibrillation are the two most frequent causes of sudden death. Chronic heart dysfunction can be caused by ventricular arrhythmias (1). Medicinal plants have considerable roles in the treatment and prevention of diseases, and they have fewer side effects than chemical drugs (2). *Melissa officinalis* L. is a perennial shrub of the lamiaceae family; it has better antioxidant effects than other members of the family, and it is commonly known as 'lemon balm.' Recently,

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high levels of polyphenols and polysaccharides were identified in lemon balm. Biochemical analyses have shown that rosmarinic acid is the major compound in the extracts and, thus, may drive the pharmacological effects (3-5). *Melissa officinalis* extract also contains other phenolic acids, including ferulic, p-coumaric, and caffeic acids, and it contains several flavonoids, such as quercetin, kaempherol, and apigenin (6). Evaluation of the effect of *Melissa officinalis* oil in human and mice cells demonstrated an anti-tumor effect, which was related to its antioxidant function (7). In addition, M. *officinalis* has been said to mitigate the effects of stress on people (8). *M*elissa *officinalis* extract has a hypolipidemic effect and causes some parameters to decrease in serum, such as total cholesterol, lipids, alanine transaminase, aspartate transaminase, and alkaline phosphatase; it also decreases lipid peroxidation and increases the glutathione levels in the livers of hyperlipidemic rats (9). *Melissa officinalis* decreases oxidative gain with the improvement of superoxide dismutase, catalase, and glutathione peroxidase plasma levels; it also decreases DNA damage, lipid peroxidation, and myeloperoxidase in plasma (10). The study of the effect of the extract from *Melissa officinalis* leaves on inotropic and chronotropic action of rats' isolated hearts indicated that lemon balm extract reduced heart rate but had no effect on the heart's contraction (11). Considering the important effect of *Melissa officinalis* extract on some cardiovascular diseases motivated us to study the effect of the hydroalcoholic extract of lemon balm (*Melissa officinalis* L.) on $CaCl_2$-induced arrhythmias in rats.

2. Material and Methods

2.1. Study design and setting

The experimental study was conducted in 2013 at the Physiology Research Center of the Ahvaz Jundishapur University of Medical Sciences in Ahvaz, Iran. Twenty-four male Sprague Dawley rats were selected according to previous studies (12).

2.2. Chemicals

Ketamine and xylazine were purchased from the Alfasan Co. (Holland) and used for anesthesia.

2.3. Animals

Adult, male Sprague Dawley rats that weighed 200-250 g were housed in standard conditions (22+2 °C with a 12/12- h light-dark cycle). The animals had free access to tap water ad libitum and standard rat chow diet (Pars Co. IR). All procedures were performed in accordance with the standards for animal care and conformed to the Guide for the Care and Use of Laboratory Animals. Twenty-four rats were divided randomly into three groups of eight rats (in accordance with previous studies), i.e., a control group (normal saline,1 ml/kg/day), extract group 1 (100 mg/kg, and extract group 2 (200 mg/kg). The extract and normal saline were gavaged for 14 consecutive days (12). After this procedure, the animals were anesthetized by intraperitoneal injection of a mixed dosage of 50 mg/kg ketamine and 10 mg/kg xylazine, and lead II electrocardiograms were recorded to determine heart rates (HRs) (13). Arrhythmia was produced by intravenous injection of $CaCl_2$ solution (140 mg/kg) into the femoral vein through a cannula. Then, lead II electrocardiograms were recorded, and the percentages of ventricular fibrillation (VF), ventricular premature beats (VPB), and ventricular tachycardia (VT) were determined (13).

2.4. Preparation of the extract

Melissa officinalis leaves were purchased from the Dezfoul Market in Dezfoul, Iran, and identified by a faculty member. The leaves were cleaned to remove dirt and coarsely powdered manually. The powder was macerated in 70% ethanol for a period of three days at room temperature, and it was stirred three times daily. The mixture was filtered, dried at room temperature to evaporate ethanol, and the powder was stored at 4 °C (14).

2.5. Statistical analysis

The data obtained for heart rates were analyzed using one-way analyses of variance (ANOVA), and they were expressed as the mean ± the standard error of the mean (SEM). The results obtained from the $CaCl_2$-induced arrhythmia were analyzed using Fisher's exact test and expressed as a percentage. P-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of Extract on Heart Rate

HRs were significantly reduced in the animals that received 100 mg/kg of the extract compared with the control group ($P<0.05$); the 200 mg/kg dose produced even more significant decreases in HR compared with the control group ($P<0.01$) (Figure 1).

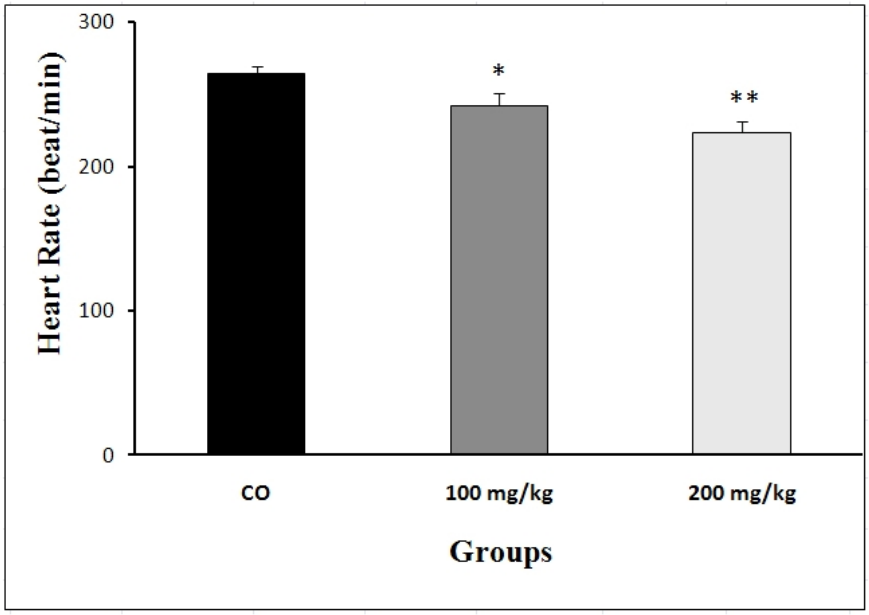

Figure 1. Comparison of the heart rates of the different groups [Control group and extract groups (100 and 200 mg/kg)]. Results are expressed as mean±SEM with eight rats per group; one way ANOVA followed by Fisher's Least Significant Difference (LSD) test was used. *P<0.05, **P<0.01 were compared with the control group.

3.2. Effect of Extract on $CaCl_2$-induced arrhythmia

The data from the control group were considered as 100. Other data were compared with the control group and the results were expressed as percentages. Evaluation of the effects of treating the rats for 14 days with the extract (100 and 200 mg/kg) showed significant decreases in the percentages of incidence of ventricular tachycardia (P<0.01), ventricular premature beats (P<0.05) and ventricular fibrillation (P<0.01 and P<0.001, respectively) compared with the control group (Table 1).

Table 1. Effects of *Melissa Officinalis* extract on $CaCl_2$-induced arrhythmia in the different groups of rats

Groups	**$CaCl_2$-induced arrhythmia**					
	VF[a]		VT[b]		VPB[c]	
	Incidence (%)	P-value	Incidence (%)	P-value	Incidence (%)	P-value
Control (Saline, 1 mg/kg/day)	100[d]		100[d]		100[d]	
Melissa Officinalis Extract (100 mg/kg)	40	< 0.01	50	< 0.01	85	< 0.05
Melissa Officinalis Extract (200 mg/kg)	20	< 0.001	33	< 0.01	71	< 0.05

[a]VF: ventricular fibrillation; [b]VT: ventricular tachycardia; [c]VPB: ventricular premature beats; [d]The data from the control group were considered as 100, and the results were compared to those of the control group expressed as a percentage data.

4. Discussion

The results presented in this study demonstrated anti-dysrhythmic effects of two different dosages of *Melissa officinalis* hydroalcoholic extract on $CaCl_2$-induced arrhythmia by decreased VF, VPB and VT. The negative chronotropic effect of *Melissa officinalis* hydroalcoholic extract also was demonstrated in this study. The important mediators of cellular injury, including hydroxyl radicals, superoxide anions, and hydrogen peroxide are known as reactive oxygen species (ROSs). ROSs participate in the process of damage to the sarcoplasmic membrane and

increased intracellular calcium during arrhythmia, thereby causing delay and early after depolarization (15). According to previous investigations, *Melissa officinalis* improves antioxidant defenses in humans (16). Studies have shown that extracts (alcohol or aqueous) from this plant can block the generation of chemically-reactive species, thereby inhibiting the initiation of lipid peroxidation. It also has been shown that *Melissa officinalis* extract can inhibit the common final pathway in the process of the peroxidation of polyunsaturated fatty acids due to its phenolic acid components, such as protocatechuic acid, caffeic acid, and rosmarinic acid, as well as its methyl esters (17). Further research investigated the potential antioxidant properties of iron (II) chelation, iron (III) reduction, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate), 1,1-diphenyl-2-picrylhydrazyl, nitric oxide free-radical scavenging, superoxide anions, and inhibition of β-carotene–linoleic acid bleaching assays (18). In in vitro tests, the extract demonstrated potential antioxidant activity in all of the assays. Our findings were in agreement with the anti-arrhythmic properties of *Melissa officinalis* (100-200 mg/kg).

In this study, *Melissa officinalis* was shown to have a negative chronotropic effect by the decreased HRs in all groups. Several polyphenolic compounds, such as caffeic acid derivatives and flavonoids, have been identified in lemon balm. The chemical composition of the essential oil of the plant leaf also has been studied, and the major compounds were citronellal, citral, and b-caryophyllene (19). Citral is a mixture of two monoterpenes, i.e., geranial and neral. Vasorelaxation is produced by citral, and it appears to be the result of the blockages of calcium channels and NO pathways that were shown in rats' aortas. Also, it has been shown that the vasorelaxation effects of the methanolic extracts of *Melissa officinalis* roots and leaves were exerted via blockage of the calcium channels (20). It has been found that calcium channels have an important role in pacemaker currents in cardiac nodal tissue. Blocking the calcium channel results in lowering the heart rate or a negative chronotropic effect is due to slowing down the conduction of electrical activity during the plateau phase of the action potential within the heart (21). In addition, the anti-arrhythmic effects of calcium-channel blockers (Class IV anti-arrhythmics) are due to their ability to decrease the firing rate of aberrant pacemaker sites and are related to their ability to decrease conduction velocity and prolong repolarization within the heart, especially at the atrioventricular node. Prolonging repolarization at the atrioventricular node could help to block reentry mechanisms, which can cause supraventricular tachycardia (22). Our findings relative to the negative chronotropic and anti-arrhythmic properties of *Melissa officinalis* extract were in agreement with these results. However, further research is necessary to prove this hypothesis. The small sample size was a major limitation in this study, because it made it difficult to generalize the results to the community. However, research is necessary to prove the hypothesis of this study.

5. Conclusions

The results of this study showed negative chronotropic effect of *Melissa officinalis* hydroalcoholic extract by decreased heart rate and also demonstrated an antidysrhythmic effect by decreased VF, VPB, and VT in rats. The results of this study indicated that *Melissa officinalis* could be considered an effective agent in the prevention of various cardiovascular diseases associated with oxidative stress, including arrhythmia. It is recommended that future studies investigate the effect of *Melissa officinalis* hydroalcoholic extract on other diseases associated with oxidative stress.

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Conflict of Interest:

There is no conflict of interest to be declared.

Authors' contributions:

All authors contributed to this project and article equally. Both authors read and approved the final manuscript.

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