Original Article

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The Evaluation of the Protective Effects of the Hydro-alcoholic Extract of Rosemary (Rosmarinus Officinalis L.) on Ventricular Arrhythmias in Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Arrhythmia is considered as a leading cause of sudden death among patients with cardiac diseases. Since antioxidant agents play a pivotal role in cardiac protection, many of them are used in the form of medicinal plants in traditional medicine. Rosmarinus Officinalis L., commonly known as Rosemary, is a medicinal herb with potent antioxidant properties. This study aimed to evaluate the protective effects of the hydro-alcoholic extract of Rosemary on ventricular arrhythmias in rats.

METHODS: In this experimental study, 32 Sprague-Dawley rats weighing between 250-200 g were divided into two main groups of control and receivers of hydro-alcoholic rosemary extract. The rats received the hydro-alcoholic extract of rosemary (50, 100 and 200 milligrams per kilogram per day via gavage) or Saline (1 ml/kg per day via gavage) for 14 days. Cardiac lead II was recorded in both groups after fourteen days of saline or rosemary extract administration. To induce arrhythmias, CaCl2 (140 kg) solution was injected intravenously, and the incidence of premature ventricular beats (PVB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were also calculated.

FINDINGS: The anti-arrhythmic effects of the hydro-alcoholic extract of rosemary (doses of 100 and 200 mg/kg) were observed following a significant decrease in the occurrence of PVB (75% and 50%, respectively), VT (71.4% and 42.9% %, respectively), and VF (50% and 25%, respectively) compared to the control group. In addition, the highest rate of this reduction occurred in the dosage of 200 mg/kg.

CONCLUSION: According to the results of this study, the hydro-alcoholic extract of rosemary could be used as a protective substance providing cardiac pre-conditioning against ventricular arrhythmias.

KEY WORDS: Hydro-Alcoholic Extract, Rosemary, Ventricular Arrhythmias, Rats.

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Introduction

Cardiovascular diseases are considered as the leading cause of death in developed and industrial countries. Research suggests that approximately half of all the deaths caused by cardiovascular diseases are sudden, and particularly due to arrhythmias (1). Cardiac arrhythmia is a heart rhythm disorder which is manifested through heart beat irregularities, increased heart rate (Tachycardia) or slow heart rate (Bradycardia). Arrhythmias are generally divided into two main types of atrial fibrillation and ventricular arrhythmia (2) which could occur in a variety of diseases such as coronary artery diseases and cardiomyopathy.

Furthermore, cardiac arrhythmias might lead to heart failure and sudden death (2). Atrial fibrillation is the most common cardiac arrhythmia, which is defined as a supraventricular tachycardia, and its prevalence increases with age (3). Rosemary (Rosmarinus Officinalis L.) is a perennial herb which belongs to the aromatic Lamiaceae family. This herb is native to the Mediterranean region and the Middle East, and lately, it has been planted in almost all countries in the world as an ornamental and medicinal herb. The stems, leaves and flowers of this plant contain essential oils, most of which are stored in the leaves (4). Several compounds have been discovered in the extract of rosemary, and the main components of the essential oil of rosemary include alpha-Pinene, Limonene, Camphor, Terpineol and 1,8-Cineole, all of which have unique practical uses in medical practice (4, 5).

Moreover, rosemary contains prominent antioxidant agents such as Carnosic acid, Rosmarinic acid, labiatic acid and Caffeic acid (4-6). The rosmarinic acid in this plant contributes to the production of Prostaglandin E2, reduces the production of Leukotriene B4 and inhibits the activity of the complement system (5).

According to the literature, rosemary has numerous other properties which are as follows: astringent, anti-inflammatory, anti-microbial, antiviral, antifungal and anti-parasitic, anti-rheumatic, anti-allergic, antidepressant, anti-diabetic, anti-tumor and anti-angiogenesis. Furthermore, rosemary is known to improve respiratory disorders, Parkinson's disease, Alzheimer's disease and memory loss, while providing liver protection, stimulating hair growth and preventing hair loss, and finally, improving blood pressure and atherosclerosis (4, 5, 7-9).

Several studies have confirmed the antioxidant properties of rosemary (9). This plant is widely known for its numerous antioxidant components, and is it mostly due to these agents that rosemary is able to inhibit lipid peroxidation and the production of free radicals (10-12). Moreover, the pharmacological effects of rosemary have mainly been attributed to the existence of antioxidant agents in this plant (13, 14).

Since previous studies have investigated the antioxidant and vasoactive properties of rosemary as well as the medicinal effects of this plant on the treatment of atherosclerosis and reduction of blood pressure, the present study aimed to evaluate the role of rosemary in reducing the risk factors of cardiovascular diseases such as arrhythmia. Furthermore, we investigated the protective effects of the hydro-alcoholic extract of rosemary on ventricular arrhythmias in rats.

Methods

Collection and Extraction of the Plant: The rosemary leaves were purchased from a market in Behbahaan city, Iran, and the material was identified by a faculty member of the university. After washing the leaves with water and drying them in the shade, they were milled into a powder and stored in a freezer until the time of extraction. Following that, 50 grams of the powder was dissolved in 200 ml of 70% ethanol, and the obtained mixture was preserved at room temperature for 3 days.

During this period, the solution was stirred several times on a daily basis and after 72 hours, it was filtered and the obtained solution was spread on a glass surface in order for the solvent to evaporate at room temperature. Finally, the dried extract was scraped off the surface of the glass, and the obtained powder was stored at 4◦C until it was dissolved in saline and was orally administered to the animals (15).

The Animals: In this study, 32 male Sprague-Dawley rats weighing between 250-200g were purchased from the proliferation section and maintenance of laboratory animals, Ahwaz University of Medical Sciences, Iran, and they were divided into groups of 8 (16). During the examinations, the animals were kept in standard cages in a ventilated room with a temperature of 24-23°C, within a photoperiod of 12 hours of light and 12 hours of darkness. The animals had open access to sufficient food and water, and the study was conducted

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following the ethics of working with laboratory animals.

Animal Preparation and Recording of ECG: Initially, the rats were anesthetized using a mixture of Ketamine Hydrochloride (50 mg/kg) and Xylazine (10 mg/kg) which were administered via intraperitoneal injection, and 70% volume alcohol was also used for skin disinfection. Following that, we made a longitudinal incision in the groin of the animals with a scalpel, and the thigh muscle and the skin were pulled back in two steps as to

find the femoral vein. Afterwards, an incision was made using corneal scissors in order to create a canal for sending a polyethylene catheter into the vein and tightening the surrounding tissue for injection.

Finally, using the Bio Amp and Power Lab devices (manufactured by AD-Instruments), cardiac electrical signals were recorded, and by using surface electrodes, standard bipolar limb lead II was also recorded for checking the electrocardiogram (ECG) (fig 1) (16).

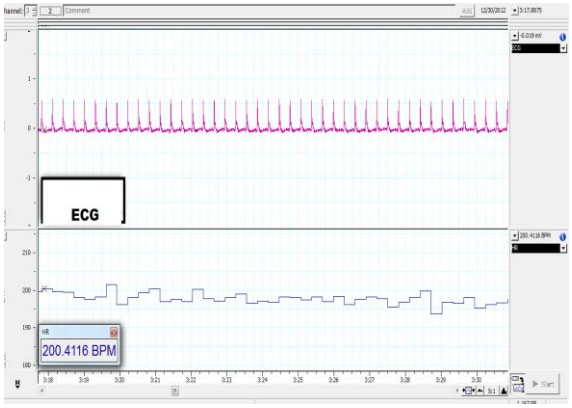


Figure 1. A Sample of ECG and heart rate diagram before the induction of arrhythmia

Grouping of the Animals: The animals were randomly divided into the four following groups:

1) The arrhythmia control group receiving normal saline (via gavage for 14 days)

2) The group receiving rosemary hydro-alcoholic extract (50 mg/kg via gavage for 14 days)

3) The group receiving rosemary hydro-alcoholic extract (100 mg/kg via gavage for 14 days)

4) The group receiving rosemary hydro-alcoholic extract (200 mg/kg via gavage for 14 days) (17).

Creation of the in vivo model of Ventricular arrhythmias: In this study, we used chemical techniques and intravenous injection of CaCl2 (140 mg/kg) for creating the in vivo model of ventricular arrhythmias (18). In order to stabilize the hemodynamic parameters, we recorded the standard bipolar limb lead II in all the study groups 15 minutes after anesthesia for three minutes as to check ECG.

Afterwards, CaCl2 injection was performed in both groups (control and receivers of the extract), and lead II was recorded again. In addition, the percentage of premature ventricular beats (PVB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were measured (fig. 2 and 3), and calculated as 100% in the control group. Finally, the incidence rate of these three types of arrhythmia was reported in the study groups receiving different doses of the hydro-alcoholic extract of rosemary (18).

Statistical Analysis: To evaluate the test data and compare the incidence of arrhythmias between the study groups, we used SPSS and Fisher's exact test, and $p<0.05$ was considered as significant.

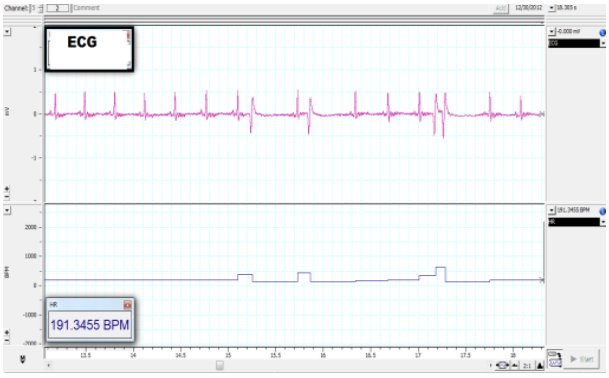


Figure 2. A Sample of the ECG and heart rate diagram after the induction of arrhythmia and observation of PVB

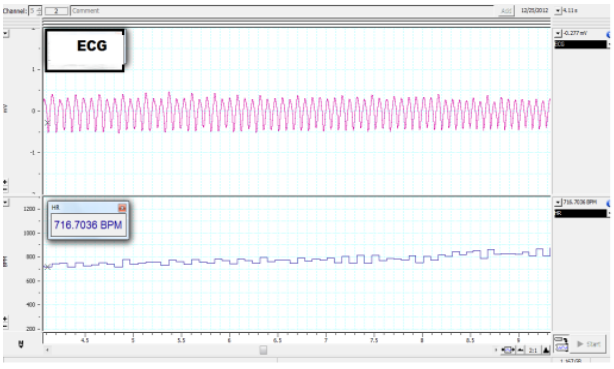


Figure 3. A Sample of ECG and heart rate diagram after the induction of arrhythmia and observation of VT

Result

Antiarrhythmic Effects: The incidence of PVB in the group receiving 50 mg/kg of hydro-alcoholic extract of rosemary (87.5%) showed no significant differences in comparison to the control group, whereas a significant

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reduction of 75% and 50% in the incidence rate was observed in the groups receiving 100 and 200 mg/kg of the extract, respectively ($p<0.05$, $p<0.01$) (fig 4).

Furthermore, the rate of VT in groups treated with doses of 100 and 200 mg/kg of rosemary extract showed a significant reduction of 71.4% and 42.9%, respectively ($p<0.05$, $p<0.01$), whereas the incidence of VT in the patients receiving 50 mg/kg of the extract (85.5%) showed no significant difference compared to the control group (fig 5).

On the other hand, doses of 100 and 200 mg/kg of the hyrdo-alcoholic extract of rosemary resulted in the significant reduction of VF by 50% and 25%, respectively compared to the control group ($p<0.01$, $p<0.001$) (fig 6). No significant difference was observed in the incidence of VF in the group receiving 50 mg/kg of the extract (100%) compared to the control group (fig 6).

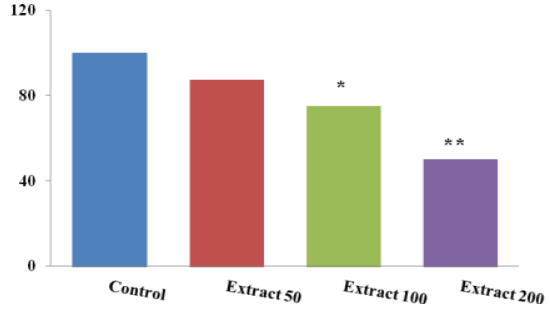


Figure 4. Incidence rate of PVB after the induction of ventricular arrhythmia in rats

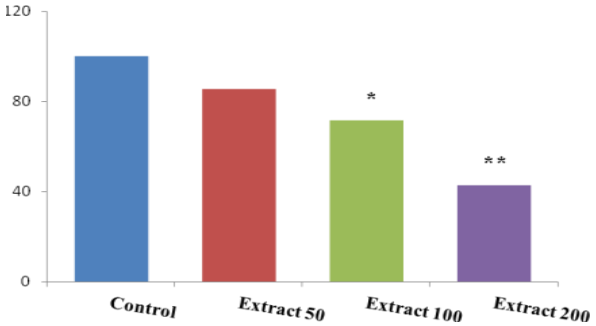


Figure 5. Evaluation of the incidence of ventricular tachycardia (VT) after the induction of ventricular arrhythmia in rats

The collected data from the control group (normal saline) were considered as 100%, and the results of the other groups (receiving hydro-alcoholic rosemary extract of 50, 100 and 200 mg/kg for 14 days) showed a significant difference compared to the control group ($p<0.01$, $p<0.05$).

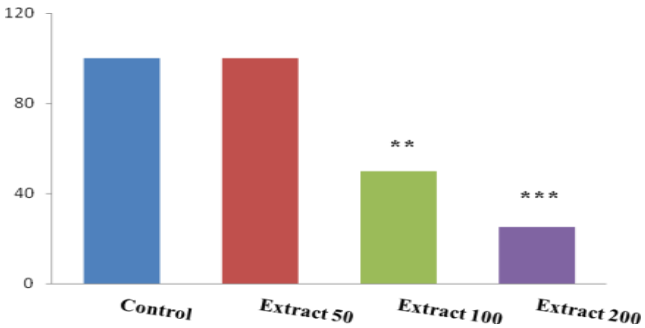


Figure 6. Evaluation of the incidence of VF after the induction of ventricular arrhythmia in rats

The collected data from the control group (normal saline) were considered as 100%, and the results of the other groups (receiving hydro-alcoholic rosemary extract of 50, 100 and 200 mg/kg for 14 days) showed a significant difference compared to the control group ($p<0.001$, $p<0.01$).

Discussion

In this study, the hydro-alcoholic extract of rosemary was administered via gavage at doses of 50, 100 and 200 mg/kg for 14 days and resulted in a significant reduction in the rate of ventricular arrhythmias (VT, VF, PVB) induced by the intravenous injection of CaCI2 in male rats.

Given their availability, limited side effects and favorable prices, medicinal herbs have always been considered as alternatives to chemical agents. Therefore, they play a pivotal role in the prevention and treatment of numerous diseases.

Previous studies have indicated that CaCI2 is able to cause arrhythmias by exerting a direct effect on the myocardium of the heart, while it may also indirectly affect the sympathetic nervous system (19).Therefore, the intravenous injection of CaCI2 was performed as to induce different ventricular arrhythmias in the current study. In addition, the anti-inflammatory and antioxidant properties of rosemary extract could result in the treatment of various diseases (4-9).

Other compounds found in this herb, such as carnosic acid and rosmarinic acid, have lead to the potent antioxidant features of rosemary (9). According to the results of this study, the anti-arrhythmic effects of the hydro-alcoholic extract of rosemary is probably caused by the reduced availability of calcium ions in the myocardial cells, which is due to the antioxidant agents found in this plant. On the other hand, increased action potential duration and recovery time are able to

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convert arrhythmia to normal sinus. In addition, cardiac enzymes are among the most prominent underlying elements likely to cause arrhythmias (19-21). In a study by Ibarra et al., rosemary extract was observed to reduce plasma cholesterol levels and blood glucose during the fasting state. Furthermore, it was found that the rosemary extract enriched with carnosic acid could be used in the prophylactic treatment of metabolic disorders since it is able to decrease cholesterol levels (22). In another study, Afonso et al. indicated that the phenolic compounds found in rosemary were able to reduce oxidative stress in rats with induced hypercholesterolemia (23).

On the other hand, the results obtained from the cardiac tissue samples, cerebral cortex and hippocampus in a study by Posadas et al. were indicative of a significant decrease in the lipid peroxidation in the cortex and hippocampus, as well as reduced levels of catalase activity in the heart and cortex of rats receiving enriched rosemary supplementation (20% of carnosic acid) (24).

In another study, Anadon et al. considered high doses of rosemary extract (more than 2000 mg/kg) as oral lethal doses in male and female rats (25); therefore, we used doses of 50, 100 and 200 mg/kg in this study. Although all the three types of arrhythmia in the groups treated with doses of 100 and 200 mg/kg of the hydro-alcoholic extract showed significant regression, the dose of 50 mg/kg could not affect the incidence of arrhythmias.

Further results of the present study were indicative of the dose-dependent effects of the the hydro-alcoholic extract of rosemary in rats after 14 days. Several other studies have also confirmed the positive effects of similar doses, which is indicative of the dose-dependent effects of this extract. In this regard, Zendehdel et al. demonstrated that rosemary extract (50, 100 and 200 mg/kg via intraperitoneal injection) could induce dose-dependent analgesic effects (17). In another study by Aqel et al., the dose-dependent relaxant effects of rosemary oil on the tracheal smooth muscle of rabbits and guinea pig were demonstrated in vitro (26).

In their study, Hamta et al. indicated that the alcoholic extract of rosemary was able to destroy cancer cells in a dose-dependent manner (27). Moreover, they claimed that the extract could provide cardiac protection against different types of ventricular arrhythmias such as VT, VF, PVB. Further research is required as to determine the exact mechanism of the rosemary extract in larger sample sizes and with a more comprehensive understanding of the cardiac cells and the related channels.

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