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Sage (*Salvia officinalis* L.) Protects Against Cardiac Arrhythmias and Electrocardiogram Irregularity in Rats

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Introduction: Cardiac arrhythmia is one of the most common types of heart diseases, which is a main cause of mortality. Drugs used to treat the cardiac arrhythmia are called antiarrhythmic drugs. Drugs such as amiodarone, can induce some of the side effects. Antioxidant agents play an important protective role in cardiovascular diseases. Therefore, medicinal herb with antioxidant properties is regarded as an alternative to chemical drugs. *Salvia officinalis* is an evergreen perennial shrub commonly seen all over the Mediterranean and south-eastern Europe regions. The aim of this study was to evaluate the electrophysiology and antiarrhythmic properties of Sage (*Salvia officinalis* L.) on $CaCl_2$ - induced arrhythmias in rats.

Materials and Methods: Forty male Sprague-dawley rats (200-250 g) were divided into 5 groups: control (N/S, 1 ml/kg, gavage, 28 days), hydroalcoholic extract of all part of Sage (100, 150, 200 mg/kg, gavage, 28 days), and amiodarone (10 mg/kg). Lead II electrocardiogram was recorded for calculating HR and voltage of QRS complex. The arrhythmia was produced by i.v. injection of a solution $CaCl_2$ (140 mg/kg). Percentages of Ventricular arrhythmias (tachycardia, fibrillation and premature beats) were recorded. Results were analyzed using one-way ANOVA, t-test and Fisher's exact test.

Results: Results showed positive inotropic and negative chronotropic effects in all groups in comparison with the control group. Administration of *Salvia officinalis* showed that incidence of Ventricular premature beat, Ventricular tachycardia and Ventricular fibrillation were significantly reduced compared to amiodarone group.

Conclusions: The results suggest a protective role of *Salvia officinalis* against cardiac diseases. This effect may have to do with the antioxidant properties of phenolic compounds in Sage.

Keywords: Sage, Arrhythmia, Electrocardiogram, Amiodarone, Rat.

Introduction

An abnormality of the cardiac rhythm is called a cardiac arrhythmia. In industrialized countries, cardiac arrhythmias are one of the most common causes of sudden death. Cardiac arrhythmias are disturbances in the rhythm of the heart, manifested by irregularity or by abnormally fast rates (tachycardia) or abnormally slow rates (bradycardia). Arrhythmias, including atrial fibrillation (AF) and ventricular tachycardia (VT), are important public health issues (Huikuri et al., 2001).

Due to the severity of heart attacks and disorders, drug and non-drug therapy is required. The drugs used to treat the cardiac arrhythmia are called antiarrhythmic drugs. Examples are quinidine, amiodarone, propafenone, veramapril and lignocaine. In most cases, medical therapies may intensify the side effects making the treatment less effective (Hohnloser et al., 2000). Human studies indicate that the use of amiodarone for the prevention of postoperative atrial fibrillation increases the risk of developing hypotension (10% to 30%), asystole/cardiac arrest (3.5%), cardiogenic shock (3%), congestive heart failure (2.2%), ventricular tachycardia (1.8%), second- and third-degree AV nodal block (less than 2%), AV heart block (1%), and Torsades de pointes (twisting of the points) (0.7%) (Weinberg et al., 1993).

Herbal medicinal products have been always regarded as an alternative to chemical drugs mainly because of their ease of access, low side effects as well as their low cost, (Ekor, 2013).

Sage (*Salvia officinalis* L.) has been an important herbal medicinal product since very early times and is still in wide use today. Sage is a plant of the family *labiatea*, native to the Mediterranean coasts of Europe, which is also cultivated in Iran. 30-60 cm tall, with bright green leaves and has a network of capillary interactions long. Its fruits are light or dark brown (Mozaffarian, 2007). Sage is the most valuable herbal medicinal in the mint family and is an important therapeutic specificity. In addition, Sage hasproperties facilitates digestion, diuretics; it is anticonvulsants, antiseptic and reduces the

amount of blood sugar (Arzi et al., 2011). Sage tea was effective in the improvement of lipid profile, antioxidant defenses, and lymphocyte Hsp70 protein expression. The usefulness of Sage for the treatment of gout, chronic rheumatism, Alzheimer's disease, vertigo, headaches, abdominal pains and colds has been shown by a multicenter open clinical trial (Bommer et al., 2009).

Although the underlying mechanisms of its treatment properties are unknown, polyphenols such as carnosol, carnosic acid, rosmanol, apigenin, hispidulin, caffeic acid, and ursolic acid have been discussed as active compounds for these pharmacological effects. An excellent review on Sage polyphenols was provided by Lu and Foo (Imanshahidi and Hosseinzadeh, 2006). Other compounds identified in the essential oil of this plant are thujon, 1 -8 cineole or cineol, and borneol (Walch et al., 2011).

Animal experiments and in vitro studies have substantiated that a vast amount of circumstantial evidence implicates oxygen-derived free radicals (especially superoxide and hydroxyl radical) and high-energy oxidants (such as peroxynitrite) play an important role in various pathological conditions of the cardiovascular, renal, diabetes, cancer, immunodeficiency and aging (Valko et al., 2007).

Recent studies demonstrate that antioxidant treatment inhibits the activation of free radicals and prevents the organ injury associated with shock, inflammation, and ischemia/reperfusion (Venardos et al., 2007).

In this study, we have focused on the beneficial effects of Sage hydro alcoholic extract on $CaCl_2$ induced arrhythmias.

Materials and Methods

Animals

Forty adult male Sprague-dawley rats (200-250 g) were purchased from animal house of Ahvaz Jundishapur University of Medical Sciences. Animals were housed in polyethylene cages at a room under the same conditions such as temperature controlled room 22±2 ˚C, adequate ventilation, with a 12 h dark- light cycle supplied with food and water ad libitum. The animals were divided into 5 groups (8 rats in each): control (normal saline, 1mL/kg, gavage, 28 days), Sage (100, 150, 200 mg/kg, gavage, for 28 days) (Ahmadi, 2012), and amiodarone (10 mg/kg) (Dianat and Akbari 2014). This study was approved by the animal care and ethical committee of the Ahvaz Jundishapur University of Medical Sciences (Grant No. 93S28).

Extract preparation

In this experimental study, dry Sage (all its parts) was purchased from Ahvaz green-groceries and after being authenticated by botany experts were powdered by grinder. For hydro-alcoholic extract, the fine powder obtained was firstly macerated in 70/30 methanol and distilled water for 72 h. Then, the mixture was filtered with whatman grade No.1 filter paper and centrifuge with 3500 rpm for 20 min. In the end, supernatant was removed and the solid remainder was collected and dried at room temperature and extracted powder was kept at 4 °C until used (Akhondali et al., 2015).

Experimental protocol

Heart rate and QRS complex recording

The animals were operated under anesthesia with combination of xylazine (10 mg/kg) and ketamine (50 mg/kg) via intraperitoneal (ip) route (Akhondali et al., 2015). Lead II electrocardiogram (ECG) was recorded by Bio Amp and monitored by a Power Lab system (AD-Instruments, Australia). Heart rate (as a chronotropic property) and QRS complex (as an inotropic property) were calculated from ECG recording during the first day and after the experiment (28 days).

The manner of induced and recording of arrhythmias

After anesthesia, ECG was recorded in all groups for 15 min, before the induction of chemical- arrhythmia to allow hemodynamic equilibration. Prep & drep with alcohol were done. Then, an incision was created in area of groin; a poly ethylene catheter was inserted in femoral vein. In this study, arrhythmia was induced by intravenously injection of $CaCl_2$ (140 mg/kg), the percentage of incidence of premature ventricular beats (PVB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were calculated after injection of $CaCl_2$ (Akhondali et al., 2015).

Statistical analysis

Data were analyzed using SPSS (V 17.0) and expressed as Mean ± SEM. Comparisons among groups were performed using t-test, one way ANOVA and fisher exact test. P-values of less than 0.05 were considered significant statistically.

Results

Effect of *Salvia officinalis* hydroalcoholic extract on inotropic properties of heart in rats

Twenty four hours before (study day zero) and after (28 day) Sage administration, the ECG was recorded and the baseline inotropic properties were measured. Results in this study showed that, in Sage (100-150-200 mg/kg) groups, inotropic effect significantly increased compared to control groups and increase in cardiac contractility in Sage 150 and200 mg/kg was shown to be more than the lower dose (Fig.1).

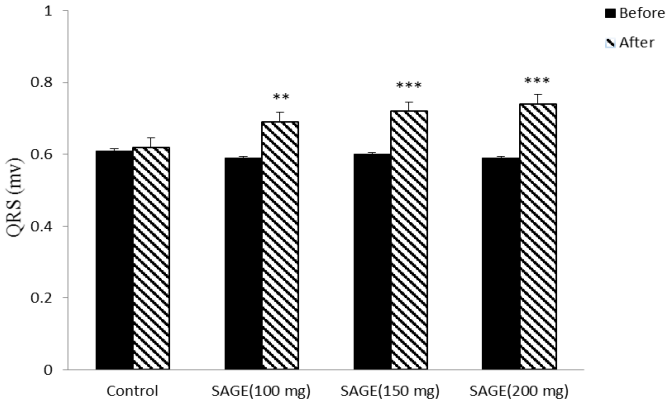

Figure 1: Effect of Sage (100, 150 and 200 mg/kg) on voltage of QRS complex in rats. Results were expressed as Mean ± SEM of 8 rats per group, used t-test; **P<0.01, ***P<0.001.

Effect of *Salvia officinalis* hydroalcoholic extract on chronotropic properties of heart in rats

This experiment also examined the effects of Sage on heart rate in rat groups. The results showed that, in Sage (100,150 and200 mg/kg) groups, chronotropic effect was significantly reduced compared to the control groups (Fig. 2) Also, decrease in heart rate in Sage (150-200 mg/kg) was shown to be more than Sage (100 mg/kg).

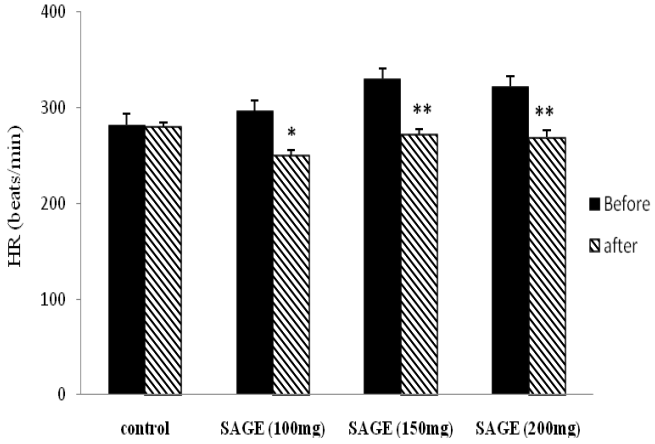

Figure 2: Effect of Sage (100-150-200 mg/kg) on heart rate in rats. Results were expressed as Mean ± SEM of 8 rats per group, analyzed by t-test; *P<0.05, **P<0.01.

Effects of *Salvia officinalis* hydroalcoholic extract and amiodarone on $CaCl_2$-induced arrhythmia

A record of lead II electrocardiogram showed changes such as shortened QT interval, prolonged PR and QRS intervals, increased QRS voltage, T-wave flattening and widening, and notching of QRS. In Sage Groups (100,150 and 200 mg/kg), intravenous injection of 140 mg/kg showed no alterations in the P-QRS-T waves (data not shown). Finally, animals were recovered and there was no mortality. Besides, evaluation of effects of 28-days Sage (100-150-200 mg/kg) and amiodarone (10 mg/kg) showed that incidence of ventricular premature beat, ventricular tachycardia and ventricular fibrillation were significantly reduced in all groups compared to control groups. Comparison of three doses of Sage and amiodarone reduction effects on $CaCl_2$-induced arrhythmia showed that Sage (150 and 200 mg/kg) was more effective (Table 1).

Table 1: Effects of *Salvia Extract* and amiodarone on $CaCl_2$-induced arrhythmia

Groups	arrhythmia		
	VF (%)	VT (%)	PVB (%)
Control (saline) (1 mL/kg, po, 28 days)	100	100	100
Salvia Extract (100 mg/kg, po, 28 days)	40**	85*	75*
Salvia Extract (150 mg/kg, po, 28 days)	25***	50**	45**
Salvia Extract (200 mg/kg, po, 28 days)	30***	40**	50**
Amiodarone (10 mg/kg)	40**	40**	45**

The data from the control group (saline) was considered as 100 % and the results were compared to those of other groups and are expressed as a percentage (Mean ± SEM; VT: Ventricular tachycardia, VF: Ventricular fibrillation; PVB: Ventricular premature beats; po: Orally; IV: Intravenously, *P<0.05, **P<0.01, ***P<0.001 vs control group).

Discussion

The results presented in this study demonstrate antidysrhythmic effects of *Salvia officinalis* Hydro alcoholic extract on $CaCl_2$-induced arrhythmia by decreased VF, PVB and VT in rats.

In earlier studies, it has been shown that antioxidant therapy is useful in the management of cardiovascular problems. Our finding of the positive influence of Sage on inotropic-chronotropic property and antiarrhythmic effect in $CaCl_2$ induced arrhythmia model, accords with previous research on cardioprotective properties of natural antioxidants (Hung et al., 2000).

$CaCl_2$ increased calcium and sodium levels while decreased potassium levels in blood. During the period of arrhythmias induced by $CaCl_2$, calcium overload lead to an increase in free radicals. The free radicals cause damage to the sarcoplasmic membrane of cardiocytes and increased intracellular calcium during arrhythmia, resulting in early and delay after depolarization (Hanna et al., 2004). In line with previous

investigations, Sage improves antioxidant defenses in humans. It was shown that *Salvia officinalis* increased activation of superoxide dismutase (SOD) glutathione peroxidase and catalase and as a scavenger to eliminate free radicals and therefore, Sage exerts has protective effects against destructive role of free radicals (Sá, 2009). Further research also shows the antioxidant activities of the Sage polyphenols, consisting of flavone glycosides and a range of rosmarinic acid derivatives scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide anion radicals. It can also reduce molybdenum (VI) to molybdenum (V). Also the *Saliva officinalis* derivatives all showed potent antioxidant activity and their capacity to reduce molybdenum (VI) to molybdenum (V) and their superoxide radical scavenging activities, with increased SOD activity (Lu and Foo, 2001). These results are in agreement with those in previous research on Sage 100,150 and 200 mg/kg (Lu and Foo, 2001). Since the effect of *Salvia officinalis* on antioxidant enzymes activities are well documented in previous studies, it is possible that in the present experiment, Sage was able to protect antioxidant enzymes inactivation and reduce oxidative stress in $CaCl_2$ model and protect heart against arrhythmias.

The Sage protective effects were similar to the effect of the amiodarone. Sometimes they appeared to have even more effective properties. In this study, Sage was shown to have positive inotropic effect by increased QRS complex amplitude in all groups.

As antioxidant agent, Sage probably eliminates factors that decreased the amplitude of QRS complex. Also previous studies have shown that beneficial cardiovascular effects of flavonoids and phenolic compounds are not caused only by direct antioxidant activity (Mladenka et al., 2010). Sage is a polyphenols compound (Lu and Foo, 2002). Tadano's study on therapeutic potential of polyphenols compound demonstrated, a cardiotonic activity by sensitizing troponin-C (TN-C) to Ca^{++}, that led to increase contractility of heart (Tadano et al., 2010). Thus, further researches are necessary to verify this hypothesis. Changes in inotropic property alter the rate of force and pressure development by the ventricle, and therefore change the rate of ejection.

In this study, Sage was shown to have with negative chronotropic effects. Perhaps increase in cardiac output stretches the baroreceptors and the increased firing results in the vasomotor center inhibiting sympathetic drive and increasing vagal tone on the SA node of the heart. The SA node is slowed by the acetylcholine and heart rate slows to correct the increase in cardiac output. The mechanism underlying such functions is unknown to the author and we are not certain as to how it creates its therapeutic effects. Thus, it is a matter in need of further investigation.

The results of this study showed antidysrhythmic effect by decreased VF, VPB, and VT in rats and also demonstrated negative chronotropic and positive dromotropic of *Salvia officinalis.* hydroalcoholic extract by decreased heart rate and increased QRS voltage complex, respectively. The major finding of this study is that *Salvia officinalis* could be considered an effective agent in the prevention of various cardiovascular diseases associated with oxidative stress, including arrhythmia. Also, due to dromotropic properties, it can be used for future development in the management of heart failure. It is recommended that future studies investigate the effect of Sage hydroalcoholic extract on antioxidant enzymes activities and plasma levels of oxidative stress markers in $CaCl_2$-induced arrhythmias.

Conclusion

The results of this study indicated that *Salvia 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 Sage 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.

References

1. Ahmadi R, Abdollahy E. 2012. Effects of salvia officinalis extract on serum level of creatine kinase and alkaline phosphatase in male rats. RJMS, 19 (96): 20-25.
2.Akhondali, Z., Dianat, M., & Radan, M. 2015. Negative chronotropic and antidysrhythmic effects of hydroalcoholic extract of lemon balm (*Melissa Officinalis* L.) on cacl2-induced arrhythmias in rats. Electronic Physician, 7(1): 971-980.
3. Arzi, A., Sarkaki, A., Aghel, N., Nazari, Z., & Zarei, N M. 2011. The effect of saliva officinalis hydroalcoholic extract on analgesic effect of morphine in rat. AJUMS, 5(47): 505-513.
4.Bommer S., Klein P., Suter A. 2009. A multicentre open clinical trial to assess the tolerability and efficacy of Sage tablets in menopausal patients with hot flushes. Planta Med, 75(10): 1070-1076.
5.Dianat M, Akbari GH. 2014. Protective effect of hydroalcoholic extract of hawthorn fruit on $cacl_2$- induced arrythmias in rats. Jundishapur Sci Med J, 12(6):693-703.
6. Ekor M. 2013. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol, 4:177.
7. Hanna, J., Chahine, R., Aftimos, G., Nader, M., Mounayar, A., Esseily, F., & Chamat, S. 2004. Protective effect of taurine against free radicals damage in the rat myocardium. Experimental and Toxicologic Pathology, 56(3), 189-194.
8. Hohnloser, S. H., Kuck, K. H., & Lilienthal, J. 2000. Rhythm or rate control in atrial fibrillation—pharmacological intervention in atrial fibrillation (PIAF): a randomised trial. The Lancet, 356(9244): 1789-1794.
9. Huikuri HV, Castellanos A, Myerburg RJ. 2001. Sudden death due to cardiac arrhythmias. New England Journal of Medicine, 345(20):1473-82.

10.Hung LM, Chen JK, Huang SS, Lee RS, Su MJ. 2000. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovascular Research, 47(3):549-55.
11.Imanshahidi M., Hosseinzadeh H. 2006. The pharmacological effects of Salvia species on the central nervous system. Phytother. Res, 20(8) 427–437.
12.Lu, Y., Foo, L. Y. 2001. Antioxidant activities of polyphenols from Sage (Salvia officinalis). Food Chemistry, 75(2), 197-202.
13.Lu YR., Foo LY. 2002. Polyphenolics of Salvia – a review. Phytochemistry, 59(4): 117–140.
14. Mladenka, P., Zatloukalová, L., Filipský, T., & Hrdina, R. 2010. Cardiovascular effects of flavonoids are not caused only by direct antioxidant activity. Free Radical Biology & Medicine, 49(6), 963.
15.Mozaffarian V. 2007. A dictionary of Iranian plant names. Tehran: Farhany moaser, P. 617.
16.Sá C M., Ramos AA., Azevedo MF., et all. 2009. Sage tea drinking improves lipid profile and antioxidant defences in humans. Int. J. Mol. Sci, 10(4): 3937–3950.
17.Tadano N., Du, CK., Yumoto, F., et all. 2010. Biological actions of green tea catechins on cardiac troponin C. British Journal Of Pharmacology, 161(5): 1034-1043.
18. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology, 39(1): 44-84.
19. Venardos, K.M., & Kaye, D.M. 2007. Myocardial ischemia-reperfusion injury, antioxidant enzyme systems, and selenium: a review. Current Medicinal Chemistry, 14(14): 1539-1549.
20. Walch, S.G., Tinzoh, L.N., Zimmermann, B.F., Stühlinger, W., & Lachenmeier, D.W. 2011. Antioxidant capacity and polyphenolic composition as quality indicators for aqueous infusions of Salvia officinalis L. (Sage tea). Frontiers in Pharmacology, 79(2): 122-134.
21.Weinberg BA, Miles WM, Klein LS, et al. 1993. Five year follow-up of 589 patients treated with amiodarone. Am Heart J, 125(23): 109-20.