Nerol Attenuates Ouabain-Induced Arrhythmias

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Nerol (C10H18O) is a monoterpene found in many essential oils, such as lemon balm and hop. In this study, we explored the contractile and electrophysiological properties of nerol and demonstrated its antiarrhythmic effects in guinea pig heart preparation. Nerol effects were evaluated on atrial and ventricular tissue contractility, electrocardiogram (ECG), voltage-dependent L-type Ca2+ current (ICa,L), and ouabain-triggered arrhythmias. Overall our results revealed that by increasing concentrations of nerol (from 0.001 to 30 mM) there was a significant decrease in left atrium contractile force. This effect was completely and rapidly reversible after washing out (∼2 min). Nerol (at 3 mM concentration) decreased the left atrium positive inotropic response evoked by adding CaCl2 in the extracellular medium. Interestingly, when using a lower concentration of nerol (30 μM), it was not possible to clearly observe any significant ECG signal alterations but a small reduction of ventricular contractility was observed. In addition, 300 μM nerol promoted a significant decrease on the cardiac rate and contractility. Important to note is the fact that in isolated cardiomyocytes, peak ICa,L was reduced by 58.9 ± 6.31% after perfusing 300 μM nerol (n = 7, p < 0.05). Nerol, at 30 and 300 μM, delayed the time of onset of ouabain-triggered arrhythmias and provoked a decrease in the diastolic tension induced by the presence of ouabain (50 μM). Furthermore, nerol preincubation significantly attenuated arrhythmia severity index without changes in the positive inotropism elicited by ouabain exposure. Taken all together, we may be able to conclude that nerol primarily by reducing Ca2+ influx through L-type Ca2+ channel blockade lessened the severity of ouabain-triggered arrhythmias in mammalian heart.

1. Introduction

Cardiac arrhythmia is caused by changes in the coordinated electrical activity of the heart and is surely among the leading causes of sudden death in the modern world [1, 2]. Arrhythmias are generally divided into two major types: (1) abnormalities in impulse generation and (2) conduction disturbances [3]. Reductions in repolarizing outward K+ currents and/or augments in depolarizing inward Na+ or Ca2+ currents can lead to distinct types of cardiac arrhythmias. Cardiac myocytes exhibit an exquisite dynamic control of intracellular Ca2+ homeostasis. Perturbations in this control process have been recognized as a major contributor to life-threatening ventricular arrhythmias [4]. In this very aspect the model of arrhythmias induced by exposure of heart tissue to ouabain (which is per se a natural product) is an excellent and straightforward approach for the study of the putative antiarrhythmic activity of natural products. Mechanistically, ouabain-triggered arrhythmias is based on disturbances in the intracellular Ca2+ handling, overload of Ca2+, and unbalance of Na+/Ca2+ exchanger activity (NCX) [5]. Recent studies have reported that cardiac hypertrophy and heart failure may be associated with increased arrhythmogenic risk by the enhanced NCX activity [6, 7].

Terpenes complain about a large variety of plant-derived substances. Several terpene compounds in the essential oils are monoterpenes and sesquiterpenes [8]. Chemically, monoterpenes are generally characterized by having 10 carbon atoms linked together and two isoprene units [9].
Nerol (C_{10}H_{18}O) is a monoterpane found in many essential oils [10]. It was originally isolated from the oil of neroli, an oil similar in scent to bergamot oil, which produced orange blossom bergamot (Citrus aurantium var. Loved or Bergamia) and is widely used in the production of perfumes [10]. Nerol is the cis-isomer of geraniol [11].

Neroli essential oil, containing nerol in its composition, is considered one of the most important aromatics for aromatherapy treatment of heart palpitations, anxiety, and depression resulting from stress and anxiety [12]. It is also effective in diminishing the amplitude of heart muscle contraction, thus benefiting people who suffer from palpitations or coronary artery spasms [13, 14]. Evidence pointed out that neroli essential oil is used in aromatherapy to treat heart palpitations, therefore suggesting its putative effect on the mechanical and/or electrical features of the mammalian myocardium. However, the precise mechanism of action and which compound would be the main responsible remained elusive. The aim of the current study was to investigate which compound would be the main responsible for triggering cardiac arrhythmias in an ex vivo model. We, then, hypothesized that nerol could prevent cardiac arrhythmias by modulating Ca\(^{2+}\) current and stabilizing intracellular Ca\(^{2+}\) homeostasis. In order to test this hypothesis, nerol was administered in isolated mammalian heart preparations and cardiomyocytes.

2. Materials and Methods

2.1. Animals. To evaluate the effects of nerol on cardiac contractility and on electrocardiogram waveform of intact mammalian heart, experiments were designed and performed using male guinea pigs (Cavia porcellus). This investigation was approved by the local Animal Research Ethics Committee of the Federal University of Sergipe, Brazil (Protocol No. 28/12).

2.2. Measurement of Contractility in Guinea Pig Left Atrium. Guinea pigs were sacrificed by decapitation and hearts were removed and isolated left atrium mounted vertically in an organ bath containing Tyrode's solution of the following composition (mM): NaCl 120, KCl 2.7, MgCl\(_2\) 0.9, NaHCO\(_3\) 11.9, CaCl\(_2\) 1.37, glucose 5.5, NaH\(_2\)PO\(_4\) 0.4, pH 7.4. The atria preparations were subsequently connected to Grass FI'-03 (Grass Instruments, USA) force displacement transducers to record changes in atrial contractile force. Each muscle was stretched to the length at which contractile force was maximal (1.0 gf). The atria were electrically paced at 1 Hz with pulses of 1.5 ms duration and stimulated by 70 V pulses. All preparations were allowed to equilibrate for 30 min until complete mechanical stabilization had been achieved. Nerol (≥97%, Lot#MKBP7755V, Sigma-aldrich) was freshly solubilized in 0.5% DMSO and cumulatively added to bath chambers. After each observation, muscles were washed several times and allowed to recover for 30 minutes until their mechanical function completely returned to control values. DMSO at this concentration did not show any significant effect on the variables measured (data not shown, n = 5).

2.3. Effect of Nerol on Ca\(^{2+}\) Influx. A pharmacological approach was used to investigate the effect of nerol on Ca\(^{2+}\) influx. Indeed, concentration-response curves for extracellular CaCl\(_2\) (0.5 – 8 mM) were obtained before and after preincubating with nerol (300 μM) during 15 min. The results were expressed as percentage of the maximum atrial contractile attained response to a given extracellular CaCl\(_2\) concentration.

2.4. Electrocardiogram Waveform and Left Ventricular Pressure Measurements. Guinea pigs were heparinized (1,000 IU, i.p.) and the hearts excised via an anterolateral thoracotomy and immediately immersed in Tyrode’s solution. The aorta was quickly cannulated to the Langendorff apparatus, and the heart was retrogradely perfused (8 mL/min) using a peristaltic pump (Milan Peristaltic Pump, Curitiba, Brazil). Tyrode’s solution was maintained at 34.0 ± 1°C (HAAKE F3, Berlin, Germany) and gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\) (pH ~ 7.4). To measure the Left Ventricular Developed Pressure (LVDP), a water filled latex balloon was placed into the left ventricular cavity and connected to a pressure transducer (FE221, Bridge Amp, ADInstruments, Colorado, USA). The system was calibrated using a column of mercury 15 cmHg. ECG waveform was recorded through three electrodes (Ag/AgCl/NaCl 1 M) that were placed near the heart surface to detect and record macroscopic electrical signals. All signals were amplified and digitalized sampling frequency of 400 Hz (PowerLab 4/35, ADInstruments, Colorado, USA) and stored in a computer (PC-compatible) for offline processing using dedicated software (LabChart 8 Pro, ADInstruments, Colorado, USA). To measure ECG parameters, such as the PR interval, QT interval, and the duration of the QRS complex, the heart was electrically stimulated by Grass stimulus isolation unit (SIU5) connected to a Grass S48 stimulator (100 V, 1 Hz, 2 ms pulse duration). For QT correction, Bazett’s formula was used (QTc = QT/√RR). To measure heart rate (beats per minute), the isolated heart was allowed to beat spontaneously following sinus rhythm.

2.5. Effects of Nerol on the L-Type Ca\(^{2+}\) Channel in Isolated Ventricular Cardiomyocytes. Using an EPC-9.2 patch-clamp amplifier (HEKA Electronics, Rheinland-Pfalz, Germany) we recorded voltage-dependent L-type Ca\(^{2+}\) current (I\(_{Ca,L}\)) in the whole-cell voltage-clamp configuration of the patch-clamp technique [15, 16]. Ventricular cardiomyocytes were isolated from guinea pig heart by enzymatic digestion as described by Shioya [17] with minor modifications (reduction of enzyme time in the first and second tubes for 6 min). The recording electrodes had uncompensated tip resistances of 1-3 MΩ. Cardiomyocytes presenting a series resistance (Rs) above 10 MΩ were promptly discarded from the analysis. The composition of pipette solution was as follows (in mM): 120 CsCl, 20 TEACl, 5 NaCl, 10 HEPES and 10 EGTA, and 1 MgCl\(_2\) (pH was set to 7.2 using CsOH) and external solution was as follows (in mM); 150 TEACl, 0.5 MgCl\(_2\), 1.8 CaCl\(_2\), 10 HEPES, and 11 glucose (pH 7.4 set using TEA(OH)). The holding potential was set at -80 mV andprepulses were elicited from -80 mV to -40 mV for 50 ms (every 10 s) to inactivate any
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Figure 1: Effects of nerol on the contractility of guinea pig left atrium and on the calcium influx. (a) Concentration-response curve for the negative inotropic effect of nerol (EC\textsubscript{50} = 1.94 ± 0.2 mM, n = 5). (b) Concentration-response curves of CaCl\textsubscript{2} in control situation and in the presence of nerol (n = 5). (c) Representative recording showing the L-type calcium currents (I\textsubscript{Ca,L}) in control and 300 μM nerol. (d) Average effect of nerol on the I\textsubscript{Ca,L} density (n = 7, *p < 0.05). Data are represented as mean ± SEM. Paired Student’s t-test.

2.6. Effects of Nerol on the Ouabain-Triggered Arrhythmias. Antiarrhythmic effect of nerol on the cardiac arrhythmias induced by ouabain was evaluated in spontaneously beating isolated heart [18, 19]. After stabilization (30 min), the hearts were perfused with ouabain (50 μM, [20]) for 10 min. The hearts were preincubated with nerol (either 30 or 300 μM) 10 min before ouabain or nifedipine (0.35 μM). Parameters investigated were maximal inotropic effect, maximal tonotropic effect, inotropic response rate (dP/dt), and onset time of arrhythmias evoked by ouabain. Furthermore, we investigated the occurrence of subtypes of ventricular arrhythmias, such as ventricular premature beats (VPB), ventricular tachycardia (VT), and/or ventricular fibrillation (VF). Arrhythmia scores were evaluated by subtypes and arbitrarily classified as previously validated by Curtis and Walker [21]. Then, The experiment was then divided into 5 intervals of 2 minutes and, depending on the type of arrhythmia, was assigned a score. Episodes of VPB < 10 events/2 min were classified as score 0 and > 10 events/2 min scored 1; episodes of VT < 30 s were 2 and > 30 s were 3; episodes of VF < 30 s scored as 4 and > 30 s as 5.

2.7. Statistical Analysis. Results were expressed as mean ± SEM. Statistical significance was determined by using Student’s t-test or one-way ANOVA followed by Tukey’s post hoc test and Chi-squared test. p < 0.05 was considered significant.

3. Results

Our initial series of experiments were aimed at determining the baseline effects of nerol on cardiac contractility. As shown in Figure 1, nerol decreased contractile force of the isolated guinea pig atria (Figure I(a), n = 5). This effect was dependent on nerol concentration (Figure I(a), EC\textsubscript{50} = 1.94 ± 0.2 mM, n = 5) and was almost completely reversible upon washout recovering up to 83.9 ± 4.1%.

Because the development of contractile force is known to rely on Ca\textsuperscript{2+} influx into the sarcoplasm from the extracellular milieu, we investigated whether nerol prevent Ca\textsuperscript{2+} activated force production. As depicted in Figure I(b), the positive inotropic maximal response produced by increasing cumulatively extracellular Ca\textsuperscript{2+} was significantly diminished by nearly 25% by 3 mM nerol. Interestingly, EC\textsubscript{50} which is a measure of responsiveness to Ca\textsuperscript{2+} was significantly altered by the presence of nerol (Figure I(b), 2.2-fold increase). The EC\textsubscript{50} of CaCl\textsubscript{2} in control conditions was 1.7 ± 0.4 mM (Figure I(b), closed squares, n = 8) and in the presence of nerol was 3.7 ± 0.5 mM (Figure I(b), Open squares, n = 5).
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Figure 2: Effect of nerol on the electrocardiographic parameters and left ventricular developed pressure (LVDP) in guinea pig isolated heart. (a) Representative traces of ECG in control (A), 30 μM (B), and 300 μM nerol (C). (b) Representative traces of LVDP in control (A), after 10 min of perfusion with 30 μM (B) and 300 μM (C) nerol. (c) Effect of nerol on the PR interval, (d) QTc interval, (e) QRS complex duration, (f) LVDP, and (g) heart rate (BPM). Data are represented as means ± SEM (n = 4-9, *p < 0.05 versus control, #p < 0.05 versus 30 μM nerol). One-way ANOVA followed by Tukey’s post hoc test.

p<0.05). This enhancement of the EC_{50} of Ca^{2+} activation could be indicative of a reduction in Ca^{2+} entry.

We next considered whether nerol would inhibit L-type Ca^{2+} channels. Accordingly, L-type Ca^{2+} current (I_{Ca,L}) was measured using whole-cell voltage-clamp technique in isolated guinea pig ventricular cardiomyocytes. As illustrated in Figure 1(c), 300 μM nerol caused a significant decrease in peak I_{Ca,L}. After 1.15 minutes of superfusion with nerol, a decrease of peak I_{Ca,L} from -4.0 ± 0.5 A/F to -1.6 ± 0.4 A/F was observed. The average decrease of peak I_{Ca,L} induced by nerol was 58.9 ± 6.3% (Figure 1(d), p<0.05, n = 7). These results indicate that Ca^{2+} influx was reduced by nerol through inhibition of L-type Ca^{2+} channels in guinea pig cardiomyocytes.

We also investigated the effects of nerol in isolated heart recording simultaneously electrocardiogram (ECG, Figure 2(a)) and left ventricular developed pressure (LVDP, Figure 2(b)). ECG was recorded in electrically stimulated heart to permit measurement of ECG intervals and in spontaneously beating heart to evaluate heart rate. Figure 2(a) displays typical traces of ECG in control (Trace a) and during 30 μM (Trace b) and 300 μM nerol perfusion (Trace c). The results showed that 30 μM nerol did not change PR interval (Figure 2(c)), QTc interval (Figure 2(d)), QRS complex duration (Figure 2(e)), and heart rate (Figure 2(g)). Important to note is the fact that at this concentration nerol decreased LVDP by only 17% (Figure 2(f)).

On the other hand, when using higher concentration, nerol (300 μM) evoked an increase of PR interval (from 78.5 ± 5.7 ms to 153.2 ± 7.7 ms, p<0.05, Figure 2(c)) and QTc interval (from 404.9 ± 6.0 ms to 462.6 ± 8.8 ms, p<0.05, Figure 2(d)) without altering QRS complex duration (Figure 2(e)). Furthermore, this concentration promoted reduction of LVDP to 30.1 ± 6.0% (Figure 2(f)) and heart rate (from 133.4 ± 4.9 bpm to 90.9 ± 5.8 bpm, p<0.05, Figure 2(g)).
As L-type calcium channel blocker drugs are used as cardiac antiarrhythmics we reasoned that nerol could be of interest to study possible antiarrhythmic properties. The protective effects of nerol on ouabain-induced arrhythmias were examined in guinea pig hearts. Figure 3(a) shows representative recording of ouabain (50 μM) effects on LVDP (Top panel). As one would expect ouabain elicited a progressive increase in ventricular inotropic response. The systolic contractions became irregular (arrhythmic events) after 3.04 ± 0.25 min (onset of arrhythmia, n = 6). After this period, contractile force amplitude significantly decreased; arrhythmia then became severe and associated with strong diastolic contracture (tonotropic effect). Finally, cardiac arrest occurred at the end of 5 min. However, when ouabain (50 μM) was added 15 min after the perfusion of 30 or 300 μM nerol, the onset time of arrhythmia considerably delayed to 5.3 ± 0.28 min (p<0.05, n = 6) and 5.00 ± 0.35 min (p<0.05, n = 6) (Figure 3(a), middle panel), respectively. We took advantage of a well-known L-type Ca^{2+} channel inhibitor, nifedipine, used at 0.35 μM to match a similar L-type Ca^{2+} current blockade as observed with nerol at 300 μM. Therefore, this concentration of nifedipine would cause about 60% reduction of the L-type Ca^{2+} current [22]. This maneuver was done in order to evaluate the involvement of similar reduction of L-type Ca^{2+} current on antiarrhythmic effect (Figure 3(a), bottom panel). Importantly, nifedipine promoted a delay of

**Figure 3:** Protective effects of nerol on ouabain-induced arrhythmias in guinea pig hearts. (a) Representative recording of effects of ouabain (Ouab, 50 μM) on left ventricular developed pressure (LVDP, Top panel), nerol (30 and 300 μM) + ouabain (middle), and nifedipine (0.35 μM) + ouabain (bottom). Summary of effects of nerol or nifedipine preincubation on the onset time of arrhythmia (b), inotropic response (c), tonotropiceffect (d), and inotropic response rate (dP/dt) induced by ouabain (e), respectively. Data are represented as means ± SEM (n = 5 - 6, *p<0.05 versus Ouab). One-way ANOVA followed was by Tukey’s post hoc test.
5.90 ± 0.50 min (n = 4) on the onset time of ouabain-triggered arrhythmia which is strikingly similar to that provoked by nerol. Figures 3(b), 3(c), 3(d), and 3(e) summarize the effects of nerol or nifedipine preincubation on the onset time of arrhythmia, inotropic response, tonotropic effect, and inotropic response rate (dP/dt) induced by ouabain, respectively. We conclude that nerol can mimic the nifedipine in abolition of responses induced by ouabain.

The data above are consistent with the idea of proposing nerol as an antiarrhythmogenic agent. The remaining experiments aim to clarify this possibility further. By analyzing electrocardiographic tracings, it was possible to classify the major types of arrhythmic events induced by ouabain. As demonstrated in Figure 4(a), 50 μM ouabain evoked the occurrence of ventricular premature beatings (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF). The severity score of arrhythmias increased from 2.5 ± 0.6 a.u (control) to 43.6 ± 4.5 a.u after exposure to ouabain (Figure 4(b), *p < 0.05). Notably, preincubation with 30 and 300 μM nerol decreased significantly the severity score of arrhythmia to 6.6 ± 1.8 a.u and 12.2 ± 3.8 a.u, respectively (Figure 4(b), *p < 0.05). Figure 4(c) shows that in control conditions the VPB is by far the prevalent (100%) form of arrhythmia whereas when hearts were perfused with ouabain more severe types of arrhythmias were observed such as VT (50% occurrence) and VF (42% occurrence). Importantly, preincubation with nerol (30 and 300 μM) attenuated the development of more severe arrhythmias as VT and VF being the ventricular arrhythmia; VPB was more prevalent. Ouabain, by itself, provoked an increase of PRI and QTc and QRS complex duration (21%, Figure 4(e)) associated with reduction of QTc (30%, Figure 4(f)). Noticing that those ECG alterations are reported associated with reduction of QTc (30%, Figure 4(e)) to increase of PRi (54%, Figure 4(d)) and QRS complex duration (21%, Figure 4(e)) and alterations previously described in ECG major parameters but promoted a small reduction on QTc (30%, Figure 4(f)). Noticing that those ECG alterations are reported to be typical of digitalis toxicity [23, 24]. To our surprise, the ECG major parameters were prevented by preincubation with 30 and 300 μM nerol.

4. Discussion

The development of cardiac arrhythmias result from a number of different causes from genetic mutations to acquired cardiac diseases. Unequivocally they constitute a major cause of sudden death in the world [25, 26]. In the past few decades, it has become clear that cardiac arrhythmogenesis is related to ion channel dysfunctions and uncontrolled intracellular Ca²⁺ dynamics. It is, therefore, imperative to motivate studies looking for pharmacological agents that present cardioprotective effects against cardiac arrhythmias [5, 27, 28].

In this study, we used a combination of different approaches to investigate the mechanisms by which nerol acts controlling Ca²⁺ influx and the subsequent impact on ouabain-triggered arrhythmias. Our major findings are as follows: (a) nerol in lower concentrations (30 μM) promotes small reduction of contractile response without alter ECG parameters and pacemaker activity; (b) nerol at 300 μM inhibits L-type Ca²⁺ current by 60% and affect ECG, heart rate, and left ventricular performance; (c) nerol, in both concentrations investigated, suppressed ouabain-triggered arrhythmias; and (d) nerol protects the heart against ventricular tachycardia and ventricular fibrillation. These findings, altogether, are consistent with the idea of proposing nerol as an antiarrhythmogenic agent.

To our surprise, there are fewer studies using nerol (trans isomer) than geraniol (cis isomer). Nonetheless, there are numerous reports on the antiitumoral [29], antioxidant [30], and anti-inflammatory properties of these monoterpenes. Specifically in the case of nerol limited information is available on its effect on cardiac myocytes [31].

In mammalian heart, several routes can lead to reduction of cardiac contractility and as it is well known, intracellular Ca²⁺ is central to regulate contractile force in the heart. In fact, our results show that nerol decreases Ca²⁺ entry into cardiomyocytes through inhibition of L-type Ca²⁺ channels reducing contractile force. Important to note at this point that nerol at 300 μM mediates 70% of reduction on contractile force and 60% blockade of L-type Ca²⁺ current. This allowed us to study further to better characterize the pharmacological effects of nerol. Furthermore, in this concentration, a decrease in pacemaker activity (31%) associated with increase of both PRI and QT¢ at baseline conditions was observed. These results were somehow expected because previously Menezes-Filho et al. [9] reported that geraniol (a nerol geometric isomer) elicited PR and QT interval increase, reduced pacemaker activity (~16%), and markedly reduced LVDP (~83%). A novel finding in this study is that nerol, in lower concentration, did not affect pacemaker activity or ECG major parameters but promoted a small reduction on LVDP (17%).

Stability of intracellular Ca²⁺ dynamics is intimately connected to I_{Ca,L}, Na⁺/Ca²⁺ exchange (NCX), Sarco(Endo)plasmic Reticulum Ca²⁺-ATPase (SERCA2a), and RyR2 in cardiomyocytes, being the key regulators of cardiac contractility. However, any source of instability can provoke arrhythmias [32]. Arrhythmia as induced by ouabain is originated by several mechanisms. The most accepted starts with Na⁺/K⁺ pump blockade by cardiac glycosides (such as ouabain) which increases [Na⁺]i, which enhances cell contractility by increasing intracellular [Ca²⁺] due to NCX [33]. This may be explained by reduced Ca²⁺ efflux by the NCX as [Na⁺]i rises. In other words, less Ca²⁺ efflux for a given Ca²⁺ influx would increase intracellular Ca²⁺. The increased [Na⁺]i could also increase Ca²⁺ influx via reverse-mode of NCX [34, 35] producing Ca²⁺ overload. During Ca²⁺ overload oscillatory Ca²⁺ releases from the SR lead to membrane depolarization favoring triggered arrhythmias. To gain more insight into nerol antiarrhythmogenic potential we made use of the ouabain-triggered arrhythmia model in guinea pig.

Our results indicate that tonotropic effect caused by ouabain is completely abolished by nerol or nifedipine suggesting that ouabain-elicted tonotropic effect does depend on Ca²⁺ influx through L-type Ca²⁺ channels. We can conclude that nerol interacts with and inhibits L-type Ca²⁺ channels. Nerol and nifedipine slowed down LVDP rate (dP/dt) arguing in favor of the notion that L-type
Figure 4: Nerol attenuates cardiac arrhythmias and electrocardiographic (ECG) alterations induced by ouabain. (a) Representative ECG recordings with 50 μM ouabain (Ouab) evoking the occurrence of ventricular premature beatings (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF). (b) Arrhythmia score and (c) occurrence of arrhythmias (VPB, VT, VF). ECG parameters: PR interval (d), QRS complex (e), and QTc interval (f). Data are represented as means ± SEM (n = 4-7, *p<0.05 versus control, # p<0.05 versus ouabain). One-way ANOVA followed by Tukey’s post hoc test. Chi-squared test (c).

Ca^{2+} current is participating on early phase of inotropic response.

Several groups have previously demonstrated that the positive inotropism observed during the exposure to ouabain is mostly related to Ca^{2+} influx through NCX when intracellular [Na^{+}]i increases as a result of Na^{+}/K^{+} ATPase inhibition. Nerol at 30 and 300 μM delayed the onset time for cardiac arrhythmias but the ouabain-triggered inotropy remained largely intact (see Figure 3(b)). These results suggest that nerol does not affect, directly or indirectly, NCX
mediated Ca\textsuperscript{2+} dynamics. Furthermore, nerol decreased the occurrence of more severe arrhythmias such as tachycardia and ventricular fibrillation.

In our study, nerol corrected key adverse effects of ouabain-triggered arrhythmias: it abolished heart diastolic contracture, it recovered electrical activity, and it reduced the propensity to generate arrhythmias. This is the first study to actually demonstrate that, mechanistically, nerol at low concentration can preserve inotropy and the electrical properties of cardiac muscle while at the same time acting as antiarrhythmic agent. Taken all together, nerol may be useful adjunct to digitalis treatment and justifies further investigation.

5. Limitations

Small animal models are commonly used in cardiovascular research showing advantages and disadvantages [36]. The present study used guinea pig as a model because its isolated cardiomyocytes’ action potential waveform and calcium handling resembles that encountered in human ventricular cardiomyocytes. In accordance with this statement, the effect of ouabain, used to induce arrhythmias, is more pronounced in guinea pigs because of higher relative contribution of NCX (28%) in removing intracellular calcium during diastole when compared to rats (7%) [37]. Although the animal model has similarities, we must be cautious in translating the results to humans. As nerol presented antiarrhythmic effects in a very specific model (ouabain-triggered arrhythmias) we intend to continue this investigation using other models to induce cardiac arrhythmias that could be closer to that of humans and therefore establish a more general mechanism to impair arrhythmogenesis.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest in the present study.

Authors’ Contributions

José Evaldo Rodrigues de Menezes-Filho and Diego Santos de Souza contributed equally to this work.

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References

[1] K. Porthan, M. Viitasalo, L. Toivonen et al., “Predictive value of electrocardiographic T-wave morphology parameters and T-wave peak to T-wave end interval for sudden cardiac death in the general population,” Circulation: Arrhythmia and Electrophysiology, vol. 6, no. 4, pp. 690–696, 2013.
[2] J. W. Waks, C. M. Sitlani, E. Z. Soliman et al., “Global electric heterogeneity risk score for prediction of sudden cardiac death in the general population: the atherosclerosis risk in communities (ARIC) and cardiovascular health (CHS) studies,” Circulation, vol. 133, no. 23, pp. 2222–2234, 2016.
[3] M. Deo, S. H. Weinberg, and P. M. Boyle, “Calcium dynamics and cardiac arrhythmia,” Clinical Medicine Insights: Cardiology, vol. 11, p. i17954681773952, 2017.
[4] A. P. Landstrom, D. Dobrev, and X. H. T. Wehrens, “Calcium signaling and cardiac arrhythmias,” Circulation Research, vol. 120, no. 12, pp. 1969–1993, 2017.
[5] P. Baumeister and T. A. Quinn, “Altered calcium handling and ventricular arrhythmias in acute ischemia,” Clinical Medicine Insights: Cardiology, vol. 10, supplement 1, pp. 61–69, 2016.
[6] K. R. Sipido, V. Bito, G. Antoons, P. G. Volders, and M. A. Vos, “Na/Ca exchange and cardiac ventricular arrhythmias,” Annals of the New York Academy of Sciences, vol. 1099, pp. 339–348, 2007.
[7] K. R. Sipido, P. G. A. Volders, M. Schoenmakers, S. H. Marieke De Groot, F. Verdonck, and M. A. Vos, “Role of the Na/Ca exchanger in arrhythmias in compensated hypertrophy,” Annals of the New York Academy of Sciences, vol. 976, pp. 438–445, 2002.
[8] N. Dudareva, A. Klempien, J. K. Muhlemann, and I. Kaplan, “Bio synthesis, function and metabolic engineering of plant volatile organic compounds,” New Phytologist, vol. 198, no. 1, pp. 16–32, 2013.
[9] J. E. R. de Menezes-Filho, A. N. S. Gondim, J. S. Cruz et al., “Geraniol blocks calcium and potassium channels in the mammalian myocardium: useful effects to treat arrhythmias,” Basic & Clinical Pharmacology & Toxicology, vol. 115, no. 6, pp. 534–544, 2014.
[10] A. E. González-Ramírez, M. E. González-Trujano, S. A. Orozco-Suárez, N. Alvarado-Vásquez, and F. J. López-Muñoz, “Nerol alleviates pathologic markers in the oxazolone-induced colitis model,” European Journal of Pharmacology, vol. 776, pp. 81–89, 2016.
[11] J. Tian, Y. Gan, C. Pan et al., “Nerol-induced apoptosis associated with the generation of ROS and Ca2+ overload in saprotrophic fungus Aspergillus flavus,” Applied Microbiology and Biotechnology, vol. 102, no. 15, pp. 6659–6672, 2018.
[12] S. Galappathie, D. J. Edwards, A. G. Elliott et al., “Antibacterial nerol cinnamates from the australian plant Eremophila longifolia,” Journal of Natural Products, vol. 80, no. 4, pp. 1178–1181, 2017.
[13] K.-G. Fahlbusch, F.-J. Hammerschmidt, J. Panten et al., “Flavors and fragrances,” in Ullmann’s Encyclopedia of Industrial Chemistry, American Cancer Society, 2003.
[14] C. Stevensen, “The psychophysiological effects of aromatherapy massage following cardiac surgery,” Complementary Therapies in Medicine, vol. 2, no. 1, pp. 27–35, 1994.
of geraniol: Modulation of cancer hallmark pathways (Review),” International Journal of Oncology, vol. 39, no. 2, pp. 550–559, 2017.

[17] T. Shiroya, “A simple technique for isolating healthy heart cells from mouse models,” The Journal of Physiological Sciences, vol. 57, no. 6, pp. 327–335, 2007.

[18] A. Bakhtiar, M.-J. Hosseini, A. Pousti, R. Behzadmehr, S. Sabzeh-Khah, and F. Najar, “Effect of N6-cyclopentyladenosine on ouabain-induced toxicity in isolated guinea pig atria,” Toxicology Mechanisms and Methods, vol. 18, no. 7, pp. 581–583, 2008.

[19] G. P. Thomas and P. M. Stephen, “Protective action of clonidine against the arrhythmogenic and lethal effects of ouabain in guinea-pigs,” British Journal of Pharmacology, vol. 104, no. 4, pp. 995–999, 1991.

[20] K. Mohammadi, L. Liu, J. Tian, P. Kometiani, Z. Xie, and A. Askari, “Positive inotropic effect of ouabain on isolated heart is accompanied by activation of signal pathways that link Na+/K+–ATPase to ERK1/2,” Journal of Cardiovascular Pharmacology, vol. 41, no. 4, pp. 609–614, 2003.

[21] M. J. Curtis and M. J. A. Walker, “Quantification of arrhythmias using scoring systems: an examination of seven scores in an in vivo model of regional myocardial ischaemia,” Cardiovascular Research, vol. 22, no. 9, pp. 656–665, 1988.

[22] J. B. Shen, B. Jiang, and A. J. Pappano, “Comparison of L-type calcium channel blockade by nifedipine and/or cadmium in guinea pig ventricular myocytes,” Journal of Pharmacology and Experimental Therapeutics, vol. 294, no. 2, pp. 562–570, 2000.

[23] G. Ma, W. J. Brady, M. Pollack, and T. C. Chan, “Electrocardiographic manifestations: digitalis toxicity,” The Journal of Emergency Medicine, vol. 20, no. 2, pp. 145–152, 2001.

[24] T. J. Campbell and P. S. MacDonald, “Digoxin in heart failure and cardiac arrhythmias,” The Medical Journal of Australia, vol. 179, no. 2, pp. 98–102, 2003.

[25] E. J. Benjamin, M. J. Blaha, S. E. Chiuve et al., “Heart disease and stroke statistics—2017 Update,” Circulation, vol. 135, no. 10, pp. e146–e603, 2017.

[26] G. Monnerat, M. L. Alarcón, L. R. Vasconcellos et al., “Macrophage-dependent IL-1β production induces cardiac arrhythmias in diabetic mice,” Nature Communications, vol. 7, no. 1, p. 13444, 2016.

[27] R. M. D. Britto, J. A. D. Silva-Neto, T. R. R. Mesquita et al., “Myrtenol protects against myocardial ischemia-reperfusion injury through antioxidant and anti-apoptotic dependent mechanisms,” Food and Chemical Toxicology, vol. 111, pp. 557–566, 2018.

[28] J. Li, D. Hu, X. Song, T. Han, Y. Gao, and Y. Xing, “The role of biologically active ingredients from natural drug treatments for arrhythmias in different mechanisms,” BioMed Research International, vol. 2017, Article ID 4615727, 10 pages, 2017.

[29] M. Cho, I. So, J. N. Chun, and J.-H. Jeon, “The antitumor effects of geraniol: Modulation of cancer hallmark pathways (Review),” International Journal of Oncology, vol. 48, no. 5, pp. 1772–1782, 2016.

[30] B. Pavan, A. Dalpiaz, L. Marani et al., “Geraniol pharmacokinetics, bioavailability and its multiple effects on the liver antioxidant and xenobiotic-metabolizing enzymes,” Frontiers in Pharmacology, vol. 9, 2018.