Nerolidol, a Sesquiterpene Alcohol, Attenuates Acute Myocardial Infarction in Rats

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Abstract

Cardiovascular diseases have a high morbidity and mortality rate and their treatment is not effective in reducing the damage caused by tissue reperfusion during an acute myocardial infarction (MI). This study aimed to investigate whether nerolidol (NRD), a sesquiterpene alcohol, would attenuate the MI in isoproterenol-treated rat model. MI was induced by the administration of two doses of ISO (100 mg/kg, i.p.) in an interval of 24 h. The animals were divided into 4 groups: control (CTR) (vehicle – saline NaCl 0.9% + TWEEN 80 0.2%), ISO (ISO + vehicle), ISO + NRD (NRD 50 or 100 mg/kg). Electrocardiogram, contractile parameters, cardiac enzymes, infarction size and antioxidant parameters in the heart were measured. ISO group showed a significant rise in ST-segment, QTc and heart rate associated to a reduction of left ventricular developed pressure (LVDP), +dP/dt and –dP/dt. Increase in content of creatine kinase (CK), CK-MB, lactate dehydrogenase (LDH), TBARS and infarction size as well as fall in activities of superoxide dismutase (SOD) and catalase (CAT) were observed. NRD significantly prevented almost all the parameters of ISO-induced MI mentioned above. Our results suggests that nerolidol has a significant effect on the protection of heart through maintaining endogenous antioxidant enzyme activities.

Introduction

Cardiovascular diseases (CVDs) are among the main cause of death worldwide, more people die from cardiovascular system-related diseases than any other. Around 17 million people died from CVDs in 2017, representing 44% of all non-transmissible chronic diseases (NTCD) globally (World Health Organization 2018).

Among the CVDs, myocardial infarction (MI) is one of the most frequent afflicting almost 8 million people between 2011 and 2014 (Benjamin et al. 2017; WHO 2018). MI is a coronary arterial disease which has high level of morbimortality. It is characterized by partial or total necrosis of the heart caused by the death of myocardial cells. MI, or heart attack, begins with the occlusion of a coronary artery, and by consequence, the area which that artery supplies is deprived of oxygen leading to the death of those cells if the blood flow does not return soon (Piegas et al. 2015; Hu et al. 2017).

Nevertheless, the reestablishment of the blood flow after a period of ischemia can cause greater damage to the cardiac tissue, leading to a reperfusion lesion. This lesion result in several dysfunctional alterations, such as cellular edema, increased inflammatory process and oxidative stress (Berne et al. 2008; Yellon and Hausenloy 2009). Therefore, MI treatment consists in the restoration of the blood flow on the affected areas, and this restoration may lead even more complications, which is one of the major challenges to physicians and researchers (Yang 2018).

Natural products (NP) have been stood out for their wide pharmacological effects. Among the NP, the terpenes is one of the most studied classes of secondary metabolites produced by plants (Bakkali et al. 2008; Dewick 2009). They have been studied in several therapeutic areas, especially in the treatment of

cardiovascular disorders (Santos et al. 2011; Silva et al. 2019). In this sense, nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) (NRD), is a sesquiterpene alcohol that can be found in two isomers cis- and trans-nerolidol (Fig. 1). It can be found in several plants such as lavender, lemon grass, and ginger (Chan et al. 2016). It has shown anticancer, antinociceptive, anti-inflammatory, antioxidant properties (Lima et al. 2012; Fonsêca et al. 2016; Baldissera et al. 2018). Recently, it was demonstrated its cardioprotector effect in a preventive treatment of MI (Asaikumar et al. 2019).

Given the above, the aim of the present study was to evaluate the cardiovascular effects of NRD on the treatment of acute myocardial infarction (AMI) induced by isoproterenol in rats, its effects on cardiac lesion biomarkers and in the oxidative stress.

Material And Methods

Animals

Adult male Wistar normotensive rats (250-300 g) were used for all the experiments. The animals were randomly housed in appropriate cages (258 cm^2 base and 17,8 cm high) at a controlled temperature (23 ± 2 °C) on a 12-h light/dark cycle (6:00 a.m. to 6:00 p.m.) with free access to food (Purina®, Sao Paulo, Brazil) and tap water. In addition, all efforts were made to minimize the number of animals used and any discomfort.

Chemicals

Nerolidol (NRD, ≥ 98% purity), (-)-isoproterenol (ISO), hexamethonium bromide (HEXA), atropine sulphate (ATR), indomethacin (INDO), nifedipine and NG-nitro-L-arginine-methyl-ester (L-NAME) were all purchased from Sigma-Aldrich™ (St. Louis, MO, USA). Sodium chloride (NaCl) from Neon™, Brazil; Tween 80 from Oxiteno™, Brazil; and ketamine chloride and xylazine from SESPO™, SP, Brazil.

Myocardial infarction (MI) protocol

MI was induced by two doses of (-)-isoproterenol (ISO) (100 mg/kg; i.p.) with a 24h-interval between each dose. After 30 min of the second dose of ISO, the animals were treated with NRD (50 or 100 mg/kg; i.p.). ISO was diluted in saline solution (NaCl 0.9%), and NRD in saline solution + tween 80 (0.2%). The animals were divided randomly into four groups as follows: CTR (vehicle), MI – ISO (100 mg/kg of isoproterenol), ISO + NRD (50 mg/kg of nerolidol), and ISO + NRD (100 mg/kg of nerolidol) (Fig. 2).

Acquisition of the Electrocardiographic profile

After MI induction (3º day), ECG was recorded in anesthetized animals (ketamine 80 mg/kg and xylazine 10 mg/kg; i.p.) by subdermal electrodes placed in the DII derivation (negative pole placed in the right and left superior thoracic region, and positive pole in the left inguinal region) connected to a cardioscope (TEB Electronics, São Paulo, Brazil). The electrical signals were amplified and digitalized (PowerLab 4/35 ADInstrument, EUA). The ECG signals were registered for 5 minutes and the LabChart 8 program

(ADInstruments) was used for analysis. Measurements of heart rate (HR) corrected QT interval (QTc), QRS complex were used in all experimental groups. The QT interval was corrected normalized for rodents by HR using Bazett's modified formula (QTc = QT / √RR /f). Any abnormalities in the ST segment morphology for MI were also analysed (Preda and Burlacu 2010).

Determination of myocardial infarct size

After MI was induced, the animals were anesthetized with ketamine (80 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.), and decapitated. The hearts were quickly removed, and the myocardial infarct size was measured using the 2,3,5-triphenyl tetrazolium tetrachloride (TTC) staining method. All hearts were sectioned in a transversal fashion in the median region to obtain a better exposure of the left ventricle. The cardiac apex region was placed in a Falcon tube with TTC 1% diluted in Krebs–Henseleit solution (composition in mM: NaCl 120, KCl 5.4, $MgCl_2$ 1.2, $NaHCO_3$ 27, $CaCl_2$ 1.25, glucose 11, NaH_2PO_4 2.0, pH 7.4) for 15 min at a 37 ºC water bath. Then, after staining, the area was scanned in high resolution and analysed with ImageJ software (free software ImageJ bundled with 64-bit Java 1.8.0_112, NIH, Bethesda, MD, USA).

Measurement of biochemical markers of tissue damage - Lactate Dehydrogenase (LDH) and Creatine Kinase Total and Myocardial Band (CK-TOTAL and CK-MB)

After experiments, the animals were euthanatized and decapitated, and the blood was collected. For serum obtainment, the blood was centrifuged at 3.500 rpm (Neofuge 15R, Heal Force, Shanghai, China) for 15 min at 4 ºC. The enzymatic essays were performed in triplicate using Labtest commercial dosing kit. All following sample analysis were performed on the LABMAX 240 Premium apparatus.

Oxidative stress assays

Measurement of Lipoperoxidation by the Thiobarbituric Acid (TBARS) Method

Malondialdehyde (MDA) is a known stress oxidative biomarker, one of the oxidation products of hydroperoxides of polyunsaturated fatty acids that are formed during lipoperoxidation process (Amara et al. 1995).

To perform TBARS experiments, the hearts were weighted and homogenized at the ration of 100 mg of tissue/mL of phosphate buffer (PBS 0.1 mol/L, pH 7.4). Next, the homogenates were incubated for 45 min at 90 ºC with a solution containing thiobarbituric acid (TBA 0.37%) in acid solution (15% trichloroacetic acid and 0.25 N hydrochloric acid). Samples were centrifuged at 14.000 rpm (Neofuge 15R, Heal Force) for 30 min at 4 °C, and the supernatant was mixed with n-butanol and saturated NaCl solution. The mixture was mixed in a vortex for 30 s and again centrifuged at 14.000 rpm for 2 min. Aliquots of the supernatant were pipetted into 96-well plates to read absorbance on a microplate reader (Biotek, ELx800 absorbance microplate reader, VT, USA) at 535 nm, correcting for absorbance values at 572 nm. The amount of MDA was expressed in nanomoles per grams of tissue (nmol/g) and was

interpreted as a marker of lipid peroxidation formed by the reaction with TBARS (Esterbauer and Cheeseman 1990).

Measurement of Measurement of Protein Carbonylation

To measure the possible oxidative damage to proteins carbonylation was used, which is the formation of carbonyl groups in proteins by the reaction with 2,4-dinitrophenylhydrazine (DNPH) with reactive ketones or aldehydes to form hydrazones, which are formed from protein oxidation. The total carbonyl group concentration was expressed as nmol/mg protein (Levine et al. 1990).

Cardiac tissues were weighted and homogenized. Initially, 2 microtubes were prepared with DNPH (experimental) and control (white), 1 mg of supernatant protein was added in both microtubes and completed to 200 µl with PBS. Next, 200 µl of trichloroacetic acid (TCA) 20% was added and homogenized for 5 min, after was centrifuged at 4.000 rpm for 5 min. The supernatant was discarded, and the pellet was resuspended in 100 µl NaOH 0,2 mol/L.

Next was added 100 µl of hydrochloric acid (HCl) 2 M in the white tube and 100 µl of DNPH 10 mM in the sample following 100 µl of TCA 20 %, the tubes were centrifuged at 16.000 rpm for 5 min in triplicate. Thereafter, was added 500 µl of ethanol/ethyl acetate (1:1). The pellet was resuspended with 1 mL of urea 8 M (pH 2.3), centrifuged again at 16.000 rpm for 3 min. Finally, 200 µl was pipetted into 96-well ELISA plate to read at an absorbance from 360 to 385 nm.

Endogenous Antioxidant Enzyme Activities

Superoxide Dismutase Activity (SOD)

SOD activity was measured as previously described (Madesh and Balasubramanian 1998). Cardiac tissue was homogenized in phosphate buffer (PBS, 50 mmol/L, pH 7.4) and centrifuged at 14.000 rpm for 30 min at 4 °C. The supernatant, PBS, tetrazolium (1.25 mmol), and adrenaline (60 mmol) were transferred to a microplate and shaken for 5 min. Thereafter, DMSO was added to the mixture, and read in a spectrophotometer (ELx 800, Biotek Instruments, VT, USA) was measured at 570 nm absorbance. SOD activity was expressed as U/µg protein.

Catalase Activity (CAT)

CAT activity was measured following a protocol previously described (Nelson and Kiesow 1972). The reaction was started with the addition of H_2O_2 (0.3 mol/L) to the supernatant samples, which were equalized in phosphate buffer (50 mmol/L, pH 7.0) and centrifuged at 12.000 rpm for 30 min at 4 °C. The test was performed in a quartz cuvette without illumination. The measurements were made in a spectrophotometer (ELx 800, Biotek Instruments), with a periodicity of 15 s at 25 °C and a wavelength of 240 nm. The reaction was quantified by measuring the consumption of hydrogen peroxide (H_2O_2, 0.3 mol/L). Decomposition of H_2O_2 by catalase was monitored for 1 min at 25 °C. The activity of the enzyme was expressed by the difference in absorbance variation (ΔE)/min/µg protein.

Statistical Analysis

Data were expressed as mean ± standard error (SEM). Statistical comparisons were performed using GraphPad Prism 6.1 (San Diego, CA, USA). The normality and equality of variance were verified by the Shapiro–Wilk and Levene tests, respectively (Santana et al. 2018; Souza et al. 2019). For the statistical decision, one-way ANOVA was used, followed by the Tukey post hoc test or chi-squared test depending on the case. Values of probability of $p < 0.05$ were considered statistically significant (Supplemental data).

Results

Nerolidol decrease ECG changes in a rat model of acute myocardial infarction

ST-elevation and T-wave inversion are typical electrocardiographic changes observed in acute myocardial infarction (MI). Fig. 3a shows representative ECG records in the 4 experimental groups. It can be noted that in the IM group there was a ST-elevation characteristic of transmural ischemia. Elevation of ST was observed with less frequency in the MI groups IM + NRD 50 (50%) or IM + NRD 100 (33%) compared to the MI group (66%) (Fig. 3b). There was no change in the PR interval and duration of the QRS complex in analysed groups (Fig. 3c and d). However, a significant increase in QTc and heart rate was observed in the MI group, which was prevented by treatment with NRD (100 mg/kg, $p < 0.05$) but not with NRD (50 mg/kg) (Fig. 3e and f).

Nerolidol ameliorates ventricular contractility in a rat model of acute myocardial infarction

Fig. 4a shows representative traces of left ventricular developed pressure (LVDP) in different experimental groups. As can be seen, isoproterenol administrated for two consecutive days promoted a marked reduction in LVDP in animals in the MI group that were fully prevented by treatment with the two doses of NRD evaluated (Fig 4a and b). Administration of NRD (50 or 100 mg/kg) alleviated the reductions in +dp/dt and - dp/dt induced by MI ($p < 0.05$, Fig. 2c and d). These findings suggested that NRD could improve the cardiac function in rats submitted to MI.

Nerolidol ameliorates the level of CK, CK-MB and LDH in a rat model of acute myocardial infarction

The measurement level of CK, CK-MB and LDH (Fig. 5) were tested in this study to investigate whether the effects of NRD ameliorates cardiac function in MI. In MI group, the level of CK, CK-MB and LDH were significantly increased compared with the control group. Interesting, treatment with NRD at the dose of 50 mg/kg and 100 mg/kg significantly reduced the levels of these biochemical markers of tissue injury, compared to that in the isoproterenol-induced acute myocardial infarction group.

Nerolidol reduces infarct size in a rat model of acute myocardial infarction

In order to explore whether NRD ameliorates the infarct size in acute myocardial infarction rat model, infarct size was measurement in our study. Fig. 6a shows representative illustrations of infarction size as stained TTC in all experimental groups. While MI group indicated a large unstained area, the heart slice of

MI + NRD exhibited a major portion stained positively showing tissue viability. As shown in Fig. 6b isoproterenol induced increase of infarct size (27.76 ± 1.51%, $p < 0.05$) in compared to control group (4.12 ± 0.54%). On the other hand, treatment with NRD (50 mg/kg or 100 mg/kg) declined the infarct size to 13.57 ± 1.09% and 3.57 ± 0.53%, respectively, compared to MI group.

Nerolidol ameliorates oxidative stress in a rat model of acute myocardial infarction

To evaluate whether the cardioprotection mediated by NRD on the MI was associated with the improvement of oxidative stress, it was verified in heart samples in all experimental groups the levels of TBARS, CAT and SOD activity, SH and carbonyl groups. MI significantly increased the levels of MDA (Fig. 7a) and suppressed the levels of SOD (Fig. 7b). Notably, NRD treatment significantly reversed these changes. CAT activity was reduced in MI group but the NRD treatment was not able to reverse this change (Fig. 7c). The results showed that there was no change in the SH and carbonyl group induced by MI (Fig. 7d and e). However, the MI groups treated with 50 and 100 NRD showed a reduction in carbonyl group compared to MI group.

Discussion

High concentrations of isoproterenol administration induce the auto-oxidation of catecholamines promoting the generation of highly cytotoxic free radicals (Rathore et al. 1998; Vennila and Pugalendi 2010). The oxidative stress is involved as one of the important cause myocardial injury promoting lipidic peroxidation, changes of permeability in cardiomyocyte membrane, calcium overload and consequently leading to subendocardial myocardial ischemia, hypoxia, necrosis and impairment of contractile function (Shaheen et al. 2011; D'Oria et al. 2020). This pattern is similar to the acute myocardial infarction seen in humans.

One of the criteria used to diagnosis of MI is ECG-abnormalities such as ST-segment elevation and T-waves inversion (Coppola et al. 2013). ST-segment elevation is generally a sign of transmural infarct and occurs in the majority of acute MI that are caused by complete blockage of a coronary artery or in isoproterenol-induced myocardial infarction in rat (Klabunde 2017). T-waves inversion occurs in myocardial ischemia that shortens the action potential duration of cells, which results in repolarization occurring earlier than normal. Treatment with NRD (100 mg/kg) markedly reduced isoproterenol-induced ST-segment elevation. QTc prolongation on ECG is a marker of delayed electrical repolarization and is risk factor for occurrence of ventricular arrhythmias and all-cause mortality. Another finding was that animals treated with isoproterenol showed an increase in heart rate by activation of β-adrenergic receptors and is responsible for augmented oxygen consumption contributing for myocardial necrosis (Rona 1985; Soraya et al. 2012). However, this increase in QTc and heart rate were not observed in the animals treated with NRD at 100 mg/kg. Short-term administration of NRD strongly prevented electrocardiographic alterations in the ECG, indicating its protective effects on cell membrane function.

Isoproterenol administration induced significantly decreased of LVDP, +dP/dt and −dP/dt. Interestingly, treatment with the two doses of NRD improved all the evaluated parameters preventing the left ventricular

dysfunction induced by isoproterenol. It was reported that nerolidol showed ability to reduce the contractile response of guinea pig left atrium probably by reduction calcium influx in cardiomyocyte (Vasconcelos et al. 2018). ISO-induced myocardium dysfunctions are mediated by an increased level of intracellular Ca^{2+}, through L-type calcium channels (Singh et al. 2001). Thus, drugs that promote negative inotropism, such as L-type calcium channel blocker, has significantly diminished isoproterenol cytotoxicity and preserved the ultrastructural architecture of the ventricle tissue of rats (Hassan et al. 2016). These results suggest that NRD may be acting as a calcium blocker to induce its protective effects on the heart.

Cardiomyocyte contains a high concentration of marker enzymes (CK, CK-MB and LDH) and in the face of MI these enzymes are released into the extracellular fluid and were observed in the plasma of ISO-induced rats. Notably, in our study, administration of NRD at doses of 50 and 100 mg/kg significantly lowered the levels of marker enzymes in ISO-induced rats. Another study reported that prolonged pre-treatment with NRD at different doses (100 and 200 mg/kg) for 21 days prevented the increase of cardiac and hepatic marker enzymes induced by ISO (Asaikumar et al. 2019).

Furthermore, another important finding of this study is that the NRD administration resulted in a remarkable cardioprotection against the early complications of myocardial infarction such as observed by reduction of infarction size.

It is reported that increases in the ROS production and/or depletion of the antioxidants in the defence system result in necrotic lesions in the heart of ISO-induced rats. Free radicals produced can react with polyunsaturated fatty acids in cell membrane leading to lipid peroxidation increasing the free radical production. In ISO-induced rats was reported that the concentrations of TBARS was increased in the plasma and heart. Furthermore, activities of enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) in heart tissue were decreased in ISO-induced rats. However, NRD was able to significantly increase the SOD activity. Decreasing in concentrations of TBARS and increase of SOD activity in animal treated with NRD showed that it exert antioxidative effects important to minimizes the myocardial injury induced by isoproterenol.

Taken together, the findings of our study demonstrate that nerolidol exert antioxidative effects by suppressing myocardial tissue oxidative stress and enhancing the antioxidative defence system in a rat model of acute myocardial infarction. Other cardioprotective effects offered by nerolidol are observed by prevention of contractile and ECG alterations on the isoproterenol-induced myocardial infarction. This study provides a novel strategy of cardioprotection mediated by nerolidol, which may be helpful in limiting myocardial injury extension in the case of myocardial infarction.

Declarations

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Ethical Approval All procedures described in the present study were approved by the Animal Research Ethics Committee of the Federal University of Sergipe (Protocol #42/2018) and were in compliance with the National Council for Animal Experiments Control (CONCEA - BRAZIL).

Consent to participate Not applicable.

Consent to publish Not applicable.

Availability of data and materials Upon request.

Competing interests The authors declare no competing interests.

Author Contributions: MSSG: Methodology, Formal analysis, Investigation, Data curation. **EAPS:** Methodology, Formal analysis, Writing - Original draft and review. **DMS:** Investigation, Resources, Data curation. **IRS:** Investigation, Data curation. **DSS:** Methodology, Formal analysis, Investigation, Writing - Original draft. **AMA and LH:** Methodology, Formal analysis, Investigation. **CMLV:** Methodology, Resources, Formal analysis, Writing - Original draft and review, Supervision. **MRVS:** Resources, Supervision, Project administration, Funding acquisition. **RSSB:** Resources. **LJQJ:** Resources, Supervision, Project administration. **ASB:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration.

All authors read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Figures

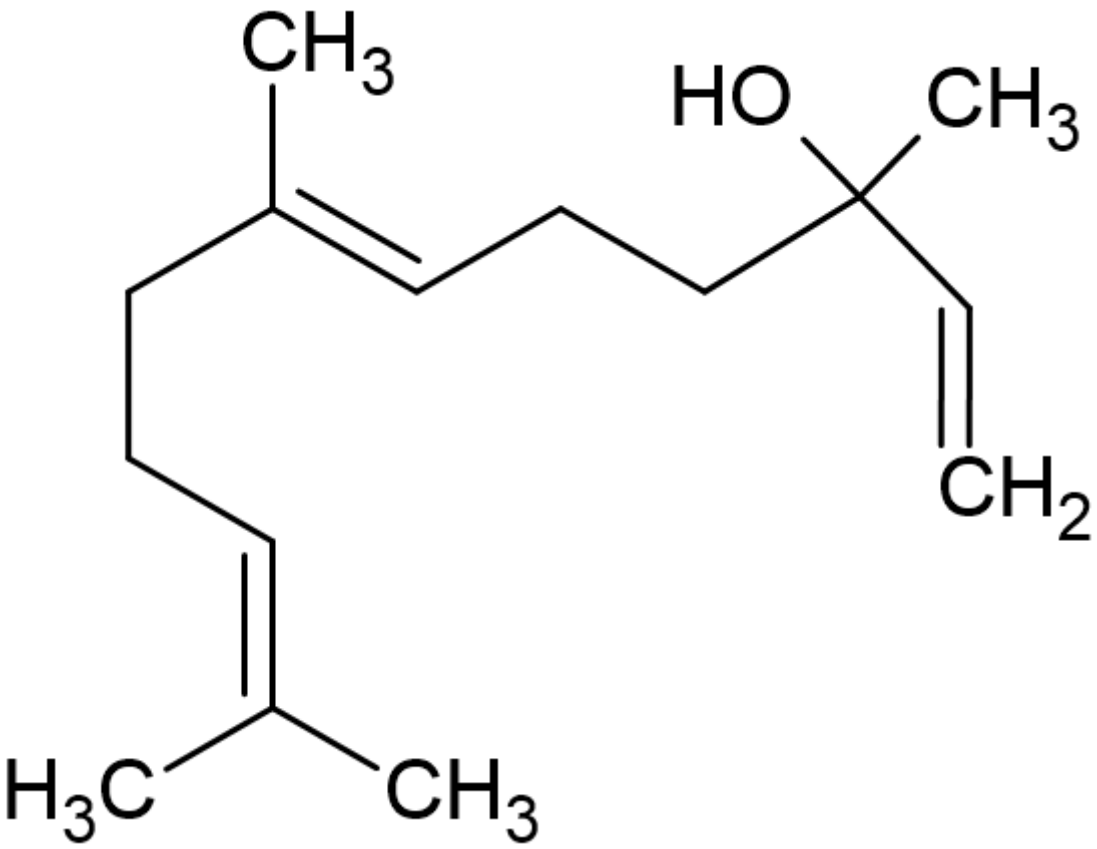

Figure 1

Chemical structure of nerolidol (NRD).

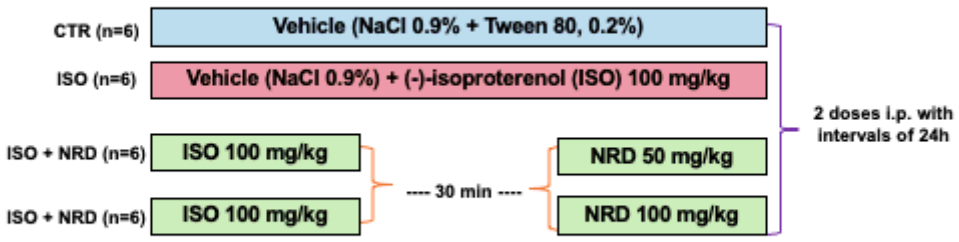

Figure 2

Design of experimental in vivo model of myocardial infarction induced by isoproterenol.

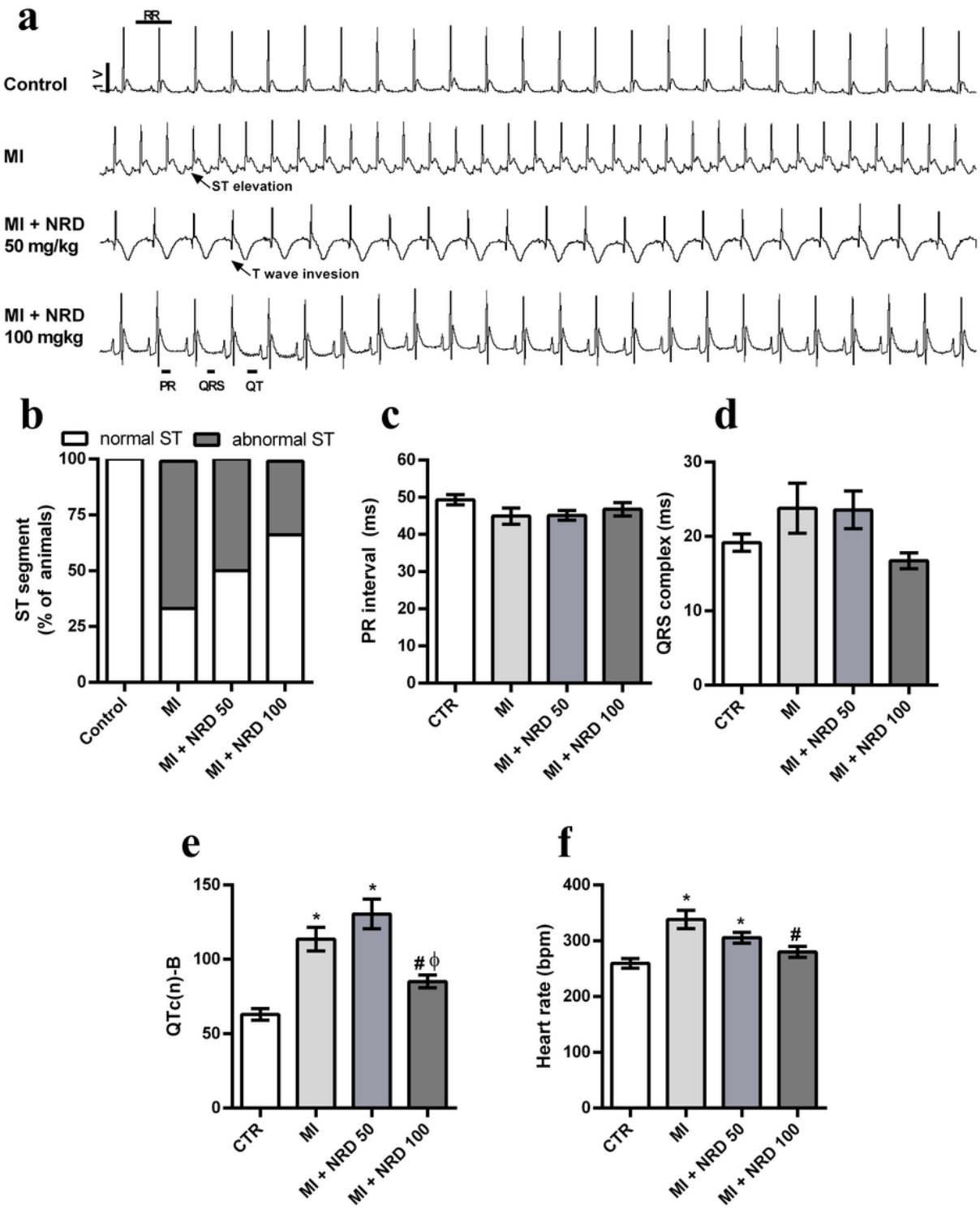

Figure 3

Nerolidol (NRD) prevents electrocardiographic alterations induced by isoproterenol-induced myocardial infarction (MI) in rats. a ECG recording in control (a), MI (b), MI + 50 mg/kg NRD (c) and MI + 100 mg/kg NRD (c). b ST segment elevation, c PR interval, d QRS complex, e QTc(n)-B and f heart rate (n = 3-5). *p < 0.05 vs. Control; # p < 0.05 vs. MI; ϕ p < 0.05 NRD 50 vs. NRD 100. One-way ANOVA followed by Tukey's post-test.

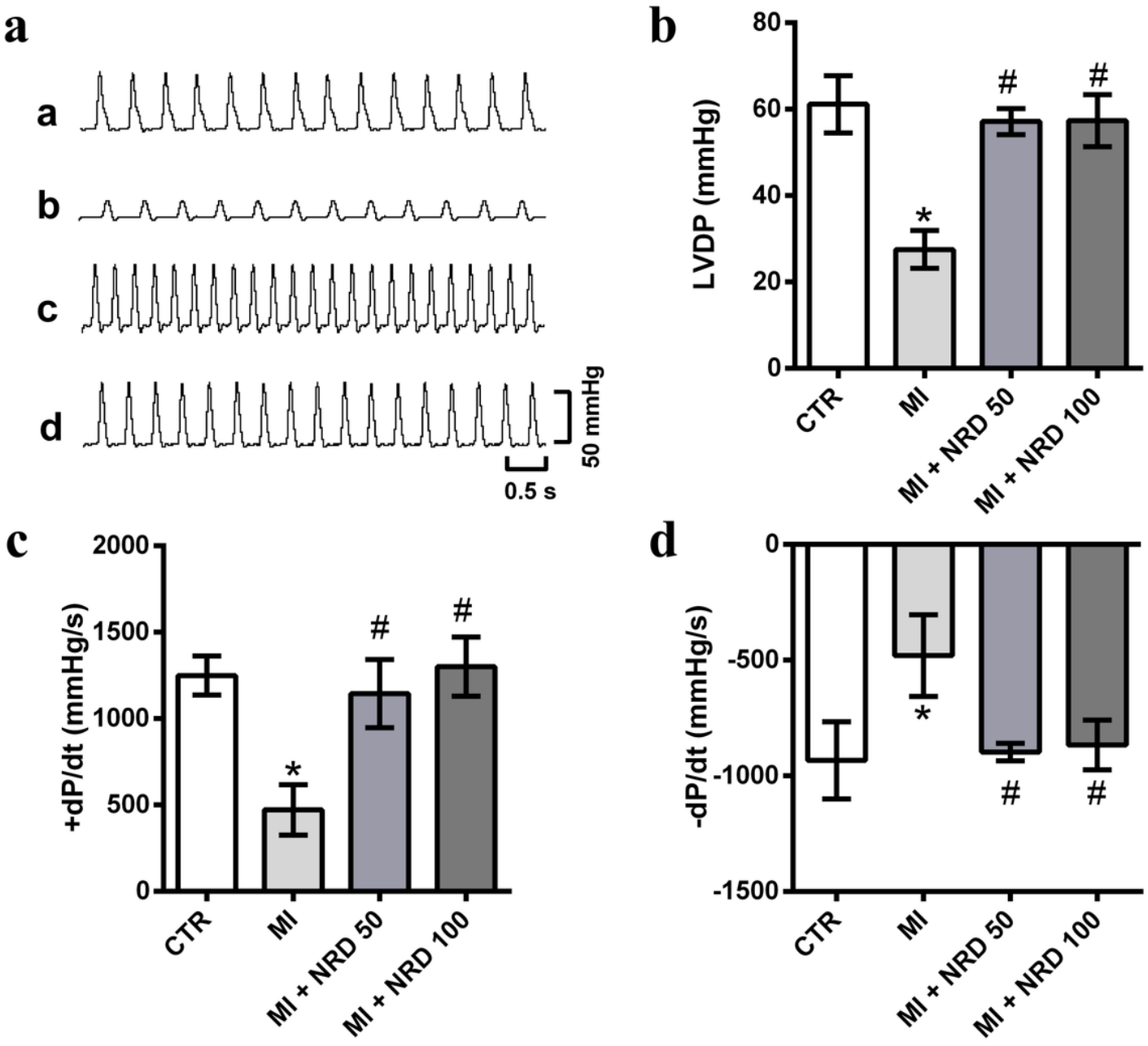

Figure 4

Nerolidol (NRD) prevents reduction of the left ventricular developed pressure (LVDP) induced by isoproterenol-induced myocardial infarction (MI) in rats. a Representative traces of LVDP in control (a), MI (b), MI + 50 mg/kg NRD (c) and MI + 100 mg/kg NRD (c). b LVDP, c maximum derivative of left ventricular pressure (+dP/dt) and d minimum derivative of left ventricular pressure (-dP/dt) (n = 3-5). *p < 0.05 vs. Control; #p < 0.05 vs. MI. One-way ANOVA followed by Tukey's post-test.

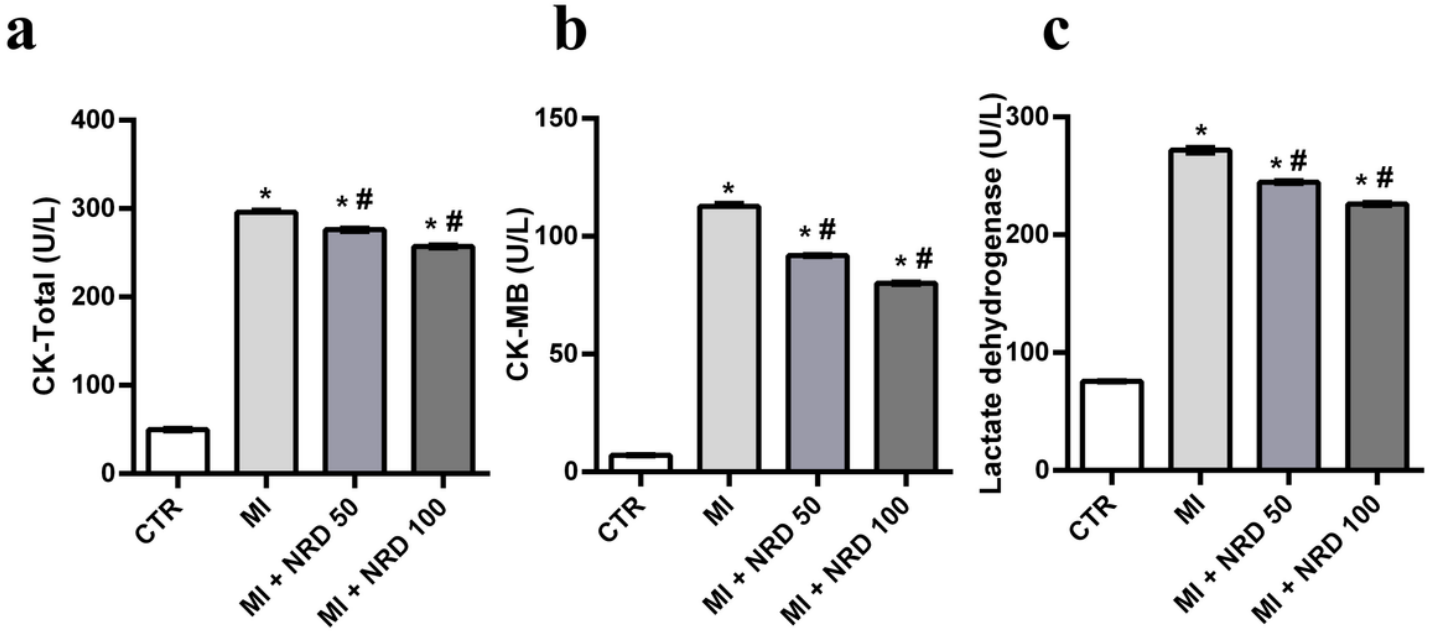

Figure 5

Nerolidol (NRD) ameliorates the level of CK-total, CK-MB and lactate dehydrogenase (LDH) induced by isoproterenol-induced myocardial infarction (MI) in rats. a CK-total, b CK-MB and c LDH (n = 3-5). *p < 0.05 vs. Control; #p < 0.05 vs. MI. One-way ANOVA followed by Tukey's post-test.

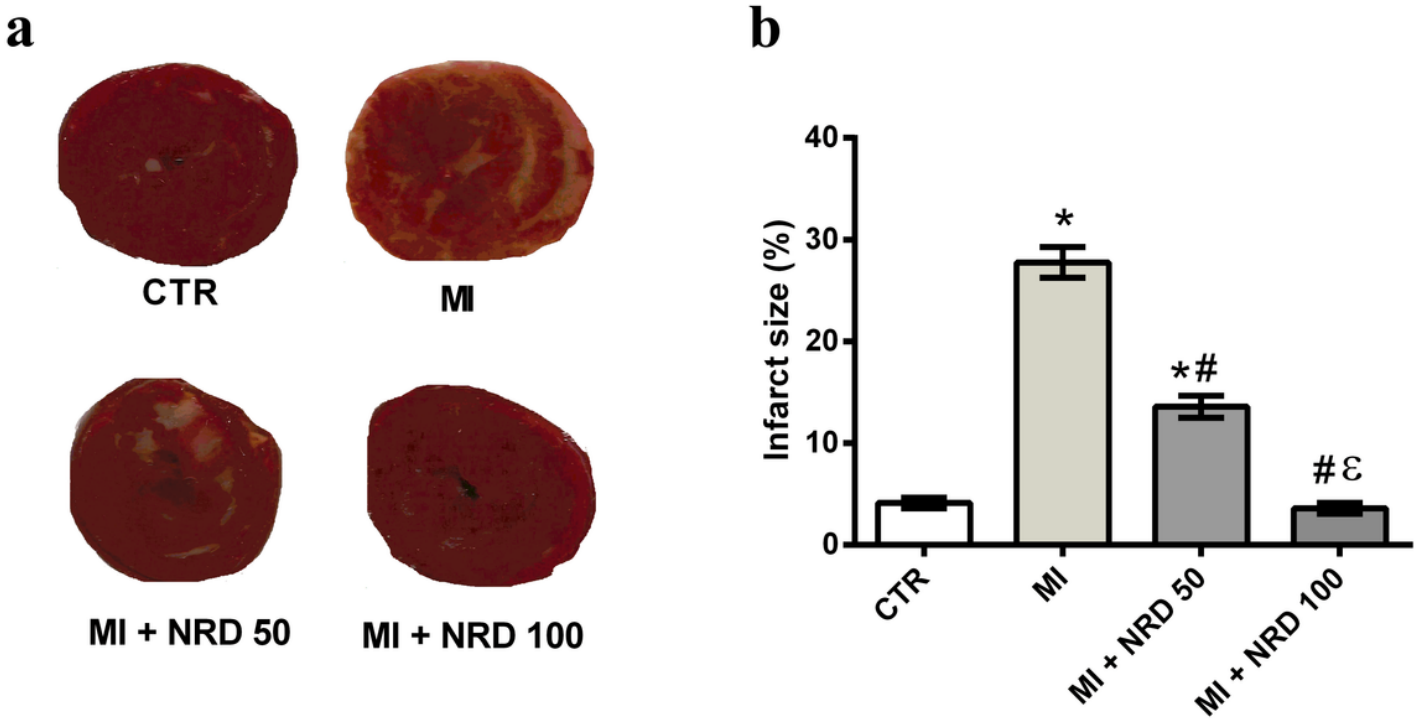

Figure 6

Nerolidol (NRD) reduces the infarct size induced by isoproterenol-induced myocardial infarction (MI) in rats. a Representative images of heart slices stained with TTC in control (a), MI (b), MI + 50 mg/kg NRD (c) and MI + 100 mg/kg NRD, b Infarct size (n = 3-5). *p < 0.05 vs. Control; #p < 0.05 vs. MI; €p < 0.05 NRD 50 vs. NRD 100. One-way ANOVA followed by Tukey's post-test.

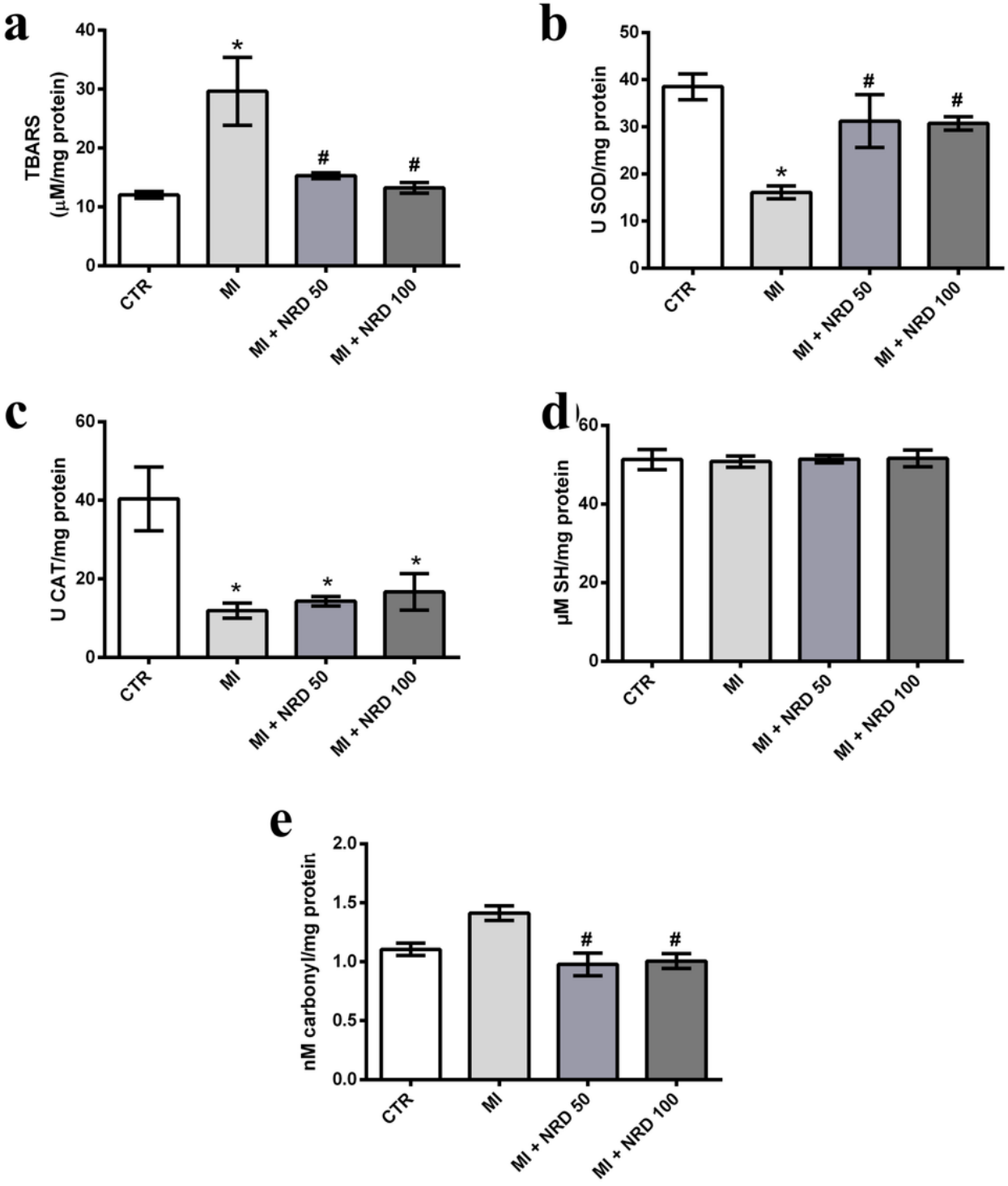

Figure 7

Nerolidol (NRD) reduces reduced the oxidative stress induced by isoproterenol-induced myocardial infarction (MI) in rats. a Lipid peroxidation as indicated by the thiobarbituric acid reactive substances (TBARS) level, b superoxide dismutase activity (SOD), c catalase activity, d SH group and e carbonyl group (n = 3-5). *p < 0.05 vs. Control; #p < 0.05 vs. MI. One-way ANOVA followed by Tukey's post-test.

Supplementary Files

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