

The role of nitric oxide on the antiarrhythmic effects of ketamine/xylazine in a rat model of acute cardiac ischemia-reperfusion

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ABSTRACT

The prevalence of ventricular arrhythmias during general anesthesia is about 70%. In experimental studies on the antiarrhythmic effects of different agents, using anesthetic drugs that do not have any protective properties are preferable. The present study was conducted to investigate molecular mechanisms involved in the antiarrhythmic effects of ketamine/xylazine (K/X).

Sixty male rats were assigned to eight groups: K/X, L-NAME (25–35 mg/kg) with thiopental (TP), L-NAME (25–35 mg/kg) with ketamine/xylazine, L-arginine (100 mg/kg) with thiopental, L-arginine (100 mg/kg) with ketamine/xylazine. After anesthetic induction using TP or K/X, the animals were subjected to 30 min of ischemia. Hemodynamic parameters, ventricular arrhythmias during ischemia, the incidence of ventricular tachycardia (VT), and ventricular fibrillation (VF) were measured. Additionally, in order to assess nitrite/nitrate ratio and LDH after ischemia, serum samples were collected and used.

Our results showed that in the K/X group, the number of VT and VF, duration of VT ($p = 0.006$), the severity of arrhythmias ($p = 0.0179$). There was no VF incidence in this group.

These protective effects were faded by administration of L-NAME with K/X. The combination of L-Arginine in the TP group decreased the number and duration of VT ($p < 0.001$, $p = 0.0013$) with no incidence of VF in comparison with TP. L-arginine with K/X groups increased the number and duration of VT ($p < 0.0001$, $p < 0.001$) compared to K/X and VF was seen (100%). However, there was no significant difference between TP and K/X groups in terms of this nitrite/nitrate ratio. These findings suggest that the antiarrhythmic effects of ketamine/xylazine might be partially relative to the nitric oxide synthesis pathway.

1. Introduction

The incidence of ventricular arrhythmias during anesthesia has been reported roughly 70%, which is considered a highly prevalent cardiovascular complication (Kwon and Kim, 2017). Although arrhythmias during surgery might result from various reasons such as pain, surgery procedure, and anesthetic agents (Shekarforoush et al., 2016; Tsukamoto et al., 2018; Jiron et al., 2019; Lotz et al., 2020) the underlying causes of ventricular arrhythmias are poorly understood. Since anesthetics exert various effects on hemodynamic parameters, which can cause arrhythmias by themselves, choosing anesthetic agents in a myocardial ischemic-reperfusion injury model is crucial. Furthermore,

to gather more reliable experimental data, anesthetics' side effects should be decreased (Lubberding et al., 2020). For instance, halothane, an inhalation anesthetic agent used for induction and maintenance of general anesthesia, sensitized the heart to plasma catecholamine, which leads to an increased risk of ventricular arrhythmias (Johnson and Loushin, 2015).

Thiopental and pentobarbital are the most routinely used barbiturates in operating rooms, which can cause respiratory depression as well as circulatory dysfunction (Abdalla, 2018). Ketamine hydrochloride, on the other hand, is a rapid-acting general anesthetic and NMDA receptors antagonist as glutamic acid antagonists, which has a sympathomimetic effect via preventing norepinephrine reuptake from the postganglionic

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sympathetic system. Xylazine has been known as one of the α_2 adrenergic receptor agonists, widely used in veterinary medicine, which brings about sedation, analgesia, and muscle relaxation. In combination with ketamine, it could effectively lessen ketamine side effects such as muscle stiffness. Several studies showed that alpha-receptors' activation of the sympathetic nervous system before an ischemic condition had reduced ischemic ventricular arrhythmias (Gonca, 2015; Shekarforoush et al., 2016; Munif et al., 2020). Based on these findings, it can be concluded that using the combination of ketamine/xylazine has a preconditioning effect to decrease ischemic ventricular arrhythmias (Svorc et al., 2017).

Nitric oxide (NO) is a gaseous lipophilic free radical cellular messenger generated by three isoforms of nitric oxide synthase (NOS) neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). NO controls the heart rate, and contractility hence plays a crucial role in preconditioning the effects of some pharmacological agents, including alpha-adrenergic agonists. It means inhibiting NO synthesis will prevent the antiarrhythmic effects of these agents during ischemia (Paulus, 2020). It has been reported that norepinephrine administration before ischemia significantly reduced ventricular arrhythmias during myocardial ischemia by stimulating alpha-1 receptor and NO production (Imani et al., 2011; Mavropoulos, 2019). Furthermore, NO-releasing agents such as sodium nitroprusside during coronary artery bypass graft significantly decrease ventricular arrhythmias (Ariafar et al., 2021). To sum up, reducing the incidence of ventricular arrhythmias, as the most severe complications of anesthesia, should be a priority when choosing anesthetic agents, especially in patients with cardiac ischemia-reperfusion who have been already predisposed to develop ventricular arrhythmias. Therefore, in the present study, we investigated the role of NO on the antiarrhythmic effects of ketamine/xylazine in a rat model of acute cardiac ischemia-reperfusion.

2. Materials and methods

2.1. Materials

Thiopental sodium, ketamine, and xylazine, LNAME (a non-specific NOS inhibitor, N-nitro-L-arginine methyl ester), and L-arginine (donor of NO) were obtained from Sigma Chemical Co (St, Louis, Mo, USA).

2.2. Animals and ethical statement

Sixty adults pathogen-free male Wistar rats weighing 250–300 g were used in this study. Animals were kept in a regulated temperature and humidity with a light/dark period of 12 with free access to food and water. Animal procedures and experimental protocols were performed under the NIH guidelines (Guide for the care and use of laboratory animals) and authorized by the Tehran University of Medical Sciences (IR.). The rats were randomly assigned to eight groups as follow:

- 1) Thiopental sodium: animals intraperitoneally (IP) received 80 mg/kg IP thiopental sodium following ischemia induction.
- 2) Ketamine/xylazine: animals received K/X (70, 10 mg/kg, IP) following ischemia induction.
- 3) L-NAME (25 mg/kg) - thiopental sodium: animals received 25 mg/kg L-NAME 20 min before IP injection of thiopental sodium following ischemia induction.
- 4) L-NAME (25 mg/kg) - ketamine/xylazine: animals received 25 mg/kg L-NAME 20 min before IP injection of K/X following ischemia induction.
- 5) L-NAME (35 mg/kg) - thiopental sodium: animals received 35 mg/kg L-NAME 20 min before IP injection of thiopental sodium injection IP following ischemia induction.
- 6) L-NAME (35 mg/kg) - ketamine/xylazine: animals received 35 mg/kg L-NAME 20 min before IP injection of thiopental sodium injection IP following ischemia induction.

- 7) L-arginine- thiopental sodium: animals received 100 mg/kg L-arginine 10 min before IP injection of thiopental sodium injection IP following ischemia induction.
- 8) L-arginine- ketamine/xylazine animals received 100 mg/kg L-arginine 10 min before IP injection of K/X following for ischemia induction.

2.3. Experimental procedure

The rats were anesthetized using either thiopental sodium or ketamine/xylazine. In L-NAME groups, animals received L-NAME 20 min before inducing anesthesia. In L-arginine groups, animals received L-arginine 10 min before anesthesia.

2.4. Myocardial ischemia protocol

Midsternal tracheotomy was performed after endotracheal intubation. Rats were ventilated using room air by Parvalux rodent respirator (15 mL/kg stroke volume and 60–70 breaths/minute). The right carotid artery was cannulated, and then connected to a pressure transducer to record mean arterial blood pressure (MBP). Electrocardiogram (ECG) was monitored using a standard limb lead II with subcutaneous stainless-steel electrodes. A Power Lab monitoring system (ML750 Power lab/4sp, castle hill, Australia) was used to record MBP, heart rate (HR), and ECG. A left lateral thoracotomy was performed between the fourth and fifth intercostal space to expose the heart. The pericardium was incised, and a 6-0 silk suture was placed around the left anterior descending coronary artery (LAD). Both ends of the silk thread were passed through a polyethylene tube. Applying such tension to the suture caused LAD occlusion so caused regional ischemia. Ischemia was confirmed by ST elevation, increased R-wave amplitude in ECG, and decreased arterial blood pressure. All animals were subjected to 30 min coronary artery occlusion. Animals that failed to anesthetize with a predetermined dose of drugs were excluded (diagram 1)

2.5. Area at risk assessment

At the end of ischemia, Evans blue dye (3 mL of 2% solution) was injected via the carotid artery to stain the perfused region of the heart, whereas the area at risk (AAR) remained unstained. Then, the rats were sacrificed; hearts were excised and frozen at -70°C for 1 h; afterward, hearts were transversely cut into 2 mm sections from apex to base. In each slice, the AAR was determined using an image processing software program (Photoshop, ver.7.0, Adobe system, San Jose, CA, USA).

2.6. Ventricular arrhythmias assessment

The ventricular arrhythmia was determined during ischemia, in accordance with the guidelines of Lambeth (Imani et al., 2011).

Ventricular ectopic beat (VEB), ventricular tachycardia (VT), and ventricular fibrillation (VF) were diagnosed as follows, respectively: distinctive and identifiable premature QRS complex, a run of four or more consecutive VEBs, unidentifiable and low voltage QRS complexes. Other forms of VEBs such as salvo and bigeminy were not assessed separately. VF lasting for more than 5 min was considered irreversible.

In order to measure the severity of the arrhythmia, we used a scoring system. In this system, hearts with 0–50 VEBs were given a score of 0, 50–500 VEBs a score of 1, more than 500 VEBs or one episode of reversible VT or VF a score of 2. In addition, 2–30 episodes of spontaneously reversible VT and VF received a score 3, more than 30 episodes of reversible VT and/or VF a score four and irreversible VF was given a score of 5 (Imani et al., 2011).

2.6.1. Plasma Lactate Dehydrogenase Measurement (LDH)

Before Evans blue injection, blood samples were collected in a heparinized tube at the end of ischemia. The samples were centrifuged, and

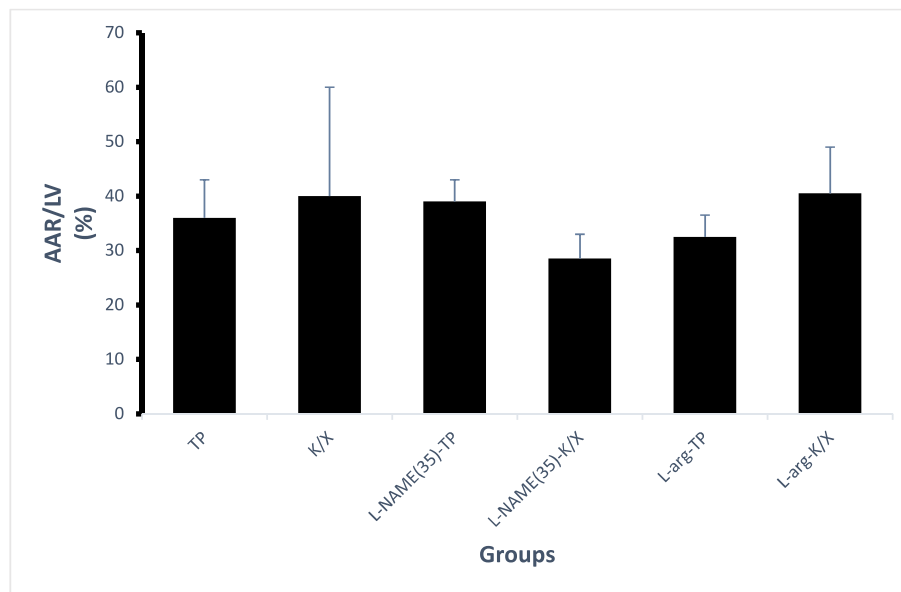


Fig. 1. The ratio of area at risk to left ventricle (AAR/LV), k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium (n = 6), (mean ± S.E.M).

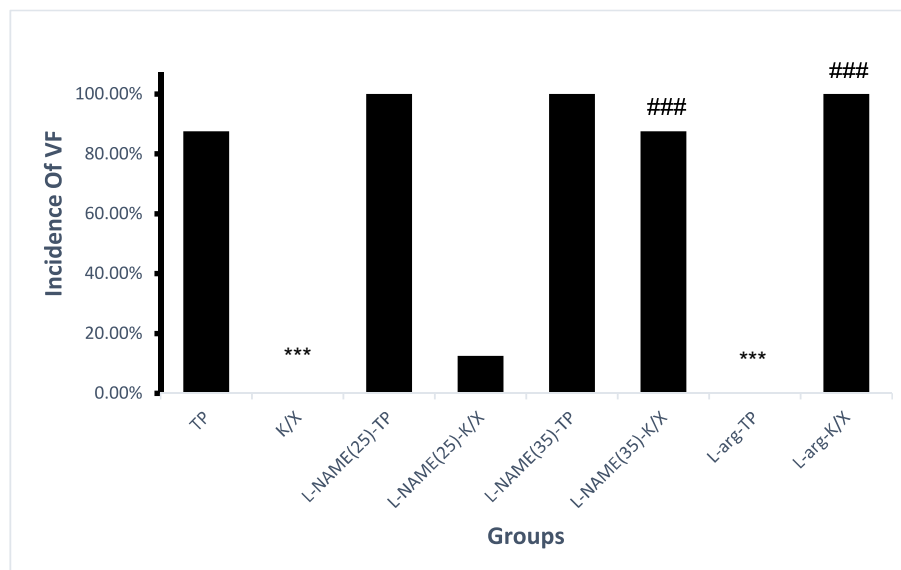


Fig. 2. Incidence of ventricular fibrillation in ischemia period, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium. *** $p < 0.001$ compared to thiopental sodium, ### $p < 0.001$ compared to ketamine/xylazine (n = 6), (mean ± S.E.M).

plasma was collected and frozen for assaying LDH with a commercially available kit.

2.7. Nitrite to nitrate measurement

Blood samples were collected and centrifuged to collect plasma. Nitrite levels were measured using Griess reagent. The standard curve was determined using 0–1000 m Mof NaNO₂.

2.8. Statistical analysis

In the present study, results are expressed as the standard error of the mean (SEM). SPSS 20.0 (SPSS, Inc.; Chicago, IL, USA) statistical software was used to perform the statistical analyses. One-way analysis of variance (ANOVA) and Tukey post hoc test for multiple group comparisons were utilized. The results were accepted to be statistically significant

when p value was less than 0.05.

3. Results

3.1. Hemodynamic parameters

As depicted in Table 1, during baseline and ischemia, HR was significantly less in animals ketamine/xylazine group than in the thiopental ($p < 0.0001$). There was no significant difference between groups in the baseline and ischemia period.

MBP also did not differ between thiopental and ketamine/xylazine groups. However, the L-NAME group showed markedly elevated BP compared to the control groups (thiopental sodium and ketamine/xylazine) ($p < 0.000$). L-arginine administration did not change MBP statically compared to control groups.

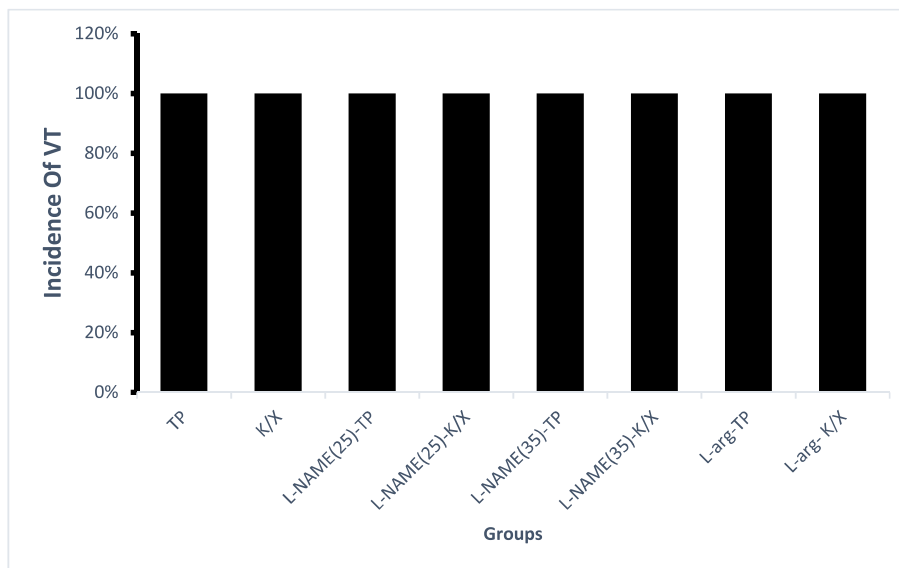


Fig. 3. Incidence of Ventricular Tachycardia, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium (n = 6), (mean ± S.E.M).

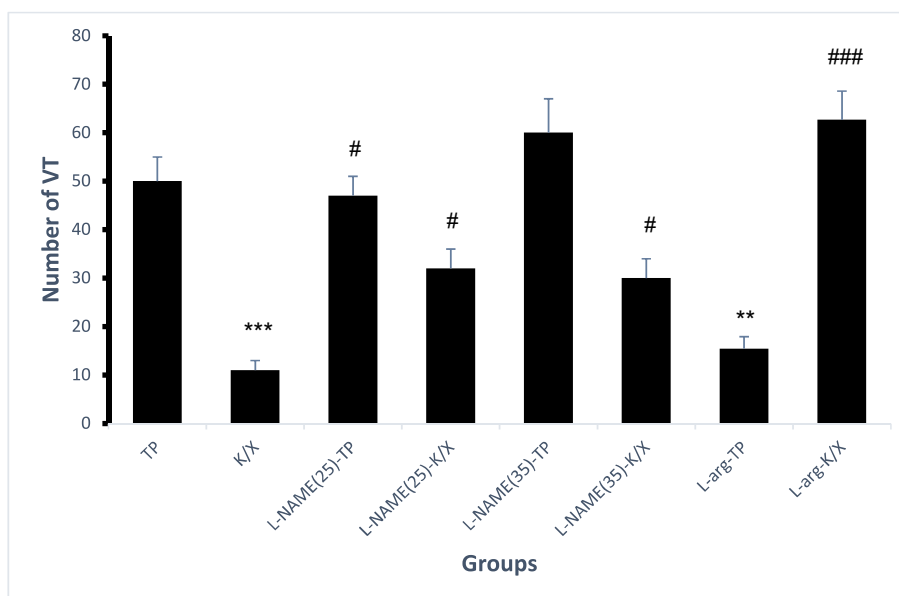


Fig. 4. Number of ventricular tachycardia, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium. ** $p < 0.01$ compared to thiopental sodium, # $p < 0.05$ compared to ketamine/xylazine, *** $p < 0.001$ compared to TP, ### $p < 0.001$ compared to K/X (n = 6), (mean ± S.E.M).

3.2. The area at risk to left ventricle ratio (AAR/LV)

There were no significant differences among groups in the AAR/LV, which confirms that LAD was ligated from the same place in all groups (Fig. 1).

Ventricular arrhythmias during ischemia.

3.3. VF incidence

In the K/X group, there was no VF incidence during ischemia compared to TP (0 vs. 85.5%). In addition, LNAME administration (35 mg/kg) caused VF incidence in animals receiving K/X (87.5% vs. 0). L-arginine administration before K/X injection also increased VF incidence (100% vs. 0). A rate that received L-arginine before TP injection did not have VF incidence during ischemia (Fig. 2).

3.4. VT incidence

During 30 min ischemia, VT was detected in all groups (Fig. 3).

3.5. VT number

In this study, animals anesthetized using K/X had fewer arrhythmias than the TP group (11 ± 2 vs. 50 ± 5, $p < 0.001$). L-NAME (25,35 mg/kg) before K/X injection significantly increased VT compared to the K/X (32 ± 4, 30 ± 4 vs. 11 ± 2, $p = 0.050$). Interestingly, the combination of L-arginine and K/X also increased VT numbers (62.67 ± 5.97 vs. 11 ± 2, $p < 0.001$) compared to the ketamine/xylazine, whereas L-arginine before TP significantly decreased VT numbers (15.43 ± 2.5 vs. 50 ± 5, $p < 0.0001$) compared to TP (Fig. 4).

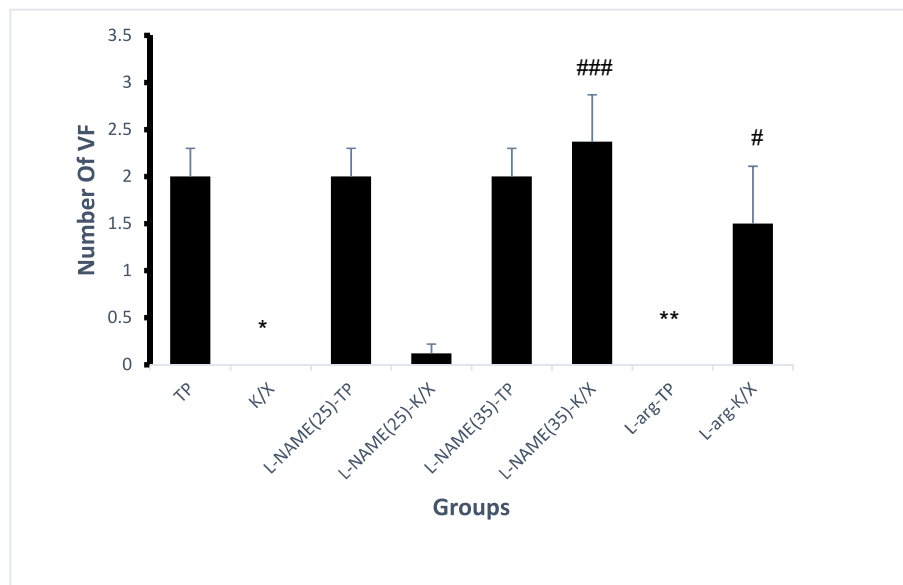


Fig. 5. Number of ventricular fibrillation, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium. ** $p < 0.01$ compared to thiopental sodium, ### $p < 0.001$ compared to ketamine/xylazine * $p < 0.05$ compared to TP, # $p < 0.05$ compared to K/X (n = 6), (mean ± S.E.M).

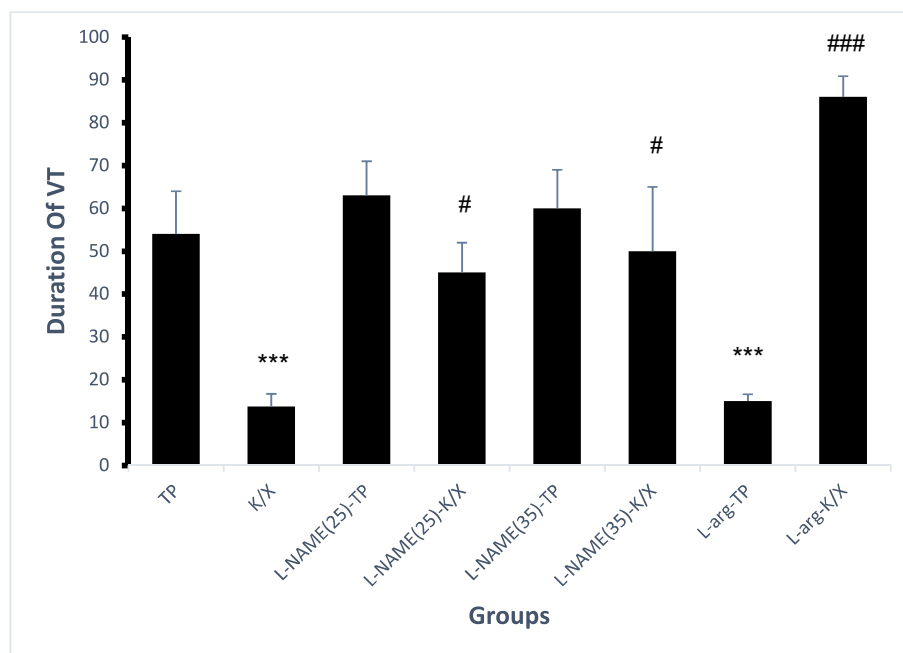


Fig. 6. Duration of ventricular tachycardia, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium. ** $p < 0.01$ compared to thiopental sodium, # $p < 0.05$ compared to ketamine/xylazine, ### $p < 0.001$ compared to K/X (n = 6), (mean ± S.E.M).

3.6. VF number

In the K/X group, there was no ventricular fibrillation, while LNAME administration (35 mg/kg) before K/X caused significant VF events (2.37 ± 0.5 vs. 0, $p = 0.0008$). L-arginine significantly reduced thiopental-induced VF (0 vs. 2 ± 0.3 , $p = 0.039$). L-arginine before K/X increased the VF event compared to the K/X (1.5 ± 0.61 vs. 0, $p < 0.05$) (Fig. 5).

3.7. VT duration

In the K/X group, a fewer duration of VT was recorded, compared with TP (13.70 ± 3 vs. 54 ± 10 , $p = 0.006$). L-NAME (25,35 mg/kg)

before K/X injection increased duration of VT, compared to the K/X (45 ± 7 , 50 ± 15 vs. 13.70 ± 3 , $p = 0.03$). L-arginine before TP decreased VT duration compared to the TP (15 ± 1.6 vs. 54 ± 10 , $p = 0.0013$). L-arginine administration before K/X caused a significant reduction in VT duration compared to the K/X group (86 ± 4.87 vs. 13.70 ± 3 , $p < 0.001$) (Fig. 6).

3.8. Scoring of arrhythmia

In the K/X group, the severity of arrhythmia significantly decreased compared to the TP group (3 ± 0 vs. 3.75 ± 0 , $p = 0.0179$). The severity of arrhythmia was as comparable in the LNAME-TP group as in the LNAME-K/X. L-arginine injection before K/X increased the severity of

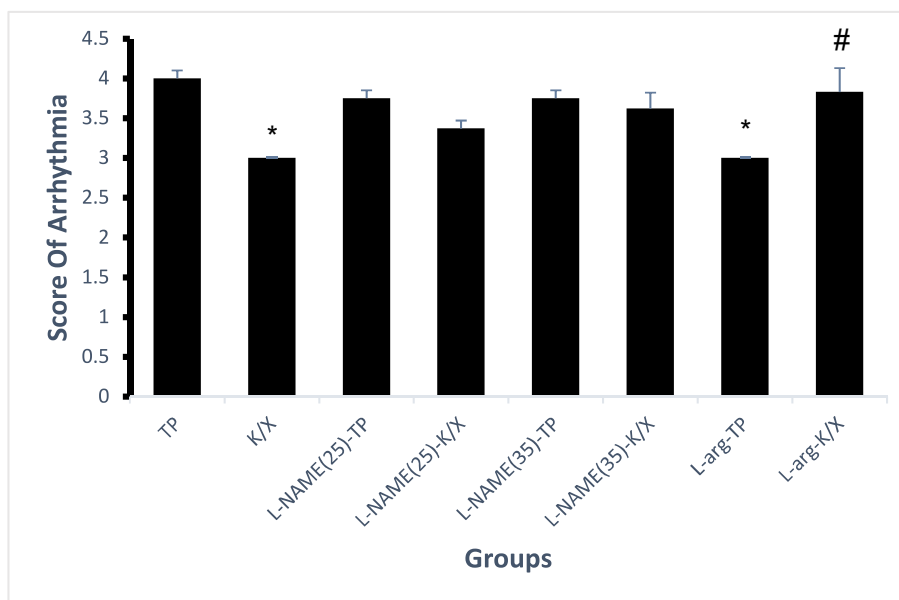


Fig. 7. Severity of ventricular Arrhythmias, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium, * $p < 0.05$ compared to thiopental sodium, # $p < 0.05$ compared to K/X (n = 6), (mean ± S.E.M).

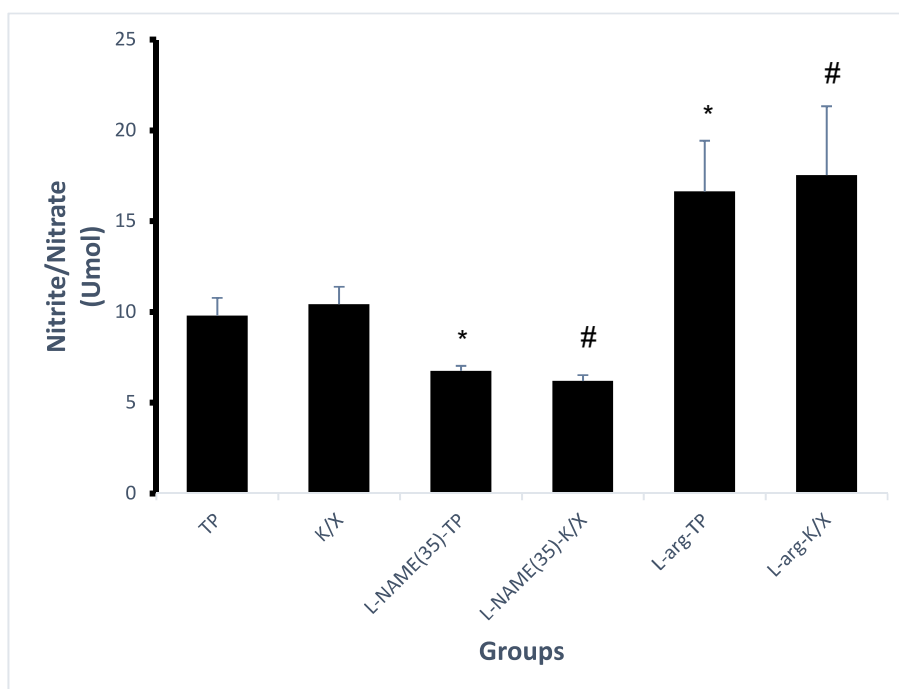


Fig. 8. The amount of nitrite/nitrate, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium, * $p < 0.05$ compared to thiopental sodium, # $p < 0.05$ compared to K/X (n = 6), (mean ± S.E.M).

arrhythmia compared to the K/X (3.8 ± 0.3 vs. 3 ± 0 , $p = 0.0466$). Conversely, L-arginine injection before TP decreased the severity of arrhythmia compared to the thiopental sodium (3 ± 0 vs. 3.75 ± 0.1 , $p = 0.0247$) (Fig. 7).

3.9. Nitrite/nitrate ratio

In the group which received L-NAME (35 mg/kg), nitrite/nitrate significantly decreased compared to the control groups (LNAME (35)-TP: 6.45 ± 0.37 , LNAME (35)-K/X: 6.19 ± 0.33 vs. TP: 10.42 ± 0.96 , K/X: 9.80 ± 0.97 , $p = 0.035$). L-arginine injection increased this amount in

comparison with the control (L-arginine-TP: 16.63 ± 2.8 L-arginine-K/X: 17.54 ± 3.79 vs. TP: 10.42 ± 0.96 , K/X: 9.80 ± 0.97 , $p = 0.0125$). There were no differences between K/X and TP groups in the amount of nitrite/nitrate (Fig. 8).

3.10. LDH level

There was not a significant difference in plasma LDH among groups (TP: 987 ± 90 , K/X: 1337 ± 146 , LNAME (35)-TP: 2101 ± 1273 , LNAME (35)-K/X: 1137 ± 169 , L-arginine-TP: L-arginine-K/X) (Fig. 9).

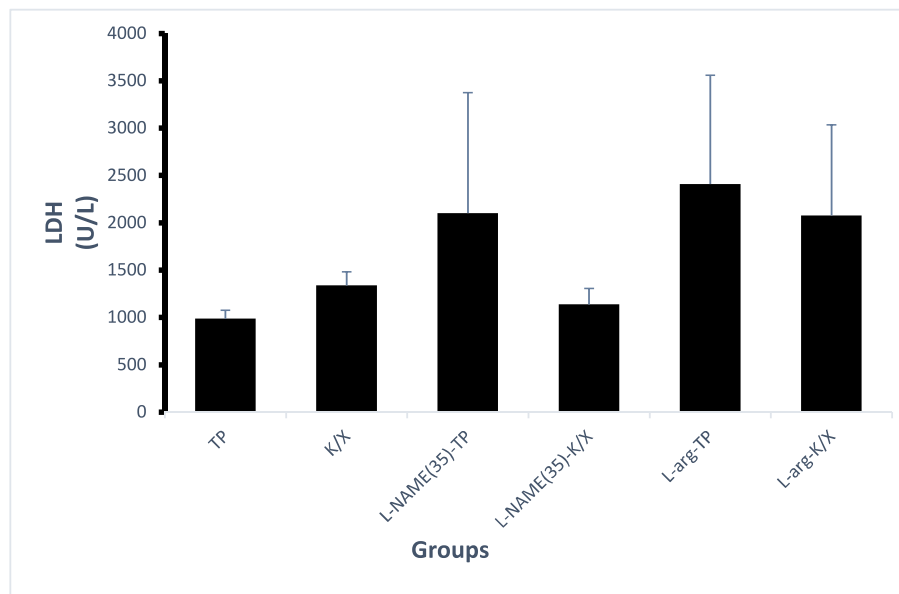


Fig. 9. Plasma concentration of LDH, k/x: ketamine/xylazine, L-arg: L-arginine, TP: thiopental sodium (n = 6), (mean ± S.E.M).

4. Discussion

Choosing the appropriate anesthetic agents is critical in myocardial ischemia-reperfusion models (Shekarforoush et al., 2016). Preventing ischemia during surgery and adequately treating it has become a significant challenge in modern anesthesia. There is solid evidence that some anesthetics and narcotic drugs have cardiac protection properties. Researchers have been suggesting that the administration of these agents during ischemic conditions might be worthwhile. In cardiac surgery, patients are more vulnerable to develop malignant arrhythmias, in which hemodynamic instability demands medical treatment during surgery and can lead to irreversible complications (Khan et al., 2019).

Our result confirmed that, firstly, anesthesia with ketamine/xylazine combination reduced the number of VT and VF, VT duration, the severity of arrhythmias, and incidence of VF during ischemia. Secondly, using L-NAME and L-arginine before anesthesia with ketamine/xylazine reduced the cardioprotective effects while L-arginine injection before thiopental was able to develop protective effects against arrhythmias. Moreover, there was no significant difference among groups in LDH levels. However, the nitrite to nitrate ratio in the L-NAME groups significantly decreased. This ratio was not statistically significant in the thiopental sodium group compared to the ketamine/xylazine group. L-arginine could increase the amount of nitrate level compared to the control groups. Anesthesia with ketamine/xylazine (with or without L-NAME and L-arginine) caused a significant decrease in heart rate compared to thiopental. There was no significant difference in arterial blood pressure between the two anesthesia groups with ketamine/xylazine and thiopental, whereas L-NAME injection increased arterial blood pressure. Evans Blue staining, in which the size of the AAR/LV was assessed, showed no significant difference between the groups, which means the extent of the exposed area in all groups was the same.

As confirmed by our results, the combination of ketamine/xylazine reduced heart rate and ventricular arrhythmias compared to the thiopental sodium group, which is in line with the previous studies (Piccolo et al., 2012; Gonca, 2015; Shekarforoush et al., 2016). The reduction in heart rate can be attributed to xylazine since it was reported that heart rate increased when only ketamine was injected, while the mixture of xylazine or diazepam with ketamine reduced the number of beats (Liebe et al., 2017). In our study, the 10 mg/kg dose of xylazine has diminished the effect with a dose of 70 mg/kg of ketamine, resulting in a decreasing

heart rate. One of the mechanisms by which ketamine could increase heart rate and arterial pressure through increasing sympathetic activity is its ability to inhibit baroreceptors (Gonca, 2015). Probably adding xylazine inhibited this effect of ketamine and thus prevented increasing heart rate and arterial pressure. Xylazine, an α -agonist with peripheral and transient effects, can cause primary vasoconstriction, leading to increased blood pressure and reduce reflexes in heart rate. However, its prolonged effect causes a reduction in the sympathetic tone, leading to decreased heart rate and blood pressure.

Since NO is a vasodilator, inhibiting NO production via L-NAME results in vasoconstriction and increased arterial pressure. L-NAME has been shown to reduce heart rate and increase arterial blood pressure (Miguel-Carrasco et al., 2008; Zayed et al., 2021). Therefore, the combination of L-NAME with ketamine/xylazine was supposed to decrease heart rate, but it did not observe in this study. It can be concluded that the effects of xylazine on the vasomotor center outweigh the reducing L-NAME impact on heart rate. Since thiopental has been shown to increase heart rate by inhibiting acetylcholine receptors (Weber et al., 2005), it seems that in the L-NAME thiopental-induced groups, the effect of thiopental on heart rate was more efficient than the L-NAME. This might explain why the number of heartbeats did not decrease in our study. The effect of NO on heart rate is intricate.

Additionally, endogenous and exogenous NO have different effects on heart rate. NO has been shown to have a chronotropic effect dependent on sinus depression. Sodium nitroprusside, at low concentrations, increases and vice versa at high concentrations, reduces the heart rate (Tamargo et al., 2010).

Not surprisingly, L-NAME (25–35 mg/kg) administration with ketamine/xylazine and thiopental was associated with a significant increase in arterial blood pressure. This consequence, as noted earlier, probably resulted from inhibiting the production of NO and diminishing its vasodilation effect. There was no significant difference in arterial blood pressure during baseline and of ischemia. However, in L-arginine groups, the reduction of mean arterial blood pressure was not statistically significant.

In this study, the role of NO in the protective effect of ketamine/xylazine on ventricular arrhythmias was studied. Although anesthesia with ketamine/xylazine cocktail significantly reduced ischemic ventricular arrhythmias compared to the thiopental group, L-NAME 35 mg/kg administration 20 min before ketamine/xylazine increased the number and the duration of tachycardia and the incidence of ventricular

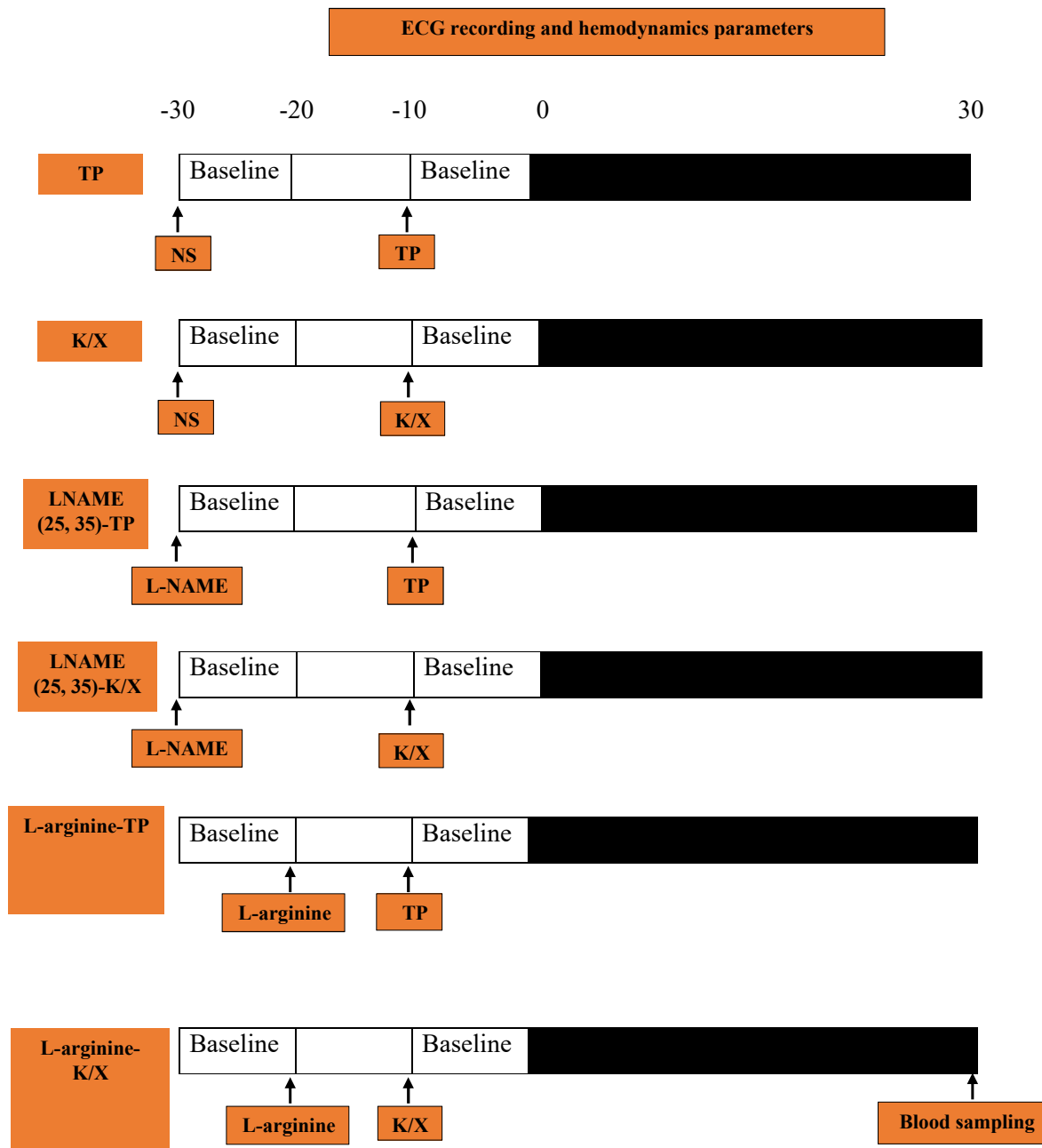


Diagram 1. Myocardial ischemia protocol, K/X: ketamine/xylazine, NS: Normal saline, TP: thiopental sodium.

fibrillation. First, we injected 25 mg/kg (IP) L-NAME 20 min before ketamine/xylazine. This dose significantly increased the number and duration of ventricular tachycardia compared to the ketamine/xylazine group during ischemia. However, ventricular fibrillation, the most dangerous ventricular arrhythmias, did not occur in this dose; then, we increased the dose of L-NAME by 35 mg/kg. Our result showed that a dose of 35 mg/kg has been able to reduce the amount of NO, which eliminates the protective effects of ketamine/xylazine on ventricular fibrillation. It means L-arginine could fade the antiarrhythmic effect of ketamine/xylazine. It may be due to the rise in NO amount, which might bring about adverse cardiac consequences since several studies reported that NO plays a double-edged sword role in the heart. At low concentrations, NO produces a small amount of cGMP, inhibits phosphodiesterase III, and causes to prevent cAMP hydrolysis. Subsequently, a protein-kinase, A, activates, leading to sarcolemma voltage-operated and sarcoplasmic ryanodine receptor Ca^{2+} channels opening. As a

result, myocardial contractility increases. At concentrations, NO causes to produce more amounts of cGMP, by which cardiodepression occurs in response to protein kinase G (PKG) activation with blockade of sarcolemma Ca^{2+} channels (Rastaldo et al., 2007; Wang et al., 2021). In our study, using L-arginine resulted in increasing NO level so that it exerted a destructive effect and increased arrhythmia in the ketamine/xylazine group. As shown in the results, L-arginine caused a significant decrease in the number and duration of VT and VF incidence in the thiopental group. It can be explained that L-arginine leads to the production of a high concentration of NO so that could dominate the inherent arrhythmogenic effects of thiopental. This phenomenon causes protective effects against arrhythmias induced during ischemia. Several mechanisms have been proposed to explain the antiarrhythmic effects of NO. Reilly et al., in 2016, reported that atrial-specific up-regulation of microRNA-31 (miR-31) decreases the rate of AF via promoting nNOS mRNA degradation. These findings recognize the atrial-specific miR-31

Table 1

Hemodynamic parameters. TP: Thiopental sodium, k/x: ketamine/xylazine, HR: heart rate, BP: blood pressure. Data are expressed as mean \pm SEM, *** $p < 0.001$ compared with Thiopental group in ischemia period, ### $p < 0.001$ compared with K/X in ischemia period, \$\$ $p < 0.01$ compared with Thiopental group in Baseline period, && $p < 0.01$ compared with K/X in Baseline period.

	Baseline ischemia			
	HR	BP	HR	BP
TP	300 \pm 15	100 \pm 2.2	295 \pm 17	84 \pm 2.7
K/X	95 \pm 10 ^{\$\$\$}	95 \pm 1.3	116 \pm 7.6 ^{***}	83 \pm 2.9
LNAME(25)-TP	270 \pm 17	123 \pm 2.7 ^{\$\$}	307 \pm 11	123 \pm 3
LNAME(25)-K/X	100 \pm 9 ^{\$\$\$}	122 \pm 1.2 ^{&&}	108 \pm 6 ^{***}	120 \pm 2.7 ^{##}
LNAME(35)-TP	310 \pm 6	125 \pm 2.3 ^{\$\$}	293 \pm 9	122 \pm 3.5 ^{**}
LNAME(35)-K/X	110 \pm 8 ^{\$\$\$}	130 \pm 2 ^{&&}	110 \pm 7 ^{***}	127 \pm 2.2 ^{##}
L-arginin-TP	300 \pm 15	70.7 \pm 3.84	278 \pm 15.34	75.7 \pm 3.84
L-arginin-K/X	150 \pm 25 ^{\$\$}	65 \pm 5.04	119 \pm 9.25 ^{***}	77.83 \pm 4.04

up-regulation as the primary mechanism associated with human AF. In which atrial dystrophin and nNOS depletion occurs, which eventually brings about AF. Their findings highlight the role of NOS in arrhythmias development (Reilly et al., 2016). Although there is ample evidence, NO has protective properties, high doses expressed by iNOS induce apoptosis, whereas low doses by eNOS have a protective effect. Altogether the notion that NO has a dual role in the heart is widely accepted. More importantly, the iNOS/NO signaling has the most influential role in the myocardium (Sloan et al., 2011; Yu et al., 2018). The results show that the nitrite/nitrate ratio in the two ketamine/xylazine and thiopental groups was not statistically significant. In both groups, the use of L-NAME reduced the NO level significantly. It was expected that ventricular arrhythmias would increase in both groups through inhibiting NO production, which was merely observed in ketamine/xylazine. Therefore, despite the high NO levels in the thiopental group, other arrhythmogenic mechanisms prevent the protective effects. One mechanism is that acetylcholine canals neuronal in the intra-cardiac ganglion (nACh) might close, which is typically observed due to thiopental sodium injection as well as ketamine. These channels are responsible for entering Ca^{2+} into the heart to initiate depolarization (Harper et al., 2020). Thiopental and ketamine act as an ATP-associated potassium channels inhibitor in cardiomyocytes, which reduce the protective effects of diazoxide, a potassium channel activator (Zaugg et al., 2002). In contrast, combining xylazine as an α -agonist with ketamine eliminates the ketamine-related myocardial suppression effects. We assessed the serum samples' NO level, which means the results would differ if the ratio were measured in the heart tissue.

To investigate the effect of ketamine/xylazine and thiopental sodium on the severity of myocardial infarction, LDH was evaluated. There was no significant difference in serum levels of LDH between groups. However, a study conducted by Shekarfouroush et al. reported that infarct size was reduced by 0.1% in ketamine/xylazine compared to other groups using tetrazolium chloride staining (Shekarfouroush et al., 2016), a high dose of ketamine (200 mg/kg) - xylazine (60 mg/kg) has been shown to protect the isolated guinea-pig heart from the ischemic-reperfusion injury (Sloan et al., 2011). The combination of ketamine/xylazine or ketamine/diazepam has antiarrhythmic properties in ischemic/reperfusion animal models; even arrhythmias were less than pentobarbital or thiopental sodium (Munif et al., 2020). An in vivo study by Zornik et al. (2010) was performed to investigate the effect of three anesthetics, thiopental sodium, pentobarbital, and urethane, on ischemic ventricular arrhythmias. The result showed that thiopental was more arrhythmogenic compared to another two agents. This result was in agreement with our finding, where the combination of ketamine/xylazine was more efficient in reducing the duration of ventricular tachycardia (Zornik et al., 2010). This study includes some limitations. For example, the

antiarrhythmic effects of ketamine/xylazine in acute cardiac ischemia-reperfusion may be associated with the proteins that activate the endothelial NO synthase (Mattagajasingh et al., 2007; I. Martins, 2018; Martins, 2018, 2018; I. J. Martins, 2018; Ministrini et al., 2021). Thus, more studies are needed to understand molecular mechanisms involved in the antiarrhythmic effects of ketamine/xylazine in acute cardiac ischemia-reperfusion.

5. Conclusion

In this study, we showed that the antiarrhythmic properties of ketamine/xylazine could be through the NO synthesis pathway. The injection of L-NAME before ketamine/xylazine could probably increase the number and duration of ventricular tachycardia and ventricular fibrillation via reducing NO. Conversely, L-arginine administration caused decreased protective effects of ketamine/xylazine due to increasing NO production.

Compliance with ethical standards

The Ethics Committee of the Tran University of Medical Sciences approved all the experiments and protocols. All the efforts were carried out to minimize animals suffering.

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Not applicable.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Credit author statement

A.I and M.N designed and performed the experiments. A.I, S.F.R, and M.N carried out the data A.I, S.F.R analysis and wrote the manuscript. M. F, K.R and T.P provided edits.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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