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Original article

Valeriana officinalis extract and 7-Nitroindozole ameliorated seizure behaviours, and 7-Nitroindozole reduced blood pressure and ECG parameters in pentylenetetrazole-kindled rats

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Abstract

Nitric oxide (NO) is known to be a neuromodulator with dual proconvulsive and anticonvulsive action. *Valeriana officinalis* (VAL) was previously believed to be antiepileptic, but is today known as a sedative and sleep regulator. Seizures may be associated with abnormal electrocardiographic changes and cardiac dysfunction arising from epilepsy may be related with neuronal nitric oxide (nNO). This study was aimed to investigate the effects of the neuronal nitric oxide synthase (nNOS) inhibitor 7-Nitroindazole (7-NI) and VAL on seizure behaviours and electrocardiographic parameters in the pentylentetrazole (PTZ)-kindled seizure model.

Wistar rats were randomised into saline control, PTZ-kindled, 7-NI, VAL and VAL+PTZ, 7-NI+PTZ and VAL+7-NI+PTZ groups. Latency, stage, frequency of seizures, blood pressure (BP), heart rate (HR) and corrected QT (QTc) values were evaluated.

Frequency and stage of seizures, BP and HR increased, while seizure latency decreased and QTc was prolonged in the PTZ-kindled group. 7-NI and VAL had no effects on BP and HR variables under normal conditions, but ameliorated the seizure stage and frequency of seizures. 7-NI treatment also resulted in a reduction of the increased BP and prolonged QTc values observed in PTZ-kindled rats.

Considering these results, QTc prolongation may be used as a predictor for recurrent seizures. 7-NI and VAL exhibited different effects on seizures and ECG variables. 7-NI shows potential as an anticonvulsant drug agent in epileptic patients with cardiac dysfunctions and those additional studies including in-vivo experiments are essential.

Key words: Seizure, 7-Nitroindazole, Valerian, PTZ kindling, Heart rate, QTc

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Introduction

Epilepsy is a common chronic neurological disorder that affects 70 million individuals and status epilepticus (SE) is a common, life-threatening neurologic disorder associated with significant morbidity and mortality (Trinka et al. 2015). Epileptic discharge leading to cardiac arrhythmias, hypoxia and autonomic imbalance results in damaged autonomic regulation and seizure-induced cardiorespiratory changes (Brewster et al. 2016). Because the cardiorespiratory system is regulated by the autonomic nervous system, autonomic disorders and an imbalance between the sympathetic and parasympathetic nervous systems may play important roles in the development of electrocardiographic (ECG) and respiratorical abnormalities during epileptic seizures (Ryvlin et al. 2013). These abnormalities may play a role in sudden unexpected death in epileptic patients (SUDEP). SUDEP affects 1/4500 children and 1/1000 adults with epilepsy every year and its underlying mechanisms are multifactorial (Vitorino et al. 2019). Experimental and clinical studies have observed cardiovascular changes, especially an increased heart rate, in both patients and experimental epilepsy models (Opherk et al. 2002, Vitorino et al. 2019). The autonomic activity and/or dysfunction can be measured and analyzed using heart rate (HR) and HR variables (Page and Rugg-Gunn 2018).

Cardiac rhythm and conduction disturbances frequently occur during prolonged or generalized seizures (Damasceno et al. 2012). During the ictal and postictal seizure period, determination of abnormal electrocardiographic (ECG) changes including T-wave inversion, ST depression and QTc prolongation suggest that myocardial ischemia may contribute to lethal arrhythmias (Damasceno et al. 2012). Previous studies have demonstrated tachycardia and QTc interval prolongation at 1-2 weeks following SE (Metcalf et al. 2009, Bealer and Little 2013) and similar ECG alterations have been reported in chronically epileptic animals (Powell et al. 2014). Nitric oxide (NO) is one of the many causes of cardiac arrhythmias (Atabay and Uzun 2009).

NO is a diffusible gaseous messenger synthesized from the amino acid L-arginine by the different isoforms of nitric oxide synthase (NOS), including neuronal NOS (nNOS), inducible NOS (iNOS), endothelial NOS (eNOS) and mitochondrial NOS (mtNOS). nNOS is expressed in the neurons of the cerebellum, hypothalamus, striatum, cerebral cortex, hippocampus (Guix et al. 2005) and peripheral tissues, as well as in the heart and large arteries (aorta) (Zhang 2017). mtNOS is an isoform of nNOS localized in the inner mitochondrial membrane. NO is known to play a role in the pathogenesis of mechanisms underlying seizure initiation and/or propagation. In vivo and in vitro studies examining the role of NO in epileptogenesis provide controversial evidence for either anti-convulsant (Noh et al. 2006, Yahyavi-Firouz-Abadi et al. 2006) or pro-convulsant (Riazi et al. 2006) effects in different seizure paradigms. Studies on the effects of NOS inhibitors show variation depending on dose, method of administration and animal species (Wojtal et al. 2003). Under normal and pathological conditions, NOS isoforms mediate systolic, diastolic and chronotropic cardiac functions (Umar and van der Laarse 2010). NO inhibits L-type Ca^{2+} channels and stimulates Ca^{2+} release from the sarcoplasmic reticulum (SR), resulting in different effects on myocardial contraction. nNOS targets cardiac SR and stimulates the release of Ca^{2+} from SR (Barouch et al. 2002). Both mediate the contraction and relaxation of cardiomyocytes (Umar and van der Laarse 2010). Expression of nNOS has been detected in the human aorta, carotid, mammary arteries and lung capillary endothelial cells (Costa et al. 2016), and nNOS-derived NO plays a physiological role in coronary vasculature (Seddon et al. 2009). 7-nitroindazole (7NI) is a more selective inhibitor of nNOS than the other two NOS isoforms, eNOS and iNOS, and does not cause changes in cerebral blood flow and cortical excitability (Brozickova and Otahal 2013). So, 7-NI is a more suitable inhibitor to establish the role of nNOS in the sympathetic and parasympathetic nervous system (Anaeigoudari et al. 2015) and vasculature (Umar and van der Laarse 2010).

Valeriana officinalis contains more than 150 chemical constituents and many of them are active physiologically. Due to its sedative effect, extracts of Valerian (Val) root are widely used as dietary supplements to treat insomnia, anxiety and heart palpitations caused by nervousness (Sharma et al. 2010). Small clinical studies have yielded controversial results on its efficacy (Bent et al. 2006, Taibi et al. 2009, Fernandez-San-Martin et al. 2010). In vivo studies mostly agree on its anxiolytic and anti-convulsive effects (Rezvani et al. 2010). In vitro assays have showed that crude extracts and isolated constituents like valerenic acid, alkaloids and lignans can interact with GABAA, adenosine and serotonin receptors (Rezvani et al. 2010). In addition, antihypertensive and preventive effects on coronary spasms have been observed (Circosta et al. 2007). Valerenic acid and valerian extracts have anticonvulsant properties in adult zebrafish (Torres-Hernández et al. 2015). Valerian extract has coronary dilatation and antiarrhythmic effects on rabbits, mice and cats. It has also been reported that the compounds it contains prevent vasopressin-induced arrhythmia and cause short-term blood flow increase in acute coronary insufficiency (Sharma et al. 2010).

The cardiomyopathy consequent to the development of SE occurs, at least in part, from a centrally evoked activation of cardiac sympathetic nerves. In this study we aimed to evaluate the effects of valerian extract and 7-NI, a nNOS inhibitor, on animal seizures and behavior, as well as the relationship between nitric oxide and ECG changes in kindled rats.

Materials and Methods

Animals and experimental protocol

Wistar albino rats (48 males; 320-350 g) were obtained from the University of Istanbul Animal Resource Unit. The animals were kept in a 12 h light/ /dark cycle at 50% humidity and 22°C with food and water provided ad libitum. Experiments were performed in accordance with the University of Istanbul Committee on Ethics in the Care and Use of Laboratory Animals and conducted following the guidelines for the Care and Use of Laboratory Animals (03.04.2009.44).

Rats were randomly divided into eight groups of six as follows: (1) Control group (n=6): intraperitoneal (i.p.) saline injection every day for 25 days; (2) PTZ group (n=6): PTZ (40 mg/kg, i.p., Sigma Aldrich, St. Louis, MO) injection every other day, a total of 13 doses; (3) 7-Nitroindazole (7-NI) group (n=6): 7-NI (25 mg/kg, i.p., Sigma Aldrich, St. Louis, MO) injection every day for 25 days; (4) Valerian (VAL) group (n=6): VAL extract (200 mg/kg, Münir Şahin Pharmaceutical Industry, Turkey) administered orally by gavage every day for 25 days; (5) VAL+7-NI group (n=6): 7-NI injected every day 30 minutes after VAL (200 mg/kg/gavage/every day) administration for 25 days; (6) 7-NI +PTZ group (n=6): PTZ injected every other day 30 minutes after 7-NI (25 mg/kg, i.p./every day) injection for 25 days; (7) VAL+PTZ (n=6): PTZ injected every other day an hour after VAL (200 mg/kg/gavage/every day) administration for 25 days; (8) VAL+7-NI+PTZ group (n=6): 7-NI injected every day 30 minutes after VAL (200 mg/kg/ /gavage/every day) administration and PTZ injected 30 mins after 7-NI injection for 25 days. 200 mg/kg VAL dose and 25 mg/kg 7-NI dose were chosen to avoid side effects and overdose.

Induction of PTZ kindling

According to the NIH/National Institute of Neurological Disorders and Stroke Anticonvulsant Drug Development Program, the PTZ model is an animal seizure model (Stables et al. 2002) exhibiting quantifiable seizure onset latency, easily recognized behavioral seizures and predictive value for anticonvulsant efficacy against generalized seizures in humans. In the present study, seizures were induced by i.p. administration of a subconvulsive dose of PTZ, which is a GABA-A receptor antagonist, at 48 h intervals for 25 days (13 doses of PTZ injected) (Postnikova et al. 2017, Wang et al. 2017). Each animal was placed into an individual plastic cage for observation. After the administration of PTZ, seizure behaviors, including seizure severity (stage) and seizure latency, were observed. Seizure stages were assessed using scores based on a modified Racine's scale (Lüttjohann et al. 2009) as follows: 1, ear and facial twitching; 2, single to repeated myoclonic jerks; 3, partial clonic forelimb convulsions in a sitting position; 4, major seizures (generalized clonic and/or tonic seizures while lying on the belly); 5, generalized tonic-clonic seizures that begin with running, followed by the loss of righting ability and then a short tonic phase (flexion or extension of fore-and hindlimbs), progressing to the clonus of all four limbs. Seizure latency was measured as the time between injection of PTZ and appearance of the first clonic convulsion, which was indicated by a sudden twitching of head or jerky movement of body. PTZ injection was conducted between 10:00 and 12:00 in order to minimize possible complicating effects of the animals' circadian rhythms on behavior.

Surgical procedure and ECG recordings

Thirty minutes after the 13th dose of PTZ, animals were anesthetized with ketamine-xylazine mixture (90/10 mg/kg, i.p.). Following a tracheotomy, the animals were mechanically ventilated at a FiO2 of 0.4. Body temperature was maintained at 37 ± 0.5°C using a heating pad. A polyethylene catheter (outer diameter = 0.9 mm; Braun, Melsungen, Germany) was inserted into the femoral artery and connected to a pressure transducer to monitor mean arterial blood pressure (MAP). After the surgical procedure (approximately 45 min), hemodynamic data were recorded for one hour, followed by a 30-min stabilization period, using Power-Lab 2/25 signal conditioner and Lab-Chart v.6 Pro software (AD Instruments, Sydney, Australia) in anesthetized rats. The standard bipolar limb lead II electrocardiogram was recorded to determine heart rates (to assess chronotropic effect) and voltage of QRS (to assess inotropic effect). Since QT interval varies with heart rate, the corrected QT (QTc) was calculated. The most frequently used formula to correct QT for heart rate (QTc) is the Bazzet's formula.

Analysis of recorded data

Recorded data were post-analyzed blindly using pre-established/fixed algorithms within Lab Chart 6 Pro

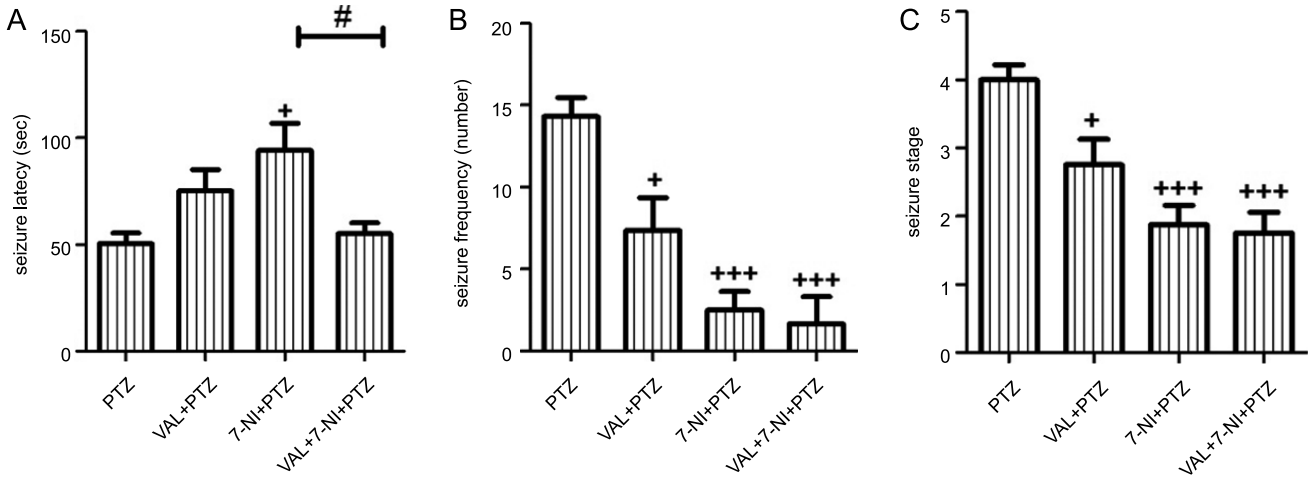

Fig. 1. Comparison of seizure latencies (A), frequencies (B) and stages (C) between PTZ and 7-NI+PTZ, VAL+PTZ, VAL+7-NI+PTZ at the end of the experiment. Data are presented as mean ± SEM. + vs PTZ kindling group, + $p<0.05$, +++ $p<0.001$; # vs VAL+7-NI+PTZ, # $p<0.05$.

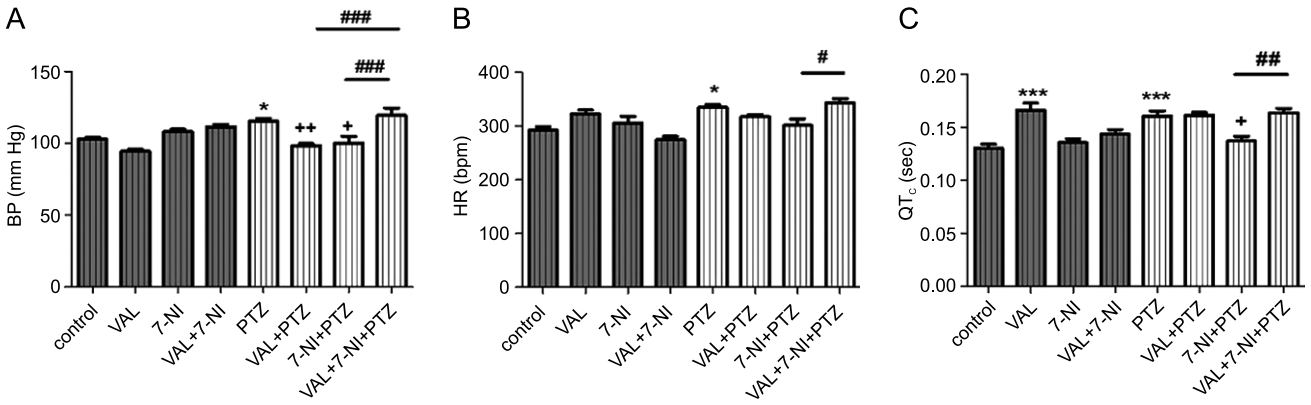

Fig. 2. The values of BP, HR and QTc parameters of all groups. Data are presented as mean ± SEM. * vs control group, * $p<0.05$, *** $p<0.001$; + vs PTZ kindling group, + $p<0.05$, ++ $p<0.01$; # vs VAL+7-NI+PTZ, # $p<0.05$, ## $p<0.01$, ### $p<0.001$.

Software. Blood pressure (BP) and ECG data were analyzed using the ECG Analysis module software in order to assess BP, heart rate (HR) and QT intervals. Data were analyzed at the end of the experiment. The QTc was calculated according to Bazzet's formula () by applying Mitchell algorithm (Roussel et al. 2016). This algorithm is designed to correct for the higher HR and altered QRS-T wave morphology in rodents.

Biochemical analysis in plasma

Blood samples collected from the artery were centrifuged at 1500 x g at 4°C for 10 minutes to get plasma. Total nitric oxide (Arbor Assays, K023-H1) and superoxide dismutase (SOD, Arbor Assays, K028-H1) levels in plasma were determined using commercial ELISA (Enzyme-Linked Immunosorbent Assay) kits according to the manufacturer's instructions.

Statistical analyses

The statistical analyses were performed using Instat Statistical Software (Instat Graphad Software 5.02, San Diego, CA, USA) and the results are expressed as the means±standard error of mean (SEM). All data were evaluated using one-way analysis of variance followed by a post-hoc Tukey comparison test. The value $p<0.05$ was considered statistically significant in all analyses.

The effects of 7-NI on behaviors in PTZ-kindled rats

Seizure latency showed an increment in the 7-NI+PTZ group compared with the PTZ group and VAL+7-NI+PTZ group (Fig. 1A, $p<0.05$). Valerian extract did not have a meaningful effect on seizure latency after 13 PTZ injections. We analyzed the seizure stage and frequency. As shown in Fig. 1B, the animals in the PTZ group showed a high number of seizures after 13 injections. The number of seizures (in stage 3)

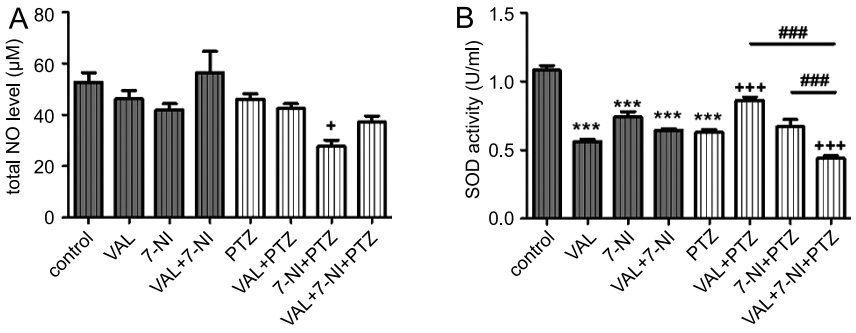

Fig. 3. Comparison of the nitric oxide metabolites levels and super oxide dismutase activity in plasma in groups. Data are shown as mean ± SEM. * vs control group, *** p<0.001; + vs PTZ kindling group, + p<0.05, +++ p<0.001; # vs VAL+7-NI+PTZ, ### p<0.001.

was lower in VAL treatment (VAL+PTZ group) (p<0.05), 7-NI treatment (7-NI+PTZ) (p<0.001) and combined treatment (VAL+7-NI+PTZ) groups (p<0.001) in comparison with the PTZ group (Fig. 1B). VAL treatment (VAL+PTZ group) (p<0.05), 7-NI treatment (7-NI+PTZ group) (p<0.001) and combined treatment (VAL+7-NI+PTZ group) (p<0.001) also caused a significant decrease in seizure stage compared to the PTZ group (Fig. 1C).

The blood pressure and ECG analysis

Blood pressure recorded three hours after PTZ injection was higher in kindled rats (p<0.05) than the control group (Fig. 2A). Also, there were no significant changes in BP of the control groups (Val group, 7-NI group and Val+7-NI group). The BP of animals in the VAL+PTZ (p < 0.01) and 7-NI+PTZ (p < 0.05) groups were lower than those of the PTZ group (Fig. 2A). The BP of the VAL+7-NI+PTZ group was higher than that of the VAL+PTZ (p<0.001) and 7-NI+PTZ (p<0.001) groups (Fig. 2A).

The heart rate of the PTZ kindling group increased (p<0.05) compared with the control group (Fig. 2B). 7-NI and VAL did not alter HR variables compared with the control group or kindling group. The HR of the VAL+7-NI+PTZ group was higher than that of the 7-NI+PTZ group (p<0.05) (Fig. 2B).

The QTc interval increased in the VAL (p<0.001) and PTZ kindling (p<0.001) groups (Fig. 2C), but did not change in the 7-NI and VAL+7-NI groups, when compared with the control group. The QTc interval decreased in 7-NI+PTZ compared with the PTZ group; however, 7-NI did not cause any changes in the VAL+7-NI+PTZ versus PTZ group. The QTc duration of the VAL+7-NI+PTZ group was higher than that of the 7-NI+PTZ group (p < 0.01) (Fig. 2C).

Biochemical results

There were no significant effects on plasma NO levels in the PTZ, VAL, 7-NI group and VAL+7NI groups in comparison with the control. When we compared the plasma total NO level with the PTZ group, only the 7-NI+PTZ group showed a significantly lower level of NO (p<0.05) (Fig. 3A).

The VAL, 7-NI, VAL+7-NI and PTZ groups exhibited significantly lower SOD activity than the control group (p<0.001) (Fig. 3B). Interestingly, VAL treatment combined with PTZ led to a higher SOD activity level than in the PTZ group (p < 0.001) (Fig. 3B). While 7-NI treatment combined with PTZ did not alter SOD activity, SOD activity decreased in the VAL+7-NI+PTZ (p<0.001) compared with the PTZ group. The combined treatment (VAL+7-NI+PTZ group) lowered SOD activity in comparison with both VAL+PTZ and 7-NI+PTZ treatments (p<0.001) (Fig. 3B).

Discussion

Our study documents that kindling seizure induction of SE constitutes a model of convulsive stress with persistent ECG changes, characterized by prolongation of the QTc interval, and higher HR and blood pressure. Severe and recurrent convulsive stress can lead to potentially fatal cardiac dysfunction by a mechanism that is not fully understood. We evaluated the effect of 7-NI and valerian extract on seizures and cardiovascular parameters.

The pentylenetetrazole-induced lowered seizure threshold is a well-established animal model that allows successful screening of anti-convulsant drugs effective at different stages of seizure generation and propagation. PTZ acts as the antagonist of the aminobutyric acid-A (GABA-A) receptor, and PTZ-induced convulsive seizures occur with increased glutamatergic neuronal activity (Watanabe et al. 2013). The PTZ-induced seizure in our study had a seizure stage of 4-5 and a frequency of 12-15 in the PTZ group. NO acts as a neuromodulator with glutamate, GABA, monoamine and cholinergic neurotransmission (Brožíčková and Otáhal 2013), and has broad neuronal, glial and vasculature effects (Guix et al. 2005). nNOS-derived NO

generation was observed to play a role in PTZ-induced convulsive seizures (Costa et al. 2016). 7-NI, a nNOS inhibitor, suppressed seizure initiation in a low-magnesium seizure model, in acute and organotypic hippocampal slice preparations (Brožíčková and Otáhal 2013). In our study, 7-NI treatment also prolonged seizure latency, and reduced the frequency and stages of PTZ-induced seizures. It is suggested that NO increases synaptic GABA release and releases presynaptic glutamate with GABA-B activation (Xing et al. 2008). Reduction in plasma NO concentration in the 7-NI+PTZ group demonstrates its ameliorative effects on seizure behaviors (latency, frequency and stage of seizures). Torres-Hernandez et al. (2015) reported that extracts of valerenic acid and valerian significantly prolonged the latency period to the onset of convulsion in adult zebrafish. Valerian extract (500 mg/kg) had an anticonvulsant effect in the temporal lobe epilepsy model in rats (Rezvani et al. 2010). Our results corroborate these findings. The stage and frequency of seizures decreased in the VAL+PTZ group and in the combined group (VAL+ 7-NI +PTZ).

Seizures directly affect HR, mean arterial pressure (MAP), respiration and cardiac function, while autonomic dysfunction also occur interictally in chronic epilepsy (Brewster et al. 2016). MAP increased and HR decreased during PTZ-induced seizures at the >3 seizure stage (Beig et al. 2009). Seizures frequently cause transient increases in HR and BP; however, it is not clear whether having active epilepsy can also affect these vital signs interictally. Recurrent seizures on a chronic basis might increase sympathetic tone interictally and affect HR or BP during a resting state. Such a state of chronically heightened sympathetic tone occurs in patients at high risk of sudden cardiac death (Vaseghi and Shivkumar 2008), and chronic tachycardia can lead to cardiomyopathy (Umana et al. 2003). In our study, seizures induced by PTZ resulted in an increase in BP and HR parameters consistent with other studies.

Insufficient NO causes an increase in blood pressure, and only inhibition of nNOS in nerve tissue (central or peripheral) can induce changes in the structure of the cardiovascular system in Wistar rats without affecting blood pressure (Brožíčková and Otáhal 2013). The inhibition of nNOS by 7-NI did not change cerebral blood flow in rats (Brožíčková and Otáhal 2013). Huang et al. (2002) confirmed that shear stress activated endothelial nNOS-derived NO release in the coronary arteries of eNOS knockout mice to compensate for eNOS absence-derived NO decrease. According to our study, treatment with 7-NI did not cause a significant change in BP or HR. These data are in agreement with previous studies, where treatment with 7-NI caused no significant change in blood pressure (Cacanyiova et al. 2009, Kristek et al. 2015). nNOS inhibitors mostly inhibit the stimulation of renin secretion (Paliege et al. 2004, Kristek et al. 2015). Angiotensin II induces blood pressure increase while having strong proliferative and hypertrophic effects on the cardiovascular system (Mehta and Griendling 2007). Furthermore, BP were lower in the 7-NI+PTZ group when we compared them with the PTZ group. Valerian is known to be a sedative and it interacts with GABA receptors, which produce the calming effect. Valerian may cause a slight reduction in blood pressure and HR (Sharma et al. 2010). VAL treatment alone did not change BP and HR in our study. BP did decrease in the VAL+PTZ group, but HR remained unchanged. Interestingly, we did not observe a significant change between VAL+7-NI+PTZ group and PTZ group.

Prolonged or recurrent seizures may induce physiological changes in the heart (Auzmendi et al. 2018). Cardiac arrhythmias with any combination of heart failure, hypoxia, atrial fibrillation or bradycardia can result in ventricular tachycardia (Brotherstone et al. 2010). Hypoxia and ischemia-associated seizures are possible causes of corrected QT prolongation, cardiac arrhythmia and sudden death (Massey et al. 2014). Vagal activation results in the stimulation of cardiac muscarinic acetylcholine receptors (Bonaz et al. 2013). In our model, prolonged QTc was observed in the PTZ group and ECG changes might be caused by parasympathetic activation. Changes in ECG examinations, atrial conduction and ventricular repolarization processes were observed after PTZ-induced seizures, while QT intervals and corrected QT increased after amygdaloidal kindled seizures (Goodman et al. 1990). Autonomic conditions affect the sinus node, which in turn affects HR. QT duration is an HR-dependent parameter. A prolonged QT interval is a sensitive marker and a potential death marker for ventricular arrhythmias (Okin et al. 2000). Long QT syndrome has been linked to seizures and/or sudden death, and long QT may affect perturbations in potassium channels and induce recurrent seizures in long QT syndrome patients (Johnson et al. 2009). Clinical findings have reported prolonged QTc after seizures (Brotherstone et al. 2010), while other studies report prolonged QT intervals (Shah et al. 2005, MacCormick et al. 2009). In our study, QTc was prolonged in kindled rats and 7-NI treatment ameliorated QTc values. Inhibition of L-type Ca^{2+} channels and stimulation of Ca^{2+} release from the SR by NO result in different effects on myocardial contraction (Barouch 2002). nNOS-derived NO stimulates cardiac SR to release Ca^{2+} (Umar and van der Laarse 2010). 7-NI treatment prevents Ca^{2+} release from SR, and this may cause changes in the threshold of excitability. According

to our study, 7-NI treatment reduced prolonged QTc probably by suppressing NO releasing. Also, QTc increased in the VAL group, while the QTc of VAL+PTZ group was similar to that of the PTZ group. This increment may be related with the perturbative effect of valerian on the heart.

To elucidate the effect of VAL and 7-NI against ROS generation, plasma level of SOD antioxidant enzyme was determined. Kola et al. (2018) reported that oxidative enzymes markers were increased while antioxidative stress enzymes (including SOD) were reduced in cortex of PTZ kindling model and SOD activity also decreased in brain tissue in PTZ- kindling mice model (Faghihi and Mohammadi 2017). Kim et al. (2015) reported that Valeriana radix extract (100 mg/kg for five days) didn't affect SOD level of plasma in stressed mice. Another study reported that *Valeriana officinalis* caused a reduction in increased SOD mRNA level by rotenone induced toxicity in Drosophila melanagoster (Sudati et al. 2013). 7-NI (25 mg/kg) increased malondialdehyde levels when reduced total SOD level in traumatic brain injury rat model (Dong et al. 2017). The studies are controversial and there haven't been many references to explain the relationship between plasma SOD level and 7-NI and VAL in kindling seizure model. In our study, all treatments caused in decrement plasma SOD activity in control animals, and only VAL treatment to the kindling animals increased the SOD activity levels. Reduced in SOD activity may be related the dose and time of drugs administration.

In conclusion, QTc prolongation may be used as a predictor for recurrent seizures and epileptogenesis. 7-NI and VAL have no effects on BP and HR variables under normal conditions. Both 7-NI and VAL treatments ameliorated the seizure stage and frequency of seizures, while 7-NI treatment resulted in a reduction of increased HR, BP and prolonged QTc in PTZ-kindled rats. The results of 7-NI treatment on BP, HR and QTc may be related with Ca^{2+} release and the renin-angiotensin system. These effects of 7-NI on seizures and cardiac function parameters suggest that 7-NI can be used as an anticonvulsant drug agent in epileptic patients with cardiac dysfunctions. We conclude that 7-NI and Valerian extract exhibit different effects on seizures. We also found that separate 7-NI and VAL administration is much more effective than applying both valerian extract and 7-NI to the epileptic rats.

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