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Lifesaving effect of lidocaine on *Odontobuthos Doriae* scorpion envenomation in mice.

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ABSTRACT

Odontobuthos doriae, a native scorpion in southern tropical parts of Iran, can cause serious health threats and wide ranges of pharmacological disturbances. α -toxins in its venom cause prolongation of Na^+ channels activity. In this study, reversing effects of lidocaine, as a Na^+ -channel blocker, was studied on mice following exposure to venom. Lidocaine (up to 500 mg/kg) and *O. doriae* crude venom (up to 12 $\mu\text{g}/\text{mice}$) was used in a 14-day acute toxicity test, to yield LD_{50} s of 110mg/kg and 10 $\mu\text{g}/\text{mice}$, respectively. Afterward, different sub-acute amounts of lidocaine (25%, 50% and 80% of LD_{50}) were used in the presence of venom (80%, 100% and 120% of LD_{50}). Our results show 80% LD_{50} of lidocaine, and not higher concentrations, could cause 50% reduction in lethality rate induced by *O. doriae* venom at LD_{50} concentration, showing the Na^+ -channel function in this event. Reducing the amount of lidocaine to safer doses show no significant effect in this aspect. Finally, lidocaine (80% LD_{50}) can partially decrease the *O. doriae* venom mortality. However, due to some other systemic dangerous lidocaine adverse effects, it is doubtful that it can be a relevant life-saving agent in this case. Further to lidocaine failure in reversing the complete venom toxicity, it would be explained that high Na^+ current induced by venom might prevent lidocaine effect at above doses, while higher concentrations also can cause lidocaine toxicity, which restricts our further investigation. Preferably, it would be suggested to use other medical approaches and medication to save the envenomed victim's life.

Keywords: Lidocaine, *Odontobuthus doriae*, Scorpion, Venoms, Sodium Channel.

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INTRODUCTION

Poisonous and venomous animals are the noteworthy cause of mortality and morbidity in the world.¹ Scorpions, with about 1500 species as a large group are spread out all over the world²⁻⁴ as well as Iran.⁵ Among them *Odontobuthus doriae* (*O. doriae*) is one of the most dangerous scorpions available in southern and central tropical parts of Iran.^{3,6} Their poisonous glands contain different compounds such as enzymes, bio-active molecules, ions, free amino acids, nucleotids, lipids, mucoproteins and other organic and inorganic compounds.^{7,8} Envenomation by *O. doriae* scorpion might lead to wide range of clinical disturbances such as local pain and inflammation at the site of sting, necrosis, cardiovascular disorders, muscle paralysis and death especially in children.^{6,9,10} So far no medication is available to control the scorpion envenomation symptoms and save life. Although anti-venoms remain the most routine treatment for management of scorpion envenoming, due to hypersensitivity reactions and systemic anaphylaxis caused by polyvalent available serums their use is subject to controversies¹¹ that their usage might be even more deadly than scorpion envenoming. Further, their usage is also associated with time interval following envenoming.

Scorpion venoms mostly exert their effects through selective ion channels.¹² α -toxins from scorpion venom slow the inactivation site of Na^+ channels, leading to longer action potential, increased intracellular Na^+ ion and some changes in the kinetics of sodium entry to the cells.¹³ Thus, excitable cell membranes are the reliable target for the neurotoxins isolated from scorpion venoms. ODI, a fraction isolated from the *O. doriae* venom, could affect on Na^+ -gated voltage channels.⁶ Therefore, Na^+ channel blockers seem to be an interesting candidate to prevent envenomation symptoms caused by scorpion sting.

Lidocaine, a local anesthetic, along with its antiarrhythmic effect¹⁴, exerts its function through blockage of Na^+ channels.^{15, 16} Accordingly, it has been used in the treatment of cardiac disturbances caused by *Tityus serrulatus* scorpion venom.¹⁷ The aim of the present study was to investigate lidocaine effectiveness against the lethality induced by *O. doriae* envenomation.

MATERIAL AND METHOD

Materials

Male swiss albino mice (Pasteur Institute of Iran) were housed every five animals per each cage. They were kept under controlled conditions of temperature ($25\pm 2^\circ\text{C}$), air conditioning, 12-hour light/dark environment and changing the bedding of the cages every other day. Protocol of the study was approved by ethics committee of Shahid Beheshti University of Medical Sciences,

Tehran, Iran. 200mg of freeze-dried *O. doriae* crude venom (from Razi Institute, Iran) and also lidocaine (from Caspian Co., Iran) were dissolved in normal saline when needed to use and respected doses were injected to 16-hours fasted mice, aged 6 weeks old with mean weight of 20 ± 2 g.

Methods

In order to evaluate the effect of lidocaine on reversing the symptoms of *O. doriae* envenomation, LD₅₀ of lidocaine and scorpion toxin were calculated using single dose sub acute toxicity test.(18) Then lidocaine was used at its non-lethal effective doses along with the venom at LD₅₀ dose in the experiments. Furthermore, the effect of lidocaine on higher doses of scorpion toxin was also studied, as described below:

Measuring the LD₅₀ of *O. doriae* venom

20 mice in 4 groups received 6, 8, 10 and 12 µg/mice of intra peritoneal (I.P.) venom, while the control group received equivolume of i.p. normal saline.

Measuring the LD₅₀ of lidocaine

100 mice in 10 groups received 0.5, 5, 50, 100, 110, 130, 200 and 500 mg/kg (I.P.) lidocaine and the 10th group received I.P. normal saline as control. The mice were observed at 2, 4 and 6 hours after injection and then daily check-up was carried out for 14 days. The total lethality was calculated by the end of day 14.

Calculating non-lethal dose of lidocaine

80% of the calculated LD₅₀ of lidocaine was used, based on the following formula as a non-lethal dose:

%of lidocaine LD₅₀ with non-lethal effects= $100 \times (\text{LD}_{50} \text{ of lidocaine} \div \text{maximum nonlethal dose})$

The effect of lidocaine on lethal dose of *O. doriae* scorpion venom

To evaluate the effect of interval time between venom and lidocaine injection, along with the effect of different sites of injection, a two-step study was designed.

35 mice in 7 groups received the treatments as below;

1. Scorpion toxin: 10 µg/mice
2. Lidocaine: 90 mg/kg
3. Lidocaine: 90 mg/kg+ scorpion venom: 10 µg/mice, simultaneously, at the same side of body
4. Lidocaine: 90 mg/kg+ scorpion venom: 10 µg/mice, simultaneously, on either sides of body
5. Lidocaine: 90 mg/kg, 2 min after scorpion venom: 10 µg/mice
6. Lidocaine: 90 mg/kg, 5 min after scorpion venom: 10 µg/mice
7. Normal saline as a control

40 mice in 4 groups received the treatments as below;

1. Scorpion venom: 10 µg/mice
2. Lidocaine: 90 mg/kg
3. Lidocaine: 90 mg/kg, 5 min after scorpion venom: 10 µg/mice
4. Normal saline as a control

Effects of sub acute doses of lidocaine

Since lidocaine, even in non-lethal doses, causes various side effects such as heartbeat, lethargy, and tremor, the dose was reduced to diminish the undesired effects while keeping the therapeutic effects. 30 mice in 3 groups received the treatments as below;

1. Scorpion venom: 10 µg/mice + lidocaine: 90 mg/kg (80% LD₅₀ of lidocaine)
2. Scorpion venom: 10 µg/mice + lidocaine: 55 mg/kg (50% LD₅₀ of lidocaine)
3. Scorpion venom: 10 µg/mice + lidocaine: 30 mg/kg (25% LD₅₀ of lidocaine)

Effect of lidocaine on different doses of the venom

Scorpion venom at doses higher than LD₅₀, along with lidocaine was injected to 60 mice in 6 groups as below;

1. Scorpion venom; 8 µg/mice
2. Scorpion venom; 8 µg/mice and lidocaine: 90 mg/kg
3. Scorpion venom; 10 µg/mice
4. Scorpion venom; 10 µg/mice and lidocaine: 90 mg/kg
5. Scorpion venom; 12 µg/mice
6. Scorpion venom; 12 µg/mice and lidocaine: 90 mg/kg

Statistical analysis

Statistical analysis for calculation of LD₅₀ for each compound using Probit analysis, and the comparisons using logistic regression were performed using SPSS[®] Software, version 21.

RESULTS AND DISCUSSION

In order to calculate the LD₅₀ of lidocaine with the least possible animal sacrifice, four doses of 0.5, 5, 50 and 500 mg/kg of lidocaine were injected to mice. Based on the results of each step, further doses were applied and the final LD₅₀ was calculated to be 110 mg/kg, using SPSS[®] software (figure 1). The same method was applied to determine the LD₅₀ of *O. doriae* venom, resulting in LD₅₀ of 10 µg/mice (figure 2).

In order to apply the dose of lidocaine with no lethality, 80% of the calculated LD₅₀ (90 mg/kg) was applied in the experiments.

According to our data, the best antagonizing effect of lidocaine is anticipated when it is injected exactly after injection of the venom (figure 3). In addition, since the site of injection of lidocaine (injection at the same side of venom injection or the other side of the body) had no statistically significant effect on the final lethality (figure 3), all the lidocaine treatments were injected on the opposite side of the venom injection site, to reduce animals' suffering from pain.

The results of our study showed that injection of *O. doriae* venom (10 µg/mice) could lead to 60% lethality. This mortality was reduced by 50% when lidocaine (90 mg/ml) was injected (fig. 4), showing the effectiveness of lidocaine in the presence of the venom. However, it is worth mentioning that although lidocaine at this dose has no significant lethality and can effectively reduce the mortality caused by the venom, some non-specific adverse effects of lidocaine, including lethargy, tachycardia, and drowsiness are still observable in mice. Thus, other doses of lidocaine (50% and 25% of LD₅₀ dose) were also studied. Although 80% of lidocaine LD₅₀ (90 mg/kg) showed to be effective, 50% of lidocaine LD₅₀ (55 mg/kg) was not as effective as 90 mg/kg of lidocaine (40% lethality) and 25% of lidocaine LD₅₀ (30 mg/kg) had no effect on lethality induced by venom (figure 5). However, based on statistical analysis, protection effect of various doses of lidocaine against lethality induced by venom was not significantly different.

Furthermore, the effect of lidocaine at less or more doses of venom (80% and 120% of venom LD₅₀) was investigated. The results of this test showed that this dose of lidocaine (90 mg/kg) can successfully control the lethality caused by 8 µg/mice of the venom (as expected), it could only partially control the lethality of 12 µg/mice of venom (40% lethality was observed) (figure 6). These results indicate that high amounts of venom are too powerful than lidocaine can ameliorate the lethality effectively.

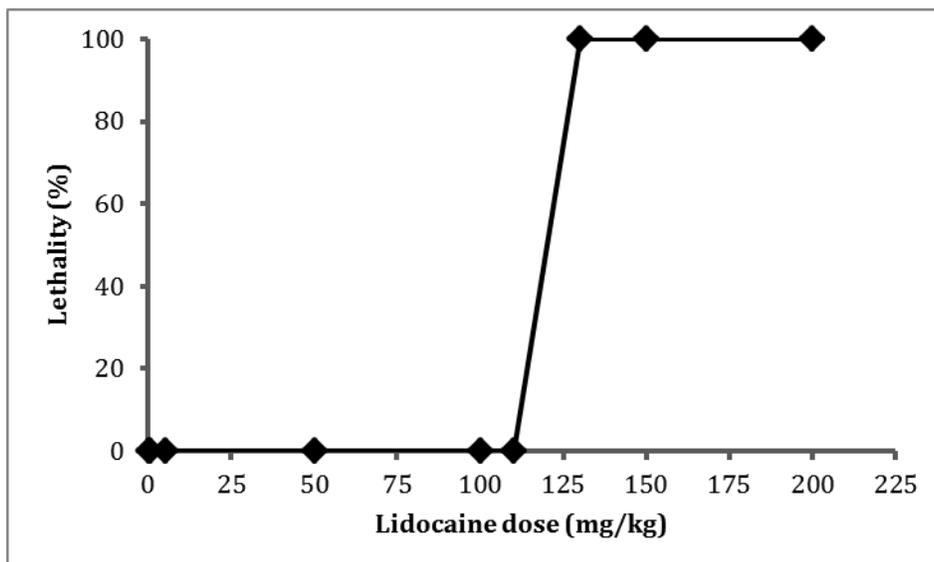


Figure 1: Lethality (%) of understudy mice vs. lidocaine dose (mg/kg) is illustrated. The lethal dose for 50% of the understudy population was calculated using SPSS software ($LD_{50}=110$ mg/kg).

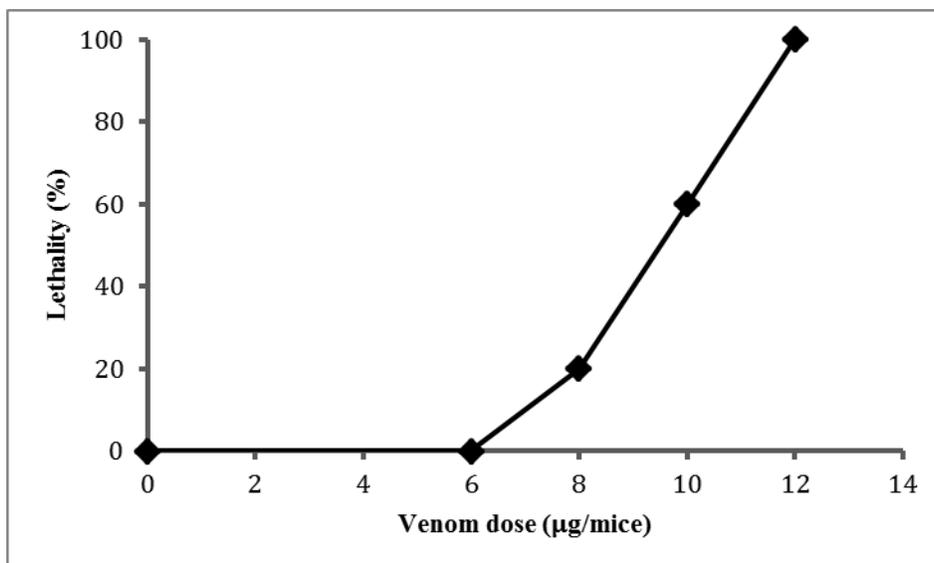


Figure 2: Lethality (%) of understudy mice vs. *O. doriae* venom dose (μ g/mice) is illustrated. The lethal dose for 50% of the understudy population was calculated using SPSS software ($LD_{50}=10$ μ g/mice).

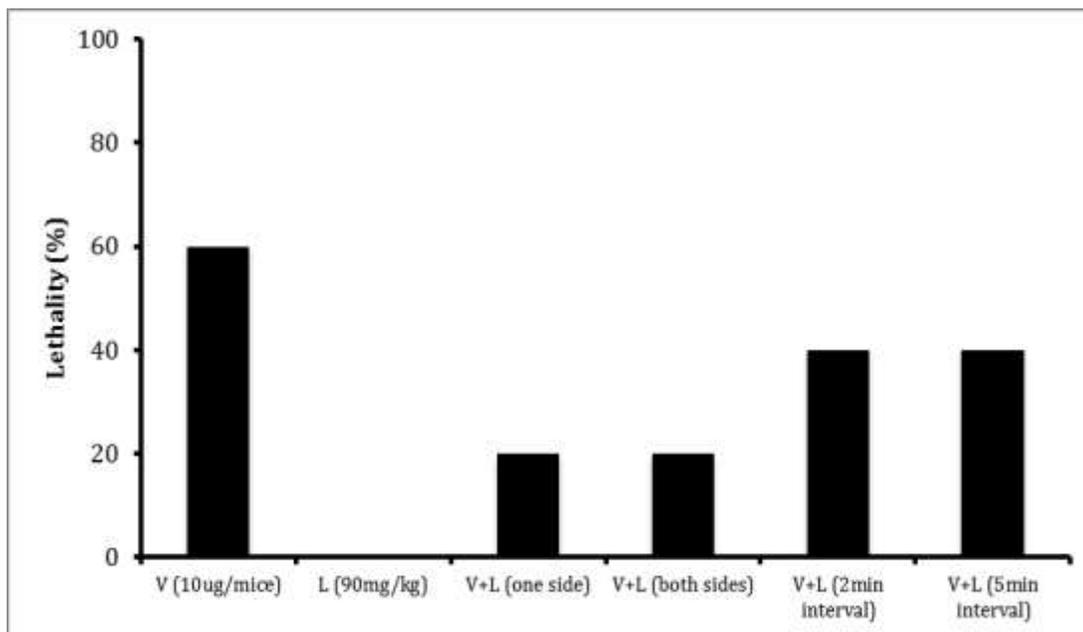


Figure 3: The effect of time interval injection between lidocaine (L) at the dose of 90 mg/kg and *O. doriae* venom (V) at the dose of 10 μ g/mice, and the effect of site of injection (injection of lidocaine at the same site of venom injection, or injection of lidocaine on the other side of the body) on the lethality.

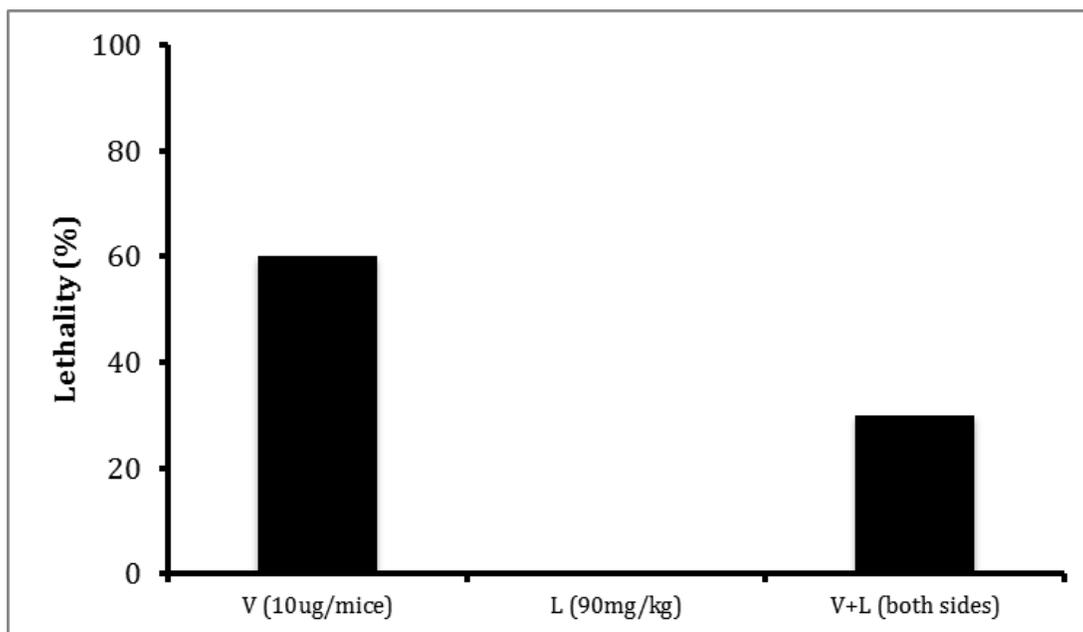


Figure 4: Effect of lidocaine (L) at the dose of 90 mg/kg on the lethality induced by *O. doriae* venom (V) at the dose of 10 μ g/mice, when injected alone or simultaneously on both sides of mice body.

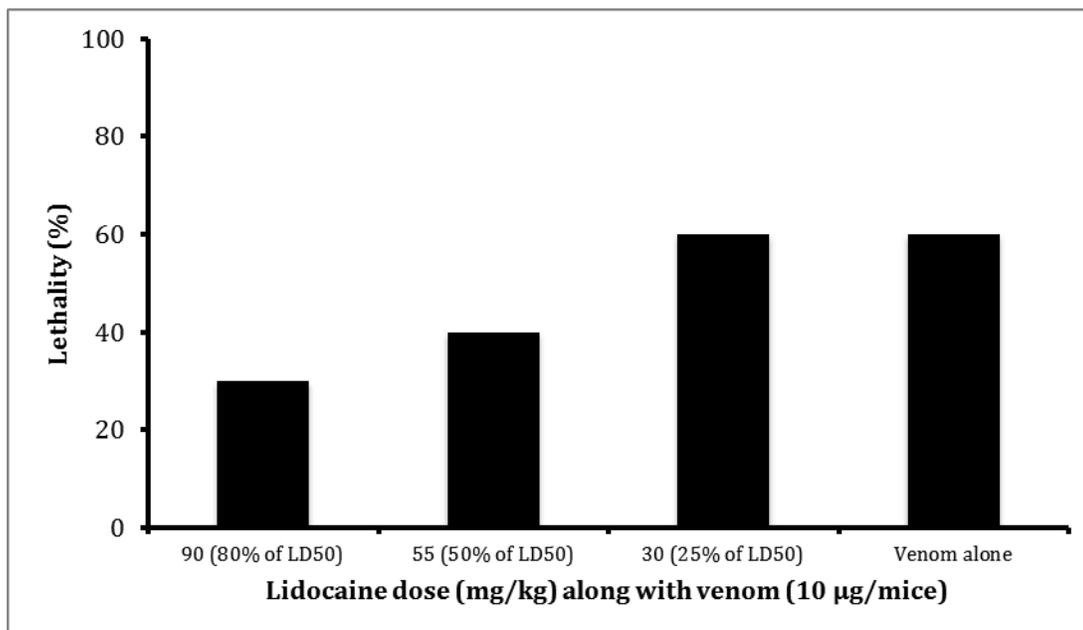


Figure 5: Effect of safer doses of lidocaine (50% of LD₅₀ (55 mg/kg) and 25% of LD₅₀ (30 mg/kg)), in comparison with 80% of lidocaine LD₅₀ (90 mg/kg), on the lethality induced by *O. doriae* venom (10 µg/mice).

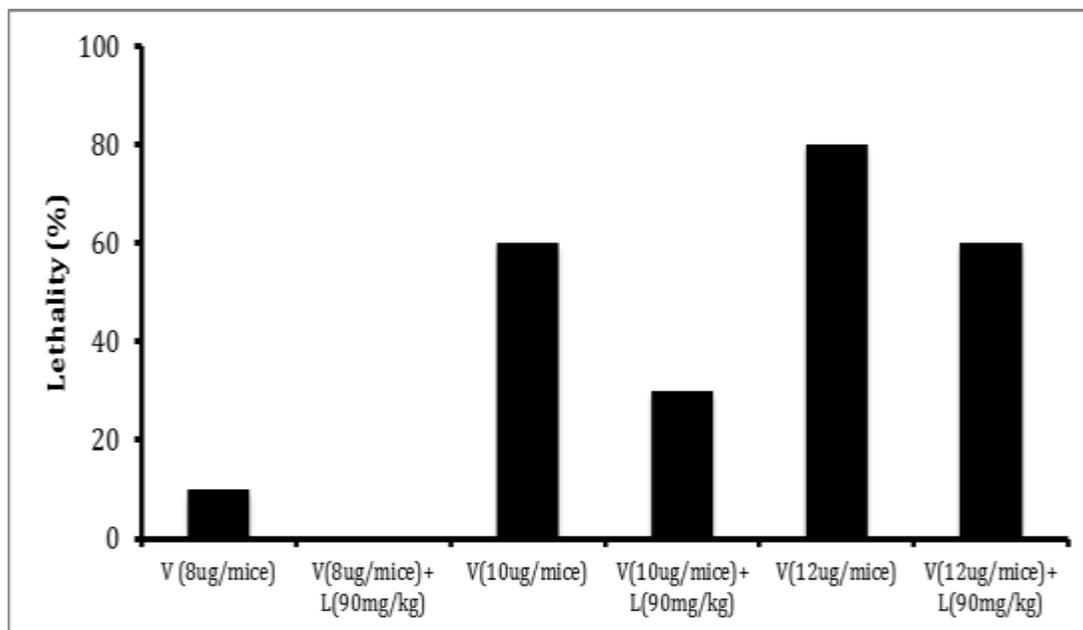


Figure 6: Effect of lidocaine (L) at the dose of 90 mg/kg on 8, 10 and 12µg/mice of *O. doriae* venom (V) induced lethality.

DISCUSSION:

Many of biological toxins modify action potential generation in excitable cell membranes through voltage sensitive sodium channels.^{19, 20} These channels have a fundamental function in generation and propagation of action potentials²¹, keeping the electrical excitability of excitable cells.²² Local

anesthetics had been shown to have a different binding site on the Na⁺ channel compared to the scorpion toxin binding-site²³, and thus might be considered a good treatment for envenomation.

Lidocaine is a member of local anesthetics, a group of chemicals that can block such action potential.²⁴ This medication might block sodium current in a state-dependent manner²⁵ and increase the steady-state outward current of the ion and decrease inward current.^{26,27}

Lidocaine can prevent the uterine contractions induced by scorpion *Tityus serrulatus* venom²⁸ and had been shown to be a beneficial treatment for arrhythmias and hypertension induced by *Buthinae* scorpion envenomation²⁹, and hence has been indicated to control the arrhythmias induced by scorpion envenoming.^{30,31} It was also suggested to be the treatment of choice, as a pain reliever in adults stung with scorpion.³²

O. doriae scorpion sting is a major health concerns in central and southern tropical parts of Iran. α -toxins isolated from its venom activate Na⁺ ion channels.³³ Formerly, Fatani *et al.* could successfully use lignocaine, another local anesthetic agent, to ameliorate the toxic effects of *Leiurus quinquestriatus* envenomation on chick biventer cervicis, guinea pig ileum, and rat vas deferens preparations.³⁴ It was also reported that lidocaine could reverse the effects of *Centruroides limpidus* scorpion venom at LD₅₀ concentration. It could increase the LD₅₀ of crude *Centruroides limpidus* venom and significantly ameliorate the intra-alveolar hemorrhage, myocardial edema and brain congestion.³⁵ Thus, based on literature, lidocaine can effectively improve the toxic effects of scorpion venom, however, the main concern of the present study was to investigate the effect of lidocaine on the lethality induced by *O. doriae* scorpion venom.

According to our results, lidocaine LD₅₀, 110 mg/kg, and *O. doriae* scorpion crude venom LD₅₀ was obtained 10 μ g/mice. Further, 80% LD₅₀ of lidocaine (90mg/kg was examined as a non-lethal dose of lidocaine in our investigation. Lidocaine 90 mg/kg exposure to 10 μ g/mice venom administered-mice, decreased the venom lethality rates by 50%, showing the performance of Na⁺ channel in this event, although this decrease was not statistically significant. In fact, other symptoms of envenoming, such as restlessness, screams and repetitive jumps were diminished with lower doses of lidocaine. Increasing lidocaine concentration would, on the other hand, lead to other side effects. It seems that high Na⁺ current induced by venom may prevent lidocaine, at above doses, however high concentrations can cause lidocaine toxicity, which restricts our further studies.

Venom at high concentrations can hurdle the effect of lidocaine on controlling lethality induced by the venom, suggesting the requirement of higher doses of lidocaine, which due to side effects such as tachycardia and lethargy, it is doubtful that it can be a relevant life saving agent in this case

specially while there are some other medical approaches and medication available more safer to use for this propose.

CONCLUSION:

Further to lidocaine failure in reversing the complete venom toxicity, it would be explained that high Na⁺ current induced by venom might prevent lidocaine effect at above doses, while higher concentrations also can cause lidocaine toxicity, which restricts our further investigation. Thus, lidocaine does not seem to be a good medication candidate for treatment of this envenomation.

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