Partial Purification and Characterization of Antimicrobial Effects from Snake (*Echis carinatus*), Scorpion (*Mesosobuthus eupeus*) and Bee (*Apis mellifera*) venoms

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**ABSTRACT**

**Background:** Some venoms and their isolated compounds have been shown to have antibacterial properties. Snake, scorpion and bee venoms are a complex mixture of proteins such as phospholipase and melittin, which have an effect on bacterial growth inhibition. This study aimed to investigate of antibacterial effect of three different venoms against selected bacterial strains.

**Materials & Methods:** Crude venoms obtained from snake (*Echis carinatus*), scorpion (*Mesosobuthus eupeus*) and bee (*Apis mellifera*) were selected. The crude venoms from these species was purified by using gel filtration chromatography and the molecular weights of the compounds in these venoms estimated by using SDS-PAGE. The approximate lethal dose values of venoms were determined. Antibacterial activity of venoms against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* were evaluated. Venoms and its isolated fractions and standard antibiotic were tested by using the disc diffusion method.

**Results:** *E. carinatus* crude venom and fraction 2 were effective against *S. aureus* and *E. coli*. *M. eupeus* crude venom and fraction 1 and 4 were effective against *B. subtilis*. *A. mellifera* crude venom demonstrated antibacterial activity against *E. coli*, *S. aureus* and Fraction 3 of this venom has an inhibition effect for *E. coli* and *S. aureus*.

**Conclusion:** Snake, scorpion and bee venoms inhibit the growth and survival of bacterial strains and that these venoms can be used as a complementary antimicrobial agent against pathogenic bacteria.

**Keywords:** *Echis carinatus*, *Mesosobuthus eupeus*, *Apis mellifera*, antibacterial activity, chromatography, LD₅₀

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Introduction

Infectious diseases have increased in recent years. These diseases are caused by pathogens such as bacteria, viruses, etc. Due to the lack of useful and effective drugs for the treatment of infectious diseases, they have spread worldwide (1). Antibiotic treatment is currently used for bacterial infections. But nowadays, it has been found that the effectiveness of many antibiotics has diminished due to their overuse. This phenomenon is known as antibiotic resistance. Antibiotic resistance is a serious public health problem and this resistance is increasing in today’s world. In 2014, the World Health Organization described drug resistance to antibiotics as a “major global threat.” (2).

As antimicrobial resistance is spreading throughout the world, the discovery of new substances is mandatory to fight against it. This will cause researchers to conduct more studies on various natural resources in order to discover newer and more effective antibiotics (3). In fact, the vast diversity of bioactive molecules in nature has long inspired scientists in their search for potential therapeutic agents (4).

More recently, there has been a resurgence in the use of antimicrobial peptides due to the decrease in the efficiency of common treatments. Antimicrobial peptides are able to target a broad spectrum of microbes with little resistance and can have a synergistic effect with antibiotics. Animal venom is thus a particularly promising source in this search for new antimicrobial compounds. Many antimicrobial peptides from the venom have shown high efficacy in vitro and in vivo, but challenges to overcome their host toxicity (5, 6), hemolytic activity (7-11), as well as the bioavailability and stability of these peptides are still present.

With more than 100,000 venomous animals, naturally occurring antimicrobial agents present in venomous species, thus hold promises for the development of novel therapeutic agents. Currently, only few antimicrobial agents are present on the market for tropical use (12).

Venoms from some animals, including snakes, scorpions, spiders, bees, etc. can be interesting and powerful alternatives to antibiotics (13). In venoms of these animals, bioactive proteins and peptides are found that have various useful pharmacological properties and are stored in large quantities (14).

One of the important reasons for the effectiveness or ineffectiveness of different animal venoms on various bacterial species is their mechanisms of action on the bacterial cell envelope. Bacterial cytoplasmic membrane is the primary target of the antibacterial peptides in venoms. Antibacterial peptides form channels in the bacterial cell membrane or disrupt phospholipid bilayers of bacterial membrane, thereby influencing its numerous functions that are necessary for the survival of the bacteria and thus cause bacterial cell death. As pointed out in some of these studies, some of the differences in the effects of these peptides stem from the differences in bacterial cell envelopes. Since these envelopes in Gram-positive bacteria consist of fewer layers compared to Gram-negative bacteria, antibacterial peptides must be more powerful in order to affect Gram-negative bacteria (15).

Today, many studies have been conducted using molecular methods on a variety of antimicrobial peptides and how they work (16-18).

The findings indicate that some of peptides present in the venom of these animals have antimicrobial properties and prevent the growth of pathogens. Antimicrobial peptides have been shown to inhibit the growth of many resistant pathogens. However, many antibiotics do not show such efficacy (19). They can be useful and valuable as pharmacological tools in drug research, as potential drug design templates, and as therapeutic agents (20).

Here we have characterized and investigated antimicrobial effect from Snake (Echis carinatus), scorpion (Mesobuthus epues) and bee (Apis mellifera) venoms.

Materials and Methods

Bacterial Strains

Four clinical isolates of bacteria, including Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli O157:H7 (ATCC 25923), Bacillus subtilis (ATCC: 6633) were purchased from the China Center of Type Culture Collection (CCTCC).

Experimental Animals

Animal studies were performed in compliance with the regulations of Razi vaccine and serum research institute (RVSRI), and with generally accepted guidelines governing such works. For this aim, normal male mice, weighing between 25 and 30 g were injected with venoms and investigated.

Other Materials and Equipment

The following Equipment and materials were used for laboratory work; Millipore filter (Biofil 0.45μm, China), Centrifuge (Herme Z513K, Germany), Freeze dryer (Christ alpha 1-4 Isc, Germany), UV spectrophotometer (UNICO SQ2800, USA), Electric heater (Electrothermo M105, England), Electrophoresis and protein markers (Bio-Rad, USA), Incubator (Mermert, Germany), Sephadex G-50 (Pharmacia, Sweden), and the Standard antibiotic gentamicin (Liofilchem S.r.1, Italy). Other reagents and chemicals were of analytical grade from Merck and Fluka.
**Venoms Preparation**

Lyophilized crude venom of *Echis carinatus* (Lot No. V8250) and *Apis mellifera* (Lot No. V3375) were purchased (Sigma Aldrich, Germany). Crude venom of *Mesobuthus eupeus* scorpion was obtained by the electrical stimulation at the end of the tail (128 Hz, 20 V). After lyophilization, it was stored at -20°C. The freeze-dried venoms were dissolved in distilled water or a suitable buffer and then venom solutions were centrifuged at 12000 g for 4 mins and the supernatant was collected.

**Venoms Purification**

Lyophilized crude venoms (200 mg) were dissolved in 4 mL of 0.1 M ammonium acetate buffer (pH 8.6) and the insoluble material was removed by centrifugation (12000 g, 4 min) and filtration. Supernatant was applied to a column of sephadex G-50 (2.5×150 cm) equilibrated with 0.1 M ammonium acetate buffer (pH 8.6). The elution was carried out with the same buffer at a flow rate of 60 mL/h. Volumes of 10 mL were collected and each fraction was identified by UV spectrophotometer (280 nm), mixed and lyophilized (5).

**Venoms protein concentration**

Protein content in the crude venom was determined by Lowry (6) and Kjeldahl method with some modifications (21). Fourteen mL of distilled water and 2 mL of trichloroacetic acid (100% w/v) was added to 5 mL of protein solution. The solution was mixed and allowed to stand for 5 min and then centrifuged for 10 min at 2000 g. The supernatant liquid was discarded and the residue was dissolved in 0.5 mL of 10 N NaOH. Dissolved residue was adjusted to 25 mL with distilled water. About 0.9 g of K2SO4, 0.1 g of CuSO4 and then 10 mL of dissolved residue was added to Kjeldahl flask. Then 7 mL of sulfuric acid 98% and 1 mL of H2O2 30% was added. The flask was heated to about 80°C for 48 h using an electric heater. After 48 h, the digested solution was cooled and about 10 mL of distilled water was added to the flask. The contents of the flask were poured into the Kjeldahl machine. Subsequently, 25-30 mL of NaOH 10 N was added and distillation was started. The reagent (10 mL of boric acid 4% with four drops of methyl red and methylene blue mixture) was placed under the outlet of the Kjeldahl distiller. One hundred mL of the output solution was titrated with 0.01 N sulfuric acid. The following formula was used to calculate the protein content (mg/mL).

\[ \text{Protein volume} = \frac{\text{Titration volume} \times 0.14 \times 25 \times 6.25}{50} \]

**Venoms Electrophoresis**

Electrophoresis was performed to check the protein profile of the venoms and its quality. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli with modifications (22). 12% Separating gels and 4% stacking gels were used (the total volume was enough for two gels with 0.75 mm spacer). Glass plates were cleaned with ethanol and casting stand was assembled by following manufacturer’s instructions (BioRad, USA). 12% separating gels was prepared by adding the solution (3 mL, 30% Acrylamide/Bis; 1.9 mL 1.5 M Tris-HCl (pH 8.8); 75 μL 10% SDS; 2.5 mL ddH2O; 37.5 μL 10% ammonium persulfate; 10 μL TEMED). The solution mixed well and quickly transferred by using pipette to the casting chamber between the glass plates and filled up to about 1.5 cm below the top of longer plate. A layer of distilled ddH2O was added over the top of the resolving gel to prevent polymerization. After 20 min, once the separating gel has polymerized, the ddH2O layer was removed by using filter paper. 4% separating gel was prepared by adding the solution (1 mL, 30% Acrylamide/Bis; 1.9 mL 0.5 M Tris-HCl (pH 6.8); 75 μL 10% SDS; 4.5 mL ddH2O; 37.5 μL 10% ammonium persulfate; 10 μL TEMED). The solution mixed well and quickly transferred by using a pipette until the space was full, and then the comb was inserted to the top of the spacers. After 20 min, once the separating gel has polymerized, the comb carefully removed. The gel cassette from the casting stand was removed and the clamping frame was put into the electrophoresis tank (the short plate was placed on the inside). Running buffer 1X (3 g Tris-Base; 14.4 g Glycine; 1 g SDS; 990 mL ddH2O) was poured into the electrophoresis tank. 25 μL of the sample buffer (10 mL 0.5 M Tris-HCl (pH 6.8); 5 mL glycerol; 1 g SDS, 2 mL 2-mercaptoethanol; 1 mL 1% bromphenol blue; 1 mL ddH2O) was added to 75 μL protein samples (0.5-1.5 mg/mL). Protein samples heated for 10 min in a boiling water bath and then centrifuged at 13000 rpm for 60 S. 15 μL of each protein samples were then loaded onto each gel well as well as load 10 μL of protein MW marker and electrophoresis was carried out at a constant voltage (100 V, 2 h). The gel was fixed with 30% methanol and 10% acetic acid, until the background became clear.

**Lethal Dose of Venoms**

Lethal dose (LD50) of venoms, which is equivalent to death in 50% of mice within 24 h after venom injection, was determined by Spearman-Karber Finney methods (23). One mg/mL stock of each venom was prepared and centrifuged for 5 min at 10000 g and then filtered. Thirty NIH mice (25-30 g) were selected. The mice were maintained at an appropriate temperature (23±2°C) with free access to water and food. Five groups of mice were treated with different doses of venom (1, 1.5, 2, 2.5, 3 mg/kg) and normal saline was injected into a control group.

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Antibacterial Effects of Venoms

Lyophilized crude venoms (25, 50, 75, 100 μg) and its fractions dissolved in 1 mL of 50 mM Tris-HCl buffer (pH 7.4), were filtered using 0.22 μm syringe filter and stored at 4°C for the assay. Antibacterial susceptibility tests were performed by the disc diffusion assays (19). First, to prepare the disks, different concentrations of venoms were poured onto blank discs and it took 3 h for the discs to dry completely. Then plates containing Mueller Hinton Agar were cultured with a swab soaked in a bacterial suspension equivalent to half a McFarland and the prepared discs were placed on the surface of the plate. The plates were incubated for 24 h at 37°C. Then, the effects of different concentrations of venoms on bacteria were investigated. In this experiment, gentamicin antibiotic disc (10 μg/disk) was used as a positive control.

Statistical Analysis

Means and standard deviations of the zone inhibition data were collected and calculated using Microsoft Excel. Student’s t-test was used to determine statistical significance. P-value<0.05 was considered statistically significant.

Results

The protein content was improved in the antibacterial active crude venoms of *E. carinatus* (1.7 mg/mL), *M. eupeus* (1.2 mg/mL), *A. mellifera* (0.4 mg/mL), respectively. Electrophoresis revealed that the range of *E. carinatus* proteins was distributed in the light, medium and heavy molecular weight; However, most *M. eupeus* venom proteins were in the average molecular weight range and proteins of *A. mellifera* venom was in the light molecular range (Figure 1).

Chromatography showed that *E. carinatus* and *A. mellifera* had three fractions (Figures 2 and 4) and *M. eupeus* had four fractions (Figure 3).

The numbers of dead mice within 24 h were recorded for each venom. After the registration of deaths, the LD<sub>50</sub> of each venom was determined, which are as follows:

**E. carinatus > M. eupeus > A. mellifera**

| Venom         | LD<sub>50</sub> μg/mouse |
|---------------|--------------------------|
| *E. carinatus*| 11.1                     |
| *M. eupeus*   | 46                       |
| *A. mellifera*| 177.8                    |

Figure 1. SDS-PAGE profile of *E. carinatus*, *M. eupeus* and *A. mellifera* crude venoms

Figure 2. *E. carinatus* venom chromatogram

Figure 3. *M. eupeus* venom chromatogram

Figure 4. *A. mellifera* venom chromatogram
E. carinatus crude venom and its fractions has shown no antibacterial effects against P. aeruginosa and B. subtilis. In contrast, the crude venom was effective against S. aureus (50, 75 and 100 µg/mL) and E. coli (75 and 100 µg/mL). In addition, F2 was effective against S. aureus and E. coli. However, standard antibiotics were shown to be effective against all bacteria (Figure 5 and Table 1). The examination showed that the antibacterial activity of F2 against E. coli was more significant than it was for the gentamicin at 10 µg/mL (Figure 5).
**M. eupeus** crude venom and its fractions has shown no antibacterial effects against *P. aeruginosa, S. aureus* and *E. coli*. In contrast, **M. eupeus** crude venom was effective against *B. subtilis* (50, 75 and 100 µg/mL). In addition, F₁ and F₄ was effective against *B. subtilis*. However, the standard antibiotic gentamicin was effective against those bacteria (Figure 6 and Table 1).

The examination showed that the antibacterial activity of **M. eupeus** crude venom (at the 75 and 100 µg/mL concentrations) and F₁ and F₄ against *B. subtilis* were more significant than it was for the standard antibiotic gentamicin at 10 µg/mL (Figure 6).

![Figure 6. Antibacterial effect of M. eupeus crude venoms and fractions against B. subtilis](image)

**A. mellifera** venom demonstrated antibacterial activity against *Escherichia coli, S. aureus* at all four concentrations. Moreover, with increasing **A. mellifera** venom concentration, the inhibition zone increased. Fraction 3 (F₃) of **A. mellifera** crude venom have inhibition effect for *E. coli* and *S. aureus* (Figure 7).

The venom concentration of 100 µg/mL showed the highest inhibition zone against *E. coli* (29.06±1.31 mm) and *S. aureus* (17.51±1.07 mm) (Table 1). **A. mellifera** crude venom and F₃ had a more significant antibacterial activity against *E. coli* in the medium than it did against either of the three other strains of bacteria.

The present examination also showed that the antibacterial activity of **A. mellifera** crude venom at the 100 µg/mL concentrations against *E. coli* and *S. aureus* was more significant than it was for the standard antibiotic gentamicin at 10 µg/mL (Figure 7). However, the antibacterial activities of **A. mellifera** crude venom against *S. aureus* (25 and 75 µg/mL) and *E. coli* (25 and 50 µg/mL) were less than the effect of the standard antibiotic gentamicin at 10 µg/mL (Figure 7). Furthermore, **A. mellifera** crude venom and its fractions was found to have no observable effect on the *P. aeruginosa* and *B. subtilis* whereas the standard antibiotic gentamicin was effective against those bacteria (Table 1).

![Figure 7. Antibacterial effect of A. mellifera crude venoms and fractions against E. coli and S. aureus](image)
Table 1. Values of growth inhibition zones due to crude venom and their fractions of *E. carinatus*, *M. eupeus* and *A. mellifera* for bacterial strains

| Venoms          | Bacterial strains | S. aureus (μg/mL) | P. aeruginosa (μg/mL) | E. coli (μg/mL) | B. subtilis (μg/mL) |
|-----------------|-------------------|-------------------|-----------------------|----------------|---------------------|
|                 |                   |                   |                       |                |                     |
| *Echis carinatus* |                   |                   |                       |                |                     |
| Crude venom     |                   | 25                |                       | 25             | 25                 |
|                 |                   | 50                | 12.92 ± 1.03          | 25             | 25                 |
|                 |                   | 75                | 15.21 ± 1.56          | 21.08 ± 0.13   | 21.08 ± 0.13       |
|                 |                   | 100               | 22.65 ± 2.1           | 23.91 ± 0.13   | 23.91 ± 0.13       |
| Gentamicin      |                   | 25.65 ± 1.64      | 21.08 ± 1.08          | 22.11 ± 0.22   | 19.21 ± 0.97       |
| Fractions       |                   |                   |                       |                |                     |
| F1              |                   |                   |                       |                |                     |
| F2              |                   | 17.2 ± 1.42       |                       |                |                     |
| F3              |                   |                   |                       |                |                     |
| Gentamicin      |                   | 16.1 ± 0.98       | 19.11 ± 1.06          | 22.54 ± 1.04   | 18.83 ± 1.17       |
| *Mesobuthus eupeus* |               |                   |                       |                |                     |
| Crude venom     |                   | 25                |                       | 25             | 25                 |
|                 |                   | 50                |                       | 29.20 ± 0.88   | 29.20 ± 0.88       |
|                 |                   | 75                |                       | 29.41 ± 0.81   | 29.41 ± 0.81       |
|                 |                   | 100               |                       | 30.83 ± 1.04   | 30.83 ± 1.04       |
| Gentamicin      |                   | 17.03 ± 0.81      | 11 ± 0.44             | 20 ± 0.22      | 4.22 ± 0.83        |
| Fractions       |                   |                   |                       |                |                     |
| F1              |                   |                   |                       |                |                     |
| F2              |                   |                   |                       |                |                     |
| F3              |                   |                   |                       |                |                     |
| F4              |                   |                   |                       |                |                     |
| Gentamicin      |                   | 20.4 ± 0.19       | 31.1 ± 1.07           | 16.5 ± 0.39    | 25.8 ± 1.02        |
| *Apis mellifera* |                   |                   |                       |                |                     |
| Crude venom     |                   | 25                | 8.1 ± 0.76            | 20.06 ± 1.50   | 20.06 ± 1.50       |
|                 |                   | 50                | 13.2 ± 0.87           | 25.30 ± 1.02   | 25.30 ± 1.02       |
|                 |                   | 75                | 9.2 ± 0.98            | 28.31 ± 0.67   | 28.31 ± 0.67       |
|                 |                   | 100               | 17.51 ± 1.07          | 29.06 ± 1.31   | 29.06 ± 1.31       |
| Gentamicin      |                   | 11.3 ± 0.47       | 19.17 ± 0.21          | 26.11 ± 0.74   | 9.19 ± 0.08        |
| Fractions       |                   |                   |                       |                |                     |
| F1              |                   |                   |                       |                |                     |
| F2              |                   |                   |                       |                |                     |
| F3              |                   | 8.91 ± 1.12       |                       | 29.51 ± 1.41   | 29.51 ± 1.41       |
| Gentamicin      |                   | 13.7 ± 0.89       | 16.5 ± 0.47           | 24.36 ± 1.09   | 9.8 ± 0.29         |

**Discussion**

The venom of animals such as scorpions, snakes and bees can prevent the growth of microorganisms. For example, scorpions spray their venom on own bodies to prevent the growth of bacteria and fungi (24). In general, the venom of these animals is a good source for pharmaceutical compounds (25). Although some of venoms and compounds derived from them have antibacterial properties, most of them have not been studied for such activities.

The present study provides evidence that venoms of different animals have antibacterial effects against bacteria. Among the venoms examined, those from snake (*E. carinatus*), scorpion (*M. eupeus*) and bee (*A. mellifera*) showed strong antimicrobial effects. These venoms exhibited greater zones of inhibition, equivalent to that shown by the gentamicin.

With respect to venoms in current study, *A. mellifera* was the most effective against the two microorganisms, among the venoms examined. All
concentrations of *A. mellifera* venom showed strong antimicrobial effects against *S. aureus* and *E. coli*. Venoms of *E. carinatus* and *M. eupeus* have got medium effects, presenting only three significant venom concentrations.

Compared with the *M. eupeus* venom, which was more specific against *B. subtilis*, the *A. mellifera* and *E. carinatus* venoms, on the other hand, exhibited a broader spectrum of antibacterial activity.

A strong activity was shown against *B. subtilis* by the *M. eupeus* venom, while venoms of *A. mellifera* (25 and 75 μg/mL) and *E. carinatus* (50 and 75 μg/mL) exhibited only a weaker activity against *S. aureus*. With respect to microorganism susceptibility, The Gram-positive cocci *S. aureus* bacterium appeared to be the most sensitive to venoms. In contrast, the venoms had no effect on *P. aeruginosa*. The results were consistent with Perumal Samy *et al.* (26). Previously, snake venoms were reported to exhibit a strong inhibitory effect against *P. aeruginosa* (27, 28).

The antibacterial effect of the venom derived from scorpions has been demonstrated in various studies. In a study by Zhao *et al.* in 2009 on antibacterial effect of the Chinese scorpion *Isometrus maculates*, it was found that the venom of this scorpion had an inhibitory effect on the growth of Gram-positive bacteria but no effect on Gram-negative bacteria *P. aeruginosa* and *E. coli*. A comparison of the results of this study with those of the present one suggests that the mechanism of the antibacterial effect of the Chinese scorpion venom is similar to that of the Iranian scorpion venom (29).

In this study, *M. eupeus* crude venom was effective against *B. subtilis* showed zone of inhibition 30 mm. These results are similar to spider venom activity reported by Benli and Yigit (30) and Ahmad *et al.* (31).

In 2009, in a study on different sources of animal venoms, various species of snakes including *Bothrops jararaca, Bothrops moojeni* and *Bothrops jararacussu* were studied for their antibacterial effects. *B. jararaca* had the strongest antibacterial effect on *S. aureus* (32).

Jami al ahmadi reported that, *E. carinatus* venom has not a wide spectrum antibacterial effect against the mentioned bacteria, although a significant activity against *S. aureus* in comparison with the standard antibiotics has been observed (33).

A study of antibacterial effect of honey bee venom on several bacteria species in 2016 reported that it had a considerable inhibitory effect on *P. aeruginosa* and *E. coli* (19). In the present study, bee venom was exhibited a strong inhibitory effect against *E. coli*. While in the present study, no antimicrobial activity of bee venom against *P. aeruginosa* was observed.

**Conclusion**

Finally, it should be noted that comparison of the antibacterial effects of the venoms with gentamicin suggested that these venoms had stronger inhibitory effects. However, this comparison was a laboratory estimation carried out without a formulation. Therefore, the results obtained in the preliminary stage seem to be valuable. The results of this study indicate that the use of these venoms, especially associated proteins and peptides has promising results. Further research in the future on other bacterial species and on animal models may allow industrial introduction of these venoms into the pharmaceutical market and help solve the drug resistance problem when treating bacterial infections.

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**Conflict of Interest**

Authors declared no conflict of interests.
خالص‌سازی نسبي و تعيين خصوصيات اثراً ضد ميكروبي زهر مار (Apis mellifera) و زنبور عسل (Mesosobuthus epues)

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چکیده

زیست‌ها و اهداف: نشان داده‌شده است که در بخش‌های زهر و ترکیبات جاذبه‌ای از آنها در خصوصیت ضد باکتری‌ای هستند.

در این مطالعه با هدف بررسی اثر ضدباکتری‌ای ماده سنگین وارد می‌شود و در برابر باکتری‌ها و قاتل، به کار می‌رود.

در این مطالعه به منظور ارزیابی اثر ضدباکتری‌ای ماده سنگین، در برابر باکتری‌ها و قاتل مورد بررسی قرار گرفت.

نتایج: قاتل و فراکسیون دوم در برابر باکتری‌های اپسیلرون، بالسانس، سدوموناس انتروزیوس و اشیرینی‌ها مورد استفاده قرار گرفتند.

کلید واژه‌ها: زهر خام، E. carinatus، Apis mellifera، ماده سنگین، SDS-PAGE، فراکسیون، باکتری‌ها، میکروب‌ها، باکتری‌های مجاور، باکتری‌های انتروپیک، باکتری‌های اپسیلرونی، فراکسیون‌ها، باکتری‌های اسپورسی، باکتری‌های شیمی‌افزایندگان، آنتی‌بیوتیک‌ها، باکتری‌های انترکریا، باکتری‌های نانو، باکتری‌های نانو، باکتری‌های نانو، باکتری‌های نانو، باکتری‌های نانو، باکتری‌های نانو، باکتری‌های نانو، باکتری‌های نانو

مقدمه

در حال حاضر درمان آنتی‌بیوتیک‌های عفونت‌های باکتری‌ای استفاده می‌شود، اما مهارت مشخص شده است که اثر بیماری از موجب می‌شود. بنابراین، آنتی‌بیوتیک‌های که در این مطالعه توسط باکتری‌های میکروژیک متأثربه هستند، به‌عنوان یک مدل علمی مشخص شده است.

انثی‌پوئزین‌ها با دلیل مصرف بیش از حد آنها که باهوشی بافت و کالبدی وارد به پایه‌های بافت و کالبدی می‌گردد. این دست نیست به مقاومت آنتی‌پوئزینی معرفی است. مقاومت آنتی‌پوئزینی که در نیم‌ایام از آنها به‌طور مداوم و به‌طور مشکل جدی بهداشت عمومی محور است و در سال 2014 سازمان بهداشت جهانی مقاومت داری در برابر آنتی‌پوئزین‌ها را یک "تهدید بزرگ جهانی" توصیف کرده است.

بیماری‌های عفونتی که در اثر عوامل بیماری‌زا مانند باکتری‌های، ویروس‌ها و غیره ایجاد می‌شوند، در حالی‌که این افراد با پیش‌بینی یافته است. به دلیل کمیتی درآموزی مقدی و مؤثر برای درمان بیماری‌های عفونی، این بیماری‌ها در سراسر جهان در حال گسترش می‌باشند (1).

در حال حاضر درمان آنتی‌بیوتیک‌های عفونت‌های باکتری‌ای استفاده می‌شود، اما مهارت مشخص شده است که اثر بیماری از موجب می‌شود. بنابراین، آنتی‌بیوتیک‌های که در این مطالعه توسط باکتری‌های میکروژیک متأثربه هستند، به‌عنوان یک مدل علمی مشخص شده است.

مجله میکروب شناسی پزشکی ایران

Majallah-i mikrub/shināsī-i pizishkī-i Īrān

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روش پژوهش:
سومهای باکتری، جویانات آزمایشگاهی و سایر مواد (Echis carinatus (Lot No. V8250) و Mesobuthus euperus (Lot No. V3375) در حجم ۴ میلیلیتر به عنوان خوراک نظامی برای حیوانات آزمایشگاهی استفاده می‌شود.

آماده‌سازی زهرها:
زهرا خام یوپیلیزه نشده (سیگما، آلما) خربدار (Echis carinatus) و آمپیلیفیر (Mesobuthus euperus) در حجم ۴ میلیلیتر به عنوان خوراک نظامی برای حیوانات آزمایشگاهی استفاده می‌شود.

ماده‌های مواد مکمل:
۴ میلیلیتر به عنوان خوراک نظامی برای حیوانات آزمایشگاهی استفاده می‌شود.

نرفته‌های مشخصات زهرها:

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به تعداد رسیده، بوده، تزریق گردید. شستشو با همان بی‌افر با سرعت 60 انجام گرفت. فراکسیون با حجمی در حداکثر 10 چریان میلیول (mL/h) جمع آوری شد و توسط اسپکتروفوتومتر (280 nm) تنظیم و P-value < 0.05 استفاده شد.

**ماتریس پروتئین مربوط به زهره**

میزان پروتئینی موجود در زهرهای خام با استفاده از روش (Kjeldahl) و Lowry سنجی شد.

**الکتروفوروز زهرها**

برای بررسی روفاکسیون پروتئینی زهرها و کیفیت آن از الکتروفوروز استفاده شد. الکتروفوروز (SDS-PAGE) با واحد کیلیلا (Laemmli) میزان پروتئینی موجود در زهرهای خام با استفاده از روش کنترل نسجی 7 (SL) لیزر گزینی کرده شد.

**اثرات ضد باکتریایی زهرها**

از هر یک از زهرهای پروتئینی بر روی باکتری نوری دیسک گرفته شد. نتایج نشان داد که در این آزمایش سه از زهرهای پروتئینی به صورت ساده و نازک، میزان پروتئین موجود در زهرهای خام با استفاده از روش کنترل نسجی 7 (SL) لیزر گزینی کرده شد.
در برای استافیلوکوکوس اورئوس و اشیریشیا کلونی موثر بود. با این حال، آنتیبیوتیک استاندارد در برای همه باکتری‌ها اثر مهاری داشت (شکل 5 و جدول 1). آزمایشات نشان داد که فعالیت ضد باکتری‌ای فراکسیون F۲ در برای استافیلوکوکوس اورئوس و اشیریشیا کلونی جنتامایسین با ۱۰۰ μg/mL غلظت و فراکسیون F۲ در برای E. carinatus و اشیریشیا کلونی جنتامایسین با ۱۰۰ μg/mL غلظت (معنی‌دار) است (شکل 5).
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شکل 5. اثر ضد باکتریایی زهر خام و فراکسیون‌های آن در برابر استافیل kokوس اورئوس و اشریشیا گلی E. carinatus (Fractions) و E. carinatus (Crude venom)

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شکل 6. میزان میزان ناحیه مهاری زهر خام M. eupeus و فراکسیون‌های آن در مقابل باکتری های S. aureus (µg/mL 100 و 75 و 50 و 25) در شکل و جدول 1 نشان داد که فعالیت ضد باکتریایی زهر خام E. carinatus (Fractions) و اثر ضد باکتریایی فراکسیون‌های آن به ویژه در مقابل باکتریای E. carinatus (Crude venom) در مقایسه با استاندارد جنتامایسین بهتر بود (شکل 5 و جدول 1). در مقایسه با استاندارد جنتامایسین بهتر بود (شکل 5 و جدول 1).
اثر آنتیبیوتیک استاندارد جناتامایسین با غلظت ۱۰۰ μg/mL (شکل ۷) علاوه بر این، سم خام A. mellifera با غلظت ۱۰ μg/mL؛ استافیلوکوکوس اورونوس از آنتیبیوتیک استاندارد جناتامایسین با غلظت ۱۰۰ μg/mL، با این حال، فعالیت ضد جناتامایسین در برابر آن باکتری‌ها مؤثر بود (جدول ۱). علاوه بر این، سم خام A. mellifera و فراکسیون‌های آن هیچ اثر قابل توجهی بر روی سودوموناس آئروژینوزا و باسیلوس سوبتیلیس نداشتند. در حالی که آنتیبیوتیک استاندارد جناتامایسین با غلظت ۱۰ μg/mL، بر روی استافیلوکوکوس اورونوس، اشریشیا کُلی و باسیلوس سوبتیلیس مشترک و تأثیر معنی‌داری را داشت (جدول ۱). در جدول ۱، مقادیر نواحی مهار رشد ناشی از زهر خام و فراکسیون‌های E. carinatus، M. eupeus و A. mellifera برای سویه‌های باکتریایی استافیلوکوکوس اورونوس، اشریشیا کُلی و باسیلوس سوبتیلیس با غلظت ۱۰۰ μg/mL به ثبت شده است.

جدول ۱. مقادیر نواحی مهار رشد ناشی از زهر خام و فراکسیون‌های E. carinatus، M. eupeus و A. mellifera برای سویه‌های باکتریایی استافیلوکوکوس اورونوس، اشریشیا کُلی و باسیلوس سوبتیلیس با غلظت ۱۰۰ μg/mL به ثبت شده است.
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| باسیلوس سوئتیلس | اشریشیا کلی | استافیلوكوس اورلوس | سودوموناس ابرزینوزا | جنتامایسین |
|------------------|-------------|-------------------|------------------|-------------|
| 75/8 ± 1/012 | 75/3 ± 5/018 | 75/2 ± 7/018 | 75/1 ± 9/018 | 75/0 ± 1/018 |
| 75/3 ± 5/018 | 75/2 ± 7/018 | 75/1 ± 9/018 | 75/0 ± 1/018 | 75/0 ± 1/018 |
| 75/2 ± 7/018 | 75/1 ± 9/018 | 75/0 ± 1/018 | 75/0 ± 1/018 | 75/0 ± 1/018 |
| 75/1 ± 9/018 | 75/0 ± 1/018 | 75/0 ± 1/018 | 75/0 ± 1/018 | 75/0 ± 1/018 |

در شکل 5، اثر ضد باکتریایی زهر خام A. mellifera و فراکسیون‌های آن در برابر اشریشیا کلی و استافیلوكوس اورلوس نشان داده شده است.
بحث

زهر حیواناتی مانند عقرب و تندیسی و زنبورها می‌توانند از رشد میکرو‌گانمی‌ها چلونگیکی کنند. عقربها برای چلونگیکی از لنز باکتری‌ها قارچ‌های آری بروی دن دخود اسیری می‌کنند. این اثر از لحاظ میکروبی، مطالعه آزمایشگاهی مهاری ضد ترین مورد، با استفاده از آزمایشگاهی همکارانش در مورد گونه (Jararca) از زهر آنها نشان داد که زهر اثر قابل ملاحظه‌ای مقابل باکتری‌های مورد بررسی، (M. eupeus) (E. carinatus) (E. carinatus) (A. mellifera) بیشتر از آنها قابل توجه داشت. زهر گونه (E. carinatus) با طوی خاص در در برخی مطالعات سه گونه سپتی‌لاس استفاده گردیده است. در برخی مطالعات مورد بررسی، زهر اثر قابل بررسی در مطالعات حاضر و آنها اختصاص داده نشان داد که زهر اثر قابل ملاحظه‌ای مقابل باکتری‌های مورد بررسی در بررسی، (M. eupeus) (E. carinatus) (A. mellifera) اثر مهاری داشت و از این نتایج نشان داد که زهر اثر قابل توجه داشت.

نتیجه‌گیری

در نهایت، لازم به ذکر است که مقایسه اثرات ضد باکتری‌ای زهرها با جنگل‌پیم‌های می‌تواند در زمره‌های مورد بررسی باعث می‌شود که اثرات ضد باکتری‌ای زهرها را در مطالعه‌ها با اهمیت بیشتری در نظر بگیریم.

در نهایت، لازم به ذکر است که مقایسه اثرات ضد باکتری‌ای زهرها با جنگل‌پیم‌های می‌تواند در زمره‌های مورد بررسی باعث می‌شود که اثرات ضد باکتری‌ای زهرها را در مطالعه‌ها با اهمیت بیشتری در نظر بگیریم.

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پژوهش در منافع این مقاله یئوهشی مستقل است که بدون حمایت مالی سازمان انجام شده است. در انجام مطالعه حاضر، نویسندگان هیچ گونه تضاد منافعی نداشتند.

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 referencia

1. Aslam B, Wang W, Arshad MI, Khurshid M, Muzamml M, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018;11:1645-58. [DOI:10.2147/IDR.S173867] [PMID] [PMCID]

2. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-18. [DOI:10.1179/2047773215Y.0000000030] [PMID] [PMCID]

3. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem. 2014;6:25-64. [DOI:10.4137/PMC.S14459] [PMID] [PMCID]

4. Perumal Samy R, Stiles BG, Franco OL, Sethi G, Lim LHK. Animal venoms as antimicrobial agents. Biochem Pharmacol. 2017;134:127-38. [DOI:10.1016/j.bcp.2017.03.005] [PMID]

5. Babaie M, Zolfagharian H, Salmanizadeh H, Mirakabadi AZ, Alizadeh H. Isolation and partial purification of anticoagulant fractions from the venom of the Iranian snake Echis carinatus. Acta Biochim Pol. 2013;60(1):17-20. [DOI:10.18388/abp.2013.1945] [PMID]

6. Babaie M, Zolfagharian H, Zolfaghami M, Jamili S. Biochemical, hematological effects and complications of Pseudosynanceia Melanostigma Envenoming. J Pharmacopuncture. 2019;22(3):140-6.

7. Babaie M, Salmanizadeh H, Zolfagharian H, Alizadeh H. Properties of biological and biochemical effects of the Iranian saw-scaled viper (Echis carinatus) venom. Bratsil Lek Listy. 2014;115(7):434-8. [DOI:10.4149/BLL_2014_085] [PMID]

8. Babaie M, Salamizadeh H, Zolfagharin H. Blood coagulation induced by Iranian saw-scaled viper (Echis Carinatus) venom: Identification, purification and characterization of a prothrombin activator. Iran J Basic Med Sci. 2013;16(11):1145-50.

9. Salamizadeh H, Babaie M, Zolfagharin H. In vivo evaluation of homeostatic effects of Echis carinatus snake venom in Iran. J Venom Anim Toxins incl Trop Dis. 2013;19(3):21-9. [DOI:10.1186/1678-9199-19-3] [PMID] [PMCID]

10. Babaie M, Ghaempanah A. Evaluation of hemolytic activity and biochemical properties of Apis mellifera bee venom on NIH laboratory mice. J Neyshabur Univ Med Sci. 2020; 8(3):25-34.

11. Babaie M. Snakes venom proteins and coagulopathy caused by snakebite. J Birjand Univ Med Sci. 2020;27(4):1-11.

12. Yacoub T, Rima M, Karam M, Sabatier JM, Fajloun Z. Antimicrobials from venomous animals: An overview. Molecules. 2020;25(2402):1-19. [DOI:10.3390/molecules25102402] [PMID] [PMCID]

13. Almeida JR, Palacios ALV, Patiño RSP, Mendes B, Teixeira CAS, Gomes P3, et al. Harnessing snake venom phospholipases A2 to novel approaches for overcoming antibiotic resistance. Drug Dev Res. 2019;80(1):68-85. [DOI:10.1002/ddr.21456] [PMID]

14. Liu G, Yang F, Li F, Li Z, Lang Y, Shen B, et al. Therapeutic potential of a scorpion venom-derived antimicrobial peptide and its homologs against antibiotic-resistant gram-positive bacteria. Front Microbiol. 2018;9:1-14. [DOI:10.3389/fmicb.2018.01159] [PMID] [PMCID]

15. Malanovic N, Lohner K. Antimicrobial peptides targeting gram-positive bacteria. Pharmaceuticals (Basel). 2016;9(3):1-33. [DOI:10.3390/ph9030059] [PMID] [PMCID]

16. Chen CH, Lu TK. Development and challenges of antimicrobial peptides for therapeutic applications. Antibiotics. 2020;9(24):1-20. [DOI:10.3390/antibiotics9010024] [PMID] [PMCID]

17. Bahar AA, Ren D. Antimicrobial peptides. Pharmaceuticals (Basel). 2013;6(12):1543-75. [DOI:10.3390/ph6121543] [PMID] [PMCID]

18. Jenssen H, Hamill P, Hancock REW. Peptide antimicrobial agents. Clin Microbiol Rev. 2006;19(3):491-511. [DOI:10.1128/CMR.00056-05] [PMID] [PMCID]

19. Zolfagharin H, Mohajeri M, Babaie M. Bee venom (Apis Mellifera) an effective potential alternative to gentamicin for specific bacteria strains. J
Pharmacopuncture. 2016;19(3): 225-30. [DOI:10.3831/KPI.2016.19.023] [PMID] [PMCID]

20. Chen Na, Xu S, Zhang Y, Wang F. Animal protein toxins: origins and therapeutic applications. Biophys Rep. 2018;4(5):233-42. [DOI:10.1007/s41048-018-0067-x] [PMID] [PMCID]

21. Maehre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen IJ. Protein determination-method matters. Foods. 2018;7(1):1-11. [DOI:10.3390/foods7010005] [PMID] [PMCID]

22. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227:680-5. [DOI:10.1038/227680a0] [PMID]

23. Zolfagharian H, Mohajeri M, Babaie M. Honey bee venom (Apis mellifera) contains anticoagulation factors and increases the blood-clotting time. J Pharmacopuncture. 2015; 18(4):7-11. [DOI:10.3831/KPI.2015.18.031] [PMID] [PMCID]

24. Ahmed U, Mujaddad-ur-Rehman M, Khalid N, Fawad SA, Fatima A. Antibacterial activity of the venom of Heterometrus xanthopus. Indian J Pharmacol. 2012;44(4):509-11. [DOI:10.4103/0253-7613.99332] [PMID] [PMCID]

25. Munawar A, Ali SA, Akrem A, Betzel C. Snake venom peptides: Tools of biodiscovery. Toxins (Basel). 2018;10(11):1-29. [DOI:10.3390/toxins10110474] [PMID] [PMCID]

26. Perumal Samy R, Gopalakrishnakone P, Thwin MM, Chow TK, Bow H, Yap EH, Thong TW. Antibacterial activity of snake, scorpion and bee venoms: a comparison with purified venom phospholipase A2 enzymes. J Appl Microbiol. 2007;102(3):650-9. [DOI:10.1111/j.1365-2672.2006.03161.x] [PMID]

27. Talebimehrdar M, Madani R, Hajhosseini R, Moradi bidhendi M. Antibacterial activity of isolated immunodominant proteins of Naja Naja (Oxiana) Venom. Iran J Pharm Res. 2017;16(1):297-305.

28. Al-Asmari AK, Abbasmanthiri R, Abdo Osman NM, Siddiqui Y, Al-Bannah FA, Al-Rawi AM, et al. Assessment of the antimicrobial activity of few Saudi Arabian snake venoms. Open Microbiol J. 2015;9:18-25. [DOI:10.2174/1874285801509010018] [PMID] [PMCID]

29. Zhao Z, Ma Y, Dai C, Zhao R, Li S, Wu Y, Cao Z, et al. Imcroporin, a new cationic antimicrobial peptide from the venom of the scorpion Isometrus maculates. Antimicrob Agents Chemother. 2009;53(8):3472-7. [DOI:10.1128/AAC.01436-08] [PMID] [PMCID]

30. Benli M, Yigit N. Antibacterial activity of venom from funnel web spider Agelena labyrinthica (Araneae agelenidae). J Venom Anim Toxin Incl Trop Dis. 2008;14:641-50. [DOI:10.1590/S1678-91992008000400007]