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VIROCIDAL ACTIVITY OF EGYPTIAN SCORPION VENOMS AGAINST HEPATITIS C VIRUS

Xojimurodov Davronjon Ikramaliyevich
Angren University, Angren City, Tashkent Region Teacher of the Faculty of Medicine Angren University, Angren City,
Tashkent Region Teacher of the Faculty of Medicine
Mirzayev Furqat Farhod o'g'li

Anotation: Hepatitis C virus (HCV) is a major global health problem, causing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Development of well-tolerated regimens with high cure rates and fewer side effects is still much needed. Recently, natural antimicrobial peptides (AMPs) are attracting more attention as biological compounds and can be a good template to develop therapeutic agents, including antiviral agents against a variety of viruses. Various AMPs have been characterized from the venom of different venomous animals including scorpions.

Keywords: Keywords: Hepatitis C virus, HCV, Antiviral activity, Scorpion venom, Scorpio maurus palmatus, Egypt, HCV-Hepatitis C virus, SVR-Sustained virological response, AMPs-Antimicrobial peptides, CC50-50% cytotoxic concentration,IC50 -50% inhibitory concentration.

Hepatitis C virus (HCV) infection is a major global health problem, with estimated more than 170 million infected individuals worldwide. HCV is an enveloped, positive-strand RNA virus that belongs to the *Hepacivirus* genus of the *Flaviviridae* family. HCV infection is the serious cause of chronic hepatitis, hepatic steatosis, liver cirrhosis and eventually hepatocellular carcinoma after a few decades. There is no anti-HCV vaccine available and therapeutic options are still limited. The current standard therapy, which is based on pegylated interferon and ribavirin, is only partially effective, resulting in a sustained virological response (SVR) in about 50% of patients and has considerable side effects. Recently, HCV NS3 protease inhibitors and NS5A inhibitors have been approved for clinical use and SVR rates have improved to reach 70% or higher.

However, these therapies are quite expensive and will probably not be accessible for all patients worldwide. For this reason, the development of new classes of safe and inexpensive antiviral compounds with improved efficacy is still needed for treatment of HCV infections.Recently, natural antimicrobial peptides (AMPs) are attracting more attention as therapeutic agents against a variety of microbes including antibiotics-resistant strains. Most AMPs share certain common features such as being small peptides of 10 to 50 amino acid residues, containing positive charge of 2 to 9 residues and an amphipathic structure. These peptides exhibit a broad spectrum of antiviral and antibacterial activities, with direct or indirect microbicidal activities. AMPs have been isolated from venomous animals including scorpions. Scorpion venoms consist of a cocktail of biologically active peptides that represent a tremendous potential for use in drug design and development.

Most scorpion venom peptides are composed of 20 to 75 amino acid residues while certain proteins, enzymes, consist of 120 to 370 residues. Scorpion venom peptides show a vast array of biochemical activities and pharmacological functions. They can be classified into two classes, i.e., disulfide-bridged and non-disulfide-bridged peptides. AMPs found in scorpion venoms are paid

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more and more attention due to their unique biological activities that can potentially be used as broad-spectrum antiviral agents. Scorpion venom AMPs are positively charged amphipathic peptides and can be divided into three structural categories: (i) cysteine containing peptides with disulfide bridges; (ii) peptides with an amphipathic α-helix but lacking cysteine residues and (iii) peptides rich in certain amino acids such as proline and glycine.The list of Egyptian scorpions currently includes 24 species classified under 13 genera within four different families, Buthidae, Diplocentridae, Euscorpiidae and Scorpionidae. In the present study, we screened crude venoms obtained from five Egyptian scorpion species, *Leiurus quinquestriatus, Androctonus amoreuxi, A. australis, A. bicolor* and *Scorpio maurus palmatus,* for possible anti-HCV activities using an HCV cell culture system. We report here that crude venoms of *S. maurus palmatus,* and *A. australis* to a lesser extent, possess antiviral activities against HCV. To our knowledge, this is the first report describing anti-HCV activities of Egyptian scorpion venoms.

Screening of anti-HCV activities of scorpion venoms.Anti-HCV activities of crude venoms of five Egyptian scorpion species were tested. As shown in Table **1**, *A. australis* and *S. maurus palmatus* showed anti-HCV activities, with IC_{50} being 88.3 ± 5.8 and 6.3 ± 1.6 μg/ml, respectively. Their CC_{50} were >300 and >100 μg/ml, respectively, with their selectivity indexes (SI; CC_{50}/IC_{50}) being >3.4 and >15.8, respectively. Crude venoms of the other three scorpion species did not exhibit significant anti-HCV activities at the concentration of 100 μg/ml. Dose-dependent anti-HCV activity of *S. maurus palmatus* is shown in Figure **1**.

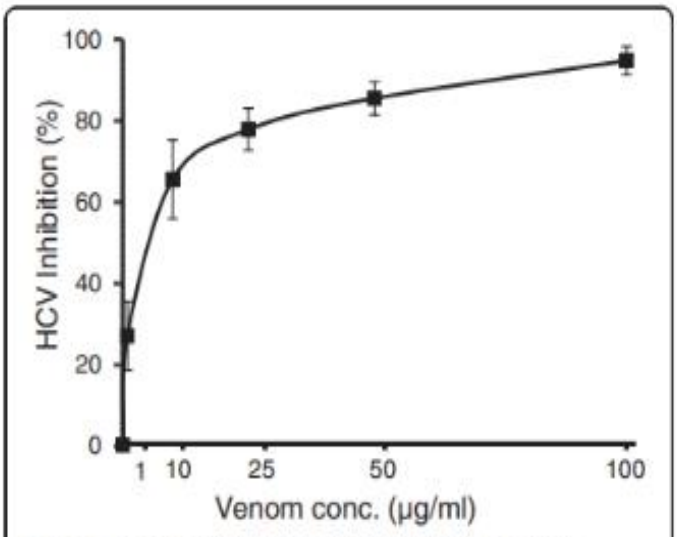

Figure 1 Dose-dependent anti-HCV activity of *S. maurus palmatus* venom. A fixed amount of HCV was mixed with serial dilutions of *S. maurus palmatus* venom and inoculated to Huh7.5 cells at a multiplicity of infection of 0.5 pfu/cell. After virus adsorption, the cells were cultured with the same concentrations of the venom for 46 hr. The culture supernatants were harvested and titrated for virus infectivity. Percent inhibitions of HCV infectivity by the venom at the concentrations of 0.1 to 100 μg/ml are shown. Data represent means ± SEM of the data obtained from two independent experiments.

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Table 1 Antiviral activity (IC_{50}) against HCV, cytotoxicity (CC_{50}) and selectivity index (SI) of crude venoms of five Egyptian scorpion species tested in this study

Species	IC_{50} (µg/ml)[a]	CC_{50} (µg/ml)[a]	SI
Leiurus quinquestriatus	>100	>100	na
Androctonus amoreuxi	>100	>100	na
Androctonus australis	88.3 ± 5.8	>300	>3.4
Androctonus bicolor	>100	>100	na
Scorpio maurus palmatus	6.3 ± 1.6	>100	>15.8

[a]: Data represent means ± SEM of the data obtained from two independent experiments using the J6/JFH1-P47 strain of HCV.
na: Not applicable.

Dose-dependent anti-HCV activity of *S. maurus palmatus* venom. A fixed amount of HCV was mixed with serial dilutions of *S. maurus palmatus* venom and inoculated to Huh7.5 cells at a multiplicity of infection of 0.5 pfu/cell. After virus adsorption, the cells were cultured with the same concentrations of the venom for 46 hr. The culture supernatants were harvested and titrated for virus infectivity. Percent inhibitions of HCV infectivity by the venom at the concentrations of 0.1 to 100 µg/ml are shown. Data represent means ± SEM of the data obtained from two independent experiments.Venomous animals including scorpions have evolved a wide variety of peptide toxins for the purpose of predation and defense. Scorpion venoms are a rich source of natural peptides and have been recognized as potential bioactive peptides. cDNA library sequencing and proteomics profiling analyses have revealed that a single scorpion venom contains more than 100 peptidic components ranging in size from 1 to 9 kDa . An increasing number of studies have shown that scorpion venoms and toxins possess antiviral activities *in vitro* and *in vivo* and are considered as a rich source for developing effective antiviral drugs.In the present study, we screened crude venoms obtained from five Egyptian scorpion species for their possible anti-HCV activities.

We observed that crude venoms of *S. maurus palmatus* and *A. australis* possessed anti-HCV activities, with their IC_{50} values being 6.3 ± 1.6 and 88.3 ± 5.8 µg/ml, respectively (Table **1**). We also demonstrated that *S. maurus palmatus* venom acts directly on HCV particles in culture supernatants to inhibit the viral infectivity, suggesting the inhibition at the entry step, the first step of HCV life cycle. On the other hand, it was unlikely that the venom exerted its antiviral activity inside the cells (Figure **2**B-D).Scorpion venoms contain a wide variety of pharmacologically active peptides and proteins. Some of them possess enzymatic activities, such as metalloproteases, while others are non-enzymatic constituents. It has been reported that the proteolytic activities of those enzymes were almost completely abolished by treatment with a matrix metalloproteinase inhibitor, 1, 10-phenanthroline.

Also, those enzymatic activities are known to be inactivated by heating at 60°C for 20 min. We made use of this information in our study and observed that treatment of the *S. maurus palmatus*

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venom with 1, 10-phenanthroline and/or heating at 60°C for 20 min did not impair its anti-HCV activity . These results suggest that the anti-HCV activity of the *S. maurus palmatus* venom is independent of its proteinase activities.Concerning the molecular mechanism(s) of the anti-HCV activity, there was a possibility that venom peptides induced disruption in virus envelope (composed of lipid bilayer) through making pores in it. If this was the case, the venom might inhibit other envelope viruses than HCV. To test this possibility, we used three different viruses, such as dengue virus type 2, another member of the family *Flaviviridae*, and measles virus and influenza virus that belong to the family *Paramyxoviridae* and *Orthomyxoviridae*, respectively.

The results obtained demonstrated that *S. maurus palmatus* venom exerted strong inhibition on dengue virus and only weak inhibition on measles virus . On the other hand, the same venom did not inhibit but rather enhanced influenza virus infectivity. These results exclude the possibility that *S. maurus palmatus* venom inhibits all the envelope viruses, suggesting that the venom preferentially inhibits HCV and dengue virus, both of which belong to the family *Flaviviridae*, but not other viruses, e.g., influenza virus.*S. maurus palmatus* venom has been reported to contain about 65 compounds, whose molecular masses vary from 413 to 14,009 Da, with a majority ranging between 3 and 5 kDa. A variety of putative bioactive molecules have been identified, such as neurotoxins (NaScTxs and KScTxs), calcines, La1-like peptides, insecticidal toxins and other AMPs.

Some of the scorpion venom peptides showed antiviral activities against certain viruses, such as measles virus, SARS coronavirus, H5N1 influenza virus, hepatitis B virus , herpes simplex virus 1 and human immunodeficiency virus. As for anti-HCV peptides from scorpion venoms, Yan et al. reported that Hp1090 screened from the venomous gland cDNA library of the scorpion *Heterometrus petersii* inhibited HCV infection by targeting the viral membrane, disrupting its structural integrity. Also, Hong et al. identified another anti-HCV peptide Ctry2459 from the venom peptide library of the scorpion *Chaerilus tryznai*. These peptides exerted a virocidal effect on HCV and some other viruses. Consistent with those results, we observed that *S. maurus palmatus* venom inhibited infectivity of HCV particles, suggesting direct virocidal activity of the venom. Interestingly, the antiviral activity of *S. maurus palmatus* venom is likely to be preferentially directed to HCV and dengue virus, both of which are members of the family *Flaviviridae*. Further studies using bioactivity-guided fractionation and purification analyses are needed to identify an active compound(s) responsible for this antiviral activity.

We screened crude venoms obtained from five Egyptian scorpion species for anti-HCV activities and demonstrated that *S. maurus palmatus* venom inhibits HCV infectivity through direct virocidal activity. In addition, this antiviral activity appeared to be independent of proteinase activities of the venom and is directed preferentially against HCV, but not equally against all the enveloped viruses. To our knowledge, this is the first report describing antiviral activities of Egyptian scorpion venoms against HCV, and has opened a new approach towards discovering antiviral compounds derived from scorpion venoms.

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