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Original Article

Inhibitory Effect of *Hemiscorpius lepturus* Scorpion Venom Fractions on Tachyzoites of *Toxoplasma gondii*

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Abstract

Background: The present study determined the effect of the fractions obtained from *Hemiscorpius lepturus* scorpion venom on the tachyzoite of *Toxoplasma gondii*.

Methods: The fractions of dried venom of *He. lepturus* scorpion of Khuzestan Province, southern Iran in 2019 were isolated through gel filtration chromatography, and then tachyzoites were exposed to fractions of venom at different concentrations. Trypan blue counting and MTT were applied to assay tachyzoite viability, and the inhibition of the cellular growth of fractions in Vero cells was evaluated.

Results: The maximum effect on tachyzoite was observed in fraction 5 of venom. To further separate the protein, fraction 5 was used in high-performance liquid chromatography assay to purify its proteins. Based on the results of HPLC of fraction 5, among which the second peak, a peptide with <10 KDa representing a more potent effect in eliminating the tachyzoite of *T. gondii*.

Conclusion: The scorpion venom-purified fractions possess anti-parasitic activity against the tachyzoite of *T. gondii* and can be used in parasite-controlling studies.

Introduction

Toxoplasma gondii is an obligate intracellular parasite with broad dispersal, which is well known due to its medical and veterinary importance. This protozoan is widely used as a model for apicomplexan cell biology. Its main and intermediate hosts are cats and all warm-blooded vertebrates contaminated through swallowing oocyst and tissue cysts (1). Toxoplasmosis does not usually need special treatment; however, it should be treated in patients with immune system deficiency and congenital toxoplasmosis (2). Todays, toxoplasmosis is treated by using pyrimethamine and sulfadiazine (pyr-sulf), despite their toxicity and side effects on the host (3). Due to the limited effect of the common treatments of toxoplasmosis in hosts and the significant side-effects of these drugs, alternative treatments are under study (4). Accordingly, conducting basic and preparatory procedures for attaining a new drug against *T. gondii* which specifically targets the parasite is essential (5).

More than 1500 known scorpion species have been described around the world. The *Hemiscorpius lepturus* scorpion belonging to the Hemscorpionidae family is a particularly significant species in Iran (6). Scorpion venom contains proteins and peptides possessing pharmaceutical and antimicrobial properties, targeting various ion channels and cell membrane components. The *He. lepturus* scorpion venom involves hemolytic, proteolytic, cytotoxic, anticancer, cardiovascular- and neoplastic disease-treating and channel-blocking compounds, which may possess therapeutic effects (7). In recent years, the antimicrobial peptides obtained from venomous animals have attracted the attention of clinical microbiologists. The range of the antimicrobial activity of cationic peptides is notable and includes activities against most Gram-positive and Gram-negative bacteria, fungi, viruses, and parasites. Among protozoans, these peptides are effective against trypanosome and plasmodium (8). Peptide compounds with phospholipase A2 activity from *Pandinus imperator* inhibit the intra-erythrocytes of *Plasmodium falciparum* which causes human malaria (9). Today, special attention has been paid to antimicrobial peptides since these natural antibiotics may overcome the increased resistance against other antibiotics (10).

Thus, the present study aimed to assess the effect of the fractions obtained from the venom of *He. lepturus* scorpion by the chromatographic method challenged with the tachyzoite of *T. gondii* and evaluate their survival at different times and concentrations by MTT and trypan blue counting methods. The peptides of scorpion venom with antibacterial activity could be adopted in preliminary studies for the development of new anti-parasitic drugs.

Methods

Extracting venom from scorpions

Mature *He. lepturus* (gadium) scorpions were collected from Khuzestan Province, southern Iran, in 2019 and then venom was extracted by using electric shock. After being lyophilized, it was stored at -20 °C.

Isolating proteins by using the gel filtration chromatography column

At first, 15 mg of the lyophilized venom of *He. lepturus* was mixed with 15 ml of ammonium acetate buffer (pH 8.5) and homogenized by using a vortex device for 1-2 min. Next, the venom content was centrifuged at 1300 gr for 5 min at 4 °C to remove mucus and excess materials. The supernatant which contained venom proteins was separated from insoluble mucoproteins and passed from a 0.45µm filter. Then, the clear fluid was slowly injected into a Sephadex G-50 column (width × length respectively 2×150 cm) and the column was connected to a tank of 0.2M ammonium ace-

tate buffer (pH 8.5) so that the intended protein would exit from the column. Subsequently, 2.5 ml of venom components were collected from the column by using a fraction collector device at the flow rate of 10 ml/h and the optical absorption of each fraction was read by using a UV-spectrophotometer at 280 nm. After plotting the diagram of optical absorption, the peaks were specified; the fractions having the maximum amount of optical absorption were lyophilized, and the protein concentration of each peak was determined via the Bradford method.

Preparing and maintaining the tachyzoite of T. gondii

Toxoplasma gondii was passed continuously with the rate of 10^6 tachyzoites per milliliter by injection into the peritoneum of BALB/c mice to prepare a high amount of tachyzoite for conducting in vitro experiments. The RH strain of the tachyzoite of *T. gondii* was used in the present study. The attenuation of the RH strain in these mice was very high, and they died in 4-5 d. After 3-4 d, the mice were euthanized and their peritoneal fluid was aspirated to collect the parasites. To prevent the bacterial contamination of the parasitic suspension, penicillin (100 IU/ml) and streptomycin (100 mg/ml) were used and the tachyzoites were kept in the refrigerator.

Exposing tachyzoite to fractions of scorpion venom by gel filtration

The tachyzoites of *T. gondii* were exposed to different concentrations of fractions (100, 80, and 60 µg protein/mg lyophilized fraction) obtained by gel filtration. Parasite viability was assessed by direct counting using a hemocytometer slide and trypan blue dye under the optical microscope at 0, 30, and 120 min.

Assaying the inhibition of the cellular growth of fraction in vitro

Vero cells of kidney fibroblast from African green monkeys (ATCC No. CCL-81) were cultured in RPMI media in 5% CO_2 at 37 ºC. Next, 200 µl of solution containing: Vero cell (cell ×104/well) and RPMI, 10% FBS medium (volume/volume) was added to each well of 96-well plate. Additionally, the fractions of *He. lepturus* with a concentration of 100, 80, and 60 µg were added into the wells, and the cells were placed in a 37 ºC, 5% CO_2 incubator for 24 hours. PBS and FBS 10% were used as the culture media and negative control in this experiment, respectively. Cellular viability in the presence of fractions of venom was determined by MTT assay. The MTT solution was added to the 96-well plates and incubated for 4 h at 37 °C in a 5% CO_2 atmosphere; then, 200 µl of DMSO was added to acquire the formazan crystals, and optical density was evaluated at 570 nm.

Assaying the inhibition of the cellular growth of fractions in tachyzoite

1×10^4 cell/well of Vero cells was added to each 96-well plate containing the RPMI and FBS10% media. After 24 h, 1×10^5 tachyzoite of RH strain *T. gondii* was added to the wells thereafter exposed to different concentrations (100, 80, and 60 µg protein/mg lyophilized fraction) of fractions of *He. lepturus* and incubated for 24 h at 37 °C in 5% CO_2. Pyrimethamine, FBS10%, and PBS were considered as positive and negative controls and culture media, respectively. The absorption of wells was measured at 570 nm. The viabilities of tachyzoite were calculated in this test compared with the control group.

High-performance liquid chromatography (HPLC)

HPLC was conducted for further purifying the peptide from the components with the maximum ability for removing parasites obtained by gel filtration chromatography. The mobile phase included acetonitrile (80%) and 1.0% acetic acid (20%) at the flow rate of 60 min and 0.8 ml/min. The fractions were separated by a C18 column (250 mm×4.6 mm), a

particle size of 5 µm, and a pore size of 100 Å. The appropriate wavelength of UV detector for fractions was considered as 214 nm. The results were obtained as curve and the area under the curve. The attained components were lyophilized at -75 ºC and under the pressure of 1 min bar for 36 h. The sub-fractions were analyzed by SDS-PAGE 12% to determine the molecular weight of the components.

Exposing tachyzoite to the most effective concentration of fraction after HPLC

Tachyzoites were exposed to the concentrations of 100, 80, and 60 µg related to the peaks obtained by HPLC from fractions with the maximum ability for removing parasites obtained by gel filtration chromatography, and tachyzoite viability was evaluated at 0, 30, and 120 min through direct counting by using a hemocytometer slide and trypan blue dye under an optical microscope. The antiparasitic activity of the sub-fraction was measured by the MTT method with the viability of the tachyzoite of RH strain *T. gondii* in Vero cells.

The experimental protocol applied in this research was approved by the Ethics Committee of Shahid Chamran University of Ahvaz (Permit number: EE/97.24.3.49892/ SCU.ac.ir).

Statistical analysis

The difference in the means of control and treatment groups was assessed in SPSS 16(Chicago, IL, USA). The Shapiro-Wilk test was first applied to ensure that the observations followed a normal distribution and the data were analyzed by using two-way ANOVA, LSD, and Dunnett's tests. The statistical judgment was based on the difference of $P<0.05$. The diagrams were provided based on the mean of data by using GraphPad Prism 8.

Results

Fraction analysis of H. lepturus venom by gel filtration chromatography

Five peaks were considered in Fig. 1, each separately used to determine protein concentration via the Bradford method.

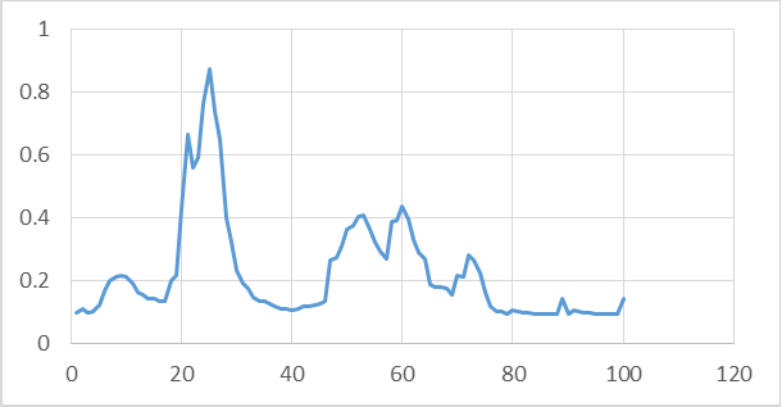

Fig.1: Gel filtration chromatogram of Hemiscorpius lepturus crude venom.

As shown in Table 1, four fractions among the five isolated ones contained protein, and their protein concentration was determined via the Bradford method. Each fraction was separately applied for the following experiments. Based on Fig. 2, the bands of fraction 2 appear from 250 to 10 kDa, and two bands at 41 and 30 kDa are related to fraction 3. The gel obtained by electrophoresis of the fractions indicated that the bands of fraction 4 were emerged in the weight range of 30, 18, and 10 kDa, while those of fraction 5 ap-

peared below 10 kDa. To achieve the concentrations of protein (100, 80, and 60 µg protein/mg lyophilized fraction), based on the protein concentration in the extracted fractions per microgram (Table 1), the amount of dried fraction according to Table 2 after measurement was dissolved in PBS and used for test (µg/µl PBS).

Table 1: Protein concentration of fractions of Hemiscorpius lepturus venom by Gel filtration chromatography

Fractions	*Fraction 2*	*Fraction 3*	*Fraction 4*	*Fraction 5*
Protein concentration (µg / mg of dried fraction)	808	760	570	308

Table 2: The amount of dried fraction of scorpion venom used to obtain the protein concentration (µg/µl PBS)

Fractions	*Protein concentration*		
	60	80	100
0.74	0.99	1.23	F2
0.79	1.05	1.31	F3
1.05	1.40	1.75	F4
1.94	2.59	3.24	F5
0.74	0.99	1.23	F2

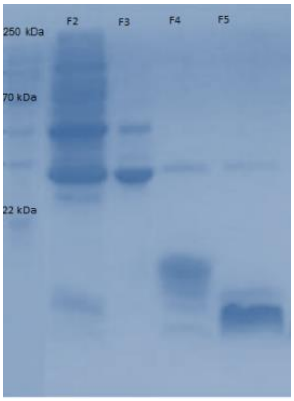

Fig.2: SDS-PAGE of fractions of *H. lepturus* venom obtained of chromatographic columns.

Evaluation of tachyzoite viability by trypan blue staining test

The tachyzoite viability exposed to different concentrations of venom fraction (100, 80, and 60 µg protein/mg lyophilized fraction) at different times of 0, 30, and 120 min measured by trypan blue staining were evaluated. All the fractions on tachyzoite viability during 120 min was significant difference was observed between the concentrations of 80 and 100 µg, while the results related to the concentrations of 60 and 100 µg significantly differed (P<0.05). As for the effect of the performance of various fractions at different times, an insignificant difference was observed between the times of 0 and 30 min. Additionally, the performance of fractions on tachyzoite viability during 120 min was significantly different from that of 0 min (P<0.05).

Evaluation of tachyzoite viability by MTT assay

The effect of different concentrations of fractions (100, 80, and 60 µg protein/mg lyophilized fraction) on tachyzoite viability significantly decreased compared to that of the control group ($P<0.05$). Further, the effect of all concentrations of fraction 5 on tachyzoite was similar to that of the effective drug, representing their identical effect. No significant difference was observed between the concentrations used for each fraction, by indicating the effect of time on this assessment.

Cytotoxicity effect of fractions of venom by MTT assay

The MTT test was conducted to survey the viability of the cells exposed to fractions of *He. lepturus* venom at the concentrations of (100, 80, and 60 µg protein/mg lyophilized fraction) (Fig. 3).

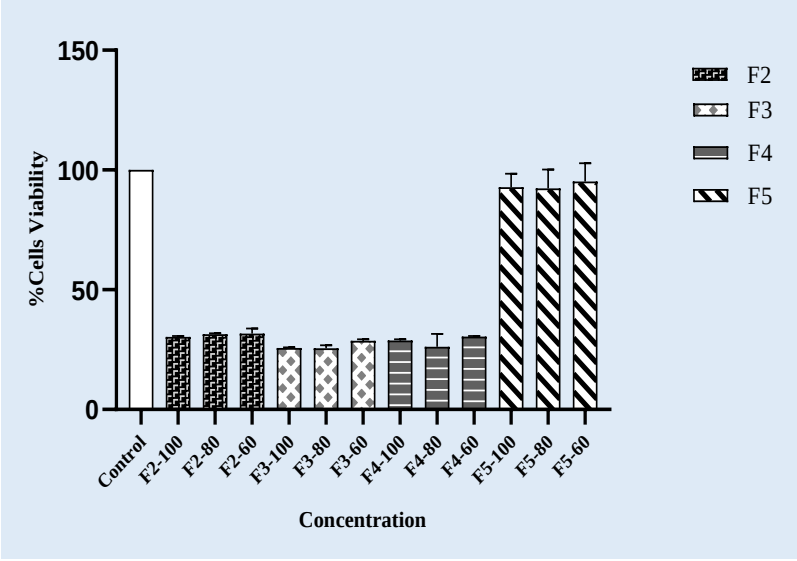

Fig.3: Effect of different fractions on Vero cell viability by MTT test

No significant difference was observed between fraction 5 and the control group regarding the challenge of Vero cell viability, whereas the other fractions significantly differed from the control group; all fractions except for fraction 5 reduced cell growth. Additionally, the percentage of the viability of the cells affected by all concentrations of fractions 2-4 was <50%, whereas different concentrations of fraction 5 acted similarly to the control group, and the percentage of cell viability was reported to be >50%.

High-performance liquid chromatography (HPLC) of fraction 5

After determining the most effective fraction on tachyzoites based on the tests performed, fraction 5 was used to prepare the sub-fraction with HPLC assay (Fig. 4). Two peaks appeared at different times by injecting fraction 5; the percentage of the first peak was 33.91% and 66.08% at 1-2 and 3-4 min, respectively. The protein components of the toxic peaks of sub-fraction5 were analyzed by SDS-PAGE electrophoresis. Both peaks possessed a molecular weight of <10 kDa. Additionally, the weight range of the bands of the first and second peaks was <10 kDa (Fig. 5).

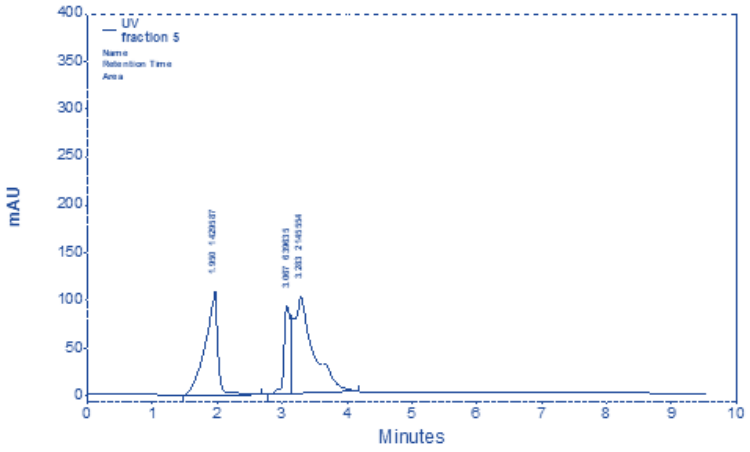

Fig.4: Peaks obtained from fraction 5 by using HPLC assay

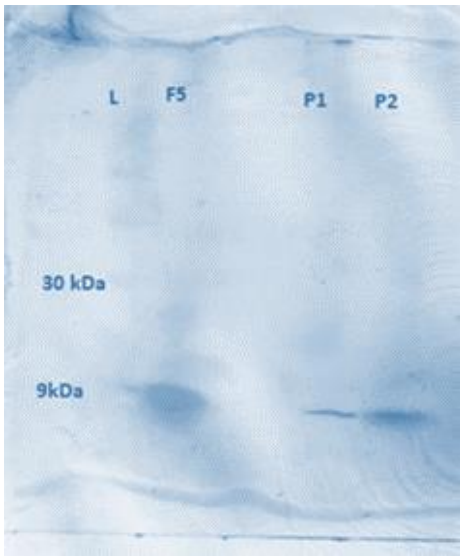

Fig.5: SDS-PAGE of toxic protein peaks from HPLC of fraction 5

Effect of sub-fraction 5 on tachyzoite viability by trypan blue staining

The peaks obtained from fraction 5 at all times significantly differed from the control group ($P<0.05$), although no significant difference was observed between the concentrations of 100 and 80 µg ($P>0.05$). In this assessment, the second peak acted better compared to the first one at all concentrations and times and represented a stronger inhibitory effect on tachyzoite viability.

Effect of sub-fraction 5 on tachyzoite viability via MTT assay

The MTT test was applied to assess tachyzoite viability by the peaks obtained from fraction 5 (Fig. 6).

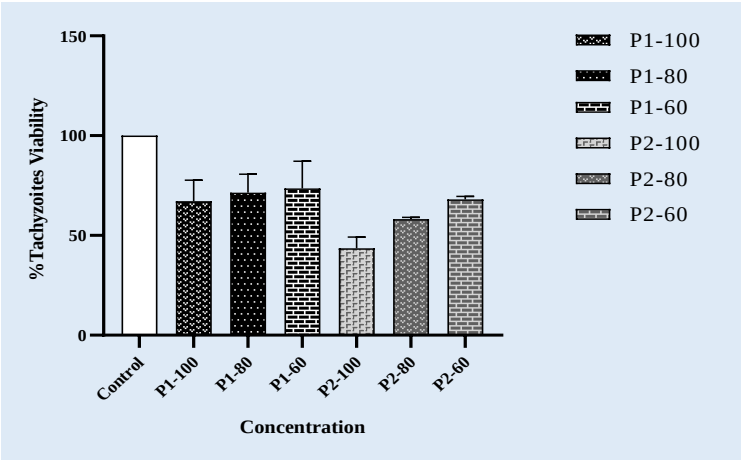

Fig.6: Effect of the peaks obtained from fraction 5 on tachyzoite viability through MTT assay

A significant difference was observed between the peaks obtained from fraction 5 and the control group, representing the effect of peaks on tachyzoites. Further, the concentrations of 100 and 60 µg were significantly different by considering the concentrations (P<0.05). Regarding the challenge of tachyzoite viability, the second peak influenced the removal more at all concentrations compared to the first one. Additionally, the percentage of the viability of the tachyzoite affected by the second peak was less at the concentration of 100 µg than at 50%, indicating that this peak at this concentration possessed the maximum effect in decreasing viability (Fig. 7).

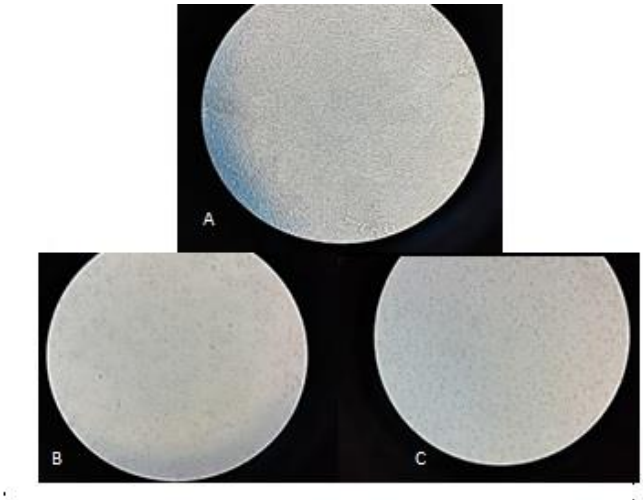

Fig.7: Tachyzoites of *Toxoplasma gondii* (A), effect of the second peak obtained from fraction 5 with the concentration of 80(B), and 100µg(C)

Cytotoxicity effect of sub-fraction 5 by MTT assay

The effect of the most effective peak obtained from fraction 5 on Vero cells, showed that insignificant difference between the second peak obtained from fraction 5 on Vero cell viability at the concentrations of 100, 80, and 60 µg, and in the control group.

Discussion

Scorpion venoms contain polypeptides and enzymes that specifically affect ion channels, enzymatic activities, and antimicrobial function (11). In recent decades, scorpion venom peptides have been isolated and purified. Peptides with ion channels pharmacological action are classified into two main types: a-toxins with Na-channel inactivation, and b-toxins that affect and open the channels' negative potentials (12). A-toxins depolarize the cell membrane and induce paralysis and cardiac arrhythmia while prolonging the action potential of excitable cells (13). Furthermore, antibacterial peptides remove pathogens by disturbing cell membranes (14). The other components of scorpion venoms are non-disulfide-bridged peptides (NDBPs) that interact with the negatively charged lipid of biological membranes. Many NDBPs peptides are cationic and have antibacterial, antifungal, cytolytic, antiviral, antimalarial, anticancer, bradykinin-potentiating, and immuno-modulating effects. Some NDBPs peptides of the scorpion venom display antimicrobial activities, so they can be considered as AMPs (15). AMPs display antibacterial activity, can inhibit the growth of both Gram-positive and Gram-negative bacteria, possess antifungal, antimalarial peptides, antiviral activity (AVP) (16-18).

The present study assessed the effect of different fractions of *He. lepturus* scorpion venom on the tachyzoite of *T. gondii*. The maximum effect on tachyzoite was observed in fraction 5 with a concentration of 100 µg after 120 min among the five fractions obtained from gel filtration chromatography. Additionally, the effect of fraction 5 on tachyzoite was similar in the MTT method, while the effect on the cell line was minimal, unlike other fractions. HPLC was conducted to further purify fraction 5 with maximum effect on tachyzoites. Two peaks at different times appeared by injecting this fraction, and the concentration of 100 µg of peak 2 had the best effect on the removal of *T. gondii*. As for the molecular weight of fractions, the weight range of fraction 5 purified through gel filtration chromatography and the peaks obtained from this fraction through HPLC was <10 kDa, which is consistent with the results of other studies.

Treating parasitic diseases by using scorpion venom has attracted the attention of many researchers. Perumal et al. (2017) found the antimalarial and anti-Trypanosoma activity of the venom of some scorpions (11). Based on the experiments, the peptides of HgeD and Hge36 derived from *Hadrurus gertschi* scorpion venom decrease the viability of *Taenia crassiceps* Cysticercus, and *Entamoeba histolytica* trophozoites. HgeD affects *E. histolytica* more than metronidazole, and their lethal effect on human lymphocytes is negligible (19). Thus, it can be a good alternative to anti-parasitic drugs. The *Mesobuthus eupeus* scorpion venom contains two linear cationic peptides named mucin 24 and mucin 25, which result in anti-*P. falciparum* activity without damaging human cells and prevent the growth of the ookinete stage of *P. berghei*. The anti-parasitic potency of these two peptides is higher compared to that of Shiva-3, the Gambicin existing in *Anopheles gambiae*, and the antimicrobial peptides isolated from the hemocyte of spider *Acanthoscurria gomesiana (20)*. Moreover, the venom of some scorpions exerts toxic effects on the larvae of *Ancylostoma caninum* and cercaria of *Schistosoma mansoni* (21, 22). Jafari et al. assessed the lethal effect of *Echinococcus granulosus* protoscoleces by using *Mesobuthus eupeus* scorpion venom for the first time. The fraction derived from this venom has scolicidal activity in 30 min. Moreover, the molecular weight of this fraction was reported to be <10 kDa, which can confirm the assessment performed in the present study (23). The effect of *Tityus discrepans* scorpion venom on *Leishmania mexicana* promastigotes showed a decreased growth in culture media. These promastigotes with the concentration of 35 mg/ml were incubated for 15 min and assessed under the optical microscope. They lost

their elongated shape and became completely round and vacuolated, and the movement of their flagellum was disturbed (24). Hadrurin is the anti-microorganism peptide of scorpion venom after scorpine B regarded as the first anti-microbial peptide with a spiral structure (25). The analogue of scorpine B named Shiva-3 prepared by chemical synthesis can interfere with *P. berghei* sporogonia. Other studies found a peptide from the venom of the scorpion *Pandinus imperator*, whose antimalarial effect was stronger compared to that of Shiva-3, while also possessing antimicrobial activity (26). The peptide of imbratoxin derived from the venom of the scorpion *P. imperator* prevents the intra-erythrocyte growth of *P. falciparum* through phospholipase A2 activity. These peptides probably react with the lipids existing in the membrane or plasma free fatty acids in infected erythrocytes and thus eliminate infection by producing lipid products such as peroxide. The low concentrations of imbratoxine I completely inhibit the formation of ookinete and reproduction in vitro (9). Moreover, the peptide of scorpine with the weight of 8.35 kDa derived from the venom of this scorpion prevents the reproduction and formation of *P. berghei* ookinete (27). The peptide of noxiustoxin derived from *C. noxius* scorpion venom possesses inhibitory effects on *P. berghei*. Additionally, the venom of the scorpion *C. sculpturatus* belonging to Buthidae family is effective on free-living non-pathogenic protozoa such as *Euglena gracilis* and *Paramecium aurelia* (28).

Conclusion

Proteins with a molecular weight of less than 10 kDa in *He. lepturus* venom factions exert anti-parasitic effects against the tachyzoite of *T. gondii,* was observed in Trypan Blue and MTT methods. The results of basic research in this field after identifying the structure of venom proteins can be used for pharmaceutical research. The presence of proteins with anti-parasitic properties in various scorpion venoms has been proven which can be important for researchers as a new approach to the control of infection.

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Conflict of interest

There is no conflict of interest to declare.

References

1. Jones L, Kruszone Moran D, Wilson M, et al. *Toxoplasma gondii* infection in the United States: Seroprevalence and risk factors. Am J Epidemiol. 2001: 154: 357-365.
2. Holland GN, Engstrom RE, Glasgow B, et al. Ocular toxoplasmosis in patients with the acquired immunodeficiency syndrome. Am J Ophthalmol. 1988; 106(6): 653-667.
3. Kim S, Fouts AE, Boothroyd JC. *Toxoplasma gondii* Dysregulates IFN-ɣ- Inducible Gene Expression in Human Fibroblasts: Insights from a Genome- Wide Transcriptional Profiling. J Immunol. 2007; 178(8): 5154-5165.
4. Bastos LM, Júnior RO, Silva DAO, et al. *Toxoplasma gondii*: Effects of neuwiedase, a metalloproteinase from Bothrops neuwiedi snake venom, on the invasion and replication of human fibroblasts in vitro. Exp Parasitol. 2008; 120(4): 391-396.
5. Hernández S, De León-Nava MA, Romero-Núñez E, et al. In Vitro Effect of the Synthetic cal14.1a Conotoxin, Derived from Conus cali-

fornicus, on the Human Parasite *Toxoplasma gondii*. Mar Drugs. 2016; 14 (4): 66.
6. Bagheri M, Babaei E, Shahbazzadeh D, et al. Development of a recombinant camelid specific diabody against the heminecrolysin fraction of *Hemiscorpius lepturus* scorpion. Toxin Rev. 2017; 36(1): 7-11.
7. Zabihollahi R, Bagheri KP, Keshavarz Z, et al. Venom components of Iranian scorpion *Hemiscorpius lepturus* inhibit the growth and replication of human immunodeficiency virus 1 (HIV-1). Iran Biomed J. 2016; 20(5): 259-265.
8. Hancock REW. Cationic peptides: effectors in innate immunity and novel antimicrobials. Lancet Infect Dis. 2001; 1(3): 156–164.
9. Guillaume C, Deregnaucourt C, Clavey V, Schrével J. Anti-*Plasmodium* properties of group IA, IB, IIA and III secreted phospholipases A2 are serum-dependent. Toxicon. 2004; 43(3): 311-318.
10. Wang Z, Wang G. APD: the Antimicrobial Peptide Database. Nucleic Acids Res. 2004; 32(Database issue):D590-2.
11. Perumal SR, Stiles BG, Franco OL, Sethi G, Lim LHK. Animal venoms as antimicrobial agents. Biochem Pharmacol. 2017; 134: 127-138.
12. Rodriguez de la Vega RC, Possani LD. Overview of scorpion toxins specific for Na channels and related peptides: biodiversity, structure-function relationships and evolution. Toxicon. 2005; 46(8): 831-844.
13. Bosmans F, Tytgat J. Voltage-gated sodium channel modulation by scorpion alpha-toxins. Toxicon. 2007; 49(2): 142-158.
14. Ehret-Sabatier L, Loew D, Goyffon M, et al. Characterization of novel cysteine-rich antimicrobial peptides from scorpion blood. J Biol Chem. 1996; 271(47): 29537-29544.
15. Almaaytah A, Albalas Q. Scorpion venom peptides with no disulfide bridges: a review. Peptides. 2014; 51: 35-45.
16. White NJ, Pukrittayakamee S, Hien TT, et al. Malaria. Lancet. 2014; 383(9918):723-35.
17. Yan R, Zhao Z, He Y, et al. A new natural alpha-helical peptide from the venom of the scorpion *Heterometrus petersii* kills HCV. Peptides. 2011; 32(1): 11-19.
18. Zeng XC, Wang S, Nie Y, et al. Characterization of BmKbpp, a multifunctional peptide from the Chinese scorpion *Mesobuthus martensii Karsch*: gaining insight into a new mechanism for the functional diversification of scorpion venom peptides. Peptides. 2012; 33(1):44-51.
19. Flores-Solis D, Toledano Y, RodríguezLima O, et al. Solution structure and antiparasitic activity of scorpine-like peptides from Hoffmannihadrurus gertschi. FEBS Lett. 2016; 590(14): 2286-2296.
20. Gao B, Xu J, Carmen Rodriguez M, et al. Characterization of two linear cationic antimalarial peptides in the scorpion *Mesobuthus eupeus*. Biochimie. 2010; 92(4): 350-359.
21. Asmar M, Swelam N, Abdel AAL, et al. Factors in the venom of scorpions toxic to *Schistosoma mansoni* cercariae. Toxicon. 1980; 18(5-6):711-5.
22. Xu ZM, Li ZS, Wen MX, et al. In vitro effect of medicinal scorpion on the larvae of *Ancylostoma caninum*. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2008; 26(5): 387-8.
23. Jafari H, Nemati M, Haddad PM, et al. Scolicidal activity of *Mesobuthus eupeus* venom against the protoscolices of *Echinococcus granulosus*. Arch Razi Inst. 2019; 74(2): 183-189.
24. Borges A, Silva S, Den Camp HO, et al. In vitro leishmanicidal activity of *Tityus discrepans* scorpion venom. Parasitol Res. 2006; 99(2): 167-173.
25. Separovic F, Luna-Ramirez K, Sani MA, et al. Membrane interactions and biological activity of antimicrobial peptides from Australian scorpion. Biochim Biophys Acta. 2014; 1838(9): 2140-2148.
26. Possani LD, Corona M, Zurita M, Rodríguez MH. From Noxiustoxin to Scorpine and Possible Transgenic Mosquitoes Resistant to Malaria. Arch Med Res. 2002; 33(4):398-404.
27. Conde R, Zamudio FZ, Rodtiguez MH, Possani LD. Scorpine, an antimalarial and antibacterial agent purified from scorpion venom. FEBS Lett. 2000; 471(2-3):165-8.
28. Johnson BD, Tullar C, Stahnke HL. A quantitative protozoan Bio-assay method for determining venom potencies. Toxicon. 1966; 3(4): 297-300.