Saudi Journal of Biological Sciences 27 (2020) 1760–1765

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

In vitro Scolicidal effects of *Androctonus crassicauda* (Olivier, 1807) venom against the protoscolices of *Echinococcus granulosus*

Esam S. Al-Malki [a,1,*], Naser Abdelsater [b,1]

[a] Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah 11952, Saudi Arabia
[b] Zoology Department, Faculty of Science, Al-Azhar University, Assuit 71524, Egypt

ARTICLE INFO

Article history:
Received 16 March 2020
Revised 30 April 2020
Accepted 2 May 2020
Available online 11 May 2020

Keywords:
Hydatid cyst
Protoscolices
Scorpion venom
Androctonus crassicauda

ABSTRACT

Hydatidosis is a zoonotic disease that commonly occurs in several places around the world, especially in the Middle East, due to infection by the larval stage of *Echinococcus granulosus*. This disease impacts an immense effect on the economic and public health of both humans and animals. Despite their effectiveness, the unacceptable side effects and progressive resistance to scolicidal agents may limit their use. According to their biopharmaceutical activity and benefits, numerous studies have reported that scorpion venom and its derivatives represent important resources for therapeutic applications. Therefore, this study was designed to investigate the in vitro scolicidal consequences of the crude venom of *Androctonus crassicauda* on *E. granulosus*. For this purpose, protoscolices from infected organs of camel containing hydatid cysts were collected, separated, and washed. The scolicidal impacts of three different concentrations of the crude venom (20, 50, and 100 μg/mL) were tested at different times of exposure (30, 60, 120, and 240 min). Particularly, eosin exclusion test was used to examine the viability of the protoscolices. The study results showed that the crude venom at 100 μg/mL destroys all protoscolices after 240 min incubation. Also, the scolicidal activity of venom increased significantly according to the time of exposure. In conclusion, the crude venom of *A. crassicauda* demonstrated high scolicidal activity in vitro against protoscolices of hydatid cysts in low concentration and short exposure time. However, the efficacy of scorpion venom remains to be evaluated in vivo for the treatment of hydatidosis in both humans and domesticated animals.

1. Introduction

Hydatidosis is a zoonotic disease caused by the larval stage of Canid small tapeworm (*Echinococcus granulosus*), affecting humans as well as domesticated and wild animals as intermediate hosts (Abdel-Baki et al., 2016; Almalki et al., 2017; Thompson, 2017). This infection has immense economic effects on the livestock industry and on human public health (Ito and Budke, 2017). For a long time, the chemotherapy drugs had been the necessary treatment to inhibit the hydatid cysts. Additionally, surgical techniques were also employed to remove larger hydatid cysts while the drugs were not enough to inhibit them (Thompson, 2017). Before and after surgery, a systematic treatment with drugs is performed to destroy the protoscolices. Also, during surgery, installing scolicidal agents were useful in preventing the risk of spillage of parasites into adjacent organs (Vidoura et al., 2017). Despite the widespread use of chemotherapy drugs, unacceptable side effects and progressive resistance to many scolicidal agents triggered the researchers to study the effectiveness of biological products in the treatment of such diseases (Musaev et al., 2017). Scorpion venom has evolved for subduing prey and for defense (Casper, 1985; Sarhan et al., 2013). Venom is a blend of several valuable components, including scorpion amino acid and enzymes that can serve as antidotes to less harmful diseases ((Jafari et al., 2019). Many studies have revealed that scorpion and snake venom can have considerable effects on humans infected with protozoan parasites such as *Plasmodium*, *Leishmania, Trypanosoma* and *Toxoplasma gondii* (Adade et al., 2012; Borges et al., 2006; Conde et al., 2000; Gao et al., 2010; Khaleghi Rostamkolaie et al., 2019; Perumal Samy et al., 2017). Scorpion venom contains multiple peptides, which have

* Corresponding author at: Department of Biology, College of Science in Zulfi, Majmaah University, Majmaah 11952, Saudi Arabia.
E-mail address: e.almalki@mu.edu.sa (E.S. Al-Malki).
[1] Equally contributed to this work.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.05.014
1319-562X/

attracted the attention of many scientists concerned with their therapeutic development (Perumal Samy et al., 2017). Jafari et al., (2019) first reported the scolicidal effects of the crude venom and its fractions of scorpion species *Mesobuthus eupeus*, against the protoscolices of *E. granulosus*. This study revealed that the venom peptides of *M. epeus* can detroy the protoscolices of hydatid cysts prompt and appropriate manner and could be used as a scolicidal agent in the management of hydatidosis. The Arabian Fat Tailed Scorpion *A. crassicauda* (Olivier, 1807) is considered as one of the medically important species belonging to the family Buthidae, and distributed across the Sinai Peninsula, the Arabian Peninsula countries and the Middle East (Kaltsas et al., 2008). Also, antimicrobial peptides (AcrAPs) isolated from *A. crassicauda* venom showed inhibitory activity against both bacterial and fungal strains (Alajmi et al., 2020; Du et al., 2014). Despite the biochemical and molecular characterization of the venom components of *A. crassicauda* being documented by several authors (Batista et al., 2002; Caliskan et al., 2006), their peptide nature has not been studied in detail utilizing biomedical and biological approaches. For this reason, this study was aimed to investigate the scolicidal effect of crude *A. crassicauda* scorpion venom against *E. granulosus* protoscolices.

2. Materials and methods

2.1. Scorpion collection, maintenance, and venom collection

One hundred individuals of *A. crassicauda* scorpions were collected from the following regions of Saudi Arabia; Khashm Ath-Thumami, (at 27.693196N, 44.987823E); Al-Kharj (at 24.132640N, 47.395228E) and Al Nuayriyah (at 27.649838N, 48.716355E), during the period between July and September 2019. The collected scorpions were kept and maintained individually in (40 cm × 40 cm) in plastic containers at 25 °C, with 10 cm deep sandy-soil substrate at Parasitology Lab, College of Science, Majmaah University. Water was provided weekly by misting the substrate. Scorpions were provided every week by water and fed insect, especially crickets and cockroaches. Remains of dead prey were regularly removed from the containers as soon as possible, these remains mold rapidly, and scorpions have been reported to become passively entrapped in the fungal hyphae and die. Venom was collected by electrical stimulation (20 V) in the articulation of the telson as described by Sarhan et al. (Alajmi et al., 2020; Sarhan et al., 2012). The venom drops were purified and gathered in an Eppendorf tube and centrifuged at 14,000 rpm at 4 °C for 15 min. The supernatant was pooled, freeze-dried and stored at −20 °C. The lyophilized samples were dissolved in distilled water and centrifuged at 15,000 rpm for 15 min at 4 °C.

2.2. Protoscolitic selection and feasibility analysis

Hydatide cysts of *E. granulosus* were collected from liver and lung of camel slaughtered in Cairo governmental slaughterhouse. The collected samples were immediately transferred to the Parasitology Laboratory, Faculty of Science, University of Al-Azhar, Cairo, Egypt. The hydatid fluid was aspirated by sterile syringe and allowed to rest for 45 min, after which the protoscolices were precipitated in a sterile falcon tube. The collected protoscolices were washed three times with normal saline and the viability of metacestodes was assessed with 0.1% eosin stain and observing their motility characteristics and muscular movements by light microscopy. Dead protoscolices absorb eosin and color red while viable protoscolices remain colorless. The protoscolices, with more than 90% viability, were selected for the following experiments (Smyth and Barrett, 1980).

2.3. Effect of crude venom on protoscolices

The parasite was transported to a sterile medium and handled as defined by (Elissondo et al., 2006) with minor modifications. Concisely, protoscolices were grown in RPMI-1640 medium, to which was added 100 IU penicillin and 100 μg/mL streptomycin. 2.5 ml of media was placed in a test tube. Approximately 5×10^3 protoscolices was then added to the tube and mixed gently. Then various concentrations (20, 50, and 100 μg/mL RPMI-1640) of scorpion venom were added, and the tube was then incubated at 37 C for 30, 60, 120, and 240 min.

At the end of each incubation period, the upper portion of the solution was discarded, taking care to avoid disturbing the settled protoscolices. One milliliter of 0.1% eosin stain was then added to the remaining settled protoscolices and mixed gently. After 5 min, the upper portion of the solution was again discarded. The remaining settled protoscolices were smeared on a glass slide, covered with a cover glass, and examined microscopically for viability. The percentages of stained (dead) protoscolices and un-stained (live) protoscolices at each concentration were estimated. To ensure the accuracy of the test and quality control, control groups containing RPMI-1640 or albendazole as negative and positive controls, respectively, were considered. Each experiment was repeated three times.

2.4. Statistical analysis

Differences among groups (study and control) were evaluated using an analytical package (Sigma Plot version 11.0).

3. Results

3.1. Viability of protoscolices

The fertility of hydatid cysts was determined by the presence of free protoscolices in the cystic fluid by wet mount drop (Fig. 1A). The viability of protoscolices was tested prior to the experiments using 0.1% aqueous eosin stain. Light microscope examination revealed live protoscolices remained colorless and also showed characteristic muscular movements and flame cell activity (Fig. 1B). When partial death occurred, dead protoscolices absorbed eosin and colored red, but live protoscolices remained colorless after exposure to albendazole or various concentrations of crude scorpion venom and staining with 0.1% eosin (Fig. 1D). All protoscolices colored red when total death occurred in both control positive groups with 100 μg/mL of crude scorpion venom and staining with 0.1% eosin (Fig. 1E).

3.2. In vitro treatment of protoscolices

The findings of this experiment showed highly significant ($P < 0.001$) scolicidal effects against protoscolices of *E. granulosus* for all of the various concentrations of crude venom, compared to the negative control group, both within the same time and for different periods, as shown in Table 1 and Fig. 2. The maximum death rates in the negative and positive control groups were 21.3% and 100%, respectively. The scolicidal activity of crude venom at a concentration of 20 μg/mL was 25.1, 31.2, 36.5, and 57.1% after application for 30, 60, 120, and 240 min, respectively, while at a concentration of 50 μg/mL it was 28.6, 39.5, 65.4, and 80.6% after application for 30, 60, 120, and 240 min, respectively. However, a concentration of 100 μg/mL killed 34.9, 71.4, 95.8, and 100% of protoscolices after 30, 60, 120, and 240 min, respectively.

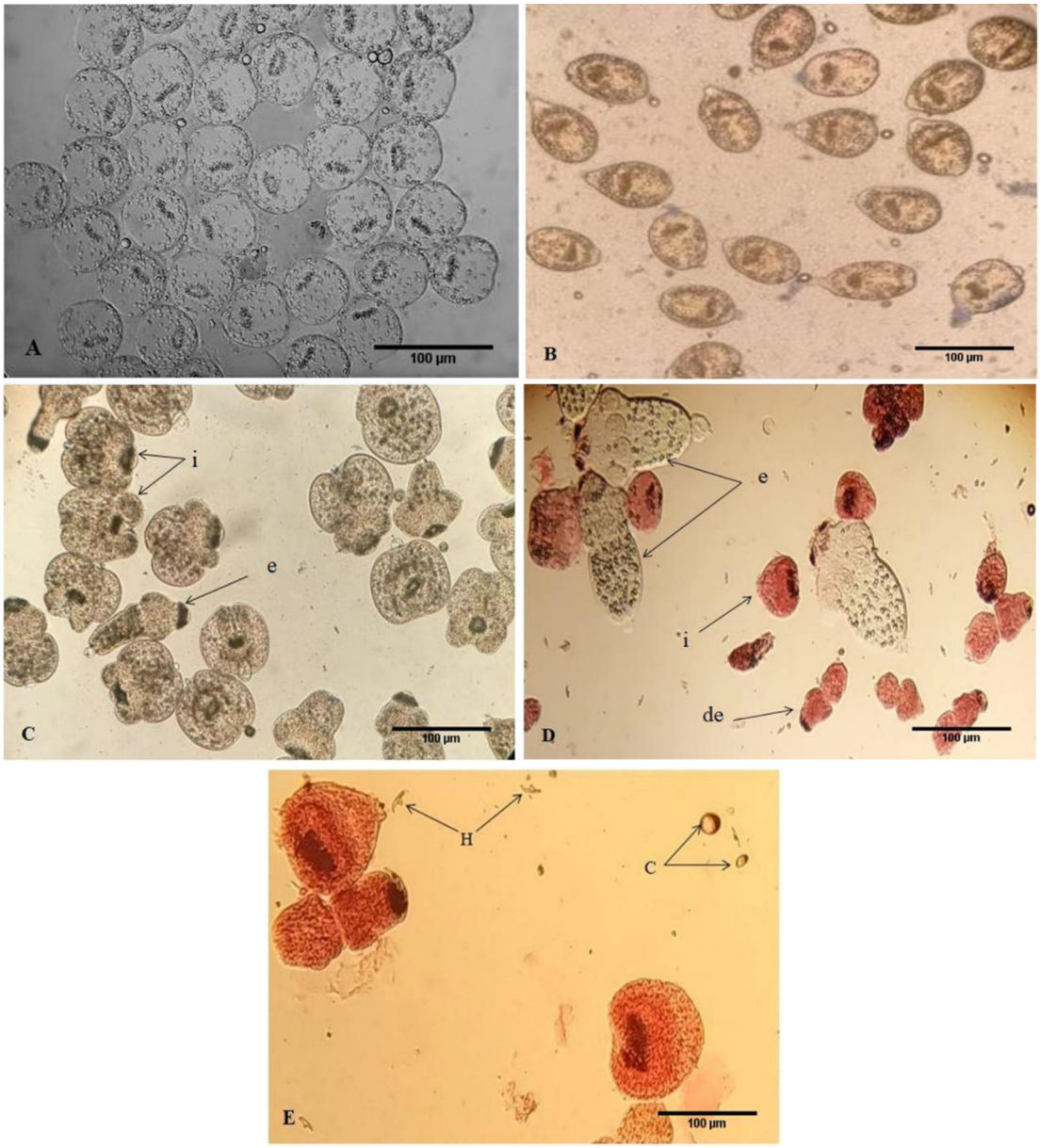

Fig. 1. Protoscolices of *E. granulosus* collected from naturally infected livers and lungs of camel. Scale bar = 100 µm. (A) Live protoscolices without staining. (B) Live invaginated colorless protoscolices after staining with 0.1% eosin. (C) Live evaginated (e) and invaginated (i) colorless protoscolices 5 min after staining with 0.1% eosin. (D) Dead evaginated colored protoscolices (de), and dead and invaginated colored protoscolices (i) and a few live colorless evaginated protoscolices after introduction to albendazole and various concentrations of crude scorpion venom and staining with 0.1% eosin. (E) Total death of invaginated protoscolices (colored) after exposure to albendazole and 100 µg/mL crude scorpion venom and staining with 0.1% eosin. Free hooks (h), and calcareous corpuscles (c).

3.3. Morphological changes of protoscolices

In the sterile medium (RPMI-1640), direct microscopic examination of viable protoscolices showed distinct movements and retained the membrane integrity and order of hooks. Most of these protoscolices were altered to an evaginated form (scolices) in time, and suckers were clearly visible (Fig. 1C). The microscopic examination of the dead protoscolices showed a distortion of their morphology and degenerative effects. These effects were characterized by loss of motility, loss of hooks, or the presence of free hooks and calcareous corpuscles (Fig. 1 D&E) after exposure to albendazole and different concentrations of crude scorpion venom and staining with 0.1% eosin.

4. Discussion

In the recent decades, several studies have been conducted on the therapeutic of the natural organic products (Aspinall et al., 2002). Such experiments demonstrated a wide range of antibiotic action of natural venom against several pathogens such as bacteria, fungi, viruses, and parasites (Alajmi et al., 2020; Bahar and Ren, 2013; Bringans et al., 2008; El-Bitar et al., 2015; Nie et al., 2012; Paniagua et al., 2012; Rodríguez De La Vega et al., 2004; Tawfik, 2018; Yan et al., 2011; Alvarenga et al., 2012; Joya et al., 2011). Scorpion venom is a rich source of active compounds, including many polypeptides (Perumal Samy et al., 2017). Caliskan et al. (2006) characterized the components of the venom of *A. crassi-*

Table 1
Scolicidal effect of *Androctonus crassicauda* scorpions.

Concentration	Exposure	Mortality rates after exposure (%)			
		30 min	60 min	120 min	240 min
20 ug/mL	1	25.9	31.5	35.1	55.8
	2	25.4	32.3	38.0	58.7
	3	24	29.8	36.5	56.8
	Average	25.1	31.2	36.5	57.1
50 ug/mL	1	31.4	39.7	63.7	81.7
	2	27.5	41.7	67.0	80.3
	3	26.9	37.0	65.5	79.7
	Average	28.6	39.4	65.4	80.6
100 ug/mL	1	37.5	72.0	94.8	100
	2	34.1	72.2	96.0	100
	3	33.2	70.1	96.6	100
	Average	34.9	71.4	95.8	100
Negative control (CN)	1	6.9	9.5	14.1	20.5
	2	6.1	10.7	15.3	21.9
	3	6.3	8.8	14.6	21.4
	Average	6.4	9.7	14.7	21.3
Positive control (CP)	1	75.0	100	100	100
	2	73.8	100	100	100
	3	76.2	100	100	100
	Average	75.0	100	100	100

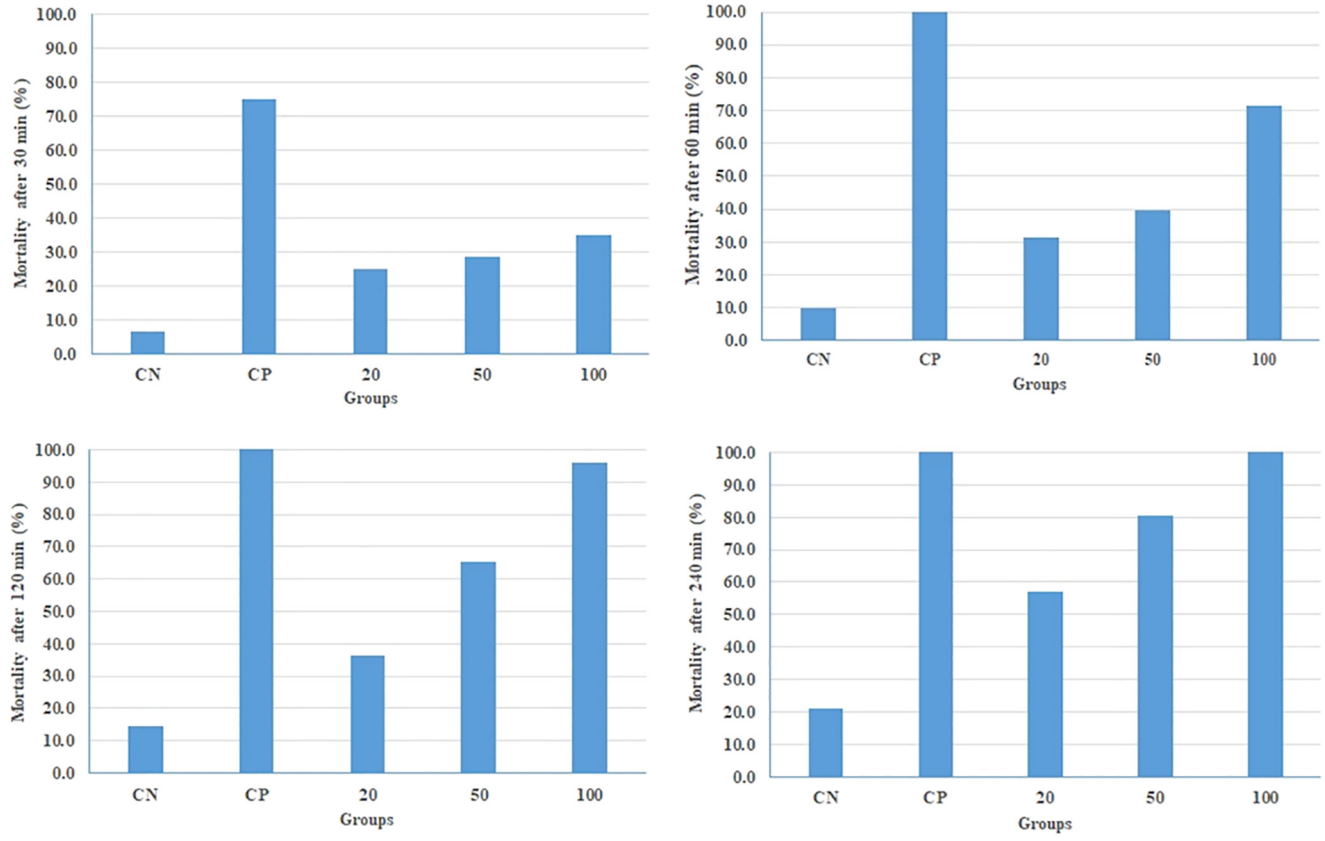

Fig. 2. Effect of different concentrations of crude venom (20, 50, and 100 μg/mL) on the viability rate of protoscolices 30, 60, 120, and 240 min after exposure. CN: negative control (RPMI1640), CP: positive control (albendazole).

cauda, for the first time based on biochemical and molecular analysis. Potential anti-tumor properties are shown by three main crassicaudal soluble venom peptides (Caliskan et al., 2009). Also, the antimicrobial peptides of the whole venom of *A. crassicauda* were identified by Altinkurt and Altan (1980). On the other hand, two linear cationic antimalarial peptides from the venom of the Iranian Buthid scorpion *M. eupeus* have been defined by Gao et al. (2010). Also, it was found that the venom of the New World scorpion, *Tityus discrepans*, significantly reduced the growth of promastigotes of *Leishmania mexicana* (Borges et al., 2006). In addition, Flores-Solis et al. (2016) identified two peptides from the scorpion venom of *Hoffmannihadrurus gertschi* that have scolicidal activities against *Taeniid cestod* and protozoan pathogens. El-Asmar et al. (1980) and Xu et al. (2008), respectively, discussed the cytotoxicity of scorpion venom on *Schistosoma mansoni cercariae and Ancylostoma caninum*.. In the present study, we screened the in vitro

scolicidal activity of crude venoms derived from the most common scorpion species in Saudi Arabia, *A. crassicauda,* against the protoscolices of *E. granulosus*. It was observed that the crude venoms of *A. crassicauda* showed a substantial impact on the mortality rate of protoscolices at all the concentrations tested compared to the negative controls, as shown in Fig. 2 and Table 1. This also study revealed that *A. crassicauda* venom was significantly induced mortality in the protoscolices of hydatid cysts at low concentrations (Fig. 1). The findings of this study showed better activity compared to those of the whole venom of *M. epeus* (Jafari et al., 2019), which showed scolicidal advantages but did not significantly affect the rate of mortality of protoscolices. However, high concentrations of *A. crassicauda* destroyed all protoscolices within 240 min, while *M. epeus* Fraction 8 induced the same effect in 30 min. The recent study showed the morphological distortion and degenerative consequences of the morphological alteration of the dead protocolices. The measurements were consistent with loss of motility, loss of hooks, or the existence of free hooks and calcareous bodies (Yones et al., 2011). It can be concluded that the scolicidal potential of *A. crassicauda* venom could be considered as an alternative strategy in hydatid cyst treatment, especially when surgery is recommended for the patient. However, the effectiveness of scorpion venom in vivo remains to be determined, and further studies are required for the identification and isolation of active compounds. In vivo trials involve with the estimation of effective doses of the *A. crassicauda* venom, their safety profile, pharmacodynamics, pharmacokinetics, and the route of their administration should be carried out.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The Authors would like to thank Deanship of Scientific Research at Majmaah University, Al Majmaah, 11952, Saudi Arabia for supporting this work under the Project Number R-1441-122.

References

Abdel-Baki, A.A.S., Almalki, E., Mansour, L., Al-Quarishy, S., 2016. In vitro scolicidal effects of salvadora persica root extract against protoscolices of echinococcus granulosus. Korean J. Parasitol. https://doi.org/10.3347/kjp.2016.54.1.61.

Adade, C.M., Chagas, G.S.F., Souto-Padrón, T., 2012. Apis mellifera venom induces different cell death pathways in Trypanosoma cruzi. Parasitology. https://doi.org/10.1017/S0031182012000790.

Almalki, E., Al-Shaebi, E.M., Al-Quarishy, S., El-Matbouli, M., Abdel-Baki, A.A.S., 2017. In vitro effectiveness of Curcuma longa and Zingiber officinale extracts on Echinococcus protoscoleces. Saudi J. Biol. Sci. https://doi.org/10.1016/j.sjbs.2016.05.007.

Alajmi, R, Al-ghamdi, S, Barakat, I, Mahmoud, A, Abdon, N, Al-Ahidib, M, Abdel-Gaber, R, 2020. Antimicrobial Activity of Two Novel Venoms from Saudi Arabian Scorpions (*Leiurus quinquestriatus* and *Androctonus crassicauda*). Int. J. Pept. Res. Ther. 26, 67–74. https://doi.org/10.1007/s10989-019-09816-4.

Altinkurt, O., Altan, M., 1980. Pharmacological effects of the scorpion (Androctonus crassicauda) venomfrom Urfa environment on laboratory animals and the antagonistic effects of streptomycin to most of these effects. J Fac Pharm Ankara 10, 41–61.

Alvarenga, E.R., Mendes, T.M., Magalhaes, B.F., Siqueira, F.F., Dantas, A.E., Barroca, T. M., Horta, C.C., Kalapothakis, E., 2012. Transcriptome analysis of the <i> Tityus serrulatus</i> scorpion venom gland. Open J. Genet. https://doi.org/10.4236/ojgen.2012.24027.

Aspinall, T.V., Joynson, D.H.M., Guy, E., Hyde, J.E., Sims, P.F.G., 2002. The Molecular Basis of Sulfonamide Resistance in Toxoplasma gondii and Implications for the Clinical Management of Toxoplasmosis. J. Infect. Dis. https://doi.org/10.1086/340577.

Bahar, A.A., Ren, D., 2013. Antimicrobial peptides. Pharmaceuticals. https://doi.org/10.3390/ph6121543.

Batista, C.V.F., Gómez-Lagunas, F., Rodríguez De La Vega, R.C., Hajdu, P., Panyi, G., Gáspár, R., Possani, L.D., 2002. Two novel toxins from the Amazonian scorpion Tityus cambridgei that block Kv1.3 and Shaker B K+-channels with distinctly different affinities. Biochim. Biophys. Acta - Proteins Proteomics. https://doi.org/10.1016/S1570-9639(02)00458-2.

Borges, A., Silva, S., Op Den Camp, H.J.M., Velasco, E., Alvarez, M., Alfonzo, M.J.M., Jorquera, A., De Sousa, L., Delgado, O., 2006. In vitro leishmanicidal activity of Tityus discrepans scorpion venom. Parasitol. Res. https://doi.org/10.1007/s00436-006-0133-z.

Bringans, S., Eriksen, S., Kendrick, T., Gopalakrishnakone, P., Livk, A., Lock, R., Lipscombe, R., 2008. Proteomic analysis of the venom of Heterometrus longimanus (Asian black scorpion). Proteomics. https://doi.org/10.1002/pmic.200700948.

Caliskan, F., Sivas, H., Sahin, Y., 2009. Purification of Ac8, Ac9 and Ac10 peptides from Androctonus crassicauda crude venom with cytotoxic effect on BC3H1 cells. AUJST 10, 515–524.

Caliskan, F., García, B.I., Coronas, F.I.V., Batista, C.V.F., Zamudio, F.Z., Possani, L.D., 2006. Characterization of venom components from the scorpion Androctonus crassicauda of Turkey: Peptides and genes. Toxicon. https://doi.org/10.1016/j.toxicon.2006.04.003.

Casper, G.S., 1985. Prey capture and stinging behavior in the Empero R scorpion, Pandinus Imperator (Koch) (Scorpiones, Scorpionidae). J. Arachnol. 13, 277–283.

Conde, R., Zamudio, F.Z., Rodríguez, M.H., Possani, L.D., 2000. Scorpine, an anti-malaria and anti-bacterial agent purified from scorpion venom. FEBS Lett. https://doi.org/10.1016/S0014-5793(00)01384-3.

Du, Q., Hou, X., Ge, L., Li, R., Zhou, M., Wang, H., Wang, L., Wei, M., Chen, T., Shaw, C., 2014. Cationicity-enhanced analogues of the antimicrobial peptides, AcrAP1 and AcrAP2, from the venom of the scorpion, Androctonus crassicauda, display potent growth modulation effects on human cancer cell lines. Int. J. Biol. Sci. https://doi.org/10.7150/ijbs.9859.

El-Asmar, M.F., Swelam, N., Abdel Aal, T.M., Ghoneim, K., Hodhod, S.S., 1980. Factor (s) in the venom of scorpions toxic to Schistosoma mansoni (intestinal belharzia) cercariae. Toxicon. https://doi.org/10.1016/0041-0101(80)90106-3.

El-Bitar, A.M., Sarhan, M.M., Aoki, C., Takahara, Y., Komoto, M., Deng, L., Moustafa, M.A., Hotta, H., 2015. Virocidal activity of Egyptian scorpion venoms against hepatitis C virus Hepatitis viruses. Virol. J. https://doi.org/10.1186/s12985-015-0276-6.

Elissondo, M., Dopchiz, M., Ceballos, L., Alvarez, L., Sánchez Bruni, S., Lanusse, C., Denegri, G., 2006. In vitro effects of flubendazole on Echinococcus granulosus protoscoleces. Parasitol. Res. https://doi.org/10.1007/s00436-005-0026-6.

Flores-Solis, D., Toledano, Y., Rodríguez-Lima, O., Cano-Sánchez, P., Ramírez-Cordero, B.E., Landa, A., Rodríguez de la Vega, R.C., del Rio-Portilla, F., 2016. Solution structure and antiparasitic activity of scorpine-like peptides from Hoffmannihadrurus gertschi. FEBS Lett. https://doi.org/10.1002/1873-3468.12255.

Gao, B., Xu, J., del Carmen Rodriguez, M., Lanz-Mendoza, H., Hernández-Rivas, R., Du, W., Zhu, S., 2010. Characterization of two linear cationic antimalarial peptides in the scorpion Mesobuthus eupeus. Biochimie. https://doi.org/10.1016/j.biochi.2010.01.011.

Ito, A., Budke, C.M., 2017. The echinococcoses in Asia: The present situation. Acta Trop. https://doi.org/10.1016/j.actatropica.2017.07.013.

Jafari, H., Nemati, M., Haddad Molayan, P., Khaleghi Rostamkolaie, L., Hamidinejat, H., 2019. Scolicidal activity of mesobuthus eupeus venom against the protoscolices of echinococcus granulosus. Arch. Razi Inst. https://doi.org/10.22092/ari.2018.121416.1213.

Joya, G., D'Suze, G., Salazar, V., Rosales, A., Sevcik, C., Visbal, G., Ferreira, A.T.S., Perales, J., 2011. Scorpion toxins modify phytopathogenic fungus physiology. A possible source of new fungicides. J. Agric. Food Chem. https://doi.org/10.1021/jf200486t.

Kaltsas, D., Stathi, I., Mylonas, M., 2008. The foraging activity of Mesobuthus gibbosus (Scorpiones: Buthidae) in central and south Aegean archipelago. Journal of Natural History. https://doi.org/10.1080/00222930701835498.

Khaleghi Rostamkolaie, L., Hamidinejat, H., Razi Jalali, M.H., Jafari, H., Najafzadeh Varzi, H., Seifi Abadshapouri, M.R., 2019. In vitro therapeutic effect of Hemiscorpius lepturus venom on tachyzoites of Toxoplasma gondii. J. Parasit. Dis. https://doi.org/10.1007/s12639-019-01113-7.

Musaev, G.K., Fatyanova, A.S., Levkin, V.V., 2017. Principles and modern trends in liver echinococcosis treatment. Khirurgiia (Sofiia). https://doi.org/10.17116/hirurgia20171290-94.

Nie, Y., Zeng, X.C., Yang, Y., Luo, F., Luo, X., Wu, S., Zhang, L., Zhou, J., 2012. A novel class of antimicrobial peptides from the scorpion Heterometrus spinifer. Peptides. https://doi.org/10.1016/j.peptides.2012.09.012.

Paniagua, D., Jiménez, L., Romero, C., Vergara, I., Calderón, A., Benard, M., Bernas, M. J., Rilo, H., De Roodt, A., D'Suze, G., Witte, M.H., Boyer, L., Alagón, A., 2012. Lymphatic route of transport and pharmacokinetics of Micrurus fulvius (coral snake) venom in sheep. Lymphology.

Perumal Samy, R., Stiles, B.G., Franco, O.L., Sethi, G., Lim, L.H.K., 2017. Animal venoms as antimicrobial agents. Biochem. Pharmacol. https://doi.org/10.1016/j.bcp.2017.03.005.

Rodríguez De La Vega, R.C., García, B.I., D'Ambrosio, C., Diego-García, E., Scaloni, A., Possani, L.D., 2004. Antimicrobial peptide induction in the haemolymph of the Mexican scorpion Centruroides limpidus limpidus in response to septic injury. Cell. Mol. Life Sci. https://doi.org/10.1007/s00018-004-4096-x.

Sarhan M, M, Fouda M, Maged, Elbitar M, A, Aly A, H, Sayed B, A, 2012. Variation of protein profile among consecutive stings of the scorpion *Parabuthus leiosoma*

(family: buthidae) from Egypt, supports the venom-metering hypothesis in scorpions. Al-Azhar Bull. Sci 23, 61–71.
Sarhan, M.M.H., Sayed, A.B., Mostafa, M.A., Yasin, A.E., 2013. Prey-capture behaviour of the Egyptian scorpion *Scorpio maurus palmatus* (Ehrenberg, 1828) (Scorpiones: Scorpionidae). Serket 13, 201–210.
Smyth, J.D., Barrett, N.J., 1980. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans. R. Soc. Trop. Med. Hyg. https://doi.org/10.1016/0035-9203(80)90157-1.
Tawfik, R.A., 2018. In vitro scolicidal fffect bf bee Venom on Echinococcus Granulosus protoscolices. J. Egypt. Soc. Parasitol. https://doi.org/10.1017/CBO9781107415324.004.
Thompson, R.C.A., 2017. Biology and Systematics of Echinococcus. Adv. Parasitol. https://doi.org/10.1016/bs.apar.2016.07.001.
Vidoura, A., Parisidou, M., Chatedaki, C., Zacharouli, D., 2017. Surgical Management of Hydatid Disease. In: Echinococcosis. https://doi.org/10.5772/intechopen.70136.
Xu, Z.M., Li, Z.S., Wen, M.X., Peng, R.Y., Sun, L., Wu, X.Y., Zhou, L.X., Tao, Y.P., Yang, L., 2008. In vitro effect of medicinal scorpion on the larvae of Ancylostoma caninum. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi.
Yan, R., Zhao, Z., He, Y., Wu, L., Cai, D., Hong, W., Wu, Y., Cao, Z., Zheng, C., Li, W., 2011. A new natural α-helical peptide from the venom of the scorpion Heterometrus petersii kills HCV. Peptides. https://doi.org/10.1016/j.peptides.2010.10.008.
Yones, D.A., Taher, G.A., Ibraheim, Z.Z., 2011. In vitro effects of some herbs used in Egyptian traditional medicine on viability of protoscolices of hydatid cysts. Korean J. Parasitol. https://doi.org/10.3347/kjp.2011.49.3.255.