IJBPAS, September, 2015, 4(9): 5980-5989

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF ANTIBACTERIAL EFFECT OF TARANTULA CUBENSISVENOME (THERANEKRON)

ARMIN GHASEMI-DIZGAH[1], NOUR AMIRMOZAFARI[2*]

[1]Department of Microbiology, Faculty of Science, Islamic Azad University of Lahijan, Lahijan, Iran (armin.ghasemidizgah@gmail.com)

[2]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

***Corresponding Author: amirmozafari@yahoo.com**

ABSTRACT

Venom of *Tarantula cubensis* as a homeopathic medicine has shown an anti-inflammatory and antitumor effect that was effective as in healing animal wounds and tumors. Some reports hinted it to having an antimicrobial activity as well. The aim of this study was to assess the antibacterial effect of *Tarantula cubensis* venom.

E.coli, *S. aureus* and *P. aeruginosa* bacteria were used. The bacteria were treated with different concentration of alcoholic extract of *Tarantula cubensis* (Theranekron) for various periods. Antibacterial effect was investigated by performing disc diffusion assay and determining of optical densityof bacterial cultures.

As result, no inhibitory effect of The ranekron on the bacterial growth was detected.

We concluded that the venom might have not significant antibacterial property. Nevertheless, we encourage more studies to clarify.

Key words: Venom, Theranekron, Antibacterial, Disc Diffusion, *Tarantula cubensis*,*E. coli*, *S.aureus*,*P. aeruginosa*

INTRODUCTION

Several venoms from both vertebrate and invertebrate venomous animals, such as snakes, spiders, insects, centipedes and amphibians, have largely shown therapeutic

applications.[1]Spiders are the largest group of venomous animals with about50,000 extant species divided into 110 families that have experienced a long history of use in primitive medical practice.[2]The majority of spiders employs venom that offers complex lethal cocktail including polypeptides, polyamine neurotoxins, nucleic acids, free acids, biogenic amines, and inorganic ions and salts to rapidly subdue and digest their preythat cause a wide range of effects in both vertebrates and invertebrates.[3] Peptides are the major compound of spider venomswith a broad spectrum of bioactivities are directed against a wide variety of pharmacological targetsandshowed antimicrobial, antifungal, antiparasitic, antiarrhythmic, analgesic, cytolytic, haemolyticand antitumor activity.[3]Antimicrobial Peptides (AMPs) are a very diverse group of small proteins that may present antibacterial, antifungal, antiparasitic and antiviral activity.[4] So far, many AMPs have been found in spider venoms.The first report of an AMP was isolated from the spider*Lycosasingoriensis* venom has been published in 1989.[5] Lycotoxins I and II from *Lycosacarolinensis*,[6]LyeTx I from *Lycosaerythrognatha*[7] and cyto-insectotoxin 1a from *Lachesanatarabaevi*[8] were the recently founded AMPs identified in spider venoms.Most AMPs derived from spider venom cause cell lysis via acting on cell membrane.[4]Bowman *et al*[9]demonstrated that peptide GsMTx4 from the venom of tarantula*Grammostolaspatulata* inhibits ion channels in cells. They discussthis peptide of spider venom can be used as a drug to treat atrial fibrillation, pain and muscular dystrophy.[9]Moreover, Gomesin is otherAMP and antitumor peptide isolated from hemocytes of Brazilian spider *Acanthoscurriagomesiana* that has been induced mammalian cell death through disruption of the cell membrane by regulation of cellular calcium influx, death signaling modulators and generation of reactive oxygen species.[10,11]

Tarantula cubensis, which may be called the MygaleCubensis and the Cuban Tarentula is natively found in Cuba and Mexico described as a large arachnid belonging to the Theraphosidae family of spiders.[2] Venom of *Tarantula cubensis* is chemically very potent and is mixture of many different toxins and digestive enzymes. The allergic reaction caused by the venom of *Tarantula cubensis* may cause many complications as aftermath like angeioneurodema, swelling, inflammation and severe allergic reaction of high magnitude. It may also cause permanent death of tissue by causing necrosis and septic

conditions and even in some predisposed and allergic subjects prove lethal and fatal. Evidences revealed that an alcoholic extract of*Tarantula cubensis* with commercial name Theranekronhas been used as a homeopathic drug in veterinary medicine. It was reported to be effective in stopping growth of canine mammary tumors,[12,13] treatment of pododermatitis,[14]bovine and canine cutaneous papillomatosis,[15,16] chronic endometritis in cows and rats[17,18]and in the treatment of foot and mouth disease lesions.[19] In addition, it reduced inflammation and tarsal bursitis volume and stimulated epithelialization in the full thickness cutaneous wounds.[20]The Venom of *Tarantula cubensis* also prevented retention secundinarium, improved uterine involution and treated the genital microbial diseases[21]and oral lesions in cattle.[22]Although,the venom of *Tarantula cubensis*wasalleged to have been used successfully to treat several ailments ranging from infections to cancers, further investigations are needed to clarify the proposed approaches. The aim of this study was to assessthe antibacterial effect of *Tarantula cubensis*venom (Theranekron).

MATERIALS AND METHODS

Theranekron

Alcoholic extract of *Tarantula cubensis* with trade name Theranekron was purchases from Richter Pharma AG (Wels, Austria).

Bacteria strains and culture

Escherichia coli strain K-12 (#10798), *Staphylococcus aureus* strain Seattle1945(#25923) and *Pseudomonas aeruginosa* strain PA01 (#47085) were purchased from American Type Culture Collection (ATCC; Manassas, VA).The bacteria from stock cultures were pre-cultured in Mueller Hinton broth (BD, New Jersey, USA) at 37°C with shaking for 16 hours prior to use.

Evaluation of Antibacterial effects

The bactericidal effect of the extracts was evaluated with the modified Kirby-Bauer disk diffusion method.[23]The bacteria cultures were grown in MuellerHinton broth medium overnight at 37°C. Optical density was compared with a 0.5 tube on the McFarland scale (10^6 UFC/ml). Using a sterilized swab, aliquots from each strain were spread on dishes with Mueller Hinton agar extract was added and incubated at 37 ºC for 24 hours. Subsequently, with the aid of a moist sterile swab the suspensions filter paper discs (6 mm in diameter) which had previously been sterilized in an oven at 100 °C for two hours, were saturated either with concentration of 100, 250 and 500 µg/ml of

Theranekron (50 µl) were placed on surface of each inoculated plate. To evaluate the efficiency of the methodology, each concentration was inserted simultaneously in a hole made (50 µl) in new plates. Discs separately soaked with distilled water and absolute ethanol was used as negative control and 10 mcg/disc ampicillin, 10 units/disc penicillin and 10 mcg/disc imipenem discs (BD, New Jersey, USA) were used as positive controls respectively for *E. coli*, *S. aureus* and *P. aeruginosa*. The plates were incubated at 37 °C for 24 hours. After incubation, inhibition haloes were measured to determine which concentrations of the extracts inhibited bacterial growth. Overall, cultured bacteria with halos equal to or greater than 6 mm were considered susceptible to either the tested extract or controls. In order to confirm the disc diffusion results we tested the extract in liquid bacterial culture. For this 100 µl (10^6 CFU/ml) of each bacterial culture in MuellerHinton broth were added in a 96-well flat-bottomed micro-plates after which appropriate volume of Theranekron or antibiotics were added to the wells. Distilled water and absolute ethanol were separately used as negative control and 100µg/ml of ampicillin, 10 units of penicillin and 10 µg/ml of imipenem (BD, New Jersey, USA) were used as positive controls respectively for *E. coli*, *S. aureus* and *P. aeruginosa*. Pre-incubation absorbance in 600 nanometer was read from a micro-plate reader (BioTek®, Winooski, USA). The micro-plates were then incubated in 37 ℃ for overnight and absorbance values were read in same wavelength. The actual absorbance value was obtained after subtracting the value read from the absorbance of blank well.

Statistical analysis

The statistical significances were considered applying two-tailed student's t test and analysis of variance (ANOVA). $P<0.05$ was considered as statistically significant.All calculation of numerical data was done using GraphPad Prism (version 5.0) software and the image basedones were analyzed using ImageJjavascript (NIH).

RESULTS

The effect of *Tarantula cubensis* venom on growth of *E. coli*, *S. aureus* and *P. aeruginosa*were investigated using disk diffusion method.As a result, there was not detected growth inhibition halo around the paper discs soaked with 100, 250 and 500 µg/ml of Theranekron on *E. coli* ($P>0.05$),*S. aureus* ($P>0.05$) and *P. aeruginosa*($P>0.05$) culture agars in comparison with negative control. In addition, no significant inhibition halo was detected around the paper discs

soaked with distilled water and ethanol on the all three bacteria culture agars. However, significant bacterial growth inhibition halo were detected around the antibiotic discs. The mean of halo sizes for *E. coli*,*S. aureus*and*P. aeruginosa*respectively observed as 6.7 mm (P<0.0001), 6.3 mm (P<0.0001) and 5.3 mm (P<0.0001).

In order to verify disk diffusion results and eliminate possible adverse effects of agarose on the venom, we tested the same concentration of Theranekron described as above, on the bacterial growth liquid culture medium. The optical density of bacterial suspension was measured in 600 nanometer wavelength. Results as shown in Figure 1 indicated high bacterial density in*E. coli*(P>0.05),*S. aureus*(P>0.05) and also*P. aeruginosa*(P>0.05) cultures each treated with 100, 250 and 500 µg/ml of Theranekron compared with negative controls. Similarly,there was not observed inhibitory effect of ethanol on the growth of the all three strains(Figure 1). In contrast, bacterial density of*E. coli* (P<0.0001),*S. aureus* (P<0.0001) and *P. aeruginosa*(P<0.0001)suspensions that were cultured in presence of respectively ampicillin, penicillin and imipenem as positive controls, were significantly lower than Theranekron treated cultures as well as negative controls (Figure 1). These results indicated that the venom did not show inhibitory effect on the growth of *E. coli*,*S. aureus* and *P. aeruginosa*.

DISCUSSION

The employing of living organisms or their products such as venom for medical purposes is as old as history. Some therapies such as leech therapy, snake and bee venom therapy can be traced back thousands of years. It was more than half a century ago to begin exploring the remarkable pharmacological diversity of spider venoms that began development quite recently. There is identified that spider venom represents a rich source of bioactive with pharmacologic and therapeutic potential.[3-5]In particular, the venom of*Tarantula cubensis*has been pharmacologically characterized as useful homeopathic medicine in the treatment of wide range of conditions such as animal wounds and tumors. Homeopathy, also known as homeopathic medicine, is an alternative medical system that was developed by Samuel Hahnemann in 1796, based on his doctrine of like cures like the notion that a disease can be cured by a substance that produces similar symptoms in healthy people.[19-22]

In the current study, we also examined the antibacterial activity of *Tarantula*

*cubensis*venom on *E.coli*, *S. aureus* and *P. aeruginosa*. The disc diffusion test results and assessment of bacterial growth under treatment with the venom showed that the venom did not indicate any inhibitory effect on the bacterial growth. This isthe only study that has examined antibacterial effect of *Tarantula cubensis* extract. First evidence regarding antimicrobial activity of the venom of *Tarantula cubensis*has been reported in 1976. May[24] observed that the cattle with wound treated with the venom had not required antibiotic therapy in order to prevent and cure the secondary infection. Habibian*et al*[25] investigated histophatological influence of *Tarantula cubensis*venom in rats and found that the venom ceased inflammation by arresting chemotaxis of immune cells to the wound. They suggested that absence of secondary infection in the wound of rats treated with the venom could be due to its antimicrobial activity.[20] In addition, another report discussed the possible antimicrobial effect of *Tarantula cubensis*venom and its role in decreasing pathological vaginal exudations and accelerating uterus involution.[21] These reports hinted to antimicrobial property of *Tarantula cubensis*venom even though this property of the venom has not been reported before. In fact, several AMPs have been indentified in wild range of venoms from different animals and are linked to antimicrobial effects. Antimicrobial peptides revealed an activity against gram-negative and gram-positive bacteria, fungi, parasites, some viruses like HIV and HSV and even cancer cells. The main action mechanism of AMPs involves their ability to cause cell membrane damage that is described in terms of several proposed models such as “barrel-stave” model, “toroidal” model and “carpet” model.Recently it has been proposed that AMP driven microbial death can be caused by others mechanisms in addition to membrane targeting followed by intracellular targets inducing cell lysis, such as the disruption of cell wall, DNA, RNA, and protein synthesis.[4]Based on the results of this study, the venom of *Tarantula cubensis* did not show antibacterial effect on *E.coli* and *P. aeruginosa* as two major gram-negative bacteria as well as *S. aureus* as a gram-positive bacterium. These species are the most common bacteria that can cause disease in animals, including humansare many qualifications to represent the pathogenic bacteria. Contrary to the ineffectiveness of the venom to inhibit the growth of bacteria, here, we witnessed that antibiotics that were used as positive controls, were quite effective on the. Thus,

we could eliminate any false negative outcomes. In short, our results do not support the antibacterial potency of *Tarantula cubensis*venomdespiteof its anti-inflammatory and antitumor activity and its effectiveness in the healing of animal wounds.

CONCLUSION

We concluded that the venom of *Tarantula cubensis* that is commercially available under the name Theranekron was not lethal to bacteria. We concluded that the venom of *Tarantula cubensis*does not have antibacterial activity at least on the strains were examined here however; further investigations are needed to clarify.

REFERENCES

[1] King GF. Venoms as a platform for human drugs: translating toxins into therapeutics. Expert OpinBiolTher. 2011 Nov;11(11):1469-84.

[2] Platnick NI. The World Spider Catalog, Version 15. American Museum of Natural History. http://platnick.sklipkani.cz (accessed on December 24, 2014).

[3] Saez NJ, Senff S, Jensen JE, Er SY, Herzig V, Rash LD, et al. Spider-venom peptides as therapeutics. Toxins (Basel). 2010 Dec;2(12):2851-71.

[4] Guilhelmelli F, Vilela N, Albuquerque P, DerengowskiLda S, Silva-Pereira I, Kyaw CM. Antibiotic development challenges: the various mechanisms of action of antimicrobialpeptides and of bacterial resistance. Front Microbiol. 2013 Dec 9;4:353.

[5] Xu K, Ji Y, Qu X. Purification and characterization of an antibacterial peptide from venom of lycosasingoriensis. ActaZoologicaSinica. 1989; 35(3): 300-5

[6] Yan L, Adams ME. Lycotoxins, antimicrobial peptides from venom of the wolf spider Lycosa carolinensis. J Biol Chem. 1998 Jan 23;273(4):2059-66.

[7] Santos DM, Verly RM, Piló-Veloso D, de Maria M, de Carvalho MA, Cisalpino PS, et al. LyeTx I, a potent antimicrobial peptide from the venom of the spider Lycosaerythrognatha. Amino Acids. 2010 Jun;39(1):135-44.

[8] Lazarev VN, Shkarupeta MM, Polina NF, Kostrjukova ES, Vassilevski AA, Kozlov SA, et al. Antimicrobial peptide from spider venom inhibits Chlamydia trachomatis infection at an

early stage. Arch Microbiol. 2013 Mar;195(3):173-9.

[9] Bowman CL, Gottlieb PA, Suchyna TM, Murphy YK, Sachs F. Mechanosensitive ion channels and the peptide inhibitor GsMTx-4: history, properties, mechanisms and pharmacology. Toxicon. 2007 Feb;49(2):249-70.

[10] Soletti RC, del Barrio L, Daffre S, Miranda A, Borges HL, Moura-Neto V, et al. Peptide gomesin triggers cell death through L-type channel calcium influx, MAPK/ERK, PKC and PI3K signaling and generation of reactive oxygen species. ChemBiol Interact. 2010 Jul 30;186(2):135-43.

[11] Paredes-Gamero EJ, Casaes-Rodrigues RL, Moura GE, Domingues TM, Buri MV, Ferreira VH, et al. Cell-permeable gomesin peptide promotes cell death by intracellular Ca(2+) overload. Mol Pharm. 2012 Sep 4;9(9):2686-97.

[12] Koch H, Stein, M. Konservativebehandlung von neoplasmen der milchdruse des hundesmitTheranekron. PraktischeTierarzt. 1980; 61:424 –30.

[13] Gultekin N, Vural MR. The effect of tarantula cubensis extract applied in pre and post-operative period of canine mammary tumors. J Istanbul Vet Sci. 2007;2(1):13-23

[14] Stampa S. A field trial comparing the efficacy of sulphamonomethoxine, penicillin, and tarantula poison in the treatment of pododermatitiscircumspecta of cattle. J S Afr Vet Assoc. 1986;57(2):91-3.

[15] Cam Y, Kibar M, Atasever A, Alalay O, Beyaz L. Efficacy of levamizole and Tarantula cubensis venom for the treatment of bovine cutaneous papillomatosis. Vet Rec. 2007;160(14):486-8.

[16] Icen H, Sekin S, Simsek A, Kochan A, TunikS.The Efficacy of Tarantula cubensis Extract (Theranekron) in Treatment of Canine Oral Papillomatosis. Asian Journal of Animal and Veterinary Advances. 2011;6:744-9.

[17] Enbergs H, Sensen B: Effectiveness of a homeopathic treatment of chronic endometritis in dairy cows. PraktTierarzt. 2007; 88:534-7.

[18] Dolapcioglu K, Dogruer G, Ozsoy S, Ergun Y, Ciftci S, SoyluKarapinar O, et al. Theranekron for treatment of endometriosis in a rat model compared with medroxyprogesterone acetate and

leuprolide acetate. Eur J ObstetGynecolReprod Biol. 2013 Sep;170(1):206-10.

[19] Lotfollahzadeh S, Alizadeh MR, Mohri M, MokhberDezfouli MR. The therapeutic effect of Tarentulacubensis extract (Theranekron®) in foot-and-mouth disease in cattle: a randomised trial in an endemic setting. Homeopathy. 2012 Jul;101(3):159-64.

[20] Sardari K, Kakhki EG, Mohri M. Evaluation of wound contraction and epithelialization after subcutaneous administration of Theranekron in cows. CompClinPathol. 2007;16(3):197-200.

[21] Kacar C, Zonturlu AK, Oral H, Yildiz S, Ari UC. The effects of Theranekron application on uterus involution and vaginal effluence in cows early puerperal period. KafkasUniv Vet FakDerg. 2007;13(1):11-5.

[22] Albay MK, Sahinduran S, Kale M, Karakuram MC, Sezer K. Influence of Tarantula cubensis Extract on the Treatment of the Oral Lesions in Cattle with Bluetongue Disease. KafkasUniv Vet FakDerg. 2010;16(4):593- 6.

[23] Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J ClinPathol. 1966;45:493-6.

[24] May J. Treatment of panaritium in cattle with Theranekron. Wein. Tieraztl. Monatsschr. 1976; 6: 228-31.

[25] HabibianDehkordi S, Nourian H, Sharifi S. Ahadian M. Taghadosi C. AkhavanTaheri M. Evaluation of Anti-Inflammatory Effects of Theranekron in Rat. Iranian Journal of Veterinary Clinical Sciences. 2009; 2:9-13.

Table 1: Average zone of inhibition of 24-hour disk diffusion test. dark lines indicate the absence of halo.

	Ethanol (Et)	Theranekron (µg/ml)			Distilled water(dw)	Antibiotic
		500	250	100		
E. coli	-	-	-	-	-	13.5(2)
S. aureus	-	-	-	-	-	12.8(1)
P.aeruginosa	-	-	-	-	-	10.5(3)

Table2: Averagevalues ofOD600microbialsuspensionstreated withvariousagentsfor 24hours.

	Ethanol (Et)	Theranekron (µg/ml)			Distilled water(dw)	Antibiotic
		500	250	100		
E. coli	6.847	6.540	6.410	6.487	7.02	0.893
S. aureus	6.497	6.643	6.953	7.130	7.153	0.730
P.aeruginosa	6.143	6.110	6.023	6.120	6.283	0.673

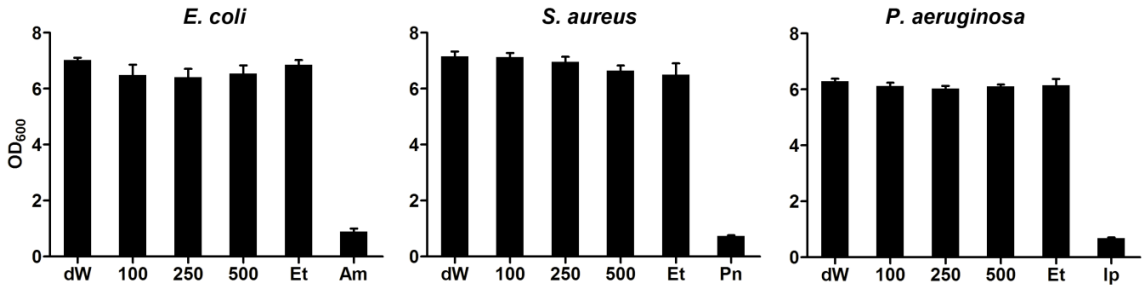

Figure 1: Inefficiency of *Tarantula cubensis* venom on bacterial growth. *E. coli, S. aureus* and *P. aeruginosa* were separately cultured in Mueller Hinton broth containing 100, 250 and 500 µg/ml of Theranekron for 24 hours and then optical density of bacterial culture was measured in 600 nanometer wavelength. Distilled water (dW) and absolute ethanol (Et) were used as negative controls and100 µg/ml of ampicillin (Am), 10 units of penicillin (Pn) and 10 µg/ml of imipenem (Ip) were used as positive control respectively for *E. coli, S. aureus* and *P. aeruginosa*. Result showed no inhibitory effect of the venom on the growth of the bacteria in comparison with negative controls while significant inhibition was detected in the antibiotics bacteria. P values are not indicated.