

PnPP-19, A Spider Toxin Derivative: New Hope for the Treatment of Sexual Dysfunction?

Silva CN, Almeida FM and de Lima ME*

Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

*Corresponding author: de Lima ME, Laboratório de Venenos e Toxinas Animais, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31.270-901, Belo Horizonte, MG-Brazil, Tel: 00-55-31-3409-2659; E-mail: melenalima@icb.ufmg.br, lima.mariaelena@gmail.com

Rec date: May 2, 2016; Acc date: May 10, 2016; Pub date: May 17, 2016

Copyright: © 2016 de Silva CN, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The incidence of erectile dysfunction (ED) is increasing every year worldwide, and affects primarily those patients that suffer from vascular diseases, such as diabetes and hypertension. There is an increasing number of patients for whom first-line and second-line pharmacotherapy are not indicated. Animal venoms and toxins have been envisaged as pharmacological tools that enable elucidating the mechanisms that lead to erectile function, as well as give hints for drug development to treat ED. Recent studies have demonstrated the promising action of the synthetic peptide PnPP-19, derived from a spider toxin (PnTx2-6), on erectile function. This peptide potentiates erection at 4 and 8 Hz *in vivo* and *ex vivo*. It shows no apparent toxicity and presents low immunogenicity to mice. It does not affect sodium channels or rat hearts. Therefore, PnPP-19 may emerge as a new pharmacological tool and shows favorable properties as a bioactive molecule in the treatment of ED.

Keywords: PnPP-19; Erectile function; PnTx2-6

Introduction

Historically, ED was first treated through intra-cavernosal/intra-urethral administration of vasoactive drugs or through surgical interventions. The discovery of nitric oxide (NO), in the late 20th century, as the main actor in corpus cavernous smooth muscle relaxation and the most important peripheral neurotransmitter in erectile function, led to the development of drugs that interfered in this mechanism. Among them, phosphodiesterase-5-inhibitors (PDE5I), which could be orally administered, revolutionized the sexual medicine field [1,2]. Today, first-line therapy for the treatment of ED comprise orally and sublingually administered drugs, while intra-cavernosal injection therapy is considered only as a second-line therapy [3].

Erectile Dysfunction

Erectile dysfunction (ED) adversely affects the life of millions of men, and is the most commonly treated sexual disorder today. ED is defined as a persistent inability to maintain or achieve a penile erection that allows sexual intercourse [4]. This comorbidity is commonly a result of the interaction of various physiologic disorders, which can have vasculogenic, neurogenic or endocrinologic basis. In addition, ED can be induced by medication or substance consumption, poor health, cardiovascular disorder, or physical disorders, as penile rupture [5]. Psychogenic causes, related to trauma from previous sexual experiences and low self-confidence may contribute to ED [6]. Patients with cardiovascular risk factors (hypertension, diabetes, hyperlipidemia, sedentariness, tabagism) and vascular abnormalities for penile blood supply present high incidence of ED [7]. According to a recent study, more than 30% of the European men surveyed, at the age of 60 or older, were affected by ED [8]. In the USA, 18.4% of adult

men reported ED and, in Brazil, the prevalence of ED was 45.1% [9,10].

The erectile process has been extensively investigated, leading to the elucidation of many of the complex molecular pathways involved [6]. These findings have enabled the design and development of drugs that target various aspects of this complex process.

Current Treatments for Erectile Dysfunction

Although PDE5I has been widely used, there is a growing need for alternative pharmacotherapy for the treatment of ED, as a result of the rise in the number of elderly people, and the recognition that a great number of ED patients do not respond to PDE5I [11].

Venoms and Toxins Active on Erectile Function: New Perspectives for the Treatment of ED

The earliest reports on erectile dysfunction treatment that are found in ancient medical literature prescribe the use of herbals and natural ingredients. Nowadays, many herbal supplements claim therapeutic effects in male sexual dysfunction [12,13]. Additionally, other substances that have been scientifically investigated over the last 20 years comprise toxins from animal venoms, mainly from scorpions and spiders [14].

Arthropod venoms have attracted attention for the development of new therapeutic drug models. Interestingly, some animal venom peptides, especially from spiders and scorpions, can cause priapism (a persistent and painful penile erection not dependent on sexual stimulation) [15]. Many studies using experimental models have shown the ability of these peptides to promote priapism and/or erection, envisaging the development of bioactive molecules to treat and/or study ED [14,16]. These molecules evoke complex effects on ion channels, chiefly sodium, potassium and calcium channels [17-19]. For

example, crude *Tityus serrulatus* venom and some of its purified toxins cause cavernosal smooth muscle relaxation *in vitro*, using rabbit and human samples [20,21]. Similarly, *Androctonus australis* and *Buthotus judaicus* scorpion venoms also relax cavernosal tissue [22].

Spider venoms and some of their purified proteins, peptides or biologically active molecules selectively target a variety of vital physiological functions, being described in the development of drugs for various applications [19,23-25]. For example, *Phoneutria nigriventer* crude venom contains potent excitatory neurotoxins [19,26,27]. Among these toxins, PnTx2-5 and PnTx2-6, which are 89% similar in their primary structure, also renamed δ -CNTX-Pn1b and δ -CNTX-Pn1c, respectively, were identified as directly responsible for priapism [25,28]. Since then, many studies have been published trying to solve the mode of action of these toxins in erection. Both toxins were described as targeting site 3 from sodium channels, slowing down the sodium current [29]. The mode of action through which these spider and scorpion toxins induce priapism in rats and mice involves the nitric oxide pathway. The involvement of nitric oxide synthase (NOS) was elucidated by using a nonselective NOS inhibitor, N(omega)-nitro-L-arginine methyl ester (L-NAME), which decreased the effect of the toxins on priapism [14,25,28].

Upon sexual stimulation, NO is released and induces relaxation of the penile smooth muscle, increases blood flow to the penile region and augments intracavernosal pressure, leading to penile erection. The NO released from penile endothelial cells or nitrergic nerves is the main mediator involved in erectile function, and PnTx2-6 injection was shown to increase NO release in corpus cavernous tissue [25]. After PnTx2-6 treatment, a microarray study using mice penile tissue showed differential expression of 10.4% of the genes involved in the NO pathway, shedding light to the effect of this toxin on the NO pathway [30]. In addition, PnTx2-5, when intra-peritoneally injected in male mice, caused penile erection and hypersalivation, severe respiratory distress and death. L-NAME partially prevented these effects and nNOS-selective inhibitor 7-Nitroindazole completely abolished them, suggesting that nNOS is the major player in the induction of erection by these toxins [19,28]. Both toxins, PnTx2-5 and PnTx2-6, are interesting pharmacological tools to study ED. PnTx2-5 has been less investigated compared to PnTx2-6, but data suggest that this toxin could also be involved in penile neuronal depolarization [28]. The mechanism of action whereby PnTx2-5 and PnTx2-6 promote cavernosal relaxation and enhance erectile function is not completely elucidated. However, the results strongly suggest that it is via NO/Cyclic Guanosine Monophosphate (cGMP) pathway [28,31].

Experiments on cavernosal strips using the pharmacological inhibitor μ -conotoxin GVIA (N-type calcium channel blocker) and knockout mice (nNOS^{-/-}, eNOS^{-/-}) suggested that the relaxation promoted by PnTx2-6 depends on N-type Ca²⁺ channels in nitrergic nerve endings, which are the main source of NO release during erection [31,32]. Thus, taking into account the action of PnTx2-6 on sodium channels, slowing down their inactivation and maintaining cell depolarization, this condition has been suggested to promote the entrance of a higher amount of Ca²⁺ into the cell, which activates nNOS and consequently increases NO availability, leading to penile erection. Additionally, the cavernosal relaxation evoked by PnTx2-6 is independent on phosphodiesterase 5 (PDE5) inhibition and this toxin restores the erectile function in hypertensive (DOCA Salt), diabetic, and elderly rats, in different proportions and prevents muscle atrophy in a rat model [25,31,33,34].

However, PnTx2-6 shows dose-dependent side effects, such as intense vascular congestion in kidney, liver, lungs and myocardium, discrete brain edema [35] and prolonged pain (unpublished data). Furthermore, obtaining large amounts of PnTx2-6 is challenging and its chemical synthesis is complex, because it contains 5 disulfide bridges. Its expression in heterologous systems is possible but costly [36,37].

The Promising PnPP-19 Peptide

PnPP-19 is a short peptide (19 amino acids) designed *in silico* from the tertiary structure of PnTx2-6 and represents a discontinuous primary structure of the epitope of this toxin. PnPP-19 supposedly represents the active core of the PnTx2-6, bearing the amino acids described as responsible for the specific interaction of the native toxin with site 3 of sodium channels [29,37]. A molecule with a shorter sequence than PnTx2-6 is expected to bring many advantages: higher specificity, lower toxicity, lower immunogenicity, lower cost and simpler synthesis. PnPP-19 peptide can be easily synthesized and, similarly to the native toxin PnTx2-6 at the same molar doses, potentiates erectile function at low frequencies *in vivo* (anesthetized rats) and *ex vivo* (cavernous strips) [37]. To illustrate this effect, Figure 1 shows a representative graph of the effect of PnPP-19 (10 nM) on rat corpus cavernous strips. Surprisingly, it does not act on any sodium channel subtypes that have been tested [37].

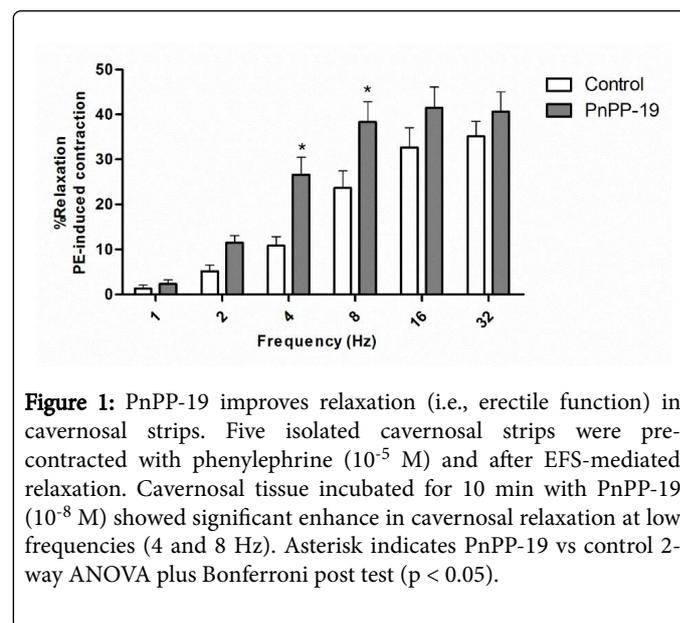


Figure 1: PnPP-19 improves relaxation (i.e., erectile function) in cavernosal strips. Five isolated cavernosal strips were pre-contracted with phenylephrine (10⁻⁵ M) and after EFS-mediated relaxation. Cavernosal tissue incubated for 10 min with PnPP-19 (10⁻⁸ M) showed significant enhance in cavernosal relaxation at low frequencies (4 and 8 Hz). Asterisk indicates PnPP-19 vs control 2-way ANOVA plus Bonferroni post test (p < 0.05).

According to the literature, the use of PDE5Is for the treatment of ED is a very attractive and effective therapy, with few side effects. However, PDE5I therapy does have limitations, especially for patients with active coronary ischemia, congestive heart failure, borderline low blood volume and pressure status, patients with multidrug antihypertensive therapy regimens and those who may need organic nitrates for the treatment of angina [7]. In addition, its effect requires intact NO relaxing nerve fibers and healthy cavernous endothelium, what impairs 30% to 35% of patients from responding to the treatment, stimulating the search for alternative drugs [38,39]. Although PnTx2-6 effectively potentiates erectile function *in vivo*, this toxin acts on isolated rat heart and slows down sodium currents in rat cardiomyocytes, besides acting on other sodium channel-subtypes

distributed in other organs. In contrast, PnPP-19 does not show effect on rat cardiomyocytes or on isolated heart, even at high concentrations (37.8 nM to 37.8 μ M) [37].

The NO relaxation response elicited by PnPP-19 is mainly dependent upon cGMP, synthesized by soluble Guanylyl Cyclase (sGC). Deficiency in NO/cGMP erection pathway is associated with many diseases. PnPP-19 increased cGMP levels in cavernous preparations in the presence of EFS (8 Hz). This effect was inhibited by L-NAME and partially prevented by 7-NI, suggesting that it partly depends on neuronal NOS activity [37]. The authors believe that it is possible that other types of NOS, besides neuronal NOS, such as endothelial or inducible NOS, may be involved in PnPP-19-mediated erectile potentiation. Functional experiments on cavernosal strips using the pharmacological inhibitor ω -conotoxin GVIA (1 μ M) suggested that the relaxation promoted by PnPP-19 does not depend on N-type Ca²⁺ channels in nitrenergic nerve endings, as previously observed with the native toxin PnTx2-6. The involvement of other subtypes of calcium channels and intracellular calcium must be checked.

Given the potential use of PnPP-19 as a drug to treat erectile dysfunction and the lack of information concerning its effect in the nociceptive pathway, the effects of PnPP-19 on nociception was determined. Recent studies of our group showed that this peptide exhibited anti-nociceptive activity, mediated by activation of both opioid and cannabinoid receptors in the peripheral nervous system and this effect appears to involve the inhibition of a neutral endopeptidase (NEP) [40]. Histopathological assays with PnPP-19 showed no signs of toxicity like vascular congestion, edema, cellular necrosis, cytoplasmic vacuolation or nuclear condensation in kidney, heart, liver, lung and brain [37]. PnPP-19 also demonstrated low immunogenicity, even at extremely high concentrations (5 mg/kg) [37]. Table 1 presents a comparison between the effects of the native toxin PnTx2-6 and PnPP-19, on erectile function, in rats.

	PnTx2-6	PnPP-19
Erectile function effect	improves	improves
Dose	10 nM	10 nM
Effects on sodium channels	Yes	No
Action on isolated rat heart	Yes	No
Side effects	Yes	Not observed
Immunogenicity	Not tested	low

Table 1: A brief comparison between the native toxin PnTx2-6 and the synthetic peptide PnPP-19.

Studies are in progress to check the efficacy of PnPP-19 on rodent animal models for ED. Preliminary experiments (unpublished data) suggest that this peptide is able to improve the impaired erectile function in spontaneously hypertensive rats (SRH). These data encourages and reveals the potential use of PnPP-19 as a pro-erectile drug model, showing promising features for erectile dysfunction treatment.

The treatment of erectile dysfunction with topical formulations has been envisaged as a tendency for the next decades, enabling a comfortable application, with possibly less systemic side effects. Some authors have reported topical formulations for the treatment of erectile

dysfunction [41,42]. Recent results of our group have shown that PnPP-19 is able to permeate human skin, enabling the development of a formulation for its topical application. Nano-formulations have been tested, aiming at protecting the peptide from possible degradation and favoring its topical administration (unpublished results).

Nowadays, the treatment of ED is expected to involve drugs that show predictable time of onset and efficacy, are well accepted and easily administered, with few side effects. Studies are in progress to verify the stability of the peptide and it seems to be stable *in vivo*, with a long half-life, since its potentiating effect was observed 15 minutes after intra-peritoneal injection in rats and lasted approximately 1 hour [37]. Besides, PnPP-19 shows no apparent toxicity and low immunogenicity. Furthermore, the synthesis of the peptide is easy and presents a good cost-value, what makes it an excellent and promising candidate for the treatment of erectile dysfunction.

Conclusions

PnPP-19, a synthetic peptide designed from the tertiary structure of a *Phoneutria nigriventer* toxin (PnTx2-6), is able to stimulate erection through NO/cGMP pathway. As it shows a new mode of action, it can be used as an alternative drug to treat ED, especially for those patients that do not respond to the first-line treatment. This peptide is stable and easily synthesized, shows no apparent toxicity and low immunogenicity and is able to permeate skin, being a good candidate for the development of topical formulations in the treatment of ED.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgement

Fellowships were awarded by the Brazilian Agencies FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and INCTTOX (Instituto de Ciência e Tecnologia em Toxinas).

References

1. Trigo-Rocha F, Aronson WJ, Hohenfellner M, Ignarro LJ, Rajfer J, et al. (1993) Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. *Am J Physiol* 264: H419-422.
2. Albersen M, Shindel AW, Mwamukonda KB, Lue TF (2010) The future is today: emerging drugs for the treatment of erectile dysfunction. *Expert Opin Emerg Drugs* 15: 467-480.
3. Davis JE, Nunes KP, Stallmann-Jorgensen I, Webb RC (2012) Current perspectives on pharmacotherapy treatments for erectile dysfunction. In: Nunes KP (ed.) *Erectile dysfunction- disease- associated mechanisms and novel insights into therapy*. InTech Europ, Croatia, 1: 146-157.
4. NIH Consensus Conference (1993) Impotence. NIH Consensus Development Panel on Impotence. *JAMA* 270: 83-90.
5. Shamloul R, Ghanem H (2013) Erectile dysfunction. *Lancet* 381: 153-165.
6. Peak TC, Yafi FA, Sangkum P, Hellstrom WJ (2015) Emerging drugs for the treatment of erectile dysfunction. *Expert Opin Emerg Drugs* 20: 263-275.
7. Evans JD, Hill SR (2015) A comparison of the available phosphodiesterase-5 inhibitors in the treatment of erectile dysfunction: a focus on avanafil. *Patient Prefer Adherence* 9: 1159-1164.

8. Corona G, Lee DM, Forti G, O'Connor DB, Maggi M, et al. (2010) Age-related changes in general and sexual health in middle-aged and older men: results from the European Male Ageing Study (EMAS). *J Sex Med* 7: 1362-1380.
9. Lindau ST, Schumm LP, Laumann EO, Levinson W, O'Muirheartaigh CA, et al. (2007) A study of sexuality and health among older adults in the United States. *N Engl J Med* 357: 762-774
10. Abdo CH, Oliveira WM Jr, Scanavino Mde T, Martins FG (2006) Erectile dysfunction: results of the Brazilian Sexual Life Study. *Rev Assoc Med Bras* 52: 424-429.
11. Hatzimouratidis K, Hatzichristou DG (2005). A comparative review of the options for treatment of erectile dysfunction: Which treatment for which patient? *Drugs*. 65: 1621-1650.
12. Abdillahi HS, Van Staden J (2012) South African plants and male reproductive healthcare: conception and contraception. *J Ethnopharmacol* 143: 475-480.
13. Shah GR, Chaudhari MV, Patankar SB, Pensalwar SV, Sabale VP, et al. (2012) Evaluation of a multi-herb supplement for erectile dysfunction: a randomized double-blind, placebo-controlled study. *BMC Complement Altern Med* 12: 155.
14. Nunes KP, Torres FS, Borges MH, Matavel A, Pimenta AM, et al. (2013) New insights on arthropod toxins that potentiate erectile function. *Toxicon* 69: 152-159.
15. Berger R, Billups K, Brock G, Broderick GA, Dhabuwala CB, et al. (2001) Report of the American Foundation for Urologic Disease (AFUD) thought leader panel for evaluation and treatment priapism. *Int J Impot Res* 13: S39-S43.
16. Nunes KP, Cardoso FL, Cardoso Jr HC, Pimenta AMC, De Lima ME (2009) Animal toxins as potential pharmacological tools for treatment of erectile dysfunction.
17. Lima ME, Pimenta AMC, Martin-Eauclaire MF, Zingali RB, Rochat H (2009) Animal toxins: state of the art. *Perspectives in health and biotechnology*. Belo Horizonte: Editora UFMG, 750.
18. Gomez MV, Kalapothakis E, Guatimosim C, Prado MA (2002) *Phoneutria nigriventer* venom: a cocktail of toxins that affect ion channels. *Cell Mol Neurobiol* 22: 579-588.
19. De Lima ME, Torres FS, Magalhães BLE, Freitas ACN (2015) Innovative perspectives for the therapeutic use of spider toxins" armadeira" *Phoneutria nigriventer* (Keyserling , 1891) in pain and erectile dysfunction. In *Biotechnology Applied to Health : Principles and Applications ; Resende RR , Soccol CR (eds)*. Blucher: São Paulo, Brazil, 1: 539-572.
20. De Lima ME, Figueiredo SG, Matavel A, Nunes KP, Silva CN, et al. (2016) *Phoneutria nigriventer* venom and toxins: A Review. In: Gopalakrishnakone P, Corzo G, De Lima ME, Diego-García E. (eds.) *Spider venom*. Springer Amsterdam, The Netherlands 1: 71-100.
21. Teixeira CE, Bento AC, Lopes-Martins RA, Teixeira SA, Von Eickstedt V, et al. (1998) Effect of *Tityus serrulatus* scorpion venom on the rabbit isolated corpus cavernosum and the involvement of NANC nitrergic nerve fibres. *Br J Pharmacol* 123: 435-442.
22. Fernandes de Oliveira J, Teixeira CE, Arantes EC, de Nucci G, Antunes E (2003) Relaxation of rabbit corpus cavernosum by selective activators of voltage-gated sodium channels: role of nitric oxide-cyclic guanosine monophosphate pathway. *Urology* 62: 581-588.
23. Teixeira CE, Teixeira SA, Antunes E, De Nucci G (2001) The role of nitric oxide on the relaxations of rabbit corpus cavernosum induced by *Androctonus australis* and *Buthotus judaicus* scorpion venoms. *Toxicon* 39: 633-639.
24. Escoubas P, Diochot S, Corzo G (2000) Structure and pharmacology of spider venom neurotoxins. *Biochimie* 82: 893-907.
25. Nunes KP, Costa-Gonçalves A, Lanza LF, Cortes SF, Cordeiro MN, et al. (2008) Tx2-6 toxin of the *Phoneutria nigriventer* spider potentiates rat erectile function. *Toxicon* 51: 1197-1206.
26. Cordeiro MN, Richardson M, Gilroy J, Figueiredo SG, Beirão PSL, et al. (1995) Properties of the venom from the South American "armed" spider *Phoneutria nigriventer* (Keyserling, 1891). *Toxin Reviews* 14: 309-326.
27. Richardson M, Pimenta AM, Bemquerer MP, Santoro MM, Beirão PS, et al. (2006) Comparison of the partial proteomes of the venoms of Brazilian spiders of the genus *Phoneutria*. *Comp Biochem Physiol C Toxicol Pharmacol* 142: 173-187.
28. Yonamine CM, Troncone LR, Camillo MA (2004) Blockade of neuronal nitric oxide synthase abolishes the toxic effects of Tx2-5, a lethal *Phoneutria nigriventer* spider toxin. *Toxicon* 44: 169-172.
29. Matavel A, Fleury C, Oliveira LC, Molina F, De Lima ME, et al. (2009) Structure and activity analysis of two spider toxins that alter sodium channel inactivation kinetics. *Biochemistry* 48: 3078-3088.
30. Villanova FE, Andrade E, Leal E, Andrade PM, Borra RC, et al. (2009) Erection induced by Tx2-6 toxin of *Phoneutria nigriventer* spider: expression profile of genes in the nitric oxide pathway of penile tissue of mice. *Toxicon* 54: 793-801.
31. Nunes KP, Cordeiro MN, Richardson M, Borges MN, Diniz SOF, et al. (2010) Nitric oxide-induced vasorelaxation in response to PnTx2-6 toxin from *Phoneutria nigriventer* spider in rat cavernosal tissue. *J Sex Med* 7: 3879-3888.
32. Nunes KP, Wynne BM, Cordeiro MN, Borges MH, Richardson M, et al. (2012) Increased cavernosal relaxation by *Phoneutria nigriventer* toxin, PnTx2-6, via activation at NO/cGMP signaling. *Int J Impot Res* 24: 69-76.
33. Nunes KP, Toque HA, Borges MH, Richardson M, Webb RC, et al. (2012) Erectile function is improved in aged rats by PnTx2-6, a toxin from *Phoneutria nigriventer* spider venom. *J Sex Med* 9: 2574-2581.
34. Jung AR, Choi YS, Piao S, Park YH, Shrestha KR, et al. (2014) The effect of PnTx2-6 protein from *Phoneutria nigriventer* spider toxin on improvement of erectile dysfunction in a rat model of cavernous nerve injury. *Urology* 84: 730.
35. Leite KR, Andrade E, Ramos AT, Magnoli FC, Srougi M, et al. (2012) *Phoneutria nigriventer* spider toxin Tx2-6 causes priapism and death: a histopathological investigation in mice. *Toxicon* 60: 797-801.
36. Torres FS, Silva CN, Lanza LF, Santos AV, Pimenta AM, et al. (2010) Functional expression of a recombinant toxin - rPnTx2-6 - active in erectile function in rat. *Toxicon* 56: 1172-1180.
37. Silva CN, Nunes KP, Torres FS, Cassoli JS, Santos DM, et al. (2015) PnPP-19, a synthetic and nontoxic peptide designed from a *Phoneutria nigriventer* toxin, potentiates erectile function via NO/cGMP. *J Urol* 194: 1481-1490.
38. Andersson KE (2011) Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. *Pharmacol Rev* 63: 811-859.
39. Gratzke C, Angulo J, Chitaley K, Dai YT, Kim NN, et al. (2010) Anatomy, physiology, and pathophysiology of erectile dysfunction. *J Sex Med* 7: 445-475.
40. Freitas AC, Pacheco DF, Machado MF, Carmona AK, Duarte ID, et al. (2016) PnPP-19, a spider toxin peptide, induces peripheral antinociception through opioid and cannabinoid receptors and inhibition of neutral endopeptidase. *Br J Pharmacol* 173: 1491-1501.
41. Foldvari M, Oguejiofor C, Afridi S, Kudel T, Wilson T (1998) Liposome encapsulated prostaglandin e1 in erectile dysfunction: correlation between in vitro delivery through foreskin and efficacy in patients. *Urology* 52: 838-843.
42. Elnaggar YS, El-massik MA, Abdallah OY (2011) Fabrication, appraisal, and transdermal permeation of sildenafil citrate-loaded nanostructured lipid carriers versus solid lipid nanoparticles. *Int J Nanomedicine* 6: 3195-3205.