MedCrave Step into the World of Research
International Journal of Complementary & Alternative Medicine

Opinion

Open Access

Recognizing antiophidian plants using the neuromuscular junction apparatus

Abstract

Here we expressed our opinion in respect to an important research area of study, the neuromuscular junction, which historically has been helped the pharmacology with numerous discoveries related to the mechanisms of action of several substances. Venomous animals, toxins, plants and other bioactive compounds can be studied using neuromuscular preparations from mammalian, avian and other species. The kind of information which is possible to extract from the data interpretation is relevant and stickling to this field of study and the teaching of new researchers should be encouraged. In the last twenty years our group has employed neuromuscular preparations for confirming the antiophidian potential of ethnobotanical plant-extracts used in folk medicine, which need scientific validation. In this view, representatives of Brazilian venomous snakes of Crotalus and Bothrops genera, Crotalus durissus terrificus and Bothrops jararacussu, respectively, have been the protagonists for these studies. Besides, other studies are of notorious importance which includes those with Ophiophagus hannah (King cobra) and Phylodrias olfersii, an opisthoglyphous colubrid. Ethnobotanical plants such as Casearia sylvestris ("guaçatonga"), Casearia gossypiosperma ("pau-de-espeto"), Curcuma zedoaroides ("Wan-Paya-Ngoo-Tua-Mia"), Camellia sinensis (tea); Dipteryx alata ("baru"), Hypericum brasiliense, Jatropha elliptica ("batata-de-teiu"), Mikania laevigata ("guaco"), Plathymenia reticulata ("vinhático"), and Vellozia flavicans ("canela-de-ema") had their antiophidian properties confirmed using the neuromuscular junction as biological preparations. In a literatures survey, the majority of studies found in on line data bank, after crossing the words "antiophidian plants and neuromuscular junction" as themes, involves the use of in vitro mouse/rat phrenic nerve-diaphragm (PND), chick biventer cervicis (BC) or the in vivo rat external popliteal/sciatic nerve-tibialis anterior (EPSTA) preparations, respectively. Such biological methods were chosen probably because the robustness and sensitivity of the neuromuscular junction to the deleterious effect of snake venoms. In the case of chick BC it has a unique value to the experiment, because the inherent sensitivity to the exogenous application of agonists such as acetylcholine and potassium chloride, which induce a contracture as a response. When the potential for clinical applications are desired, in vivo experiments such as those of EPSTA are relevant, in respect to the involvement of the neuromuscular system in the pharmacological response. In conclusion, this text reflects the potential of an indispensable physiological preparation named neuromuscular junction, which is outstanding to recognize and validate antiophidian ethnobotanical compounds. In this context, Dipteryx alata is a plant that has been studied by our group for a long time, in which neuromuscular preparations were chosen as efficient biological models.

Keywords: antiophidian ethnobotanical plants, biventer cervicis, external popliteal/sciatic nerve-tibialis anterior, phrenic nerve-diaphragm, snake venoms

Volume 5 Issue 5 - 2017

Y. Oshima Franco,[1] Ch Dal Belo[2]
[1]Post-Graduate Program in Pharmaceutical Sciences, University of Sorocaba, Brazil
[2]Laboratory of Neurobiology and Toxicology, Federal University of Pampa, Brazil

Correspondence: Y. Oshima-Franco, Post-Graduate Program in Pharmaceutical Sciences, University of Sorocaba, UNISO, Rodovia Raposo Tavares, km 92.5, Zip code 18023-000, Sorocaba, SP, Brazil, Tel +55 15 2101-7197, Email yoko.franco@prof.uniso.br

Received: February 19, 2017 | **Published:** February 21, 2017

Abbreviations: BC, biventer cervicis; EPSTA, external popliteal/sciatic nerve-tibialis anterior; HE, hydroalcoholic extract; ME, methanol extract; PND, phrenic nerve-diaphragm

Introduction

The snake accidents are a relevant issue due the number of victims affected worldwide and the severe clinical condition that the venom develops. In fact, the World Health Organization added snakebite to the list of Neglected Tropical Diseases in 2009.[1] Harrison et al.,[2] described the accidents as a disease of poverty, since the most affected people usually live in poor rural communities with few or no medical resources.

Paradoxically, contrarily to other diseases, a highly effective treatment already exists, and corresponds to the timely administration of a specific antiserum.[2] However, in the latest years, the production of antisera has declined by the public-sector manufacturers or by some private producers, as appointed by The Lancet's editorial.[3] Plants are an important source of drugs.[4] The literature is vast in the study of antiophidian plants such as those against lethal and myotoxic effects,[5–9] phospholipase A2[10–12] or hemorrhagic activities, antinucleolytic or other antiophidian properties as seen with *Pentaclethra macroloba*.[13,14]

However, there are few researchers in the world using neuromuscular junction as biological assay for studying the potential of medicinal antiophidian plants. Thus, the rationale for this matter is the involvement of mainly the lower or upper limbs in local snake bites, the majority affecting neuromuscular junctions. In folk medicine plants are used to counteract edema and hemorrhage caused by the envenomation, simply by applying the extract on the local of the bite or even by chewing the leaves or barks aiming to neutralize the venom in the blood.[15,16] No matter how much the population believes in the popular knowledge, scientific validation is necessary for confirming the medicinal property of a given plant. Table 1 shows chronologically the studies found using neuromuscular preparations for recognizing antiophidian plants.[11,17–35]

Table 1 Ethnobotanical plants with antiophidian potential under the neuromuscular junction parameter

Snake venom/toxin	Plant	Neuromuscular preparation	Authors
Bothropstoxin-I (BthTX-I) from *Bothrops jararacussu*	*Casearia sylvestris* Sw. (HE/ leaves)	Mouse PND	Oshima-Franco et al. [17]
Crotoxin from *C. durissus terrificus*, Bothropstoxin-I from *B. jararacussu*, Piratoxin-I from *B. piraja,* Myotoxin-II from *B. moojeni*	*Casearia sylvestris Sw.* (aqueous extract/leaves)	Mouse PND	Cavalcante et al. [11]
Bothrops jararacussu; Crotalus durissus terrificus	*Mikania laevigata;* HE/leaves *Plathymenia reticulata.* HE/barks	Mouse PND	Melo et al[18]
Bothrops jararacussu	*Dipteryx alata* Vogel (HE/barks)	Mouse PND	Puebla et al. [19]
Crotalus durissus terrificus	*Camellia sinensis* (HE/leaves)	Mouse PND	Rosa et al. [20]
Ophiophagus hannah (King cobra)	*Curcuma zedoaroides A.* Chaveerach& T. Tanee Isolatedcompound [2-(5,5,8a-trimethyl-2- methylene-decahydro-naphthalen-1-yl)- ethylidene]-succinaldehyde	Rat PND	Lattmann et al. [21]
Bothrops jararacussu; Crotalus durissus terrificus	*Dipteryx alata* Vogel (hexane, dichloromethane, ethyl acetate and methanol) extracts/barks	Mouse PND	Nazato et al. [22]
Bothrops jararacussu	*Casearia gossypiosperma* Briquet (HE/leves)	Mouse PND	Camargo et al. [23
Crotamine, crotoxin, *C. d. terrificus;* Bothropstoxin-I, *B. jararacussu*	*Galactia glaucescens* (Kunth) (Leguminosae) (HE/leaves)	Mouse PND	Colares et al. [24]
Philodryas olfersii	*Mikania laevigata* Sch. Bip. ex Baker (ME/leaves)	Mouse PND	Collaço et al. [25]
Bothrops jararacussu	*Plathymenia reticulata* Benth. (hexane, dichloromethane, ethyl acetate and methanol) extracts/barks	Mouse PND	Farrapo et al. [26]
Bothrops jararacussu	*Dipteryx alata* Vogel Isolated lupane triterpenoids	Mouse PND	Ferraz et al. [27]
Bothropstoxin-I; *Bothrops jararacussu*	*Camellia sinensis* L. (HE/leaves)	Mouse PND	Oshima-Franco et al. [28]
Philodryas olfersii	*Mikania laevigata* Sch. Bip. ex Baker (HE/leaves)	Mouse PND Chick BC	Collaço et al. [29]
Crotamin, crotoxin, *Crotalus durissus terrificus*	*Hypericum brasiliense* Choisy (HE/leaves)	Mouse PND	Dal Belo et al. [30]
Bothrops jararacussu	*Dipteryx alata* Vogel Isolated compound7,8,3'-trihydroxy-4'-methoxyisoflavone	Mouse PND	Ferraz et al. [31]
Bothrops jararacussu; Crotalus durissus terrificus	*Casearia gossypiosperma* Briquet (Hexane fraction/leaves)	Mouse PND	Soares-Silva et al. [32]
Bothrops jararacussu	*Vellozia flavicans* Mart. Ex Schult. (HE/leaves)	Mouse PND	Tribuiani et al. [33]
Bothrops jararacussu	*Dipteryx alata* Vogel Isolated compound betulin	Mouse PND Rat EPSTA	Ferraz et al. [34]
Bothrops jararacussu	*Jatropha elliptica* (Pohl) Oken. (HE/roots)	Mouse PND	Ferreira-Rodrigues et al. [35]

These antiophidian plants were found by using isolated neuromuscular preparations which provided a rapid screening against the neurotoxic and/or myotoxic ability. It is known that at the neuromuscular junction, snake venoms induce an *in vitro* irreversible inhibition of the muscle strength by different mechanisms of action and sensitivity. In addition, it is possible to assess biochemical parameters such as phospholipase A2, creatine kinase and other enzymatic activities by collecting samples from the bath media.

The concomitant use of mammalian (as phrenic nerve-diaphragm, PND) and avian isolated preparations (as biventer cervicis, BC) is a precious apparatus for studying the pharmacological effects and mechanisms of action of snake venoms, toxins[36] and other bioactive

compounds, including plant extracts. For example, in absence of electrical stimulation, chick BC are generally used to distinguish pre- or post-synaptic activity of venoms, by means of an exogenous application of acetylcholine chlorhydrate, while the addition of potassium chloride, unmasks an activity upon the sarcolemmal region.[36–38] In the end of each experiment, the resulting preparations can still provide an important material for assessing myotoxicity, unveiling the ability of plant-extracts to counteract the snake deleterious activity. Such effects can be identified in detail by using different techniques like light microscopy, immunohistochemistry, or other available resources.

Finally, our studies using *Dipteryx alata* Vogel, are classical representatives, that demonstrate the usefulness of the *in vitro* mouse PND[22] and the *in vivo* rat external popliteal/sciatic nerve-tibialis anterior (EPSTA)[34] assays, to validate the antiophidian potential of betulin, a novel anti-snake venom isolated compound devoided of mutagenicity, demonstrated by Salmonella/Microsome assays.[39]

Conclusion

We conclude that the neuromuscular junction is still an important tool for studying any bioactive substance, especially neurotoxic compounds such as venoms and their isolated toxins, but also all antivenin compounds in which medicinal plants are a classical illustration.

Acknowledgements

We would like to thank all students and collaborators mentioned in all manuscripts; to University of Sorocaba, and also to FAPESP for financial support (2004/09705-8; 07/53883-6; 08/52643-4; 12/0871-0).

Conflicts of interest

Author declares there are no conflicts of interest.

Funding

None.

References

1. http://www.who.int/neglected_diseases/diseases/snakebites/en/index.html
2. Harrison RA, Hargreaves A, Wagstaff SC, et al. Snake envenoming: a disease of poverty. *PLoS Negl Trop Dis*. 2009;3(12):e569.
3. Snake bite- the neglected tropical disease. *Lancet* . 2015;386(9999):1110.
4. Rates SM. Plants as source of drugs. *Toxicon*. 2001;39(5):603–613.
5. Mors WB, do Nascimento MC, Parente JP, et al.. Neutralization of lethal and myotoxic activities of South American rattlesnake venom by extracts and constituents of the plant *Eclipta prostrata (Asteraceae)*. *Toxicon*. 1989;27(9):1003–1009.
6. Asuzu IU, Harvey AL. The antisnake venom activities of *Parkia biglobosa* (Mimosaceae) stem bark extract. *Toxicon*. 2003;42(7):763–768.
7. Soares AM, Ticli FK, Marcussi S, et al. Medicinal plants with inhibitory properties against snake venoms. *Curr Med Chem*. 2005; 12(22):2625–2641.
8. De Paula RC, Sanchez EF, Costa TR, et al. Antiophidian properties of plant extracts against *Lachesis muta*venom. *J Venom Anim Toxins incl Trop Dis*. 2010;16(2):311–323.
9. Binorkar SV, Jani DK. Profile of medicinal plants with anti-ophidian property. *JPSI*. 2012;1(5):13–20.
10. Machiah DK, Gowda TV. Purification of a post-synaptic neurotoxic phospholipase A2 from *Naja naja venom* and its inhibition by a glycoprotein from *Whitania somnifera*. *Biochimie*. 2006;88(6):701–710.
11. Cavalcante WLG, Campos TO, Dal Pai-Silva M, et al. Neutralization of snake venom phospholipase A(2) toxins by aqueous extract of *Casearia sylvestris*(Flacourtiaceae) in mouse neuromuscular preparation. *J Ethnopharmacol*. 2007;112(3):490–497.
12. Hage-Melim LI, Sampaio SV, Taft CA, et al. Phospholipase A2 inhibitors isolated from medicinal plants: alternative treatment against snakebites. *Mini Rev Med Chem*. 2013;13(9):1348–1356.
13. da Silva JO, Coppede JS, Fernandes VC, et al. Antihemorrhagic, antinucleolytic and other antiophidian properties of the aqueous extract from *Pentaclethra macroloba*. *J Ethnopharmacol*. 2005;100(1-2):145–152.
14. da Silva JO, Fernandes RS, Ticli FK, et al. Triterpenoid saponins, new metalloprotease snake venom inhibitors isolated from *Pentaclethra macroloba*. *Toxicon* . 2007;50(2):283–291.
15. Oliveira LS, Muzitano MF, Coutinho MAS, et al. Plantas medicinais como recurso terapêutico em comunidade do entorno da reserva biológica do Tinguá, RJ, Brasil-metabólitos secundários e aspectos farmacológicos. *Rev Cient Int*. 2011;4 (17):54–74.
16. Ferreira PM1, Costa-Lotufo LV, Moraes MO, et al. Folk uses and pharmacological properties of *Casearia sylvestris*: a medicinal review. *An Acad Bras Cienc*. 2011;83(4):1373–1384.
17. Oshima-Franco Y, Alves CMV, Andréo Filho N, et al. Neutralization of the neuromuscular activity of bothropstoxin-i, a myotoxin from *Bothrops jararacussu*snake venom, by a hydroalcoholic extract of *Casearia sylvestris* Sw. (guaçatonga). *J Venom Anim Toxins Incl Trop Dis*. 2005;11(4):465–478.
18. Melo RS, Farrapo NM, Rocha DS, et al. Chapter 8/ Antiophidian mechanisms of medicinal plants. In: Keller RB (Ed.), Flavonoids: Biosynthesis, Biological Effects and Dietary Sources. *Nova Science Publishers, Inc*., New York, USA. 2009.pp.249–262.
19. Puebla P, Oshima-Franco Y, Franco LM, et al. Chemical constituents of the bark of *Dipteryx alata* vogel, an active species against *Bothrops jararacussu venom*. Molecules. 2010;15(11):8193–8204.
20. Rosa LJR, Silva GA, Amaral Filho J, et al. The inhibitory effect of *Camellia sinensis* extracts against the neuromuscular blockade of *Crotalus durissus terrificus* venom. *J Venom Res*. 2010;1:1–7.
21. Lattmann E, Sattayasai J, Sattayasai N, et al. *In-vitro* and *in-vivo* antivenin activity of 2-[2-(5,5,8a-trimethyl-2-methylene-decahydro-naphthalen-1-yl)-ethylidene]-succinaldehyde against *Ophiophagus hannah* venom. *J Pharm Pharmacol*. 2010;62(2):257–262.
22. Nazato VS, Rubem-Mauro L, Vieira NA, et al. *In vitro* antiophidian properties of *Dipteryx alata* Vogel bark extracts. *Molecules*. 2010;15(9):5956–5970.
23. Camargo TM, Nazato VS, Silva MG, et al. *Bothrops jararacussu* venom-induced neuromuscular blockade inhibited by *Casearia gossypiosperma* Briquet hydroalcoholic extract. *J Venom Anim Toxins incl Trop Dis*. 2010;16(3):432–441.
24. Colares AV, Santos MG, Corrado AP, et al. The anti-ophidic activities of the hydroalcoholic extract from the leaves of *Galactia glaucescens* (Kunth) (Leguminosae). *Ann Natl Acad Med*. 2010;180(2):16–25.
25. Collaço RCO, Rocha Junior DS, Silva MG, et al. Propriedade antiofídica do extrato metanólico de *Mikania laevigata* sobre as ações biológicas induzidas pelo veneno de *Philodryas olfersii* na junção neuromuscular. *REU*. 2010;36(2):105–113.

26. Farrapo NM, Silva GAA, Costa KN, et al. Inhibition of *Bothrops jararacussu* venom activities by *Plathymenia reticulata* Benth extracts. *J Venom Res*. 2011;2:52–58.

27. Ferraz MC, Parrilha LAC, Moraes MSD, et al. The effect of lupane triterpenoids *(Dipteryx alata* Vogel) in the *in vitro* neuromuscular blockade and myotoxicity of two snake venoms. *Curr Org Chem*. 2012;16(22):2717–2723.

28. http://www.intechopen.com/books/pharmacology/antibothropic-action-of-camellia-sinensis-extract-against-theneuromuscular-blockade-of-bothrops-jararacussu

29. Collaço R de C, Cogo JC, Rodrigues-Simioni L, et al. Protection by *Mikania laevigata* (guaco) extract against the toxicity of*Philodryas olfersii* snake venom.*Toxicon*. 2012;60(4):614–622.

30. Dal Belo CA, Lucho AP, Vinadé L, et al. *In vitro* antiophidian mechanisms of *Hypericum brasiliense* Choisy standardized extract: quercetin-dependent neuroprotection. *Biomed Res Int.* 2013;943520.

31. Ferraz MC, Yoshida EH, Tavares RV, et al. An isoflavone from *Dipteryx alata* Vogel is active against the *in vitro* neuromuscular paralysis of *Bothrops jararacussu* snake venom and bothropstoxin-I, and prevents venom-induced myonecrosis. Molecules. 2014;19(5):5790–5805.

32. Soares-Silva JO, Oliveira JL, Cogo JC, et al. Pharmacological evaluation of hexane fraction of *Casearia gossypiosperma* Briquet: antivenom potentiality. *J Life Sci*. 2014;8(4):306–315.

33. Tribuiani N, Silva AM, Ferraz MC, et al. Vellozia flavicans Mart. ex Schult. Hydroalcoholic extract inhibits the neuromuscular blockade induced by Bothrops jararacussu venom. *BMC Complement Altern Med*. 2014;14:48.

34. Ferraz MC, de Oliveira JL, de Oliveira Junior JR, et al. The triterpenoid betulin protects against the neuromuscular effects of *Bothrops jararacussu* snake venom *in vivo*. *Evid Based Complement Alternat Med*. 2015;939523.

35. Ferreira-Rodrigues SC, Rodrigues CM, Dos Santos MG, et al. Anti-inflammatory and antibothropic properties of *Jatropha elliptica,* a plant from Brazilian cerrado biome. *Adv Pharm Bull*. 2016;6(4):573–579.

36. Harvey AL, Barfaraz A, Thomson E, et al. Screening of snake venoms for neurotoxic and myotoxic effects using simple *in vitro* preparations from rodents and chicks. *Toxicon*. 19947;32(3):257–265.

37. Barfaraz A, Harvey AL. The use of the chick biventer cervicis preparation to assess the protective activity of six international reference antivenoms on the neuromuscular effects of snake venoms *in vitro*. *Toxicon*. 1994;32(3):267–272.

38. Vatanpour H. Effects of black scorpion *Androctonus crasicuda* venom on striated muscle preparation *in vitro*. *Ir J Pharm Res*. 2003;2(1):17–22.

39. Yoshida EH, Tribuiani N, Sabadim G, et al. Evaluation of betulin mutagenicity by Salmonella/Microsome Test. *Adv Pharm Bull*. 2016;6(3):443–447.

Citation: Franco YO, Belo CD. Recognizing antiophidian plants using the neuromuscular junction apparatus. *Int J Complement Alt Med.* 2017;5(5):130–133. DOI: 10.15406/ijcam.2017.05.00165