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 Phylodrius olfersi, an ophiphyloglyphous colubrid. Ethnobotanical plants such as Casearia sylvestris ("guacatonga"), Casearia gossypiosperma ("pau-de-espeto"), Curcuma zedoaroides ("Wan-Paya-Ngo-Tua-Mia"), Camellia sinensis (tea); Diptryx alata ("baru"), Hypericum brasiliense, Jatropha elliptica ("bata-de-teiú"), Mikania laevigata ("guaco"), Platyhymenia 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, Diptryx 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

Abstract

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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 antiser 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] |
| Crototoxin from C. durissus terrificus, Bothropstoxin-I from B. jararacussu, Piratoxin-I from B. pireja, 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. Chaveirach & T. Tanee Isolated compound [2-(5,5,8a-trimethyl-2-methylene-decahydro-naphthalen-1-yl)-ethylidene]-sucinaldehyde | Rat PND | Latmann 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/leaves) | Mouse PND | Camargo et al. [23] |
| Crotamine, crotoxin, C. d. terrificus; Bothropstoxin-I, B. jararacussu | Galactia glaucescens (Kuntl) (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 compound 7,8,3’-trihydroxy-4’-methoxyisoflavone | Mouse PND | Ferraz et al. [31] |
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 activity 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/sciotic 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.

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