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Characterization of *Daboia russelii* and *Naja naja* venom neutralizing ability of an undocumented indigenous medication in Sri Lanka

Madhushika M. Silva [a, b], Sampath S. Seneviratne [b], Devaka K. Weerakoon [b], Charitha L. Goonasekara [a, *]

[a] Faulty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, 10390, Sri Lanka
[b] Department of Zoology, Faculty of Science, University of Colombo, Colombo, 03, Sri Lanka

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ABSTRACT

Background: Indigenous medicinal practice in Sri Lanka talks about powerful compounds extracted from native plants for treating venomous snake bites which are hardly documented in literature but are used by the indigenous doctors for thousand years.
Objective: We screened the neutralizing ability of a herbal preparation practiced in indigenous medicine of Sri Lanka, consisting of *Sansevieria cylindrica*, *Jatropha podagrica* and *Citrus aurantiifolia*, for its ability to neutralize venom toxins of *Naja naja* (Common Cobra) and *Daboia russelii* (Russell's viper).
Materials and methods: The venom toxicity was evaluated using a 5-day old chicken embryo model observing the pathophysiology and the mortality for six hours, in the presence or absence of the herbal preparation. The known toxin families to exist in snake venom, such as Phospholipase A_2, Snake venom Metalloprotease, were evaluated to understand the mechanism of venom neutralizing ability of the herbal preparation.
Results: The LD_{50} of *D. russelii* venom, as measured using the 5-day old chicken embryo model, was 4.8 ± 0.865 ug (R^2 = 84.8%, P = 0.079). The pre-incubation of venom with the herbal preparation increased the LD_{50} of *D. russelii* venom to 17.64 ± 1.35 μg (R^2 = 81.0%, P = 0.100), showing a clear neutralizing action of *D. russelii* venom toxicity by the herbal medicine. Whereas the pre-incubation of venom with the 1× venom neutralizing dose of commercially available polyvalent anti-venom serum shifted the LD_{50} venom only up to 5.5 ± 1.35 μg (R^2 = 98.8%, P = 0.069). In the presence of the herbal preparation, Phospholipase A_2 activity of *D. russelii* venom was significantly reduced from 9.2×10^{-3} mM min^{-1} to 8.0×10^{-3} mM min^{-1} and that of *N. naja* from 2.92×10^{-2} mM min^{-1} to 0.188×10^{-2} mM min^{-1}. Further, the pre-incubation of *N. naja* venom with the herbal preparation significantly reduced its Metalloprotease activity from 0.069 units min^{-1} to 0.019 units min^{-1}.
Conclusion: The herbal preparation shows a clear neutralizing action against the toxicities of *D. russelii and N. naja* venoms demonstrating the potential to be used as a plant based antidote for snake envenomation.

1. Introduction

Snake bites are proven to be a major health hazard in the tropical belt especially affecting the rural communities and agricultural sector in Asia, Africa, Oceania and Latin America. Recent studies show that the annual envenoming cases around the world is as high as 421,000–1,841,000 [1] and the deaths may be as high as 24,000–94,000 [1]. The true figurers of mortality could be even higher as a proportion of the people affected do not seek formal medical attention. The burden is mostly confined to the poorer communities and mainly is an occupational hazard in farming and agricultural communities [1,2]. Sri Lanka, a developing South Asian country, falls among the countries of highest snakebite records [1–3]. Sri Lanka is inhabited by 102 species of snakes [4]; among the mentioned snake species only 21 are considered highly venomous and five species as moderately venomous. From the highly venomous species 14 are sea snakes and 2 are terrestrial species with very low

* Corresponding author.
E-mail addresses: madhushikas@gmail.com (M.M. Silva), charithalg@kdu.ac.lk (C.L. Goonasekara).
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contact with humans, and the highest weight of the morbidity and mortality are associated with snakebites of the highly venomous *Naja naja* (Common cobra) and *Daboia russelli* (Russell's viper).

The only specific treatment currently available to snake venom toxins is the hyper-immune globulins from snake venom immunized horse [5]. But the cost of anti-venom does not make it a readily accessible medication to tropical poorer regions. Therefore around the world it is an emerging trend on experimenting other possible antidotes for the snake envenomation. As a spectrum of possibilities lie within many of eastern traditional medicines extracted from plants, many of eastern herbal extracts are now under the scientific limelight.

Several studies have explored efficacy of such remedies. Extracts of *Hydrocotyle javanica* and *Gloriosa superba* gives 80–90% protection to mice treated with minimum lethal dose of venom (LD_{99}) of *Naja nigricollis* (Spitting Cobra) and has produced significant changes of membrane stabilization of human red blood cells (HRBC) exposed to hyposaline-induced haemolysis [6]. In another study, *Andrographis paniculata* and *Aristolochia indica* plant extracts were tested for neutralizing activity against *Echis carinatus* (Saw-scaled Viper) venom where both plant extracts have shown effective neutralization of venom induced lethal activity [7]. *Hibiscus aethiopicus* leaf extract completely stopped haemorrhagic activity against the venom of *Echis ocellatus* (African Carpet Viper) and *N. nigricollis* (Spitting Cobra) [8]. The methanolic root extracts of *Vitex negundo* and *Emblica officinalis ex*tracts has significantly antagonized the *D. russellii* and *Naja kaouthia* (Monocled Cobra) venom induced lethal activity in both *in vitro* and *in vivo* studies with neutralization of venom-induced haemorrhagic, coagulant, defibrinogenating and inflammatory activities [9]. In another study, snake venom neutralizing potential of *Rauvolfia serpentina* plant extract was tested by *in vitro* and *in vivo* methods against *D. russelli* venom. The *in vivo* assessment of LD_{50} in *D. russelli* venom was found to be 0.628 μg/g. *R. serpentina* plant extract effectively neutralized this venom lethality with an effective dose (ED) of 10.99 mg/3LD_{50} of venom [10].

Sri Lanka being an oriental country inheriting a great indigenous system of medicine, treating snakebites with herbal extracts is one such practice that has been widely used by indigenous doctors. Therefore, this study contributes towards the scientific evaluation of the effectiveness of a traditional herbal preparation used in Sri Lanka against *N. naja* and *D. russelli* venom toxicity and the characterization of its ethnopharmacological properties. The practice of this herbal preparation is confined to a late traditional medical practitioner from the southern coastal region of the country, who had inherited the medication through the family. The herbal preparation had been applied as a topical treatment over the bite site of the victims, who are at the early stages of envenomation by *N. naja* and *D. russelli*. The consent of the medical practitioner's family was obtained for the scientific evaluation of the herbal preparation for this study. The ethical clearance was obtained from the Institute of Biology, University of Colombo. To our knowledge, this study provides the first laboratory evidence for the venom neutralizing ability of a herbal preparation from Sri Lankan indigenous medicine. Preliminary forms of this work were presented at the 8th International Conference of General Sir John Kotelawala Defence University and at the 2nd International Conference of Traditional and Complementary Medicine [11,12].

2. Material and methods

2.1. Preparation of herbal extract

The herbal preparation was prepared by mixing together the aqueous extracts, obtained by crushing 2.5 g each of leaves of *Sansevieria cylindrica, Jatropha podagrica*, with a drop of the extract of *Citrus aurantiifolia* fruit. *S. cylindrica* is also known as the cylindrical snake plant, African spear or spear sansevieria. *J. podagrica* is known by several English common names, including Buddha belly plant, bottle plant shrub, gout plant, purging-nut, Guatemalan rhubarb, and goutystalk nettlespurge. *C. aurantiifolia* is known as the lime fruit. Fresh plant material of *S. cylindrica, J. podagrica* and *C. aurantiifolia* were collected from home gardens located off suburbs of Colombo in Kalutara district. The collected species were identified and authenticated by taxonomists from the Department of Plant Science, Faculty of Science, University of Colombo Sri Lanka. The preparation was made as a fresh aqueous extract on each day prior to testing. The volumes of the extracts individually as well as in the mixture, and the pH of the final preparation, were measured at each preparation, in order to maintain the consistency between preparations. The pH of the final preparation was 4.2.

2.2. Collection of venom samples

The venom samples of *D. russelii* and *N. naja* were collected from captive animals housed in the herpeterium of Faculty of Medicine, University of Colombo in September 2014. Samples from the two species were pooled separately and were freeze dried and were kept at −20 °C until use in the experiments.

2.3. SDS polyacrylamide gel electrophoresis (SDS PAGE) of venom

The amount of protein in the freeze dried venom samples was quantified by measuring absorbance at 280 nm wave length using Bovine serum albumin (BSA) as a standard. A 35 μg of freeze dried venom of either *D. russelii* or *N. naja*, dissolved in sample buffer was loaded on to a 12% polyacrylamide in the presence of Sodium Dodecyl Sulphate and electrophoresed at 180V for 30 min [13]. Then the gels were stained with Coomassie R-250 for 45 min followed by destaining with acetic acid and methanol, to visualize venom protein groups after 12% SDS PAGE.

2.4. Statistical analysis

Statistical analysis was carried out using Minitab 17 software. Sigmoidal dose-response curves for LD_{50} was generated using GraphPad Prism 4.03 (GraphPad Software,Inc.).

2.5. Chick embryo model for venom neutralizing activity of the herbal preparation

Freeze dried venom of *D. russelii* and *N. naja*, at varying doses, were reconstituted in PBS (7.4) to be impregnated on to 3 mm diameter of Whatman no 1 filter papers and placed over the vitelline vein on the exposed yolk sac membrane of *in vitro* cultivated 5-day old chicken embryos [14]. Pathological symptoms induced by each mentioned venom were closely monitored and recorded till death over a period of six hours. The experiment was replicated with each venom type incubated with the herbal preparation or the anti-venom (as a positive control).

2.5.1. Analysis of snake venom and treatment with herbal preparation and anti-venom

For the determination of LD_{50}, venom on 5-day old chick embryo, a gradient of 2 μg, 4 μg, 8 μg, 16 μg, 32 μg, and 48 μg of freeze dried venom dissolved in distilled water was used in the above procedure. The cut-off time for calculating number of embryo state dead/alive was six hours after treatment.

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