Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: https://www.tandfonline.com/loi/iphb20

Inhibition of the Toxic Effects of *Lachesis muta*, *Crotalus durissus cumanensis* and *Micrurus mipartitus* Snake Venoms by Plant Extracts

Vitelbina Núñez, Rafael Otero, Jacqueline Barona, Ramiro Fonnegra, Silvia Jiménez, Raúl Guillermo Osorio, Juan Carlos Quintana & Abel Díaz

To cite this article: Vitelbina Núñez, Rafael Otero, Jacqueline Barona, Ramiro Fonnegra, Silvia Jiménez, Raúl Guillermo Osorio, Juan Carlos Quintana & Abel Díaz (2004) Inhibition of the Toxic Effects of *Lachesis muta*, *Crotalus durissus cumanensis* and *Micrurus mipartitus* Snake Venoms by Plant Extracts, Pharmaceutical Biology, 42:1, 49-54, DOI: 10.1080/13880200490505483

To link to this article: https://doi.org/10.1080/13880200490505483

Published online: 03 Oct 2008.

Pharmaceutical Biology
2004, Vol. 42, No. 1, pp. 49–54

Taylor & Francis
Taylor & Francis Group

Inhibition of the Toxic Effects of *Lachesis muta*, *Crotalus durissus cumanensis* and *Micrurus mipartitus* Snake Venoms by Plant Extracts

Vitelbina Núñez[1], Rafael Otero[1], Jacqueline Barona[1], Ramiro Fonnegra[1,2], Silvia Jiménez[1,3], Raúl Guillermo Osorio[1], Juan Carlos Quintana[1] and Abel Díaz[1]

[1]Programa de Ofidismo, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia; [2]Herbario Universidad de Antioquia, Medellín, Colombia; [3]Facultad de Química Farmacéutica, Universidad de Antioquia, Medellín, Colombia

Abstract

The ethanol extracts of eight plants utilized against snakebites by traditional healers in Colombia (*Pleopeltis percussa*, *Trichomanes elegans*, *Brownea rosademonte*, *Tabebuia rosea*, *Heliconia curtispatha*, *Bixa orellana*, *Renealmia alpinia* and *Citrus limon*) were examined for their possible inhibitory ability against the venoms of *Lachesis muta*, *Crotalus durissus cumanensis*, and *Micrurus mipartitus*. Extracts were preincubated with 1.5 LD_{50} of each venom, and injected by the intraperitoneal route, in mice. Under these conditions, the lethal effect of *L. muta* venom was neutralized by all of the extracts, whereas *C. d. cumanensis* and *M. mipartitus* venoms were inhibited by six extracts, exceptions being *P. percussa* and *C. limon.* In addition, the myotoxic activity of *C. d. cumanensis* venom was neutralized by the extracts of *H. curtispatha*, *B. rosademonte*, *P. percussa*, and *T. elegans*. The neutralizing ability of the plant extracts was also evaluated by independent administration experiments. Extracts were injected by intravenous or i.m. (*in situ*) routes, immediately after the i.p or i.m. injection of 1.5 LD_{50} of venom, respectively. Under such conditions, their neutralizing efficacy was significantly reduced, and in some cases disappeared. Nevertheless, *in situ* administration of *B. rosademonte*, *H. curthispatha*, *P. percussa*, and *T. elegans* extracts still caused a partial but significant inhibition of the lethal effect of *C. d. cumanensis* venom, and the first three extracts reduced significantly the development of myonecrosis. These results identify useful plant species for future purification of venom-neutralizing components that might become helpful in the development of supplementary therapies against snakebites.

Keywords: Medicinal plants, extracts, neutralization, snake venoms, lethality, myotoxicity.

Introduction

Snakebite is a significant health problem, especially in the tropical regions of the world (Chippaux, 1998). In Colombia, 90–96% of the approximately 3000 snakebites per year are inflicted by snakes of the genera *Bothrops*, *Porthidium,* and *Bothriechis*; 2% by *Lachesis muta* (bushmaster); 1% by *Micrurus* spp. and 1% by *Crotalus durissus cumanensis* (rattlesnake). The mortality rate is estimated at 5–8%, with important sequelae observed in 6–9% of survivors (Otero et al., 1992; 2001).

Medical resources and antivenoms are especially limited in the rural communities. Therefore, up to 60% of the victims are initially attended by traditional healers, who commonly utilize plants as antidotes (Otero et al., 2000a). Although a number of plants are known to be the source of useful drugs in modern medicine (Gowda, 1997), the validity of traditional treatments must be scientifically evaluated in the laboratory. In recent studies, 85 plant species

Accepted: May 3, 2004

Address correspondence to: Vitelbina Núñez, Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica. E-mail: vnunez@icp.ucr.ac.cr

DOI: 10.1080/13880200490505483

belonging to 41 families were listed, and 13 of them were identified as capable of neutralizing the lethal and/or hemorrhagic effects of *B. atrox* venom (Otero et al., 2000b,c). Based on these screenings, in the present work we evaluated the inhibition of *L. muta*, *C. d. cumanensis* and *M. mipartitus* snake venoms, by the ethanol extracts of eight plants.

Materials and methods

Animals, venoms and plant extracts. Swiss-Webster mice of 18–20 g body weight were utilized in all experiments, under the University of Antioquia guidelines for the use of experimental animals. Snake venoms were pools obtained from 10 specimens of *L. muta* from Antioquia and Chocó, 20 specimens of *M. mipartitus* from Antioquia, and 12 specimens of *C. d. cumanensis* from the Atlantic coast and the Department of Meta, Orinoquia (Colombia), respectively. The venoms were centrifuged, lyophilized, and stored at −20 °C.

Plants were collected in rural communities of the Atrato river valley in Antioquia and Chocó (Vigía del Fuerte, Bojayá, Unguía) and on the Pacific coast of Chocó (Nuquí, Bahía Solano), Colombia, together with the traditional healers. Then, plants were identified and deposited in the Herbarium of Universidad de Antioquia, Medellín. The following plant species and parts were studied: whole plants of *Pleopeltis percussa* (Cav.) Hook & Grev, and *Trichomanes elegans* L.C. Rich; stem bark of *Brownea rosademonte* Berg., and *Tabebuia rosea* (Bertold.) DC.; rhizomes of *Heliconia curtispatha* Petersen, and *Renealmia alpinia* (Rottb.) Maas; leaves and branches of *Bixa orellana* L.; and ripe fruits of *Citrus limon* (L.) Burm. f. After drying and crushing individual samples of each part utilized, they were percolated with 96% ethanol for 2 days. Extracts were concentrated to a semisolid paste using a rotavapor, lyophilized, and stored at −20 °C (Weniger, 1991).

Lethal activity of the venoms. In order to determine the lethal dose 50% (LD_{50}) of the venoms studied, groups of four mice received intraperitoneal (i.p.) injections of varying doses, in 500 µl of phosphate buffered saline (PBS; 0.12 M NaCl, 0.04 M sodium phosphate), pH 7.2. Deaths were recorded within the following 72 hr and the LD_{50} values were calculated by the Spearman-Karber method (W.H.O., 1981). All experiments were performed three times, and the results expressed as means, with 95% confidence intervals. The lethal activity of the three venoms was also determined by the intramuscular (i.m.) route, with the aim of simulating the natural envenomation conditions. Thus, groups of four mice received varying doses of venoms by i.m. injection in the gastrocnemius, in 100 µl PBS. The corresponding i.m. LD_{50} values were calculated as described. In order to assess the possible lethal activity of the plant extracts, they were assayed similarly to the venoms, in groups of four mice, by the i.p. and i.m. routes.

Neutralization by *in vitro* preincubation experiments

Lethal activity. In order to evaluate the neutralizing ability of the extracts against the lethal activity of the venoms, variable doses of each extract were preincubated for 30 min at 37 °C with 1.5 LD_{50} (i.p.) of each venom (corresponding to 183 µg for *L. muta*, 2.7 µg for *C. d. cumanensis,* or 13.5 µg for *M. mipartitus*). Then, the mixtures were injected into groups of ten mice, by i.p. route, in 500 µl of PBS. A control group of mice received a similar injection containing venom alone. Experiments were repeated three times, and deaths were recorded for a period of 72 hr. The effective dose 50% (ED_{50}) for each plant extract was calculated by the Spearman-Karber method (W.H.O., 1981).

Myotoxicity. Extracts that showed neutralizing ability against the lethal effect of *C. d. cumanensis* venom were also evaluated for inhibition of the myotoxic activity of this venom. A venom dose of 6.2 µg, dissolved in 100 µl of PBS, was preincubated with 100 µg of each extract for 30 min at 37 °C. Then, the mixtures were injected by the i.m. route into the gastrocnemius, in groups of four mice. A control group received the venom alone. After 3 hr, mice were bled from the tail, using heparinized capillaries, and the plasma creatine kinase (CK; EC 2.7.3.2) levels were determined following a colorimetric procedure (Sigma No. 520), as described (Gutiérrez et al., 1980; Otero et al., 1995). CK activity was expressed as U/ml, one unit being defined as the phosphorylation of one nanomole of creatine per min at 25 °C.

Neutralization by *in vivo* independent administration experiments

Lethality. In order to evaluate the neutralizing efficiency of the plant extracts against the lethal activity of the venoms, independent administration experiments were conducted (Gutiérrez et al., 1990). Groups of six mice received an i.p. injection of each venom (1.5 LD_{50}) and, after 5 min, they received an i.v. injection of each extract (0.5–2 mg). In another group of experiments, mice received an i.m. injection of each venom (1.5 LD_{50}) and, immediately after, extracts (2–4 mg) were injected *in situ*, by the i.m. route. Experiments were repeated three times for each extract and deaths were recorded at 6, 24, 48, and 72 hr.

Myonecrosis. Extracts that showed *in vitro* inhibition of the myotoxic activity of *C. d. cumanensis* venom, were independently administered *in situ* (i.m.) to groups of mice that had received an i.m. venom injection (6.3 µg) into the gastrocnemius. Extracts (100 µg) were injected immediately (<1 min) after envenomation, and plasma CK activity was determined as described.

Statistical analyses. The significance of the differences observed between groups was determined by analysis of variance (ANOVA). When ANOVA values were significantly different ($p < 0.05$), the significance of differences was determined by the Newman-Keuls test. The survival rate in the groups of mice that received venom and extract was com-

pared at different times with controls (venom alone) using the F Cox test, with the aid of the STATISTICA 98 software (StatSoft, Tulsa, OK).

Results

Neutralization of lethal activity

The calculated LD_{50} values for *L. muta, C. d. terrificus*, and *M. mipartitus* venoms, by i.p. route, were 122 (99–165), 1.8 (1.2–2.5), and 9.0 (6.6–12.0) µg/mouse, respectively (ranges in parentheses represent 95% confidence intervals). By the i.m. route, the LD_{50} values were 273 (188–396), 9.0 (6.2–13.3), and 9.0 (7.2–11.3) µg/mouse, respectively. On the other hand, all the plant extracts had LD_{50} values higher than 5 mg/mouse, except for *Bixa orellana*, which showed some toxicity at 2.5 mg/mouse.

As summarized in Table 1, all of the extracts demonstrated inhibition of the lethal effect of *L. muta* venom, with somewhat different potencies, when tested by *in vitro* preincubation experiments. The venom of *C. d. cumanensis* was neutralized by seven of the extracts, in some cases with very low ED_{100} values. For example, 15.6 µg of *B. rosademonte* or *B. orellana* protected all the animals from the venom challenge (Table 1). However, the extract of *Citrus limon* did not neutralize this venom (Table 1). The lethal activity of *M. mipartitus* venom was completely neutralized by six plant extracts, negative results being obtained with *Citrus limon* and *P. percussa* (Table 1).

When the extracts were independently administered by the i.v. route after an i.p. venom injection, there was a significant drop in their neutralizing ability, in comparison to experiments of the preincubation type. Only *R. alpinia* and *B. rosademonte* extracts showed a low (33%), although significant neutralization ($p = 0.003$ and $p = 0.02$, respectively) against the lethal effect of *L. muta* venom, within 6 hr after venom injection (data not shown). None of the extracts inhibited the lethal activity of *C. d. cumanensis* or *M. mipartitus* venoms, when independently administered after envenomations by this route. Nevertheless, when the extracts of *B. rosademonte*, *H. curthispatha*, *P. percussa*, and *T. elegans* were administered i.m. *in situ* after venom injection, they showed a partial but significant ($p < 0.05$) neutralization of the lethal effect of *C. d. cumanensis* venom within 72 hr. The extract of *H. curtispatha* showed the best neutralizing ability (87%) by this route (Table 2).

Neutralization of myotoxicity

The i.m. injection of *C. d. cumanensis* venom increased plasma CK levels to 366 U/ml, after 3 hr. Preincubation of this venom with *B. rosademonte*, *P. percussa*, *H. curtispatha*,

Table 1. Plant extracts with *in vitro* inhibitory ability against the lethal effect of *L. muta, C. d. cumanensis* and *M. mipartitus* snake venoms

Family/species (Voucher specimen)[1]	Part Used[2]	µg extract / mouse					
		L. muta		*C.d. cumanensis*		*M. mipartitus*	
		ED_{50}[†]	ED_{100}[‡]	ED_{50}	ED_{100}	ED_{50}	ED_{100}
Bixaceae / *Bixa orellana* L. (RF 6485)	L,BR	140 (70–270)[a]	1000	7.8 (4.1–14.7)[a]	15.6	62.4 (31.1–125)[a]	500
Caesalpiniaceae / *Brownea rosademonte* Berg. (RF 6455)	SB	210 (120–360)[ab]	500	3.3 (1.7–6.4)[a]	15.6	52.5 (28.1–98.2)[a]	125
Rutaceae / *Citrus limon* (L.) Burm.f. (RF 6736)	RF	710 (320–1580)[bcd]	4000	(–)	(–)	(–)	(–)
Heliconiaceae / *Heliconia curtispatha* Petersen (RF 6486)	RH	350 (200–620)[abc]	1000	9.28 (5.0–17.4)[a]	62.5	125 (70–223)[a]	500
Polypodiaceae / *Pleopeltis percussa* (Cav.) Hook & Grev. (RF 6410)	WP	180 (90–360)[ab]	1500	4.6 (2.4–9.0)[a]	31.25	n.d.	n.d.
Zingiberaceae / *Renealmia alpinia* (Rottb.) Maas (RF 6456)	RH	1000 (530–1890)[cd]	4000	1680 (900–3140)[c]	4000	841 (450–1573)[b]	2000
Bignoniaceae / *Tabebuia rosea* (Bertold.) DC. (RF 6458)	SB	1400 (720–2750)[d]	4000	500 (260–950)[c]	2000	707 (396–1262)[b]	2000
Hymenophyllaceae / *Trichomanes elegans* L.C.Rich (RF 6744)	WP	250 (140–450)[ab]	1000	52.6 (30.3–91.2)[b]	125	105 (56.2–196.6)[a]	250

[1] Voucher specimen: RF, Ramiro Fonnegra; [2] Part Used: L = leaves; WP = whole plant; BR = branches; RH = rhizomes; RF = ripe fruits; SB = stem bark.

(–) no neutralizing ability. n.d.: not determined (80% neutralization). [†] Effective dose 50%: extract dose that protected 50% of mice against lethal effect of venom. Values in parenthesis are 95% confidence limits. [‡] Effective dose 100%: extract dose that protected 100% of mice against lethal effect of venom. Values with different superscripts are significantly different ($p < 0.05$).

Table 2. Inhibition of the lethal effect of *C. d. cumanensis* venom by independent administration of venom (i.m.) and extract (*in situ*)

		survival of mice (%)							
		6 h		24 hr		48 h		72 h	
Plant species	Extract Dose	**V + E**	**V**	**V + E**	**V**	**V + E**	**V**	**V + E**	**V**
Bixa orellana	2 mg	93	40	53	7	33	0	27	0
Brownea rosademonte	2 mg	86	40	60	7	60	7	60	7
Heliconia curtispatha	4 mg	93	33	87	0	87	0	87	0
Pleopeltis percussa	2 mg	97	60	50	17	43	13	43	13
Renealmia alpinia	4 mg	67	27	0	0	0	0	0	0
Tabebuia rosea	4 mg	75	25	0	0	0	0	0	0
Trichomanes elegans	3 mg	67	20	40	0	40	0	40	0

The extracts were administered immediately by i.m. route, at the same site of the venom injection (1.5 LD_{50} = 13.5 µg) by i.m. route. The mortality was recorded at 6, 24, 48 and 72 hr. Results are the mean of three determinations (n = 6 mice/group). V + E: groups of mice that received venom and extract. V: groups of mice that received venom alone.

and *T. elegans* extracts reduced plasma CK levels by more than 50% ($p < 0.001$), indicating a significant protection from myotoxicity. When these extracts were independently administered (*in situ*), immediately after venom injection, there was a drop in their neutralizing ability, but still three of them (*B. rosademonte*, *H. curtispatha* and *P. percussa*) demonstrated a significant neutralization, as judged by the reduction in plasma CK levels (Fig. 1).

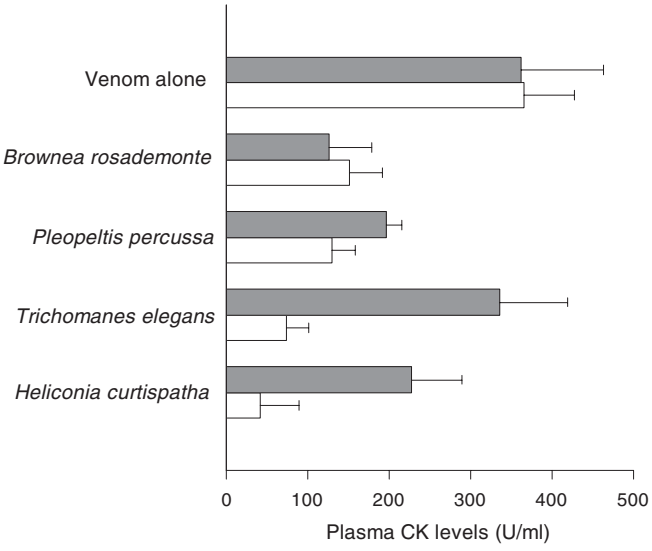

Figure 1. Inhibition of the myotoxic activity of *Crotalus durissus cumanensis* venom by plant extracts. In preincubation experiments (empty bars), each extract (100 µg) was incubated with 6.25 µg of venom, and the mixture was then injected to groups of six mice, by the i.m. route. In independent administration experiments (filled bars), extracts (100 µg) were administered *in situ* (i.m.) after i.m. venom injection (6.25 µg). Basal plasma CK activity in the control group (PBS alone) was 20 ± 6 U/ml.

Discussion

A number of plants have been reported to be effective against snake venoms (Martz, 1992; Houghton & Osibogun, 1993). For example, in preincubation-type experiments, the extract and constituents of *Eclipta prostrata* decreased the lethality and myotoxicity of *C. d. terrificus* venom, as well as the myotoxic and hemorrhagic effects of *B. jararaca*, *B. jararacussu,* and *L. muta* snake venoms (Mors et al., 1989; Melo et al., 1994; Melo & Ownby, 1999). The aqueous or ethanolic extracts of *Tabernaemontana catharinensis* (Apocynaceae), and its purified component, inhibited the lethal and myotoxic activities of *C. d. terrificus* venom (Batina et al., 2000). Our present results demonstrate that *B. rosademonte*, *B. orellana*, *P. percussa*, *H. curtispatha*, *T. elegans*, *T. rosea*, and *R. alpinia* extracts contain compounds which inhibit the lethal action of *L. muta*, *C. d. cumanensis* and *M. mipartitus* venoms from Colombia. Maximal neutralization was obtained when the extracts were preincubated with the venoms before injection. Notably, the lethal effect of *C. d. cumanensis* venom was completely abolished with very low amounts of some of the extracts (i.e., *B. rosademonte, B. orellana, P. percussa, H. curthispatha*).

Interestingly, *C. limon* extract did not neutralize the two neurotoxic venoms (*C. d. cumanensis* and *M. mipartitus*). The former probably contains crotoxin, a main toxic component of the South American rattlesnake venoms, which is a lethal, neurotoxic and myotoxic phospholipase A_2 (Harris, 1991). Recently, Otero et al. (2000b) reported that *C. limon*

did not neutralize the phospholipase A_2 activity of *B. atrox* venom. This might explain the lack of neutralization against the lethal effect of neurotoxic venoms. Although *P. percussa* extract showed phospholipase A_2 inhibitory activity (Otero et al., 2000b) *in vitro*, it was partially able to neutralize the lethal effect of *M. mipartitus* venom in this work. Gowda (1997) reported several examples in which an interaction between phospholipase A_2 and isolated plant compounds was demonstrated.

Experiments utilizing an independent i.v. injection of the extracts, 5 min after an i.p. venom injection, clearly showed that their neutralizing efficiency against lethality decreases, in comparison to preincubation-type assays. Nevertheless, when the extracts were administered *in situ*, immediately after an i.m. venom injection, mice were partially, but significantly protected from death induced by *C. d. cumanensis* venom within 72 hr. These results suggest that a rapid contact between the extract and venom is necessary to neutralize its lethal components. Similar conclusions may be obtained from the experiments of *in vitro* and *in situ* neutralization of the myotoxic action of *C. d. cumanensis* venom by the extracts.

Hyperimmune antivenoms constitute the best known therapy for envenomations by snakebites. However, they still have some limitations in neutralizing particular venom effects (Bon, 1996). It is important to search for different inhibitors, either natural or synthetic, that could complement or enhance the therapeutic action of antivenoms. The present results demonstrate that plant extracts constitute a rich source of natural compounds, which can neutralize the toxic activities of snake venoms, and identify useful species for further research. The future isolation and characterization of these inhibitory components might be of importance in the development of novel, supplementary therapies against snakebites, as well as in studies focusing on basic aspects of snake toxins and their molecular mechanisms of action.

Acknowledgements

We thank the communities of the studied region, and Ministerio del Medio Ambiente for their valuable cooperation, as well as Dr. Bruno Lomonte (Instituto Clodomiro Picado, Universidad de Costa Rica) for critical review of the manuscript. The financial support of the Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología Francisco José de Caldas (COLCIENCIAS), and Universidad de Antioquia, is gratefully acknowledged. This work was performed in partial fulfillment of the doctoral degree of Vitelbina Nuñez at the University of Costa Rica.

References

Bon C (1996): The serum-therapie was discovered 100 years ago. *Toxicon 34:* 142–143.

Batina MF, Cintra AC, Veronese EL, Lavrador MA, Giglio JR, Pereira PS, Dias DA, França SC, Sampaio SV (2000): Inhibition of the lethal and myotoxic activities of *Crotalus durissus terrificus* venom by *Tabernaemontana catharinensis*: Identification of one of the active components. *Planta Med 66:* 424–428.

Chippaux JP (1998): Snake-bites: Appraisal of the global situation. *Bull WHO 76:* 513–524.

Gowda TV (1997): Interaction of snake venom phospholipases A_2 with plant isolates. In: Kini RM, ed., *Venom Phospholipase A_2 Enzymes,* John Wiley & Sons, Chichester, pp. 205–221.

Gutiérrez JM, Arroyo O, Bolaños R (1980): Mionecrosis, hemorragia y edema inducidos por el veneno de *Bothrops asper* en ratón blanco. *Toxicon 18:* 603–610.

Gutiérrez JM, Rojas G, Lomonte B, Gené JA, Chaves F, Alvarado J, Rojas E (1990): Standardization of assays for testing the neutralizing ability of antivenoms. *Toxicon 28:* 1127–1129.

Harris JB (1991): Phospholipases in snake venoms and their effects on nerve and muscle. In: Harvey AL, ed., *Snake Toxins*, Pergamon Press, Inc: New York, pp. 91–129.

Houghton PJ, Osibogun IM (1993): Flowering plants used against snakebite. *J Ethnopharmacol 39:* 1–29.

Martz W (1992): Plants with a reputation against snakebite. *Toxicon 30:* 1131–1142.

Melo PA, Nascimento MC, Mors WB, Suarez-kurtz G (1994): Inhibition of myotoxic and hemorrhagic activities of crotalid venoms by *Eclipta prostrata* (Asteraceae) extracts and constituents. *Toxicon 32:* 595–603.

Melo PA, Ownby CL (1999): Ability of wedelolactone, heparin, and *para*-bromophenacyl bromide to antagonize the myotoxic effects of two crotaline venoms and their PLA_2 myotoxins. *Toxicon 37:* 199–215.

Mors WB, Nascimento MC, Parente JP, Da Silva MH, Melo PA, Suarez-Kurtz G (1989): Neutralization of lethal and myotoxic activities of South American rattlesnake venom by extracts and constituents of the plant *Eclipta prostrata* (Asteraceae). *Toxicon 27:* 1003–1009.

Otero R, Tóbon GS, Gómez LF, Osorio RG, Valderrama R, Hoyos D, Urreta JE, Molina S, Arboleda JJ (1992): Accidente ofídico en Antioquia y Chocó. Aspectos clínicos y epidemiológicos (marzo de 1989–febrero de 1990). *Acta Médica Colombiana 17:* 229–249.

Otero R, Núñez V, Osorio RG, Gutiérrez JM, Giraldo CA, Posada LH (1995): Ability of six Latin American antivenoms to neutralize the venom of mapaná equis (*Bothrops atrox*) from Antioquia and Chocó (Colombia). *Toxicon 6:* 809–815.

Otero R, Fonnegra R, Jiménez SL, Núñez V, Evans N, Alzate SP, García ME, Saldarriaga M, Del Valle G, Osorio RG, Díaz A, Valderrama R, Duque A, Vélez HN (2000a): Snakebites and ethnobotany in the northwest region of Colombia. Part I: Traditional use of plants. *J Ethnopharmacol 71:* 493–504.

Otero R, Núñez V, Jiménez S, Fonnegra R, Osorio RG, García ME, Díaz A (2000b): Snakebites and ethnobotany in the

northwest region of Colombia. Part II: Neutralization of lethal and enzymatic effects of *Bothrops atrox* venom. *J Ethnopharmacol 71:* 505–511.

Otero R, Núñez V, Barona J, Fonnegra R, Jiménez SL, Osorio RG, Saldarriaga M, Díaz A (2000c): Snakebites and ethnobotany in the northwest region of Colombia. Part III: Neutralization of the hemorrhagic effect of *Bothrops atrox* venom. *J Ethnopharmacol 73:* 233–241.

Otero R, Gutiérrez J, Rodríguez O, Cárdenas SJ, Rodríguez L, Caro E, Díaz A, Nuñez V, Mesa MB, Duque E, Arango JL, Gómez F, Cano F, Toro A, Martínez J, Cornejo W, Gómez LM, Uribe FL, Miranda E, Pinto ML, Pareja JI, Marquez A, García J, Ramírez A, Diosa RA, Velázquez LH, Mena I, Arbelaez HJ, Buendia H (2001): Aspectos actuales de las mordeduras de serpientes en Colombia. Propuesta de intervención para un grave problema de salud pública en Antioquia y Chocó. *Rev Epidemiol Antioquia 26:* 43–48.

Weniger B (1991): Theory and instrumentation involved with extraction, control, quality insurance and registration of natural products. In: *First International Advanced Course on Technology and Control of Drugs*, Perugia, pp. 31–40.

World Health Organization (1981): Progress in the characterization of venoms and standardization of antivenoms. *WHO Offset Publication* 58: 1–44.