Pharmacology & Pharmacy, 2014, 5, 828-837
Published Online July 2014 in SciRes. http://www.scirp.org/journal/pp
http://dx.doi.org/10.4236/pp.2014.58093

Inhibition of Toxic Effects of Viper and Cobra Venom by Indian Medicinal Plants

M. I. Alam

Department of Paramedical Sciences, Hamdard Institute of Medical Sciences & Research, Jamia Hamdard University, New Delhi, India
Email: iqbalasc@yahoo.com

Received 1 June 2014; revised 3 July 2014; accepted 20 July 2014

Abstract

The mortality rate from snakebites in West Bengal is very high and most of the deaths are caused by the *Daboia russelli* and *Naja naja* envenomation. Twenty-three plants from the seventeen families were collected from the traditional healers and explored for the first time for antisnake venom activity. In our previous report, the methanolic root extract of the Indian medicinal plants *Pluchea indica*, *Hemidesmus indicus*, *Vitex negundo* and *Emblica officinalis* significantly neutralized the Viper and Cobra venom-induced pathophysiological changes [1] [2]. In the present study, we explored four plant extracts (*Curcuma aromatica*, *Aristolochia indica*, *Androgrphis paniculata* and *Curcuma zeodaria*) for the antisnake venom activity. The plant extracts significantly antagonized *Daboia russelli*, *Echis carinatus*, *Ophiophagus hannah* and *Naja kaouthia* venom-induced lethal activity both in *in vitro* and *in vivo* studies. *Daboia russellii* venom-induced haemorrhage, coagulant, defibrinogenating and PLA_2 activity were significantly neutralized by the extracts. No precipitating bands were observed between the plant extract and venom. This observation confirmed the role of active constituents of plants and plant materials involved in snake venom inhibition. Further studies are going on in our laboratory for the identification of active molecules as well as their mechanism of venom inhibition.

Keywords

Anti-Snake Venom Activity, Medicinal Plants, Snake Venom

1. Introduction

Every year more than 100,000 people die due to snakebite worldwide. In India morality rate is very high, 40,000 - 50,000 die annually [3] [4]. *Daboia russelli*, *Echis carinatus*, *Naja kaouthia and Naja naja* are the common

snakes found throughout India and a large number of deaths occur due to envenomation by these snakes. The antiserum is the only therapeutic agent available for the treatment of snakebite and does not provide enough protection against venom inducing haemorrhage, necrosis, nephrotoxicity and often produces hypersensitive reactions [5]-[7]. Antiserum development in animal is time consuming, expensive and requires ideal storage condition. Monovalent antiserum is not available and the health center is usually far and few in number. To overcome these drawbacks, there is a great need to search, to develop new affordable and suitable antidote against snakebite.

The World Health Organization estimates that 80% of the world's population depends on traditional medicine for their primary health care needs. As long as man can remember, plants/plant materials have been used worldwide in traditional medicine for the treatment of different diseases. It is estimated that even today approximately seventy percent of the world population rely on medicinal plants as their primary source of medicines [8]. Till today many of the drugs used are derived from natural products or are dependent upon natural products for the development. In the recent discoveries of the analgesic, anti-arthritis, antimicrobial and anticancer agent (taxol) indicate the continuing importance of plant species in drug discovery. Currently, a large number of plants and plant materials are being screened for pharmacological activities especially those used in traditional/folk medicine against different diseases. Over the years, many attempts have been made for the development of snake venom antagonists especially from plant sources in spite of the existence of antiserum. Many Indian medicinal plants/plant materials are recommended for the treatment of snakebite [2] [9] [10] and some are tested, but so far no systematic analysis has been done. In our earlier studies, it was showed that four of these plants (**Table 1**), successfully neutralized the venom-induced pathophysiological changes [1] [2]. The present investigation attempts to identify those species, which are used in folk medicine in West Bengal, India and are worthy of further investigation as leads for drug development in management of snake bite.

2. Materials and Methods

2.1. Venom

Lyophilized snake venom of *Daboia russelli*, *Echis carinatus*, *Ophiophagus hannah* and *Naja kaouthia* were obtained from Calcutta snake park, Kolkata, India and were preserved in a desiccator at 4˚C for further use. It was dissolved in 0.9% saline and centrifuged at 2000 rpm for 10 min. The solution was used as venom and was stored at 4˚C until further use. Venom concentration was expressed in terms of dry weight.

2.2. Plant Extracts

Plants/plant materials were obtained from different places of West Bengal after being indicated by traditional healer and then they were identified by Dr. N. Paria, Department of Botany, University of Calcutta, West Bengal. Some of these plants were also purchased commercially from M/s United chemical and Allied products, Kolkata, and processed in the following manner.

After drying and crushing individual sample of the relevant parts of each plant species, was extracted first by refluxing with petroleum ether (50˚C - 60˚C, for 72 h) and then by methanol (60˚C - 80˚C for 72 h). Methanol extract was concentrated in vaccuo and kept in a desiccator at room temperature for further use. Before use, it was dissolved in saline and centrifuged at 2000 rpm for 10 min at room temperature. The solution was used for further investigation and kept at 4˚C. The plant extracts were expressed in terms of dry weight.

2.3. Snake Venom Antiserum

Lyophilized polyvalent snake venom antiserum (as reference serum) was obtained from Bengal chemicals and pharmaceuticals Private Ltd. Kolkata. Before use antiserum was dissolved in 0.9% saline.

2.4. Animals

Male albino mice of body weight about 18 - 20 g were used in this study after acclimatization to the laboratory condition. The study plan was approved by the Animal Ethics Committee of University of Calcutta, Kolkata; India. All animals were cared for and handled in accordance with the guideline of the ethical committee.

Table 1. Medicinal plants used by traditional healer against snake bites were tested in laboratory against snake venoms in experimental rodents.

Family and species	Parts used	Source	Antisnake venom activity (present study)
1. Acanthaceae			
Andrographis paniculata Nees	Root	Medicineman and Literature	Present
Rhinacanthus communis Nees	Root	Medicineman	Absent
2. Amaranthaceae			
Achyranthes aspera linn.	Root	Medicineman	Absent
3. Aristolochiaceae			
Aristolochia indica linn.	Root	Medicineman and Literature	Present
4. Asteraceae (compositae)	Aerial parts	Medicineman	Absent
Eclipta alba (L.) Hassk			
Syn. *Eclipta prostrata* (L.L.)			
Eupatorium triplinerve Vahl	Leaf	Medicineman	Absent
Syn. *Eupatorium ayapana* Vent.			
**Pluchea indica* (L.) Less	Root	Medicineman	Present
5. Cururbitaceae			
Trichosanthes dioica Roxb	Root	Literature	Absent
6. Crasulaceae			
Kalanchae pinnata (Lamk.) Pers	Root	Literature	Absent
Syn. *Bryphyllum calycinum*			
7. Cyperaceae			
Kyllinga nemoralis (J.R.et G. Forster)	Whole body	Medicineman	Absent
Dandy ex Hutchinson Dalziel			
Syn. *Kyllinga monocephala* Rottb.			
Syn. *Cyperas kylling* Endle.			
8. Euphrobiaceae			
Ricinus communis L.	Root	Medicineman	Absent
**Embilica officinalis* Gaertn	Root	Medicineman	Present
9. Fabaceae			
Albizzia lebbeck (L.) Beath	Stem Bark	Literature	Absent
10. Lamiaceae (Labiatae)			
Leucas lavandulifolia Sm.	Leaf	Medicineman and Literature	Absent
11. Menispermaceae			
Stephania japonica (Thunb.) Miers	Root	Medicineman	Absent
Syn. *Stephania heamandifolia* (Wild) Wslp			
12. Moringaceae			
Moringa pterygasperma Gaertn.	Stem Bark	Medicineman	Absent
Syn. *Moringa oleifera* (Lamk)			
13. Molluginaceae			
Glinus oppositifoilius L.	Whole body	Medicineman	Absent
Syn. *Mollugo spergula* (Linn.)			
14. Periplocaceae			
**Hemidesmus indicus* (L.) R.Br.	Root	Literature	Present
Syn. *Periploca indica* (L.)			
15. Theaceae			
Camellia sianensis (Linn.) Kuntz	Root	Medicineman	Absent
16. Verbenaceae			
**Vitex negundo* Linn.	Root	Medicineman and Literature	Present
17. Zingiberaceae			
Curcuma aromatica Salisb	Root	Medicineman and Literature	Present
Curcuma zedoaria Rox	Root	Medicineman	Present
Curcuma longa Linn.	Root	Medicineman	Absent

*Plant possess anti-snake venom activity as reported earlier (Alam and Gomes, 2003; Alam *et al*., 1996).

2.5. Inhibition of Venom Lethal Effect

The toxicity of snake venom was assessed by injection of different concentration of venom in 0.2 ml physiological saline into tail vein of male albino mice 18 - 20 gm [1]. To assess the *in vitro* antagonism, various concentrations of venom (1 - 300 LD_{50}) were mixed with a fixed amount of plant extract (2 mg), the mixture incubated at 37˚C for 1h, and centrifuged at 2000 rpm for 10 min. The supernatant was injected intravenously into male albino mice (18 - 20 g), ten mice per dose. The median lethal dose (LD_{50}) was calculated 24 h after injection of the venom-extract mixture. Lethal toxicity was also assessed by subcutaneously injection of various doses of venom. The neutralizing potency of each plant extract was assessed by *s.c* injection of venom (1 - 5 LD_{50}) into groups of ten mice followed by immediate injection of fixed dose of plant extract (5 mg) orally (*p.o.*).

2.6. Inhibition of Venom Haemorrhagic Activity

The minimum haemorrhagic dose (MHD) of venom (defined as the least amount of venom which when injected intradermaly (*i.d*) into mice results in a haemorrhagic lesion of 10 mm diameter 24 h later was measured [11]. Neutralization of the haemorrhagic activity was estimated by mixing a fixed amount of plant extract (2 mg) with different amounts of venom (1 - 300 MHD). The plant extract-venom mixture was incubated at 37˚C for 1h, centrifuged at 2000 rpm × 10 min, and 0.1 ml of supernatant injected (*i.d*). The haemorrhagic lesion was estimated after 24 h. To assess the antihaemorrhagic activity of venom *in vivo*, various amount of venom (1 - 6 MHD) were injected (*i.d*) followed immediately by the plant extract (5 mg, *p.o.*) and the haemorrhagic lesion measured after 24 h.

2.7. Inhibition of Venom Coagulant Activity

The minimum clotting dose of plasma (MCDP) was determined as described by Alam *et al*. [1]. Neutralization of this activity was estimated by mixing different amount of venom (1 - 200 MCD), with a fixed amount of plant extract (100 µg), incubating for 1 h at 37˚C. Different concentration of incubate were added to the experimental tube in place of 0.1 ml physiological saline, and the clotting time was recorded.

2.8. Inhibition of Venom Serum Inhibitory Activity

Inhibition of snake venom serum activity was measured in order to examine if the decreased clotting of *V. russellii* venom on goat plasma was due to venom inhibition in serum. Goat blood was collected without anticoagulant, incubated in glass tubes for 2 h at 37˚C and then centrifuged at 2000 rpm for 15 min. Pooled sera were kept frozen at −20˚C. Equal volumes of Tyrode-Hepes buffer (137 mM Nacl; 2.7 mM KCl; 12 mM $NaHC0_3$, 0.42 mM NaH_2Po_4, 10 mM Hepes; pH 7.4) or goat serum was incubated with venom solution of different concentration for 5 min at 37˚C. Aliquots of 50 µl were then added to 200 µl of goat serum (maintained for 2 min at 37˚C) and the clotting time recorded. MCDS were then calculated. Neutralization of serum inhibitory activity was estimated by mixing different amount of venom with fixed amount of plant extract, incubating for 1 h at 37˚C and centrifuged. Supernatant was then added to the experimental group and the clotting time was observed.

2.9. Inhibition of Venom Defibrinogenating Activity

Minimum defibrinogenating dose (MDD) of *V. russellii* venom is defined as the minimum amount of venom which when injected *i.v* into mice causes' incoagulable blood 1 h later [1]. Neutralization of this activity was estimated by mixing different amount of venom with fixed amounts of plant extract, incubating at 37˚C for 1 h, and centrifuge at 3000 rpm at 5 minutes. The supernatant was injected *i.v* into albino mice (18 - 20 g) as described above. For *in vivo* studies, the MDD of venom was injected *i.v* followed by the plant extract (*p.o.*) and the nature of the blood observed after 1 h.

The neutralization ability of plant extracts was expressed as effective dose 50 (ED_{50}) defined as the amount of plant extract at which the venom induced (lethal and haemorrhagic) action was reduced by 50%. The effective dose (ED) was defined as the amount of plant extract at which the venom induced MCDP and MDD activities were effectively neutralized.

2.10. Inhibition of Venom PLA_2 Effect

For PLA_2 activity experiments, egg yolk (obtained from commercially available eggs), 0.5% EDTA, 50 mM

Tris HCl buffer (pH 7.5), 2% sodium chloride, 1% calcium chloride and 0.9% sodium chloride from Sigma except otherwise stated were used.

For carrying out the experiment, 2.0 ml of egg yolk suspension and 0.2 ml of test material (venom or venom compound) were mixed in the test tube and incubated at 37˚C for 1 h. After incubation the time required for coagulation of egg yolk was recorded by placing the test tube containing test materials on the boiling water bath. A blank was run with normal saline instead of test material. One unit enzyme activity was defined as the amount of venom, which increased the coagulation time of the egg yolk control by one minute.

Neutralization of the enzyme activity was estimated on the different amount of plant extract mixed with the different amount of viper venom. The venom-extract mixture was incubated at 37˚C for 1 h. Centrifuged at 2000 rpm for 10 min, supernatant was tested in a total of 0.2 ml for the enzyme neutralization activity.

2.11. Immunological Interaction of Venom and Plant Extracts

To assess the immunological interaction of venom and plant extract, immunogel diffusion [12] and immunogel electrophoresis [13] were performed.

2.12. Statistical Analysis

All results are expressed as Mean ± SE. The significance of the difference between mean was determined by student's t test. All assays were performed ten times.

3. Results

LD_{50} of *Doboia russellii* venom was 2.2 µg per mouse (*i.v*), 45 µg per mouse (*s.c*), *Echis carinatus* venom 1.58 µg per mouse (*i.v*), 25.19 µg per mouse (*s.c*), *Ophiophagus hannah* venom was 2 µg per mouse (*i.v*), 2.51 µg per mouse (*s.c*) and *Naja kaouthia* venom was 2.8 µg per mouse (*i.v*), 4.61 µg per mouse (*s.c*). The MHD was 5 µg per mouse of *Daboia russelli* venom and 2 µg per mouse of *Echis carinatus* venom. MCDP/MCDS 1 µg/ml, PLA_2 was 2 µg and MDD was 2.5 µg per mouse of *Daboia russelli* venom. Both plant extracts (up to 1 mg/kg, *i.v*) did not produce lethal effect up to 48 h of observation.

Through collection from different rural places of West Bengal and identification, twenty three plants/plant materials of seventeen families were examined for anti-snake venom activity. Eight plant materials were found to be active against snake bite (**Table 2**). In the present paper only four plants those are not reported earlier were taken for anti-snake venom activity. The plant extracts (up to 1 mg/kg, *i.v*) did not produce lethal effect.

Table 2. Neutralization of lethal action of *Daboia russellii* and *Echis carinatus* venom by plant extracts in male albino mice.

Extract (mg)	D. russellii Venom (µg)	Fold of neutralization (LD_{50})	*ED_{50} (mg)	*E. carinatus* venom (µg)	Fold of neutralization (LD_{50})	ED_{50} (mg)
Venom extracts incubated 370˚C/60 min and injected *i.v*						
C. aromatica (2)	45	20.5	0.21 ± 0.01	50	31.6	0.22 ± 0.01
A. indica (2)	25	11.4	0.22 ± 0.01	12	07.6	0.31 ± 0.02
A. paniculata (2)	25	11.4	0.31 ± 0.01	06	03.8	0.53 ± 0.02
C. zedoaria (2)	15	06.8	0.53 ± 0.02	06	03.8	0.61 ± 0.01
Venom injected (*s.c*) ** followed immediately by extract, *p.o.*						
C. aromatica (5)	180	4	2.6 ± 0.22	100	3.96	2.7 ± 0.20
A. indica (5)	090	2	2.7 ± 0.20	060	2.38	2.6 ± 0.21
A. paniculata (5)	090	2	2.8 ± 0.22	060	2.38	2.5 ± 0.22
C. zedoaria (5)	090	2	2.8 ± 0.23	050	1.98	2.7 ± 0.22

*Defined as the effective dose of plant extract which neutralized venom dose of 2 LD_{50} *in vitro*/2LD_{50} *in vivo*. Results are expressed as mean of ten observations. **Duration of deaths time following venom exposure: *Daboia russellii* venom (1 Minimum lethal dose) = 13.24 ± 0.30 hours; *Echis carinatus* venom (1 Minimum lethal dose) = 12.20 ± 0.30 hours.

3.1. Lethal Activity

In *in vitro* study, viper venom (1 - 50 LD_{50}) was incubated with plant extracts (2 mg) and gave protection against venom-induced lethality. In *in vivo* study, viper venom (1 - 6 LD_{50}) was injected *s.c* into male albino mice followed by the plant extracts (5 mg/mouse, *p.o.*). *Daboia russellii*, *Echis carinatus*, *Ophiophagus hannah* and *Naja kaouthia* venom induced lethality was significantly antagonized by the plant extracts (**Table 2** & **Table 3**). The ED_{50} of the plant extracts of *C. aromatica*, *A. indica*, *A. paniculata* and *C. zeodaria* were found to be 0.21 mg, 0.22 mg, 0.31 mg, 0.53 mg *in vitro* and 2.6 mg, 2.7 mg, 2.8 mg, 2.8 mg respectively in *in vivo* against *Daboia russelli* venom (**Table 2**).

3.2. Haemorrhagic Activity

In *in vitro* study, venom (1 - 10 MHD) incubated with extract (2 mg) and injected (i.d) into mice, gave protection against venom induced haemorrhagic activity (**Table 4**). In *in vivo* study, venom (1 - 2 MHD) injected (*i.d*) into mice followed by plant extracts (5 mg, *p.o.*), gave protection against venom induced haemorrhagic activity. The degree of protection in *in vivo* was less than that of *in vitro*. Effective dose of *C. aromatica*, *A. indica*, *A. paniculata* and *C. zeodaria* were found to be 0.25 mg, 0.26 mg, 0.35 mg, 0.34 mg *in vitro* and 2.5 mg, 2.6 mg, 2.5 mg, 2.7 mg respectively *in vivo* against *Daboia russellii* venom (**Table 4**).

Table 3. Neutralization of lethal action of *Naja kaouthia and Ophiophagus hannah* venom by plant extracts in male albino mice.

Extract (mg)	*N. kauthia* Venom (µg)	Fold of neutralization (LD_{50})	*ED_{50} (mg)	*O. hannah* venom (µg)	Fold of neutralization (LD_{50})	ED_{50} (mg)
Venom extracts incubated 370˚C/60 min and injected *i.v*						
C. aromatica (2)	18	6.4	0.32 ± 0.01	14	7	0.41 ± 0.02
A. indica (2)	12	4.3	0.41 ± 0.01	06	3	0.51 ± 0.01
A. paniculata (2)	06	2.1	0.61 ± 0.02	06	3	0.61 ± 0.01
C. zedoaria (2)	06	2.1	0.65 ± 0.01	06	3	0.62 ± 0.01
Venom injected (*s.c*) ** followed immediately by extract, *p.o.*						
C. aromatica (5)	18	4.0	2.7 ± 0.21	12	4.78	2.7 ± 0.22
A. indica (5)	10	2.1	2.6 ± 0.15	06	2.39	2.7 ± 0.21
A. paniculata (5)	10	2.1	2.7 ± 0.21	06	2.39	2.8 ± 0.15
C. zedoaria (5)	10	2.1	2.7 ± 0.14	06	2.39	2.6 ± 0.23

*Defined as the effective dose of plant extract which neutralized venom dose of 2 LD_{50} *in vitro*/2LD_{50} *in vivo*. Results are expressed as mean of ten observations. **Duration of deaths time following venom exposure: *Ophiophagus hanah* venom (1 Minimum lethal dose) = 6.24 ± 0.20 hours; *Naja kaouthia* venom (1 Minimum lethal dose) = 10.68 ± 0.30 hours.

Table 4. Neutralization of haemorrhagic action of *Daboia russellii* and *Echis carinatus* venom by plant extracts in male albino mice.

Extract (mg)	*D. russellii* Venom (µg)	Fold of neutralization (LD_{50})	*ED_{50} (mg)	*E. carinatus* venom (µg)	Fold of neutralization (LD_{50})	ED_{50} (mg)
Venom extracts incubated 370˚C/60 min and injected *i.v*						
C. aromatica (2)	50	10	0.25 ± 0.01	30	15	0.32 ± 0.01
A. indica (2)	40	08	0.26 ± 0.01	20	10	0.35 ± 0.02
A. paniculata (2)	40	08	0.35 ± 0.02	20	10	0.36 ± 0.02
C. zedoaria (2)	30	06	0.34 ± 0.01	10	05	0.45 ± 0.02
Venom injected (*s.c*) ** followed immediately by extract, *p.o.*						
C. aromatica (5)	10	02	2.5 ± 0.18	14	07	2.6 ± 0.18
A. indica (5)	05	01	2.6 ± 0.22	06	03	2.8 ± 0.24
A. paniculata (5)	05	01	2.5 ± 0.20	06	03	2.8 ± 0.10
C. zedoaria (5)	05	01	2.7 ± 0.16	06	03	2.9 ± 0.19

Defined as the effective dose (ED) of plant extract which neutralized venom dose of 25 MHD *in vitro* and 1 MHD *in vivo*. Results are expressed as mean of ten observations.

3.3. Coagulant Activity

The effect of *Daboia russellii* venom on coagulant activity and their neutralization by plant extract were examined as estimated by plasma recalcification time. The normal value (0.9% saline + calcium induced) of minimum clotting dose of plasma (MCDP) was found to be 25 ± 0.17 seconds and venom + calcium induced MCDP was found to be 15 ± 0.20 second.

The extracts (0.2 mg each) were incubated with different amount of venom (1 - 75 µg) and tested for coagulant activity. The venom induced plasma recalcification were antagonized effectively by the plant extracts (**Table 5**).

3.4. Serum Inhibitory Activity

Daboia russellii venom was taken for the serum inhibitory activity. The normal value (calcium-induced) of minimum clotting dose of serum (MCDS) was found to be 25 ± 0.20 seconds. The plant extracts (0.2 mg each) incubated with different amount of venom (1 - 75 µg), antagonized serum inhibitory activity significantly (**Table 5**).

3.5. Defibrinogenating Activity

The methanolic root extract of the plants effectively antagonized the viper venom induced defibrinogenating activity. In *in vitro* study, the plant extracts (2 mg) gave protection up to 20 MDD against venom-induced defibrinogenation. In *in vivo* study, venom-induced defibrinogenation was antagonised by the plant extracts. The effective dose of *C. aromatica*, *A. indica*, *A. paniculata* and *C. zeodaria* was found to be 0.32 mg, 0.34 mg, 0.43 mg, 0.44 mg respectively in *in vitro* and 2.4 mg, 2.6 mg, 2.5 mg, 2.5 mg respectively in *in vivo* of *Daboia russellii* venom (**Table 6**). The fold of protection was always higher in *in vitro* studies.

3.6. Phospholipase Activity

Phospholipase A_2 activity of *Daboia russellii* venom was assessed by egg yolk coagulation method. One unit of *Daboia russellii* venom activity was found to be 2 µg which increased the coagulation time by one minute (control 0.9% saline, coagulation time was found to be 45 ± 1.16 seconds).

The extracts (*C. aromatica*, *A. indica*, *A. paniculata* and *C. zeodaria*: 0.2 mg each) were incubated with different amount of viper venom (2 - 100 µg) and tested for phospholipase A_2 activity. The venom PLA_2 was effectively neutralized by the plant extracts. The *Curcuma aromatic* extract (0.2 mg) significantly neutralized the venom (80 µg) induced PLA_2 activity. The degree of protection was found to be higher in *Curcuma aromatic*. The effective dose to be 18 µg, 20 µg, 20 µg and 20 µg by *C. aromatica*, *A. indica*, *A. paniculata* and *C. zeodaria* respectively (**Table 7**).

Table 5. Neutralization of coagulant activity of *Daboia russellii* venom by the plant extracts.

Extract (mg)	Venom (µg)	Fold of neutralization (in terms of MCDP/MCDS)	*Effective dose ED_{50}
Venom extracts incubated 370˚C/60 min added to plasma for MCDP			
C. aromatica (0.2)	75	75	20 ± 1.5
A. indica (0.2)	50	50	22 ± 1.6
A. paniculata (0.2)	50	50	22 ± 1.4
C. zedoaria (0.2)	50	50	23 ± 1.3
Venom extracts incubated 37˚C/60 min then added to plasma for MCDS			
C. aromatica (0.2)	75	75	17 ± 1.6
A. indica (0.2)	50	50	19 ± 1.6
A. paniculata (0.2)	50	50	22 ± 1.7
C. zedoaria (0.2)	50	50	21 ± 1.5

*Defined as the effecting dose of plant extract which neutralized venom dose of 15 MCDP/15 MCDS. Results are expressed as mean of 10 observations. MCDP = minimum clotting dose plasma; MCDS = Minimum clotting dose serum.

Table 6. Neutralization of defibrinogenating activity of *Daboia russellii* venom by plant extracts in male albino mice.

Extract (mg)	Venom (µg)	Fold of neutralization (in terms of MDD)	*Effective dose ED (µg)
Venom extracts incubated 370˚C/60 min added to plasma for MCDP			
C. aromatica (2)	50	20	0.32 ± 0.01
A. indica (2)	25	10	0.34 ± 0.02
A. paniculata (2)	25	10	0.43 ± 0.02
C. zedoaria (2)	15	06	0.44 ± 0.01
Venom extracts incubated 37˚C/60 min then added to plasma for MCDS			
C. aromatica (5)	3.5	1.5	2.4 ± 0.06
A. indica (5)	2.5	1.0	2.6 ± 0.05
A. paniculata (5)	2.5	1.0	2.5 ± 0.16
C. zedoaria (5)	2.5	1.0	2.5 ± 0.20

*Defined as the effective dose of plant extract which neutralized venom dose of 3 MDD *in vitro* and 1 MDD *in vivo*. Results are expressed as mean of 10 observations. MDD minimum defibrinogenating. Dose = 2.5 µg.

Table 7. Neutralization of Phospholipase A2 activity of Daboiarusselli venom by plant extracts.

Extract (mg)	Venom (µg) (Unit PLA2)	Fold of neutralization (in terms of Unit)	*Effective dose ED (µg)
Control (only venom)			
Daboia russellii	2 (1)	1	–
Venom extracts incubated 37˚C/60 min then added to egg yolk suspension			
C. aromatica (0.2)	80 (40)	40	18 ± 1.5
A. indica (0.2)	60 (30)	30	20 ± 1.6
A. paniculata (0.2)	60 (30)	30	20 ± 2.0
C. zedoaria (0.2)	60 (30)	30	20 ± 0.3

Effective dose of plant extract which neutralized venom dose of 10 units of PLA_2 activity. Results are expressed as mean of ten observations. 1 unit = one unit of enzyme activity of *Daboia russelli* venom was defined as the amount of venom which increased the coagulation time of egg yolk by one minute.

3.7. Immunological Interaction of Snake Venom and Plant Extracts

Immunogel diffusion and immunogel eclectophoresis of snake venom of *Daboia russelli*, *Echis carinatus*, *Ophiophagus hannah* and *Naja kaouthia* were produced precipitating band with polyvalent antisnake venom antiserum. However venom antigen and plant extract did not produced any precipitating band.

4. Discussion

More than hundreds of plants have been used in folk medicine throughout the world for snakebites [14] [15]. Till date few plants/plant materials have been evaluated in well controlled assays and about forty of them have been found to be effective against Cobra and Viper envenomation [2] [16]. In our earlier studies we found that the methanolic root extracts of *H. indicus*, *P. indica*, *E. officinalis* and *V. negundo* possessed potent anti-snake venom activity [1] [2]. These plant extracts effectively neutralized the snake venom inducing lethal, haemorrhagic, coagulant and anti-coagulant activity both *in vivo* and *in vitro*. An organic acid, 2-hydroxy-4-methoxy benzoic acid was isolated, purified from the Indian medicinal plant *Hemidesmus indicus* root extract, which effectively antagonized the *Daboia russelli* venom inducing pathophysiological changes both *in vitro* and *in vivo*. The mechanism of venom inhibition by the compound was due to free radical formation inhibition through lipoxygenase and cyclo-oxygenase pathway [17].

In the present work, four plants out of twenty three plant species were reported for the first time in the Indian context. The plants/plant materials were collected from the traditional healers used in snakebite in the eastern parts of India and were identified by the botanist. In the present investigation the four new plant species which were found active against snake venom were taken for further study. The four plant extracts *Aristolochia indica*, *Andrographis paniculata*, *Curcuma aromatica* and *Curcuma zeodaria* were effective against snake-venom of Cobra (*Naja kaouthia* and *Ophiophagus hannah*) and Viper (*Daboia russelli* and *Echis carinatus*) venom both *in vivo* and *in vitro*. The maximum fold of protection was found by the methanolic root extract of *Curcuma aromatica* both in Viper and Cobra venom. The *Daboia russellii* venom which induced haemorrhagic, coagulant and anticoagulant activities (both *in vitro* and *in vivo*) were significantly neutralized by all the four plant extracts.

Of all the plant compounds reported against snake envenomation, the mechanism of action was somehow similar. Some of the plant constituents like coumarin, polyphenols, flavonoids, cinnamic acid derivatives, hydroxyl benzoic acid derivatives, tannins and aristolochic acid have the protein binding and enzyme inhibiting properties [18]. This is due to their affinity to peptides, proteins (enzymes and other toxic components) and metal binding capacity present in the compounds. The PLA_2 enzyme is present in large quantity in almost all the snake venom and has been found to be neutralized by several plant compounds [17] [19] [20].

Curcumin present in *Curcuma aromatica* may be involved in venom inactivation process. Another unsaturated ketone of turmeric, ar-turmerone was shown to inhibit the lethal action of rattle snake venom [21] [22]. Aristolochia species are famous for snakebite remedies. The aristolochic acid effectively neutralized the viper venom-induced edema [23]. This compound may act through enzyme association and cause significant change in the secondary structure of the snake venom protein. The other chemical compounds present in the plant can be held responsible for the neutralizing effect of snake venom actions [24]. Many compounds identified from plants having different chemical structure were reported to be capable of interacting with peptides and proteins (enzyme) of snake venom. The mechanism of action of the plant extracts/plant compounds are still not clear [25], and they may be attributed to the blocking of receptors-structure prone to chemical attack, and may block the active site of the snake venom. Other mechanism of action of the plant compounds are inhibition of metalloproteinase present in the snake venom. This is due to the metal chellator substances present in the plant extracts/plant compounds. The presence of benzoic acid derivatives and phenolic compounds seem to act through LOX, COX and free radical formation inhibiting systems [17]. This is one of the possible mechanisms of action of venom inhibition. The above evidence may provide some clues of mechanism of venom inhibitions. In our laboratory further work is going on for the identification of pure compound(s) from the plants/plant extracts and their mechanism of venom inhibition.

Acknowledgements

The authors are obliged to Council of Scientific and Industrial Research, New Delhi, for partial financial support.

References

[1] Alam, M.I., Auddy, B. and Gomes, A. (1996) Viper Venom Neutralizing by Indian Medicinal Plant (*Hemidesmus indicus and Pluchea indica)* Root Extract. *Phytotherapy Research*, **10**, 58-61. http://dx.doi.org/10.1002/(SICI)1099-1573(199602)10:1<58::AID-PTR775>3.0.CO;2-F

[2] Alam, M.I. and Gomes, A. (2003) Snake Venom Neutralization by Indian Medicinal Plant (*Vitex negundo* and *Emblica officinalis*) Root Extracts. *Journal of Ethnopharmacology*, **86**, 75-80. http://dx.doi.org/10.1016/S0378-8741(03)00049-7

[3] Warrell, D.A. (1995) Clinical Toxicology of Snake Bites in Asia. In: White, M.A., Ed., *Handbook of Clinical Toxicology of Animal Venoms and Poisons*, CRC Press, Boca Raton, 493-588.

[4] White, J. (2002) Bites and Stings from Venomous Animals: A Global Overview. *Therapeutic Drug Monitoring*, **22**, 65-68. http://dx.doi.org/10.1097/00007691-200002000-00014

[5] Sutherland, S.K. (1977) Serum Reaction. An Analysis of Commercial Antivenom and the Possible Role of Anticomplimentary Activity in De-Novo Reactions to Antivenoms and Antitoxins. *Medical Journal of Australia*, **1**, 613-615.

[6] Corrigan, P., Russel, F.E. and Wainchal, J. (1987) Clinical Reactions to Antivenin. In: Rosenburg, P., Ed., *Animal, Plant and Microbial of Toxins*, Pergamon Press, New York, 457-464.

[7] Stahel, E., Wellamer, R. and Freyvogel, T.A. (1985) Verzidtongen Durch Einheimische (*Viper aviperaberus* and *Viper*

aaspirise): A Reterospective Studies on 133 Patients. *Schweizerische medizinische Wochenschrift*, **155**, 890-896.

[8] McChesney, J.D. (1995) The Promise of Natural Products for the Development of New Pharmaceuticals and Agrochemicals. In: Seidl, P.R., Gottlieb. O.R. and Kaplan, M.A.C., Eds., *Chemistry of the Amazon Symposium Series*, America Chemical Society: D.C, 54.

[9] Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956) Glossary of Indian Medicinal Plants. CSIR Publication, New Delhi, 330.

[10] Nazimudeen, S.K., Ramaswamy, S. and Kameswaran, L. (1978) Effect of Andrographispaniculata on Snake Venom Induced Death and Its Mechanism. *Indian Journal of Pharmaceutical Sciences*, **40**, 132-133.

[11] Theakston, R.D.G. and Reid, H.A. (1983) Development of Simple Standard Assay Procedures for the Characterization of Snake Venom. *Bulletin of the World Health Organization*, **61**, 949-956.

[12] Ouchterlony, O. (1948) *In Vitro* Methods for Testing the Toxin Producing Capacity of Diphtheria Bacteria. *Acta Pathologica Microbiologica Scandinavica*, **25**, 186-190. http://dx.doi.org/10.1111/j.1699-0463.1948.tb00655.x

[13] Graber, P. and Williams, L.A. (1954) Method for Combined Investigation of Electrophoretic and Immunochemical Properties of a Protein. *Biochimica et Biophysica Acta*, **10**, 193-197.

[14] Houghton, P.J. and Osibogun, I.M. (1993) Flowering Plant Used against Snakiebite. *Journal of Ethnopharmacology*, **39**, 1-29. http://dx.doi.org/10.1016/0378-8741(93)90047-9

[15] Samy, R.P., Thwin, M.M., Gopalkrishnakone, P. and Ignacimuthu, S. (2008) Ethnobotanical Survey of Folk Plants for the Treatment of Snakebites in Southern Part of Tamilnadu. *Journal of Ethnopharmacology*, **115**, 302-312. http://dx.doi.org/10.1016/j.jep.2007.10.006

[16] Martz, W. (1992) Plants with Reputation against Snakebite. *Toxicon*, **30**, 1131-1142. http://dx.doi.org/10.1016/0041-0101(92)90429-9

[17] Alam, M.I., Auddy, B. and Gomes, A. (1998) Viper Venom Induced Inflammation and Inhibition of Free Radical Formation by Pure Compound (2-hydroxy-4-methoxy Benzoic Acid) Isolated and Purified from Anantamul (*Hemidesmus indicus* R.Br.) Root Extract. *Toxicon*, **36**, 207-215. http://dx.doi.org/10.1016/S0041-0101(97)00070-6

[18] Alcaraz, M.J. and Hoult, J.R.S. (1985) Effect of Hypolaetin-8-Glucoside and Related Flavonoids on Soybean Lipooxygenase and Snake Venom Phospholipase A_2. *Archives Internationales de Pharmacodynamie et de théRapie*, **278**, 4-12.

[19] Gowda, T.V. (1997) Intereaction of Snake Venom Phospholipase A2 with Plant Isolates. Chapter 8. In: Kini, R.M., Ed., *Venom Phosphlipse A2 Enzyme*: *Structure*, *Function and Mechanism*, John Wiley & Sons, Ltd., New York, 205-222.

[20] Melo, P.A. and Ownby, C.L. (1999) Ability of Wedelolactone, Heparin and *para*-bromophenacyl Bromide to Antagonize the Myotoxic Effects of Two Crotalide Venoms and Their PLA_2 Myotoxins. *Toxicon*, **37**, 199-215. http://dx.doi.org/10.1016/S0041-0101(98)00183-4

[21] Ferreira, L.A.F., Henriques, O.B., Anderoni, A.A.S., Viotal, G.R.F., Campos, M.M.C., Habermehl, G.G. and Moraes, V.L.G. (1992) Antivenom and Biological Effects of Ar-Tumerone Isolated from *Curcuma longa* (Zingeberaceae). *Toxicon*, **30**, 1211-1218. http://dx.doi.org/10.1016/0041-0101(92)90437-A

[22] Mendes, M.M., Vieira, S.A.P.B., Gomes, M.S.R., Paula, V.F., Alcantara, T.M., Homsi-Brandeburgo, M.I., Dos Santos, J.I., Margo, A.J., Fontes, M.R.M. and Rodrigues, V.M. (2013) Triaconyl *p*-Coumarate: An Inhibitor of Snake Venom Metalloproteinases. *Phytochemistry*, **86**, 72-82. http://dx.doi.org/10.1016/j.phytochem.2012.10.007

[23] Tsai, L.H., Yang, L.L. and Chang, C. (1980) Inactivation of Formosan Snake Venoms *in Vivo* by Aristolochic Acid, the Chemical Component of Aristolochia Radix. *Formosan Science*, **34**, 40-44.

[24] Mors, W.B., Nascimento, M.C., Bettina, M., Ruppelt, P. and Pereira, N.A. (2002) Plant Natural Product Active against Snakebite—The Molecular Approach. *Phytochemistry*, **55**, 627-642. http://dx.doi.org/10.1016/S0031-9422(00)00229-6

[25] Dufton, M.J. and Hider, R.C. (1980) Lethal Protein Conformations. *Trends in Biochemical Sciences*, **5**, 53-56. http://dx.doi.org/10.1016/S0968-0004(80)80097-1

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.