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Indian Black Scorpion (*Heterometrus bengalensis*) Venom Action Neutralization by Indian Medicinal Plants in Experimental Animals

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Abstract

The anti scorpion venom activity of the Indian medicinal plant (*Hemidesmus indicus, Pluchea indica* and *Aristolochia indica*) root extracts (aqueous and methanol) was established in experimental animal models. Adult black scorpions (*Heterometrous bengalensis*) of both sexes were collected and the Scorpion Venom (SV) was collected by electrical stimulation, pooled, lyophilized and stored at 4 °C. Scorpion venom was expressed in terms of dry weight. Plant root extracts of *Hemidesmus indicus* (H.I) *Pluchea indica* (P.I) and *Aristolochia indica* (A.I) were (aqueous/methanol) prepared and expressed in terms of dry weight. Anti scorpion venom activity was evaluated using various *in vivo* and *in vitro* models (lethality, edema, urinary changes, plasma recalcification, cardiotoxicity, neurotoxicity, etc.). The aqueous/ methanol plant root extracts (H.I, P.I and A.I) could neutralize scorpion venom induced lethality, edema, urinary changes, plasma recalcification, cardiotoxicity, neurotoxicity, in experimental animal models. The aqueous root extracts were more effective in neutralizing scorpion venom actions as compared to methanol plant root extracts. These observations confirmed the ethnopharmacological value of the Indian medicinal plants active against scorpion venom and warrants further detailed studies.

Introduction

Scorpions are one of the venomous arthropods, widely found all over the world, especially in tropical countries, like South America, Mexico, India, etc. There are 1500 species available worldwide, and approximately 25 species are dangerous to humans [1,2] especially in children and the elderly [3]. In India, 90 species of scorpions are available like *Heterometrus swammardami*, *Buthus tamulus, Lycus leavifrons*, etc. among which *Heterometrus bengalensis* is commonly found in West Bengal (Figure 1) [4]. There are 33 species of genus *Heterometrus* around the world [5]. Envenomation by scorpion remains a serious health problem, causing child mortality and elderly morbidity [6]. Their venoms constitute a complex mixture of polypeptides exhibiting different pharmacological activities [7] along with other components, such as enzymes and cytotoxic agents [8,9]. Although only hundreds die annually from harmful species, not less than one million people around the world are predicted to suffer from symptoms at the stung site and are associated with the induction of systematic symptoms [10,11].

Treatments are commonly symptomatic since specific anti venoms are not available, especially in India. In some developed countries, like South Africa, Middle East, America, etc. antivenoms are available but due to their various side effects, their use is controversial. Thus, to emphasize has been given on ancillary treatment. Prophylactic immunisation against scorpion envenoming has also been advocated, but acceptable experimental evidences are lacking [12]. Various alternative/folk and traditional treatments are available against scorpion envenomation, among which the most common one is the usage of herbal products [13]. Plants like, *Mangifera indica* L, *Aristolochia indica, Solanum indicum* L, *Andrographis paniculata Nees, Barringtonia acutangula* L, *Hemidesmus indicus, Pluchea indica* and *Aristolochia indica* etc. [14-16], have been mentioned in traditional medicine and used by village folks for the treatment against scorpion sting. These plant products are cheap and easily available; therefore based on traditional knowledge a few of these plants were selected for their scientific effectiveness. The present investigation explored the ethnopharmacological value of the plant root extracts of *Hemidesmus indicus, Pluchea indica* and *Aristolochia indica,* in experimental scorpion envenomation.

Materials and Methods

Chemicals

All chemicals and solvents used were of Analytical Grade, unless

Figure 1: Indian black scorpion *Heterometrus bengalensis*.

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Table 1: Effect of aqueous and methanol plant root extracts of H.I., P.I and A.I. on SV induced lethal action in male albino mice.

Aqueous extracts (mg/kg)	SV mg (no. of animals died/total animals)	MLD Fold	Protection (%)
H.I (100)	0.4(0/6)	1	100
	0.8(0/6)	2	200
	1.2(6/6)	3	N.P
P.I (100)	0.4(0/6)	1	100
	0.8(0/6)	2	200
	1.2(6/6)	3	N.P
A.I (20)	0.4(0/6)	1	100
	0.8(0/6)	2	200
	1.2(6/6)	3	N.P
Methanol extracts (mg/kg)	SV mg (no. of animals died/total animals)	MLD Fold	Protection (%)
H.I (100)	0.4(0/6)	1	100
	0.8(0/6)	2	200
	1.2(6/6)	3	N.P
P.I (100)	0.4(0/6)	1	100
	0.8(6/6)	2	N.P
A.I (100)	0.4(0/6)	1	100
	0.8(6/6)	2	N.P

Results are expressed as a mean of 6 observations (n=6). ED_{50} value expressed in terms of mean SE.N.P indicates - No protection. Note that all the three aqueous plant root extracts gave maximum protection up to 200% and the methanol plant root extract of H.I gave maximum protection up to 200%.

otherwise mentioned. Petroleum ether, Chloroform, Methanol (Spectrochem, India), Potassium Chloride (SRL, India), Calcium Chloride (Merck, India), Magnesium Chloride (Qualigen, India), Sodium bicarbonate (SRL, India), Sodium biphosphate (SRL, India), Glucose (Qualigen, India).

Collection of scorpion and scorpion venom

Adult black scorpions (*Heterometrous bengalensis*) of both sexes were collected from Burdwan district of West Bengal, India, during the rainy seasons (June-September) and were kept in a wire mesh cage (Figure 1). They were provided with food (live cockroach, an egg), water ad libitum. The Scorpion Venom (SV) was collected once in a month by applying square wave electrical stimulation (15 V, 1 ms) to the telson. The venom was pooled, lyophilized and stored at 4 °C in amber colour bottle, until further use. Before use, SV was weighed, dissolve in 0.9% saline/ phosphate buffer 0.01 M, pH 7.2 and was expressed in terms of dry weight.

Collection of plant materials

The plant (*Hemidesmus indicus*, *Pluchea indica* and *Aristolochia indica*) roots were collected commercially from M/s United Chemicals and Allied Products, Kolkata and were identified by Prof N. Paria, Department of Botany, University of Calcutta. A voucher specimen (AG100699, AG100799, AG100) has been deposited at the Department of Botany, University of Calcutta, Kolkata, India.

Preparation of plant root extract

a) Aqueous plant extract: The air dried grounded plant roots (2 gm) were soaked in distilled water overnight at 8 °C. It was filtered and the filtrate was centrifuged at 2000 rpm X 10 mins. The supernatant was used for further testing and kept at 8 °C. The plant extract was expressed in terms of dry weight.

b) Methanol plant extract: Air dried plant roots were grounded and extracted by refluxing with petroleum ether (60-80 °C), chloroform (60- 65 °C) and methanol (64-65 °C) in a soxhlet apparatus. The materials were then dried in a desiccators at room temperature for further use. Before use, the extracted material was dissolved in 0.9% saline and centrifuged at 2000 rpm X 10 mins at room temperature. Supernatant was used for further investigation and kept at 4 °C. The plant extract was expressed in terms of dry weight.

Animals used

Male Swiss albino mice (20±2 gm), male guineapig (180±10 gm) and Wister albino rats (100±10 gm) were obtained from enlisted supplier of Calcutta University and were maintained in standard laboratory conditions, diet and water *ad libitum*. All animal experiments were approved by the University Animal Ethics Committee, Department of Physiology, University of Calcutta, Kolkata, India and were in accordance with the guidelines of the committee for the purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India (Ref. No.: 820/04/ac/CPCSEA dated 06.08.2004).

Scorpion venom neutralization (*in vivo*)

Neutrlization of lethal activity: MLD (Minimum Lethal Dose) of SV was defined as the minimum dose of SV, which when injected in tail vein (*i.v*) of male albino mice (20±2 g), caused death of the animal within 24 hrs [17]. Antilethal activity of plant root extracts was studied by the neutralization method [18] using incubate of certain

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Table 2: Effect of aqueous and methanol plant root extracts of H.I., P.I and A.I. on SV induced urinary constituent changes in male albino mice.

Aqueous extracts (mg/kg)	SV mg (no. of changed urinary constituents /total animals)	MDAUC Fold	Protection (%)
H.I (100)	0.1(0/6)	1	100
	0.2(6/6)	2	N.P
P.I (100)	0.1(0/6)	1	100
	0.2(0/6)	2	200
	0.3(6/6)	3	N.P
A.I (20)	0.1(6/6)	1	N.P
Methanol Extracts (mg/kg)	SV mg (no. of changed urinary constituents / total animals)	MDAUC Fold	Protection (%)
H.I (100)	0.1(6/6)	1	N.P
P.I (100)	0.1(0/6)	1	100
	0.2(6/6)	2	N.P
A.I (100)	0.1(6/6)	1	N.P

Results are expressed as a mean of 6 observations (n=6). ED_{50} value expressed in terms of mean SE. N.P indicates - No protection. Note that aqueous plant root extract of P.I gave maximum protection up to 200% and the methanol plant root extract of P.I gave maximum protection up to 100%.

Table 3: Effect of aqueous plant root extracts of H.I., P.I and A.I. on SV induced PLA_2 activity.

Aqueous extracts (mg)	SV µg (no. of coagulable egg yolk/total exp.)	PLA_2 Fold	Protection (%)
H.I (2.0)	5(0/6)	1	100
	20(0/6)	4	400
	40(0/6)	8	800
	50(6/6)	10	N.P
P.I (2.0)	5(0/6)	1	100
	20(0/6)	4	400
	40(0/6)	8	800
	50(6/6)	10	N.P
A.I (0.5)	5(0/6)	1	100
	20(6/6)	4	400
	40(0/6)	8	800
	50(6/6)	10	N.P
Methanol plant root extracts [Dose (mg)]	SV µg (no. of coagulable egg yolk/total exp.)	Fold of Protection	Protection (%)
H.I (0.2)	5(0/6)	1	100
	20(0/6)	4	400
	40(0/6)	8	800
	50(6/6)	10	N.P
P.I (0.2)	5(0/6)	1	100
	20(0/6)	4	N.P
A.I (0.2)	5(0/6)	1	100
	20(6/6)	4	N.P

Results are expressed as a mean of 6 observations (n=6). ED_{50} value expressed in terms of mean SE.N.P indicates - No protection. Note that all the aqueous plant root extract of H.I, P.I and A.I gave maximum protection up to 800 % and the methanol plant root extract of H.I and P.I gave maximum protection up to 800%.

amount of plant (aqueous and methanol) and venom, incubated at 37 °C for 30 mins and then centrifuging at 2000 rpm X 10 mins. The supernatant was injected (i.v) into male albino mice and mortality was recorded.

Neutralization of pro-inflammatory activity: The Minimum Edema Dose (MED) of SV was defined as the least amount of SV which when injected into male albino mice (20±2 g) produces edema after 2 hrs, in mice paw. Pro- inflammatory activity of plant extract was studied by using incubate of certain amount of plant (aqueous and methanol) and venom, incubated at 37 °C for 30 mins and then

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Table 4: Effect of aqueous and methanol plant root extracts of H.I., P.I and A.I on SV induced plasma recalcification.

Aqueous extracts (mg)	SV µg (no. of clotted plasma/total exp.)	MCDP Fold	Protection (%)
H.I (0.5)	30(0/6)	1	100
	60(0/6)	2	200
	90(0/6)	3	300
	120(6/6)	4	N.P
P.I (0.5)	30(0/6)	1	100
	60(0/6)	2	200
	90(0/6)	3	300
	120(6/6)	4	N.P
A.I (0.075)	30(0/6)	1	100
	60(0/6)	2	200
	90(0/6)	3	300
	120(6/6)	4	N.P
Methanol extracts (mg)	SV µg (no. of clotted plasma/total exp.)	MCDP Fold	Protection (%)
H.I (0.3)	30(0/6)	1	100
	60(0/6)	2	200
	90(0/6)	3	300
	120(6/6)	4	N.P
P.I (0.3)	30(6/6)	1	N.P
A.I (0.3)	30(0/6)	1	100
	60(6/6)	2	N.P

Results are expressed as a mean of 6 observations (n=6). ED_{50} value expressed in terms of mean SE. N.P indicates - No protection. Note that all the three aqueous plant root extracts gave maximum protection up to 300% and the methanol plant root extract of H.I gave maximum protection up to 300%.

centrifuging at 2000 rpm X 10 mins. The supernatant was injected in the Subplantor (*s.p*) region of the left hind paw. Equal amount of saline was injected in the right hind paw (*s.p*) as control. The diameter of both hind paws of each mouse was measured with the help of a digital micro caliper (Mitutua, Japan) [19].

Neutralization of urinary changes

The Minimum Dose of SV Affecting Urinary Constituents (MDAUC) was defined as the amount of SV, which when injected (*i.v*) in male albino mice, produces significant changes in urine, in 24 hrs. Urine analysis was done by injecting SV (*i.v*) into male albino mice (20±2 g) followed by a saline load of 2 ml (*i.p*) and their urine was collected after 24 hrs. The urinary constituents were measured using standard reagent strips (Multistrix SG, Bayer, Germany). Neutralization was done using a certain amount of plant (aqueous and methanol) and venom, incubated at 37 °C for 30 mins and centrifuged at 2000 rpm X 10 min. The supernatant was injected (*i.v*) for the urine analysis as stated above.

Scorpion venom neutralization (*in vitro*)

Neutralization of PLA_2 activity: Phospholipase A_2 activity was estimated by the egg yolk coagulation method [20]. One unit of PLA_2 activity was defined as the amount of SV which increases the coagulation time of egg yolk suspension by 1 min. Inhibition of enzyme activity was estimated by mixing fixed amount of plant (aqueous and methanolic) and venom and then incubating at 37 °C for 30 mins and centrifuged at 2000 rpm X 10 mins. The supernatant was used for testing PLA_2 activity [21].

Neutralization of plasma recalcification

Fresh goat's blood was collected in 3.8% trisodium citrate (1:9 v/v) and centrifuged at 3000 rpm X 15 mins. 0.1 ml of plasma, 0.1 ml of 0.5% $CaCl_2$ and 1ml of saline (control)/SV (treated) was added in a test tube and placed in a water bath at 37 °C. Clotting time of plasma was recorded with the help of a stop watch at 15 secs interval [15]. Minimum Clotting Dose of Plasma (MCPD) is defined as the minimum amount of SV, which increased the clotting time of plasma by 1 min compared to saline control. Neutralization was done using certain amount of plant root extract (aqueous and methanol) and venom incubated at 37 °C for 30 mins and centrifuged at 2000 rpm X 10 mins. The supernatant was used for plasma recalcification activity testing [21].

Neutralization of neurotoxic activity: Minimum Neurotoxic Dose (MNTD) was defined as the amount of SV which blocks Toad Gastrocnemius Sciatic nerve muscle preparation (TGS) within 1 hour. Isolated Toad Sciatic nerve preparation (TGS) was suspended (6 ml bath) in oxygenated (95% O_2 and 5% CO_2) Frog Ringer's solution (NaCl 154 mM, KCl 5.6 mM, $CaCl_2$ 2.2 mM, $NaHCO_3$ 6.0 mM and Glucose 5.55 mM) and maintained at room temperature. The preparation was stimulated with a square wave electronic stimulator (Grass, USA) at 12 V, stimulus duration 0.2 millisec and pulse at intervals of 10 sec. Contractions were recorded by Brodie's lever on

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a smoked drum. Neutralization was done using a certain amount of plant root extract (aqueous and methanol) and venom, incubated at 37 °C for 30 mins and centrifuged at 2000 rpm X 10 mins and used for neutralizing neurotoxicity on TGS preparation.

Neutralization of cardiotoxic activity: Minimum Cardiotoxic Dose (MCTD) was defined as the least amount of SV, which inhibited amplitude of contraction and heart rate (50%) within 30 mins. The guinea pig (250±10 g) heart was prepared [22], perfused with oxygenated (95% O_2 and 5% CO_2) mammalian Tyrode's solution (NaCl 137 mM, KCl 2.7 mM, $CaCl_2$ 2.6 mM, $MgCl_2$ 1.27 mM, $NaHCO_3$ 11.9 mM, NaH_2PO_4 0.4 mM and glucose 5.5 mM) containing double dextrose (2 g/l). The temperature was maintained at 37±1 °C. Contraction was recorded on a rotating drum using a spring heart lever. Neutralization of cardiotoxic activity was estimated with plant root extract (aqueous and methanol) and venom incubate kept at 37 °C X 10 mins and then centrifuged at 2000 rpm X 10 mins. The supernatant was used for neutralization of cardiotoxic activity stated as above.

Statistics

All results were expressed as mean±standard error of mean (n=6). The level of significance of difference between means was determined by Student's t test and $P<0.01$ was considered as statistically significant.

Results

Scorpion venom neutralization studies (*in vivo*)

Neutralization of SV induced lethal action: 1 MLD of SV was found to be 20 mg/kg (iv). SV (1-3 MLD) was incubated at 37 °C X 15 mins with aqueous plant root extracts (A.I, H.I and P.I at 20 to 100 mg/kg) and injected (*i.v*) in male albino mice. All extracts gave 200% protection (Table 1).

SV (1-3 MLD) was incubated at 37 °C X 15 mins with methanol plant root extracts (A.I, H.I and P.I at 100 mg/kg) and injected (*i.v*) in male albino mice. H.I gave 200% protection and P.I and A.I gave 100 % protection (Table 1).

Neutralization of SV induced pro-inflammatory action

1 MED of SV was found to be 50 µg. SV (1-2 MED) was incubated at 37 °C X 15 mins with aqueous plant root extracts (A.I, H.I and P.I at 20 to 100 mg) and injected (*s.p*) in male albino mice. All the plant aqueous extracts gave no protection.

SV (1-2 MED) was incubated at 37 °C X15 mins with methanol plant root extracts (A.I, H.I and P.I at 0.3 mg/kg) and injected (*s.p*) in male albino mice. H.I gave 100% protection and P.I and A.I gave no protection.

Neutralization of SV induced urinary changes

1 MDAUC (presence of blood and protein) of SV was found to be 5 mg/kg (i.v). SV (1-3 MDAUC) was incubated at 37 °C X 15 mins with aqueous plant root extracts (A.I, H.I and P.I at 20 to 100 mg/kg) and injected (*i.v*) in male albino mice. P.I gave 200% protection, H.I gave 100% protection and no protection was given by A.I.

SV (1-2 MDAUC) was incubated at 37 °C X 15 mins with methanol plant root extracts (A.I, H.I and P.I at 100 mg/kg) and injected (*i.v*) in male albino mice, P.I gave 100% protection and no protection by P.I and A.I. (Table 2).

Scorpion venom neutralization studies (*in vitro*)

Neutralization of SV PLA_2 activity: 1 unit of PLA_2 activity of SV was found to be 5µg. SV (1-10 PLA_2 unit) was incubated at 37 °C X15 mins with aqueous plant root extracts (A.I, H.I and P.I at 0.5 to 2.0 mg) and tested for PLA_2 activity. All the three aqueous extracts gave 800% protection. The ED_{50} doses of plants were H.I- 1.98 mg, P.I- 1.98 mg and A.I- 0.49 mg respectively.

SV (1-10 PLA_2 unit) was also incubated at 37 °C X15 mins with methanol plant root extracts (A.I, H.I and P.I at 0.2 mg) and tested for PLA_2 activity, H.I gave 800% protection and 100% protection were given by P.I and A.I. The ED_{50} doses of plants were H.I- 0.19 mg, P.I- 0.18 mg and A.I- 0.19 mg, respectively (Table 3).

Neutralization of SV induced plasma recalcification

1 MCDP of SV was found to be 30 µg. SV (1-4 MCDP) was incubated at 37 °C X 15 mins with aqueous plant root extracts (A.I, H.I and P.I at 0.075 to 0.5 mg) and tested for plasma recalcification, where all the three aqueous extracts gave 300% protection. The ED_{50} doses of plants were H.I- 0.49, P.I- 0.49 and A.I- 0.07.

SV (1-4 MCDP) was also incubated at 37 °C X 15 mins with methanol plant root extracts (A.I, H.I and P.I at 0.3 mg) and tested for plasma recalcification. H.I gave 300% protection and 100% protection was given by P.I and A.I. The ED_{50} doses of plants were H.I- 0.29 mg, P.I- 0.29 mg and A.I- 0.29 mg, respectively (Table 4).

Neutralization of Neurotoxic changes

1 MNTD of SV was found to be 2 mg/ml. SV (1-2 MNTD) was incubated at 37 °C X 15 mins with aqueous plant root extracts (A.I, H.I and P.I at 0.25 to 2.0 mg/ml) and tested for neurotoxicity in isolated toad's gastrocnemius sciatic nerve preparation. All the three aqueous extracts gave 100% protection.

SV (1-2 MNTD) was also incubated at 37 °C X 15 mins with methanol plant root extracts (A.I, H.I and P.I at 1mg/ml) and tested for neurotoxicity in isolated toad's gastrocnemius sciatic nerve preparation. H.I gave 100% protection and no protection was given by P.I and A.I.

Neutralization of cardiotoxic changes

1/2 MCTD of SV was found to be 2 mg. SV (1/2 MCTD) was incubated at 37 °C X 15 mins with aqueous plant root extracts (A.I, H.I and P.I at 0.5 to 5.0 mg) and tested for cardiotoxicity in isolated guinea pigs heart. P.I gave 93% protection (significant, $P<0.01$) in amplitude (Control- 24.67±0.33 mm; SV- 8.83±0.4 mm; P.I- 23.50±0.22 mm) and 80% protection (significant, $P<0.01$) in heart rate (Control- 142±8.2 beats/min; SV- 203±7.4 beats/min; P.I- 154±7.6 beats/min). No protection was given by H.I and A.I.

SV (1/2 MCTD) was incubated at 37 °C X 15 mins with methanol plant root extracts (A.I, H.I and P.I at 4 mg) and tested for cardiotoxicity in isolated guinea pigs heart. P.I gave 99% protection (significant, $P<0.01$) in amplitude (Control- 24.67±0.33 mm; SV- 8.83±0.4 mm; P.I- 23.83±0.31 mm) and 76% protection (significant,

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P<0.01) in heart rate (Control- 142±8.2 beats/min; SV- 203±7.4 beats/min; P.I- 157±8.1 beats/min). No protection was given by H.I and A.I.

Discussion

Till date only symptomatic treatments are available against scorpion envenomation. Countries like India, where the anti scorpion venoms are not available, people in rural areas basically rely on folk usage of herbal products either orally or as paste applied on the bitten parts. Plants like *Mangifera indica* L., *Aristolochia indica, Solanum indicum* L., *Andrographis paniculata Nees, Barringtonia acutangula* L., *Calamus* sp. etc have been used by village folks as a treatment for scorpion envenomation [14,15]. *Andrographis paniculata* have been reported to possess neutralizing activity against red scorpion venom [23]. *Mimosa tinuiflora* could effectively neutralize *Tityus serrulatus* venom induced inflammation [24]. But there is no scientific validation of neutralization activities of herbs against *Heterometrus bengalensis* scorpion venom. The present study identified three plant root extracts (aqueous/methanol) of *Hemidesmus indicus* (H.I), *Pluchea indica* (P.I) and *Aristolochia indica* (A.I) having neutralizing capacity against black scorpion (*Heterometrus bengalensis*) venom in experimental animal models. The maximum fold of neutralization was found with aqueous root extracts as compared to methanol extracts.

H.I could neutralize scorpion venom induced lethality, inflammation, PLA_2 change, etc. Further, crude extract of H.I has been already reported to possess antioxidant, hepatoprotective, antisnake venom activity [25-27]. A pure compound (lupeol acetate) isolated from H.I have been already reported to possess anti inflammatory activity induced by snake venom [21]. It shows anti-inflammatory activity by suppressing the capacity of snake venom induced oxidative stress and pro-inflammatory cytokines release, the two important inflammatory mediators. Therefore scorpion venom induced inflammation might be neutralized by the same mechanism as that of snake venom. Compounds like lupeol acetate, 2-OH- 4-Methoxy benzoic acid, isolated from H.I neutralized snake venom induced *in vivo* and *in vitro* changes [25,28]. Similar compounds might be responsible in neutralizing scorpion venom induced *in vivo* and *in vitro* changes subject to further testing.

P.I has also been reported to possess anti-inflammatory, antioxidant and antisnake venom activity [29-31]. Earlier, compounds like beta-sitosterol and stigmasterol isolated from the root extract of P.I significantly neutralized viper venom-induced lethal, hemorrhagic, defibrinogenation, edema and PLA2 activity [32]. Therefore the scorpion venom induced lethality, inflammation, etc. could be neutralized by the same or homologous compounds present in P.I. In the Ethnobotanical survey of folk plants for the treatment of snake envenomation in Southern part of India, A.I and H.I both have been used to neutralize the toxic effects of snake venoms in *in vitro* and *in vivo* models [27]. Both the plants have been mentioned in the folk medicine against scorpion envenomation. Other effect induced by SV includes systemic effects is renal toxicity. The sign of renal toxicity is the presence of high protein and blood in urine of SV treated animals. The plant root extracts of H.I, P.I and A.I (aqueous/methanol) could decrease the above mentioned parameters from the urine of SV treated animals.

Previous studies indicated the presence of alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, tannin, lignin in *Hemidesmus indicus* root extract, flavonoids, phenolic compounds, tannin, saponin, proanthocyanidins in *Pluchea indica* root extract and essential oil, aristolochic acid, isoaristolochic acid, sesquiterpene, ishwarol, aristololactam, 12-nonacosenoic acid in *Aristolochia indica* root extract [33-35]. These bioactive compounds might be responsible against experimental scorpion envenomation. Till date, no specific anti-scorpion venom (against *H bengalensis*) is available in India. Use of Prazosin (α1 adrenergic blocker) against red scorpion envenomation has been documented [36]. Plant extracts along with synthetic drugs might be of great importance to develop scorpion venom antagonists against scorpion sting.

Conclusion

From the above study it may be concluded that Indian medicinal plants (*Hemidesmus indicus, Pluchea indica* and *Aristolochia indica*) has the potential to neutralize scorpion venom activity in animal models subject to further detail investigations at molecular levels.

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