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Antisnake venom activity of ethanolic seed extract of *Strychnos nux vomica* Linn.

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The whole seed extract of *S.nux vomica* (in low doses) effectively neutralized Daboia russelii venom induced lethal, haemorrhage, defibrinogenating, PLA_2 enzyme activity and Naja kaouthia venom induced lethal, cardiotoxic, neurotoxic, PLA_2 enzyme activity. The seed extract potentiated polyvalent snake venom antiserum action in experimental animals. An active compound (SNVNF) was isolated and purified by thin layer chromatography and silica gel column chromatography, which effectively antagonised *D. russelii* venom induced lethal, haemorrhagic, defibrinogenating, oedema, PLA_2 enzyme activity and N. kaouthia induced lethal, cardiotoxic, neurotoxic, PLA_2 enzyme activity. Polyvalent snake venom antiserum action was significantly potentiated by the active compound. Spectral studies revealed it to be a small, straight chain compound containing methyl and amide radicals. Detailed structure elucidation of the compound (SNVNF) is warranted before its clinical trials as a snake venom antagonist.

Key words: *Daboia russelii*, *Naja kaouthia*, Snake venom *Strychnos nux vomica*
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Herbal medicine is still the mainstay of about 75-80% of the world population mainly in the developing countries for primary health care[1]. According to the World Health Organization (WHO) the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times[2]. Injury and death due to snakebite is a major sociomedical problem[3] and India has the highest mortality due to snakebite[4]. *Daboia russelii* (Viper) and *Naja kaouthia*(Cobra) are the two venomous snakes responsible for maximum deaths in eastern India. For the last century, antiserum is the only available specific treatment[5]. However, antiserum does not provide enough protection against venom induced haemorrhage, necrosis, nephrotoxicity and often develops hypersensitivity reactions[6-8]. Many Indian medicinal plants are mentioned in literature, which are used to treat snakebite victims specially in the rural areas[9-11]. Very few scientific reports on the active constituents from plants are there, which inhibit snake venom action[12,13]. Alam *et al.*[14] isolated an active compound, 2-hydroxy-4-methoxy benzoic acid from the root extract of *Hemidesmus indicus* R.Br, which effectively neutralized, *Daboia russelii* venom induced pathophysiological changes[14]. The present investigation explores the neutralization of viper and cobra venom by the seed extract of *Strychnos nux vomica* (Bengali: Kuchila) in experimental animals. An active compound was isolated from the seed extract and its antisnake venom activity and antiserum action potentiation have been studied.

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Materials and Methods

Lyophilised snake venoms (*Daboia russelii* and *Naja kaouthia*) were purchased from Calcutta Snake Park, Calcutta, India and preserved in a dessicator at 8°C till further use. The venom was dissolved in 0.9% saline and centrifuged at 2000 rpm for 10 min. The venom concentration was expressed in terms of dry weight (mg/ml).

Dried seeds of *Strychnos nux vomica* Linn.(Loganiaceae) was obtained commercially from M/s. United Chemicals and Allied Products, Calcutta, India. The seeds were crushed and extract prepared by refluxing it with 80% ethanol for 72 hr in Soxhlet apparatus. The extract was then concentrated in a rotary evaporator and kept in a dessicator at room temperature. Before use, the extract was dissolved (10mg/ml) in 0.9% saline and centrifuged at 2000 rpm×10 min at room temperature. The extract was designated as SNV-extract.

Lyophilised polyvalent snake venom antiserum (equine) from Bengal Chemical Pharmaceutical Pvt. Ltd., Calcutta, India was purchased (batch No. 89202). Before use, antiserum was weighed, dissolved in 0.9% saline. The antiserum concentration was expressed in terms of dry weight (20mg/ml, w/v).

Animals were purchased from M/s R. Ghosh, Calcutta. Albino mice (Swiss) and rats (Wistar) were given synthetic diet (pellets, Ashirwad Industries, Chandigarh, India) and guineapigs were given green leafy vegetables and water *ad libitum*.

Inhibition of venom lethal effect—The lethal toxicity (LD_{50}) of *Daboia russelii* and *Naja kaouthia* venom was assessed by injection of different concentration of venom (in 0.2% saline) into the tail vein of male albino mice (20g, n=10)[15]. Various doses of venom were mixed with a fixed amount of SNV-extract/active compound, incubated at 37°C × 60 min and centrifuged at 2000rpm × 10 min. The supernatant was injected (iv) into male albino mice. Median lethal dose (LD_{50}) was calculated from the number of deaths occurring within 24 hr of injection of the venom-plant extract/active compound incubate. Effective dose (ED) was calculated as the minimum dose of plant extract/active compound that neutralized the median lethal dose (LD_{50}) of venom.

Inhibition of venom haemorrhagic effect—The minimum haemorrhagic dose (MHD) of venom was defined as the least amount of venom, which when injected intradermally (id) into male albino mice (n=10) results in a haemorrhagic lesion of 10 mm diameter 24 hrs later[15]. Inhibition was estimated as the fixed amount of SNV-extract/active compound mixed with different amount of venom. The venom-SNV-extract/active compound was incubated at 37°C × 60 min, centrifuged at 2000rpm × 10 min and supernatant was injected (id) in a total volume of 0.1ml into male albino mice (20g). The haemorrhagic lesion was estimated after 24 hr and expressed in terms of MHD.

Inhibition of venom defibrinogenating activity—The minimum defibrinogenating dose (MDD) of venom was defined as the minimum amount of venom, which when injected (iv) into male albino mice (20g, n=10), causes incoaguable blood 1 hr later[15]. Inhibition was estimated by mixing different amounts of venom with fixed amount of SNV-extract/active compound, incubated at 37°C × 60 min, centrifuged at 2000 rpm × 10 min and the supernatant injected (iv) into albino mice (20g). Presence of clotted/non clotted blood was observed after 2 hr and MDD was estimated.

Inhibition of venom phospholipase activity—Phospholipase A activity was estimated by the egg yolk coagulation method. One unit of PLA_2 activity was defined as the amount of venom which increases the coagulation time of egg yolk suspension by 1 min[16]. Inhibition of the enzyme activity was estimated as fixed amount of SNV-extract/active compound mixed with different amount of venom (*D. russelii* and *N. kaouthia*), incubated at 37°C × 60 min, centrifuged at 2000 rpm × 10 min and the supernatant was tested for PLA_2 activity.

Inhibition of venom cardiotoxic activity—Minimum cardiotoxic dose (MCTD) was defined as the least amount of *N. kaouthia* venom which stopped auricular contraction within 15 minutes. Isolated guineapig auricle was prepared[17] and suspended in oxygenated (95% O_2 and 5% CO_2) Ringer's solution at 29°±1°C. The spontaneous contraction of auricle was recorded on a smoked drum through lightly sprung heart lever. One cardiotoxic dose (25μg) stopped 98±0.5% of the contraction of the isolated guinea pig auricle within 15 min. Inhibition of the cardiotoxic activity was estimated as venom/venom-SNV extract incubated at 37°C × 60 min centrifuged at 2000 rpm × 10 min and the supernatant added to a glass bath (3ml) containing the auricle and the nature of contraction was recorded.

Inhibition of venom neurotoxic activity—Minimum neurotoxic dose (MNTD) was defined as the least amount of *N. kaouthia* venom which blocked rat phrenic nerve action within 30 min. Isolated rat phrenic nerve diaphragm (RPND) was prepared[18]. The preparation was suspended in oxygenated (95% O_2 and 5% CO_2) Tyrode's solution at (29 ° ± 1°C) in a glass bath (6ml) and stimulated (10V, 0.5 ms, 0.1 Hz) with a square-wave electronic stimulator (Grass, USA). Contractions were recorded with a lightly sprung lever on a smoked drum. One neurotoxic dose (75μg) stopped 98±0.5% of the contraction within 30 min. Inhibition of neurotoxic activity was estimated as venom-SNV extract/active compound incubated at 37°C × 60 min, centrifuged at 2000 rpm × 10 min and the supernatant applied to a glass bath (6ml) containing RPND preparation. Inhibition of neurotoxicity by the incubate and MNTD was estimated.

Inhibition of venom induced edema—The minimum edema dose (MED) of venom is defined as the least amount of venom which when injected into male albino mice produces edema in mice paw. Male albino

mice (20g, n=10) treated to different doses of venom injected in the subplanter region of the right hind paw. Equal amount of saline was injected in the left hind paw (subplanter) as control. The diameter of both hind paws of each mice was measured by screw gauge caliper at 30, 60 and 120 min interval after the injection (Wang and Teng, 1988)[19]. To assess the anti edema activity, the active compound was incubated (37°C × 60 min) with various amounts of viper venom. It was centrifuged and injected (subplanter) and diameter of the hind paws was measured. For standard, proinflammatory drug, carrageenan, was used (1% carrageenan solution injected 0.02ml in subplanter surface).

Antiserum action potentiation—The lethal toxicity of *D. russelii* venom was assessed by injection (iv) of fixed doses of commercial equine antiserum (2mg) mixed with various doses of venom, incubated at 37°C × 60 min, centrifuged at 2000 rpm × 10 min and the supernatant was injected (iv) in male albino mice (20g). Antiserum potentiation of SNV-extract/active compound was estimated by injecting (iv) venom-antiserum-SNV extract/active compound incubate in male albino mice and lethality was recorded up to 24 hr of observation. Antiserum action potentiation was expressed in terms of LD_{50} value.

Isolation and purification of active compound—A mixture of silica gel (GF_{254}) was suspended in water, degassed and poured on to TLC glass plates (20cm × 10cm × 1mm). The plates were activated at 110°C for 1 hr. The ethanolic seed extract was applied and developed in a solvent system of isopropanol:0.1(N) HCl (7:3), bands developed in iodine chamber and R_f calculated. Each band was eluted and tested for venom inhibition. The band containing the active compound was rechromatographed in preparative TLC, using the same solvent system. Band with R_f 0.5 was eluted and tested for venom inhibition. The active band was eluted in methanol and applied in a silica gel (100-200 mesh) column (200 mm × 20 mm) and was eluted with petroleum-ether:chloroform (9:1, 4:1, 2:1, 1:1, 0:1, 250ml each), chloroform : methanol (95:5, 90:10, 75:25, 50:50, 250 ml each). The compounds eluted were evaporated to dryness and tested for venom neutralization.

Spectral study—IR (KBr) spectra of the active compound was recorded on a Perkin-Elmer spectrometer(Model 732). ^{13}C NMR spectra (in $CDCl_3$) were recorded on a model of FX 300 spectrometer (300 MHz).

Statistical analysis—All results were expressed as mean ± SE and mean ± SD. The significance of the difference between means was determined by Student's *t* test and *P* values < 0.05 was considered as significant.

Results

The LD_{50} of *D. russelii* and *N. kaouthia* venom in male (20g) albino mice was found to be 2.4μg and 3.09μg respectively. The minimum haemorrhagic dose (MHD) of *D. russelii* was found to be 2μg, minimum defibrinogenating dose (MDD) was 2μg, minimum oedema dose (MED) was 1 μg and venom PLA_2 activity was 5μg≡one unit. In *in vitro* studies, *N. kaouthia* venom induced minimum cardiotoxic dose (MCTD) was 25μg in isolated guineapig auricle, minimum neurotoxic dose (MNTD) was 75μg in isolated rat phrenic nerve diaphragm preparation and venom PLA_2 activity was 5μg≡one unit.

SNV extract—Yield of extract was 6.35±0.29%. The seed extract (5mg/kg, iv) did not produce any lethal effect up to 24 hr of observation, in male albino mice.

Venom neutralization by SNV extract

Daboia russelii venom—In *in vitro* studies, *S. nux vomica* seed extract was found to neutralize 4.1 fold of LD_{50} dose of venom, 3 fold of minimum haemorrhagic dose (MHD) of venom and 2 fold of minimum defibrinogenating dose (MDD) of venom. ED_{50} of the seed extract was found to be 19.8 ± 1.46 in case of LD_{50}, 20.6 ± 1.06 in case of MHD and 20.05 ± 0.76 in case of MDD (Table 1).

Naja kaouthia venom—In *in vitro* studies, *S. nux vomica* seed extract was found to neutralize 2.9 fold of LD_{50} dose of venom, 2 fold of minimum cardiotoxic dose (MCTD), and 2 fold of minimum neurotoxic dose (MNTD) of venom. ED_{50} of the seed extract was found to be 20.16 ± 1.06 in case of LD_{50}, 20.5 ± 0.5 in case of MCTD and 20.0 ± 0.57 in case of MNTD (Table 2).

Antiserum action potentiation—In *in vitro* studies, *D. russelii* venom (1-20 LD_{50}) incubated with seed extract (40μg) gave protection up to 4.1 LD_{50} of venom induced lethal action. In antiserum action potentiation study, venom (1-10 LD_{50}) incubated with seed extract (40μg) and snake venom antiserum (2mg) and incubate injected (iv) in male albino mice, gave protection up to 8.3 LD_{50} thereby indicating antiserum action potentiation of 415% with respect to protection

given by antiserum alone. Similarly, *N. kaouthia venom* (1-10 LD_{50}) incubated with seed extract (40μg) gave protection up to 2.9 LD_{50}. In antiserum action potentiation study, venom (1-10 LD_{50}) incubated with seed extract (40μg) and snake venom antiserum (2mg) and injected (iv) in male albino mice, gave protection up to 4.8 LD_{50} thereby indicating antiserum action potentiation of 240% with respect to protection given by antiserum alone (Table 3).

Isolation and purification of the active compound—Ethanolic extract of *S. nux vomica* seed was applied to TLC plates of silica gel (GF_{254}) and the plates were run in the solvent system 0.1N HCl:isopropanol 3:7, v/v. An active fraction obtained at R_f 0.6 was further fractionated on TLC. Active compound was obtained at Rf 0.5. The band eluted in methanol was filtered (millipore) and was applied in silica gel column chromatography. The pure compound was eluted with ethyl acetate:-water:-methanol (80:19:1, v/v). TLC pattern revealed a single spot at Rf 0.4 in ethyl acetate:water:methanol (80:19:1, v/v) solvent system. The isolated active compound from ethanolic seed extract of *S. nux vomica* by thin layer chromatography was designated as *S. nux vomica* neutralizing factor or SNVNF. Yield of SNVNF was found to be 0.04%.

Table 1—*V. russelii* venom action inhibition by *S. nux vomica* seed extract

	Venom properties	Venom (μg)	Venom-SNV extract (μg)	Fold of neutralization (in terms of LD_{50}, MHD/MDD)	ED_{50} (μg)
1	Lethal action	4	4(0/10)	1.6	
			10(0/10)	4.1	19.8±1.46
			15(10/10)	NP	
2	Haemorrhagic action	5	5(0/10)	2.5	
			6(0/10)	3.0	20.6±1.06
			8(10/10)	NP	
3	Defibrinogenation action	2	2(0/10)	1	
			4(10/10)	2	20.05±0.76
			6(10/10)	NP	

Results are expressed as a mean of 10 observations. ED_{50} expressed in terms of Mean ± SD;
Venom-SNV extract incubated, 37° C/60 mins, injected i.v./i.d.
MHD=Minimum haemorrhagic dose; MDD= Minimum defibrinogenating dose; NP=No protection
Data in paranthesis indicate :
1) Number of animals died/Number of animals taken
2) Haemorrhage produced in animals/Number of animals taken
3) Non clotted blood produced in animals/Number of animals taken

Table 2—*N. kaouthia* venom action inhibition by *S. nux vomica* seed extract

	Venom properties	Venom (μg)	Venom-SNVextract (μg)	Fold of neutralization (in terms of LD_{50}, CTD/MNTD)	ED_{50} (μg)
1	Lethal action	6	6(0/10)	1.9	
			9(0/10)	2.9	20.16±1.06
			10(10/10)	NP	
2	Cardiotoxic activity	25	25(0/10)	1	
			50(0/10)	2	20.5±0.5
			100(10/10)	NP	
3	Neurotoxic activity	75	75(10/10)	1	
			150(0/10)	2	20.0±0.57
			225(10/10)	NP	

Results are expressed as a mean of 10 observations. ED_{50} expressed in terms of Mean ± SD ;
Venom-SNV extract incubated, 37° C/60 mins, injected i.v.
MCTD=Minimum cardiotoxic dose; MNTD=Minimum neurotoxic dose; NP=No protection
1) Number of animals died/Number of animals taken
2) Number of auricle stopped/Number of expts
3) Number of nerve-muscle blocked/Number of expts

Spectral study of the active compound—IR and ^{13}C NMR spectra of SNVNF suggested that it was a straight chain compound ($-CH_2-CH_2$ at 1384.2cm^{-1}) with an amide radical (68.1 ppm). The compound is likely to be of small molecular mass (Figs 1 and 2).

Venom neutralization with SNVNF

Daboia russelii venom—In *in vitro* studies, SNVNF was found to neutralize 3.7 fold of LD_{50} dose of venom, 2.5 fold of minimum haemorrhagic dose (MHD) of venom, 1.5 fold of minimum defibrinogenating dose (MDD) of venom, 2 fold of minimum oedema dose (MED) of venom and 2 units of venom PLA_2 activity. The ED_{50} of SNVNF was found to be 9.4 ± 0.9, 10.1 ± 1.1, 9.6 ± 0.8, 9.5 ± 0.2 and 10.0 ± 0.74 (Table 4).

Table 3—Antiserum action potentiation by *S. nux vomica* seed extract on viper and cobra venom induced lethal action in male albino mice

Venom	Venom+AS* µg(LD_{50})	Venom+SNV extract µg(LD_{50})	Venom+SNV extract +AS* µg(LD_{50})	Potentiation (%)
RVV	5(2)	10(4.1)	20(8.3)	415
NKV	6(2)	9(2.9)	15(4.8)	240

Results are expressed as a mean of 10 observations.
* Dose of antiserum (AS) taken is 2mg which neutralized venom dose of 2 LD_{50}
ED_{50} of SNV extract was taken which neutralized venom dose of 2 LD_{50}

Naja kaouthia venom—In *in vitro* studies, SNVNF was found to neutralize 1.9 fold of LD_{50} dose of venom, 1 fold of minimum cardiotoxic dose (MCTD) of venom, 1 fold of minimum neurotoxic dose (MNTD) of venom and 1 unit of venom PLA_2 activity. The ED_{50} of SNV-NF was found to be 10.0 ± 0.7, 9.8 ± 0.7, 10.3 ± 0.6 and 11.5 ± 0.8 (Table 5).

Antiserum action potentiation—Antiserum action potentiation was studied with SNVNF. In *in vitro* studies, *Daboia russelii* venom (1-10 LD_{50}) incubated with SNVNF (20µg) gave protection up to 3.75 LD_{50} of venom induced lethal action. In antiserum action potentiation study, venom (1-20 LD_{50}) incubated with

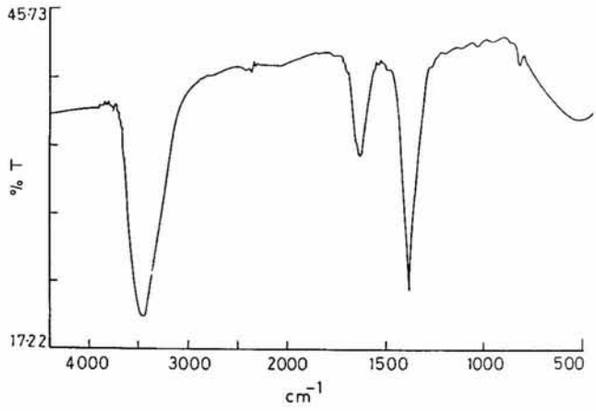

Fig. 1—IR spectra of SNVNF

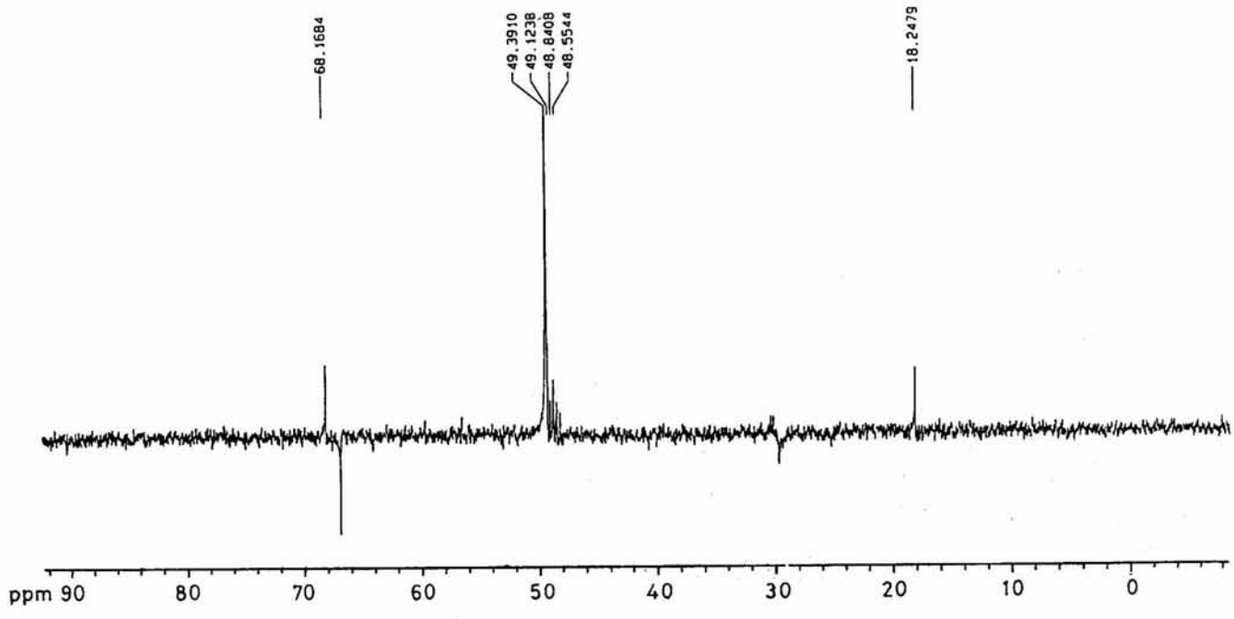

Fig. 2—^{13}C NMR spectra of SNVNF

SNVNF (20μg) and snake venom antiserum (2mg) and incubate injected (iv) in male albino mice, gave protection up to 5 LD_{50} thereby indicating antiserum action potentiation of 250% with respect to protection given by antiserum alone. Similarly, *N. kaouthia* venom (1-10 LD_{50}) incubated with SNVNF (20μg) gave protection up to 1.94 LD_{50}. In antiserum action potentiation study, venom (1-10 LD_{50}) incubated with SNVNF (20μg) and snake venom antiserum (2mg) and incubate injected (iv) in male albino mice, gave

Table 4—*V. russellii* venom action inhibition by SNVNF

	Venom properties	Venom (μg)	Venom-SNVNF (μg)	Fold of neutralization (in terms of LD_{50}, MHD/MDD/MED/unit)	ED_{50} (μg)
1	Lethal action	4	4(0/10)	1.6	
			9(0/10)	3.7	9.4±0.9
			10(10/10)	NP	
2	Haemorrhagic action	5	5(0/10)	2.5	10.1±1.1
			10(10/10)	NP	
3	Defibrinogenation action	2	2(0/10)	1	
			3(0/10)	1.5	9.6±0.8
			4(10/10)	NP	
4	Edema	1	1(0/10)	1	9.5±0.2
			2(0/10)	2	
			5(10/10)	NP	
5	PLA_2	5	5(0/10)	1	
			10(0/10)	2	10±0.74
			15(10/10)	NP	

Results are expressed as a mean of 10 observations. ED_{50} expressed in terms of Mean ± SD ;
Venom-SNVNF incubated, 37° C/60 mins, injected i.v./i.d./subplanter.
MHD=Minimum haemorrhagic dose; MDD= Minimum defibrinogenating dose
MED=Minimum edema dose; NP=No protection
Data in parenthesis indicate :
1) Number of animals died/Number of animals taken
2) Haemorrhage produced in animals/Number of animals taken
3) Non clotted blood produced in animals/Number of animals taken
4) Oedema produced in animals/Number of animals taken

Table 5—*N. kaouthia* venom action inhibition by SNVNF

	Venom properties	Venom (μg)	Venom-SNVNF (μg)	Fold of neutralization (in terms of LD_{50}, MCTD/MNTD/unit)	ED_{50} (μg)
1	Lethal action	6	6(0/10)	1.9	10.0±0.7
			8(10/10)	NP	
2	Cardiotoxic activity	25	25(0/10)	1	9.8±0.7
			50(10/10)	NP	
3	Neurotoxic activity	75	75(0/10)	1	10.3±0.6
			150(10/10)	NP	
4	PLA_2 activity	5	5(0/10)	1	11.5±0.8
			10(10/10)	NP	

Results are expressed as a mean of 10 observations. ED_{50} expressed in terms of Mean ± SD ;
Venom-SNVNF incubated, 37° C/60 mins, injected i.v.
MCTD=Minimum cardiotoxic dose; MNTD=Minimum neurotoxic dose; NP=No protection
Data in parenthesis indicate :
1) Number of animals died/Number of animals taken
2) Number of auricle stopped/Number of expts
3) Number of nerve-muscle blocked/Number of expts

protection up to 3.8 LD_{50} thereby indicating antiserum action potentiation of 190% with respect to protection given by antiserum alone (Table 6).

Discussion

S. nux vomica Linn. is one of the most popular medicinal plants used worldwide. Its uses are scripted in details in the Materia medica by Clarke[20]. There has been no report of snake venom neutralization action by the seed extract of *S. nux vomica*. The clue about the seed extract to antagonize venom was obtained from a local medicine man of Bankura district, West Bengal, India. Doubts were there since the seeds of *S. nux vomica* (SNV) are highly toxic themselves. The nonlethal dose of the SNV seed extract was worked out (5 mg/kg, iv) in mice. At this dose level, the snake venom (viper and cobra) neutralization studies were conducted. It is well known that viper venom is haemotoxic in nature. So, the viper venom neutralization studies was focused on lethal, haemorrhagic, and defibrinogenating activities in mice. Whereas, in case of cobra-venom, it is well known that venom is cardiotoxic, neurotoxic in nature and accordingly, the neutralization testing models were selected (i.e. cardiotoxicity, neurotoxicity). It was observed that SNV-seed extract effectively antagonized viper and cobra venom induced actions in different *in vivo* and *in vitro* test models. The venom neutralizing capacity of SNV seed extract was stronger in case of viper venom than the cobra venom (lethal action neutralization fold 4.1:2.9, viper vs cobra venom, Tables 1 and 2). Another interesting information obtained from the present study, is that SNV-seed extract alone not only could neutralize viper and cobra venom but also potentiated the venom neutralizing capacity of polyvalent snake venom antiserum (% potentiation 415:240, viper:cobra venom, Table 3). This information is of great importance for therapeutic management of snake bite as combination therapy, as stated earlier by Alam and Gomes [21].

In the present study, the snake venom neutralizing factor was tried to purify from the SNV-seed extract. A small straight chain molecule (SNVNF) (molecular weight likely to be <500 dalton) was isolated and purified by TLC, silica gel column chromatography, having a low yield (0.04%). SNVNF antagonized both viper and cobra venom induced actions (*in vivo* and *in vitro*) in animal models. SNVNF was found more effective against viper venom than cobra venom (lethal action neutralization fold was 3.7:1.9, viper vs cobra venom, Tables 4 and 5). SNVNF was also effective in viper and cobra venom neutralization in presence of polyvalent snake venom antiserum, indicating that this plant seed derived micromolecule may mechanistically replace or remove venom protein toxins. The actual mechanism of SNVNF induced venom neutralization is not known but it is likely that these micromole-cules hold together by Van der Waals and hydrophobic forces and inactivates the venom proteins toxins. SNV seed extract contains several alkaloids (strychnine, brucine, cantieyine, lignoceric acid, palmitic acid)[22,23]. However, in the present study, SNVNF likely to be a different compound, whose complete identity is still pending.

Several plant derived small molecules monoterpens, triterpenes, sterols, phenolic compound, benzoic acid derivatives, coumarins, flavanoids, tannins, the so called secondary metabolites, are proved to be effective in snake venom neutralization[24]. However, in most of the cases the mechanism of venom neutralization is yet to be worked out.

Since time immemorial, the medicinal values of the seeds of *S. nux vomica* have been studied and utilized. The present study revealed for the first time, the potent antisnake venom activity of this seed extract and one of its active constituents. Further studies on the snake venom neutralization by the SNV-seed extract and its constsituents are warranted for the development of a cost effective snake venom antagonist for therapeutic purposes.

Table 6—Antiserum action potentiation by SNVNF on viper and cobra venom induced lethal action in male albino mice

Venom	Venom+AS* µg(LD_{50})	Venom+SN VNF µg(LD_{50})	Venom+SN VNF+AS* µg(LD_{50})	Potentiation (%)
RVV	5(2)	9(3.7)	12(5)	250
NKV	5(2)	6(1.9)	12(3.8)	190

Results are expressed as a mean of 10 observations.
* Dose of antiserum (AS) taken is 2mg which neutralized venom dose of 2 LD_{50}
ED_{50} of SNVNF extract was taken which neutralized venom dose of 2 LD_{50}

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