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SHORT COMMUNICATION

A protective effect of *Symphorema polyandrum* Wight seeds against *Naja naja* venom- Pharmacological evaluation

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Symphorema polyandrum Wight (Family Verbenaceae), an ethnomedicinal plant, is reported for its use in the management of snake bite by the tribal people of Odisha. In this study, the efficacy of *S. polyandrum* was evaluated for its antivenom activity. Seed powder 360 mg/kg was given to envenomed albino rats with sub-lethal dose of *Naja naja* venom (0.5 mg/kg), in normal saline by intraperitonial injection. Biochemical parameters like serum cholesterol, serum triglyceride, HDL cholesterol, serum total protein, albumin, globulin, alkaline phosphatase, blood sugar, SGPT and in haematological investigation only the total count was found significantly reversing the effect of venom between venom control and test drug group. The study also showed that the envenomation of rats led to increase in lipid peroxidation in all the three tissues. Administration of the test drug to venomised rat significantly decreased the lipid peroxidation in liver and heart. Further, it also enhanced the anti-oxidant activity through enzyme catalase in liver and heart.

Keywords: Antivenom, *Naja naja*, *Symphorema polyandrum* Wight

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Introduction

Ethnobotanical surveys have reported several medicinal plants believed to be snakebite antidotes and are reported to being used for the treatment of snakebite[1-3]. Over the years, many attempts have been made for the development of snake venom antagonists from plant sources[4]. The common poisonous snakes found in India are cobra (*Naja naja*), krait (*Bangarus caeruleus*), Russell's viper (*Daboia russelli*), and saw scaled viper (*Echis carinatus*)[5]. *Symphorema polyandrum* Wight (Family Verbenaceae), an ethnomedicinal plant is reported for its use by tribal people of Odisha for snake bite management[6,7]. Hence, the present study was undertaken to validate the antivenom activity of seeds of *S. polyandrum* Wight on experimental animal model.

Materials and Methods

Snake venom and experimental animals

Freeze dried venom of *Naja naja* was procured from Haffkine Institute, Mumbai and stored at 4 °C. Wistar strain albino rats of either sex (weighing 200±60 g) obtained from the Animal House of IPGT&RA, Gujarat Ayurved University, Jamnagar, were used for the present study. The experiments were carried out after obtaining the permission from institutional animal ethics committee (Approval no. IAEC 07/2010/MD-11).

Test drug authentication and method of experimental study

Mature seeds of *S. polyandrum* were collected after proper identification of the plant from Balangir, Odisha during May 2010 by Dr. Harisha C. R., Head, Pharmacognosy laboratory, IPGT&RA, Gujarat Ayurved University, Jamnagar, Gujarat. A voucher specimen (No. 6059) has been preserved in the department for future reference. Seeds were shade dried, powdered with a mechanical grinder, passed through 120 no. mesh, and utilized for the present study. Dose of the test drug was calculated by extrapolating the reported therapeutic dose (28 seeds weigh approximately 4 g)[6]. Rat dose was fixed by referring to the table of Paget and Barnes[8]. Stock solution was prepared by keeping the powder of test drug in a mortar and macerated it with tap water being added slowly in a known dilution ratio. This solution was administered to animals by oral route depending upon the body weight. The overnight fasted animals were used for the experiment and divided into 3 groups water control (WC), venom control (VC), and venom with test drug (TD). Sub-lethal dose (0.5 mg/kg of *Naja naja* venom, in physiological saline was administered by intraperitonial injection. Finely powdered seeds in water suspension were given by oral intubation in the dose of 360 mg/kg to

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only group TD after 30 min of envenomation. The animals were sacrificed after 90 min of envenomation[9].

Blood was collected from supra orbital plexus, under mild ether anaesthesia prior to sacrifice. In biochemistry, requisite quantity of serum was fed to the auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac medicare Pvt Ltd, Mumbai), which was automatically drawn in to the instrument for estimating different parameters. The kit literature mentioning references, given on the basis of the methods on which test procedures are followed have been given below. The biochemical parameters were blood glucose (Glucose oxidase – Peroxidase method end point)[10], blood urea (Urease – glutamate dehydrogenase – fixed time, kinetic, enzymatic method)[11], serum creatinine (modified Jaffe's reaction)[12], serum total cholesterol (Cholesterol oxidase – Peroxidase end point)[10], serum HDL cholesterol (Trinder reaction)[13], serum triglyceride (Glycerophosphate oxidase method, end point)[14], serum total protein (Biurate method, end method)[15], serum albumin (Bromocresol Green dye method, end point)[16], serum alkaline phosphatase {International Federation of Clinical Chemistry (IFCC) method, kinetic method}[17], Serum Glutamic Oxaloacetic Transaminase (SGOT) (IFCC method without pyridoxal phosphate)[14] and Serum Glutamic-Pyruvic Transaminase (SGPT) activity (IFCC method kinetic without pyridoxal phosphate)[18]. Serum globulin was calculated from serum protein and serum albumin values; A/G ratio was calculated from the above values. In hematology, 0.08 mL blood was mixed with 0.02 mL of ethylenediaminetetraacetic acid (33.33 mg/mL) and fed to the auto analyzer (ERBA CHEM-5, Trans Asia), which was automatically drawn in to the instrument for estimating different hematological parameters like Total Leukocyte Count (TLC/TC), Differential Leukocyte Count (DLC), hemoglobin, Packed Cell Volume (PCV), Total Red Blood Cell, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) parameters following standard procedure. Tissue (liver, kidney, and heart) homogenate of envenomated albino rats were used to evaluate the effect of test drug on total protein[19], lipid peroxidation[20], and catalase activity[21]. Results are presented as mean±SEM, difference between the groups was statistically determined by unpaired student's t-test with the level of significance set at $P < 0.05$.

Results

Test drug produces an apparent and non-significant increase in blood sugar level and SGPT activity was observed in VC group in comparison to WC group, which is significantly attenuated by administration of test drug. Lipid profile (namely serum cholesterol, serum triglyceride, and HDL cholesterol) was significantly decreased in VC group in comparison to WC group. Administration of test drug in envenomated rats led to significant reversal of elevated lipid profile (Table 1).

Envenomation of rats led to remarkable decrease in serum protein levels in comparison to WC rats.

Table 1—Effect of the test drug on venom induced changes in various serum biochemical parameters in albino rats

Parameter	Water control	Venom control	Venom with test drug
Blood sugar (mg/dL)	128.50±03.62	136.40±03.08	126.33±02.67*
Serum cholesterol (mg/dL)	50.50±05.98	32.75±02.66$^{\#}$	70.40±12.16*
Serum triglyceride (mg/dL)	76.33±01.45	46.33±03.53$^{\#\#}$	80.50±10.18*
HDL cholesterol (mg/dL)	35.33±01.45	16.80±02.35$^{\#\#}$	37.60±07.43*
Blood urea (mg/dL)	91.75±17.43	75.00±15.50	86.00±08.54
Serum creatinine (mg/dL)	00.53±00.03	00.68±00.05$^{\#}$	00.60±00.03
SGPT (IU/l)	79.75±19.95	81.67±08.14	61.33±03.64*
SGOT (IU/l)	202.50±46.03	234.33±40.90	258.83±32.57
Total protein (g/dL)	06.65±00.12	06.15±00.15$^{\#}$	06.85±00.26*
Albumin (g/dL)	03.68±00.11	03.10±00.22	03.63±00.08*
Globulin (g/dL)	02.98±00.08	02.74±00.10	03.30±00.22*
A:G ratio	01.25±00.06	01.13±00.11	01.15±00.06
Alkaline phosphatase (IU/l)	245.25±35.71	262.50±17.84	166.60±24.75*
Bilirubin (Total mg/dL)	00.53±00.29	00.43±00.10	00.42±00.05

Values are Mean±SEM, $^{\#}P<0.05$, $^{\#\#}P<0.01$ (Compared with water control), $*P<0.05$ (Compared with venom control)

Administration of test drug in envenomated rats led to significant reversal of depleted serum protein level. A marginal increase in the serum alkaline phosphatase was also observed in VC group in comparison to WC group, which was significantly decreased by treatment with the test drug. Further, significant increase in serum creatinine level was observed in VC group, which is non-significantly decreased by the test drug. Other serum biochemical parameters were not affected to statistically significant level by venom injection (Table 1).

A significant increase in the neutrophil percentage and decrease in lymphocyte percentage was observed in VC group, in comparison to WC group. Administration of the test drug in envenomated rats, failed to reverse these parameters to statistically significant extent. Other hematological parameters were not affected to statistically significant extent by venom injection (Table 2).

Envenomation led to an apparent decrease in the total protein level in liver, kidney, and heart tissue homogenate, in comparison to WC group. Administration of the test drug in envenomated rats led to significant reversal of depleted serum protein level in kidney and heart tissues, where as test drug failed to reverse it in liver tissue (Table 3).

Envenomation led to an apparent increase in the lipid peroxidation in liver, kidney, and heart tissue homogenate in comparison to WC group. However, only the observed increase in lipid peroxidation of heart tissue was found to be statistically significant. Administration of the test drug in the envenomated rats failed to reverse the increased lipid peroxidation in all the three tissues (Table 4).

Envenomation led to an apparent but statistically non-significant decrease in the catalase activity in the liver and heart tissue homogenate, in comparison to WC group. Administration of the test drug in envenomated rats led to significant reversal of the depleted catalase activity in the liver and heart tissues (Table 5).

Table 3—Effect of the test drug on total protein in tissue homogenate of envenomated albino rats

	Total protein (mg/g tissue)		
Group	Liver	Kidney	Heart
Water control	07.53±01.88	08.20±01.51	03.90±01.54
Venom control	06.98±00.74	04.28±00.29$^{\#}$	02.62±00.58
Test Drug	05.28±00.78	07.40±01.18*	04.98±00.46*

Values are Mean±SEM, $^{\#}P$ <0.05 (Compared with water control), *P <0.05 (Compared with venom control)

Table 4—Effect of test drug on lipid peroxidation in tissue homogenate of envenomated albino rats

Parameter	Liver	Kidney	Heart
Water control	27.80±05.93	25.02±06.11	21.29±04.50
Venom control	30.31±03.06	28.65±04.75	54.19±08.79$^{\#}$
Test Drug	29.83±07.02	56.95±11.25*	52.91±06.10

Values are Mean±SEM, $^{\#}P$ <0.05, (Compared with water control), *P <0.05 (Compared with venom control); Lipid peroxidation (μ mol MDA released/g wet tissue)

Table 5—Effect of the test drug on catalase activity in tissue homogenate of envenomated albino rats

Parameter	Liver	Kidney	Heart
Water control	02.67 ± 01.11	03.07 ± 00.98	19.79 ± 06.18
Venom control	02.34 ± 00.68	04.47 ± 00.78	16.03 ± 05.19
Test Drug	04.64 ± 00.60*	03.59 ± 00.67	56.21± 12.13*

Values are Mean±SEM, *P <0.05 (Compared with venom control); Catalase (μ moles H_2O_2 consumed/mg protein/min)

Table 2—Effect of test drug on venom induced changes in various hematological parameters in albino rats

Parameter	Water control	Venom control	Venom with test drug
Total count(/mm^3)	7000±733.71	6850±1039.63	11166.7±317.9*
Total RBC (million/mm^3)	8.338±00.23	8.903±00.33	8.902±00.23
Neutrophils (%)	15.25±03.09	26.17±02.70$^{\#}$	29.67±03.79
Eosinophils (%)	02.00±00	02.33±00.33	02.50±00.22
Lymphocytes (%)	81.25±03.33	69.67±02.99$^{\#}$	66.00±04.18
Monocytes (%)	01.50±00.29	01.83±00.17	01.83±00.31
Haemoglobin (gm %)	15.15±00.49	16.30±00.53	16.08±00.42
PCV (%)	48.65±01.45	51.38±01.54	51.38±01.27
MCV	58.38±00.97	57.83±01.11	57.68±00.58
MCH	18.18±00.36	18.37±00.49	18.08±00.32
MCHC	31.10±00.24	31.72±00.35	31.33±00.26

Values are Mean±SEM, $^{\#}P$<0.05, (Compared with water control), *P <0.05 (Compared with venom control)

Discussion

As the tribes in Odisha use the test drug in crude form, the same was selected for the present study. It is well known that snake venom, the most complex poison is a mixture of enzymatic and non-enzymatic toxic compounds, which includes procoagulant enzymes, hemorrhagins, cytolytic or necrotic toxins, pre synaptic and post synaptic neurotoxins, phospholipases A_2, B, C, D, hydrolases, phosphatases leads to disruption of normal cellular functions. Tissue damage may lead to hematological disturbances like decrease in WBC count and increase in neutrophil, increase in serum biochemical parameters like urea, creatinine, alkaline phosphatase activity, SGOT enzyme, SGPT enzyme. Tissue damage leads to generation of oxidants like lipid peroxidation and superoxide ions.

The reduction in serum total proteins, albumin, and globulin in envenomated rats has been reported in laboratory animals exposed to viper snake venoms by various investigators[22-25]. However, the precise mechanisms through which the venoms cause reduction of these parameters are not fully known. It might be assumed that the reduced levels of these serum constituents could be due to disturbances in renal function as well as haemorrhages in some internal organs. In fact, increased vascular permeability and hemorrhages in vital organs due to the toxic action of various snake venoms has been reported[26,27]. Several workers reported acute renal failure characterized by vascular lesions and tubular necrosis in the renal cortex following various snake bites[28]. Another possibility for the decrease in serum proteins level of the VC may be attributed to the decrease in antibodies formation due to toxic effect of venom. Treatment with the test drug significantly reversed the total protein level indicating the protective effect of test drug. Significant increase in serum creatinine level indicates impairment of renal function. Similar observations have been reported in the rats following administration of various viper venoms[22,29,30]. Test drug non-significantly reversed serum creatinine level.

Increase in serum glucose levels has been reported by envenomation by action of venom on glycogen metabolism in the hepatocytes, muscle fibers, and medullary catecholamines that stimulate glycogenolysis and gluconeogenesis in those tissues[22,27]. Mohamed *et al*.[31] also reported the diabetogenic action of *Naja nigricolis* venom. In the present study also, non-significant increase in the serum glucose level may be through the above mechanism, treatment with the test drug significantly reversed it.

Elevated activity of SGPT, SGOT, and serum alkaline phosphatase in VC group might indicate liver and other vital organ damage brought about by the venom. Such findings are in agreement with those reported for *Bitis arietans* venoms[23,32]. Test drug significantly reversed the SGPT and alkaline phosphatase activities, confirming the cytoprotective potential of the test drug.

The intense decrease in serum lipid profile in VC group probably results from venom-induced lipolysis. This lipolytic action could have occurred either as a direct effect of the venom on the hepatic and peripheral tissue lipids or by the activation of the adrenal secretions[33]. Adrenal medullary catecholamines as well as the adrenocorticosteroids are known as potent lipolytic hormones. This conclusion is supported by the results of Mohamed *et al.*[34], who reported a stimulatory effect of *Naja haje* venom on rat adrenals. Ezzat and Abd El-Aal found that a sub-lethal dose of *Naja haje* venom decreased serum total cholesterol in rabbits[35]. These authors explained their findings by the activation of the pituitary-adrenal axis resulting in increased circulatory levels of Adrenocorticotropic hormone (ACTH) and cortisol. ACTH induces specific membrane receptors in the cells of the adrenal cortex to increase serum cholesterol uptake[36]. Treatment with test drug to envenomated rats significantly reversed serum lipid profile and the observed effect may be attributed to one or other mechanism explained above.

A significant decrease in the lymphocyte was noticed in the venom treated rats. It was reported that intermittent injections of sublethal doses of the venom of *Naja haje* of Egypt caused temporary leucopenia followed by leucocytosis in guinea-pigs and rabbits. There was always a rise in the percentage of neutrophil leucocytes and a corresponding drop in the percentage of lymphocytes[37]. Snake venom phospholipase A_2 has been implicated in a direct correlation between the degree of lipid peroxidation and extent of membrane phospholipid hydrolysis. Lipid peroxidation produces a general increase in membrane viscosity, which is associated with instability and enhanced PLA_2 attack. Lipid peroxidation causes destructive process in cells in which much of the damage occurs by disrupting

membrane structure and function. These effects are attributed to free radical induced inactivation of membrane associated enzymes and peroxidation of membrane phospholipids[38].

In the present study, envenomation of rats led to an increase in the lipid peroxidation in all the three tissues. Administration of the test drug to venomised rat non-significantly decreased the lipid peroxidation in liver and heart. Further, it also enhanced the activity of anti-oxidant enzyme catalase in the liver and heart. This shows the antioxidant effect of the test drug.

Till date, no definite answer or mechanism has been established for how the medicinal plants neutralize the toxic venom constituents within the body. Many hypotheses like protein precipitation hypothesis, enzyme inactivation hypothesis, chelation hypothesis, adjuvant action hypothesis, anti-oxidant hypothesis, and protein folding hypothesis have been proposed. The observed significant anti-venom activity of test drug may be through any one of these mechanisms. The most acceptable mechanism may be the anti-oxidant hypothesis.

Conclusion

The present investigation authenticates the folklore claim of the snake venom neutralizing capacity of the test drug. *S. polyandrum* seeds powder is effective in neutralizing the toxic effects of the cobra venom. Considering the present encouraging results, further studies may be undertaken by using purified plant extracts in different dose forms.

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