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In-vivo Study of the Efficacy of *Sanjeevani Vati* in Snake Venom Poisoining

Pradip S. Mohurle [a*#] and Rajendra Lambat [b†]

[a] Department of Agadatantra Evum Vidhivaidyaka, North Eastern Institute of Ayurveda & Homeopathy (NEIAH), Shillong, Meghalaya, India.
[b] Department of Agadtantra, Government Ayurved College, Nagpur, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: *Visha* (Poison) is a substance, which after entering the body, disturbs natural and physiological functions of body (i.e. *Dosha, Dhatu, Mala*). Due to its potency, it may potentially cause death in a relatively short period. A significant proportion of Indians live in villages distant from the city and work in agriculture with their lower extremities exposed. Snake-rat habitat is more prevalent in rice and sugarcane fields.
Aims and Objective: To study the efficacy of *Sanjeevani Vati* in common cobra venom poisoning and Russell's viper venom as a first aid measure.
Materials and Methods: The preparation of *Sanjeevani Vati*is carried out in Department of Rasashastra, Govt. Ayurved College, Nagpur and venom was collected from snake farm', Haffkine Institute for Training Research and Testing, Mumbai. Animal Experiment for efficacy of *Sanjeevani Vati* as a first aid measure on Common cobra venom and Russell's viper venom was carried out in National Toxicology Center (NTC) Pune.
Results: The results of survival period in Russell's viper venom group were proved to be

[#] Lecturer;
[†] Associate Professor;

statistically significant. P value is 0.0055(Unpaired t-test; Two tail).But in results of Common Cobra venom, it was observed that there is no delay in appearance of paralysis, convulsion & survival period. In fact all these symptoms appear near about at same time, when *Sanjeevani Vati* was given orally after ingestion of Cobra Venom.

Conclusion: *Sanjeevani Vati* property is an ophidian. If you have Russell's viper venom, it is helpful as a first aid measure since it extends its life time. Poly Valent Anti snake venom serum does not interact with it (PVASVS).

Keywords: Sanjeevani Vati; snake; venom.

1. INTRODUCTION

Visha (Poison) is a substance, which after entering the body, disturbs natural and physiological functions of body (i.e. *Dosha, Dhatu, Mala*) [1]. It also deteriorates the health of human being resulting into the death in very small time as it is more potent in small dosage.In India large number of people stay in villages far from city and engaged in manual agricultural work often with exposed lower extremities. In paddy and sugarcane farms there is more snake-rat habitat [2]. Hence snake-bite cases are more common in villages but due to lack of emergency medical facilities mortality rate is very high there. Since reporting is not mandatory in many regions of the world, snakebites often go unreported [3]. However, some estimates put the number at 5.4 million snakebites, 2.5 million envenoming, resulting in perhaps 125,000 deaths. Others estimate 1.2 to 5.5 million snakebites, 421,000 to 1.8 million envenoming, and 20,000 to 94,000 deaths. Most snake envenoming and fatalities occur in South Asia, Southeast Asia, and sub-Saharan Africa [4], with India reporting the most snakebite deaths of any country. In India almost all of these deaths are caused by the Big Four, consisting of the Russell's viper, Indian cobra, saw-scaled viper, and the common krait. Maharashtra, one of the states of India with the highest incidence, reported 70 bites per 100,000 population and mortality of 2.4 per 100,000 per year. The other states with a large number of snakebite cases include West Bengal, Tamil Nadu, Uttar Pradesh, Assam & Kerala.

In snake-bite people take primary treatment at Rural Hospital and Primary Health Centers which are also away from periphery [5]. Sometimes due to lack of awareness people even go for local or traditional treatment. Currently the only scientifically valid treatment for snake venom envenomation is serotherapy i.e. Polyvalent Anti-Snake Venom Serum (PVASVS) [6]. It is made available at Rural Hospital, Primary Health Centre and Government Hospital [7].There is limited effectiveness of serotherapy against venom components and rapid local tissue damage is not reversed. Hence, we require the primary substitution or first aid measure before serotherapy, which will increase the survival period and decrease the mortality and morbidity. The most important factor in first aid measure is that should not interact with PVASVS.

In *Ayurveda* identification of poison and symptoms, antidotes of poisons and principle of treatment with 96 anti-poisonous *kalpas* are mentioned. But experimentally and on modern science parameters are not proved. So it is very need of science of *Ayurveda* to introduce *Ayurveda* medicines all over the world with the help of research and experimental study. Most of the experimental studies were done by in-vitro or pre-incubation assay method. Neutralizing actions of extractives of plants, upon shake venom are more notable in vitro but not confirmed in vivo study [8]. Poisonous snakes are classified into neurotoxic, vasculotoxic and myotoxic. Out of neurotoxic and vasculotoxic snakes, Common Cobra & Russell's viper poisoning are more common [9]. So the efficacy of drug on common cobra venom and Russell's viper venom poisoning needs to be investigated [10].

'*Sanjeevani Vati'* as remedy for snake venom poisoning mentioned in *Sharangadhara Samhita Madhyam Khanda Cha*. 7, which is subject to the experimental study.

As compared to PVASVS the Sanjeevani Vati is cheap, easy to carry, easily available and does not require trained persons for administration. Route of administration of '*Sanjeevani Vati*' is oral as mentioned in *Sharangadhara Samhita*. It will reduce the fatality and morbidity as it will not interact with PVASVS. If this formulation will prove effective in-vivo study, then we can develop it further for clinical trials. I want to study the effect of '*Sanjeevani Vati*' as a first aid measure in snake venom poisoning. If the

efficacy of this drug will be proved then it will be a precious gift to the world of medical science and ultimately a blessing for humanity [11].

1.1 Aim and Objective

To study the efficacy of *Sanjeevani Vati* in common cobra venom poisoning as a first aid measure.

To study the efficacy of *Sanjeevani Vati* in Russell's viper venom poisoning as a first aid measure.

To study whether there is any adverse reaction between *Sanjeevani Vati* and polyvalent anti snake venom serum (PVASVS).

2. MATERIALS AND METHODS

Drug: The preparation of *Sanjeevani Vati* is carried out in Department of Rasashastra, Govt. Ayurved College, Nagpur.

2.1 Ingredients of *Sanjeevani Vati*

Table 1. Ingredients of *Sanjeevani Vati*

Sr.no	Dravya	Latin Name
1.	Haritaki	*Terminaliachebula*
2.	Bibhitaki	*Terminaliabellirica*
3.	Amalaki	*Emblicaofficianalis*
4.	Shunthi	*Zingiberofficianale*
5.	Pippali	*Piper longum*
6.	Vidanga	*Emblicaribes*
7.	Vacha	*Acoruscalamus*
8.	Guduchi	*Tinsporacardifolia*
9.	ShudhaVatsanabha	*Aconitum ferox*
10.	ShudhaBhallataka	*Semicarpus anacardium*
11.	Gomutra	--

2.2 Collection of Venom

Dried lyophilized venom from of 150mg of common cobra venom and 150 mg of Russell's viper venom was collected from snake farm', Haffkine Institute for Training Research and Testing, Mumbai.

2.3 Collection of polyvalent Anti Snake Venom Serum (PVASVS)

PVASVS was procured from Haffkine Institute for Training Research & Testing, Mumbai

2.4 Chemical Use

Dried lyophilized common cobra venom and Russell's viper venom, Polyvalent Anti Snake Venom Serum (PVASVS), *Sanjeevani Vati* with *ArdrakaSwarasa* as anupana, Distilled water.

2.5 Dose Calculation of venom

Human Fatal dose for Common Cobra is 12mg. According to conversion factor mice fatal dose for common cobra venom is 0.0312 mg. i.e. 31.2 µgm.

Dose of Common Cobra venom = 31.2 µgm
Dose of Russell's Viper venom = 345 µgm

2.6 Dose Calculation of *Sanjeevani Vati*

Dose of *Sanjeevani Vati* in '*Sharangadhara Samhita*' was given as 3-3 *vati*. But single *vati* should be prepared same as size of *Gunja* seed which is considered currently to weigh as 125mg each. Therefore therapeutic dose of *Sanjeevani Vati* in human is calculated as

125 mg × 6 = 750 mg

We can take 750 mg ideal human dose of *Sanjeevani Vati* for snake poisoning.

According to conversion factor for mice dose, the dose for mice was calculated as

Dose of *Sanjeevani Vati* in mice = 0.0026 × 750 mg

Dose of *Sanjeevani Vati* in mice = 1.950 mg ≈ 2 mg

2.7 Dose Calculation of PVASVS

1 ml of reconstitute PVASVS neutralizes 0.6 mg of Common cobra venom. For our animal experiment, we gave 31 µgm of common cobra venom. So required dose of PVASVS was 0.05 ml.

Dose of PVASVS for common cobra Group = 0.05 ml

1 ml of reconstitute PVASVS neutralizes 0.6 mg of Russell's viper venom. For our animal experiment we gave 345 µgm of Russell's viper venom so required dose of PVASVS was 0.57 ml.

List 1. Details are as per following

Common Cobra venom	Vial No771 X.	0.15 gm	Sealed at 1999.
Russell's viper venom	vial No. 785 B	0.15 gm	sealed at 1999

Dose of PVASVS for Russell's viper venom = 0.57 ml

2.8 Experimental Protocol

Animal Experiment for efficacy of *Sanjeevani Vati* as a first aid measure on Common cobra venom and Russell's viper venom was carried out in National Toxicology Center (NTC) Pune. Following Protocol is depicted in [Table 1], Grouping is depicted in [Table 2].

2.9 Procedure

First preliminary drug toxicity study for each sample of venom was done, In each group, weight of animals was taken first and noted; simultaneously dose of venom, PVASVS and *Sanjeevani Vati* were calculated. Accordingly route, venom dose was given by IM route, after 5 min drug dose was given by oral route and then PVASVS was given after 5 min by IV route. After dosing animals were observed for 24 hrs up to 7 day's, Comparative observations were tabulated.

3. RESULTS AND OBSERVATION

Lethal dose (LD_{50}) of our animal experiment, we gave 31 µgm of common cobra venom. For our animal experiment we gave 345 µgm of Russell's viper venom.

Analysis of common cobra venom are depicted in [Fig. 1].

Analysis of Russell's viper venom are depicted in [Fig. 2].

In many Ayurvedic texts it is stated that remedy of *Sanjeevani Vati* is effective in all type of snake venom poisoning.

The results of survival period in Russell's viper venom group were proved to be statistically significant. P value is 0.0055 (Unpaired t-test; Two tail).

But in results of Common Cobra venom, it was observed that there is no delay in appearance of paralysis, convulsion & survival period. In fact all these symptoms appear near about at same time, when *Sanjeevani Vati* was given orally after ingestion of Cobra Venom.

4. DISCUSSION

Any drug which acts by any other mode of action i.e. other than chemical neutralization cannot be studied by these methods. This is particularly true of drugs acting on nervous system which primarily act by blocking of receptor sites or competitive inhibition. Both these methods give results which seldom stand true in clinical situations. Thus '*in vivo*' study becomes of

Table 2. Experiment protocol and conditions

Animal species	B6 D2 F1 Black Mice
Source of Animals	National Toxicology Centre, Pune
Aug. Wt. of mice	22 gms
No. of Animals	3 mice in each group
Age of Animals	6-5 wks
Sex of Animals	Female in each group
Diet	Pelleted feed supplied by "Nav Maharashtra Chakan oil mills ltd. Pune."
Water	Community tape water ad libitum
Room Temperature	20 -24 ºC
Relative Humidity	40% to 60 %
Light cycle	12 hrs light and 12 hrs dark
Vehicle used	Water
No. of group	8
Period of Acclimatization	7 days
Period of Fasting	Overnight
Dosing	Snake venom was given by Intramuscular route; *Sanjeevani Vati* was given by oral route and PVASVS was given by Intravenous route.

Table 3. Groups for animal experiments

Group I	Only common cobra venom
Group II	Common cobra venom + *Sanjeevani Vati*
Group III	Common cobra venom + PVASVS.
Group IV	Common cobra venom + *Sanjeevani Vati*+ PVASVS
Group V	Only Russell's viper venom
Group VI	Russell's viper venom + *Sanjeevani Vati*
Group VII	Russell's viper venom + PVASVS
Group VIII	Russell's vipers venom + *Sanjeevani Vati* + PVASVS

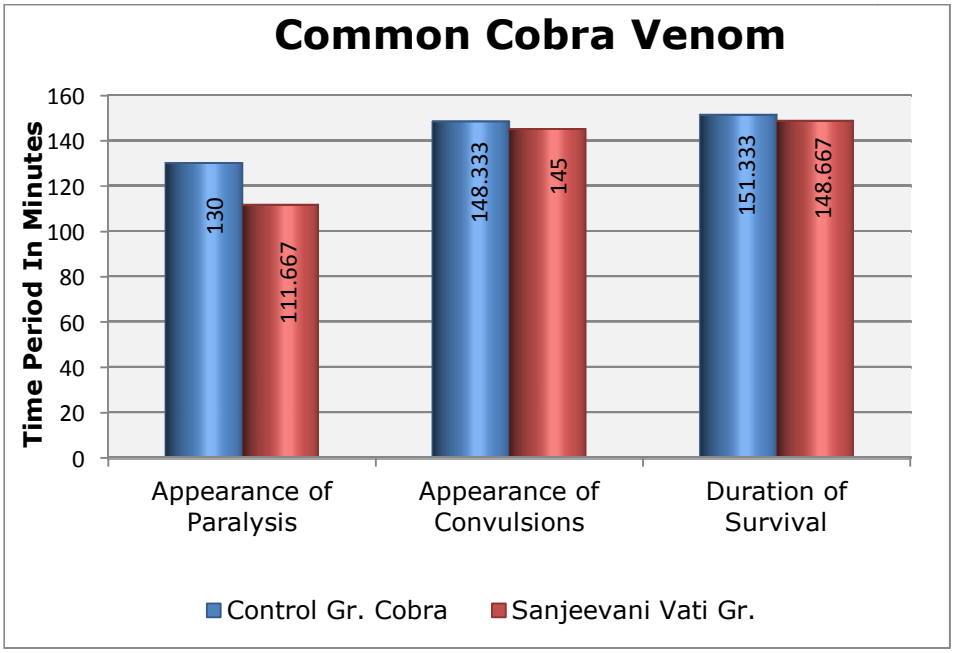

Fig. 1. Graph showed analysis of common cobra venom

paramount importance in proving the efficacy of ant ophidian drugs. As per the policy set by my institute & my guide our emphasis is on '*in vivo*' studies. Many of the difficulties in the previous studies were avoided in the current study. The previous *in vivo* studies in Tilak AyurvedMahavidyalaya, Pune have paved the way & my experiment was basically for the extension of screening process by using a different drug than previous researchers from that institute. As there are 96 formulations mentioned in *Ayurveda* as ant ophidian drugs there is a great scope for this type of study.

Drug Procurement: Theayurvedicmedicine Sanjeevani *Vati*, contains 10 ingredients, all of which are easily available. Dried samples of each ingredient were collected from local herbal drug market at Nagpur. The preparation of *Sanjeevani Vati* is carried out in Department of Rasashastra, Govt. Ayurved College, and Nagpur; while standardization is done at Qualichem Laboratories, Nagpur.

Venom Procurement: For the entire animal experiment phase, only few micrograms of the Common cobra venom and Russell's viper venom were required. Available smallest packing for Common cobra venom was 150 mg and for Russell's viper venom was 150 mg Lyophilized venom was diluted. In first dilution, I took out only 10% of venom and diluted it further. Thus I had no use of almost 90% of the venom. The remaining venom lost its potency within 2 days. In lyophilized form it can remain potent up to 10 years. Due to lack of facility for lyophilization, I had to dispose off 90% of the procured venom. I suggest that in further experiments of this type either lyophilization facility should be made available or other experiments utilizing the remaining venom should be planned simultaneously.

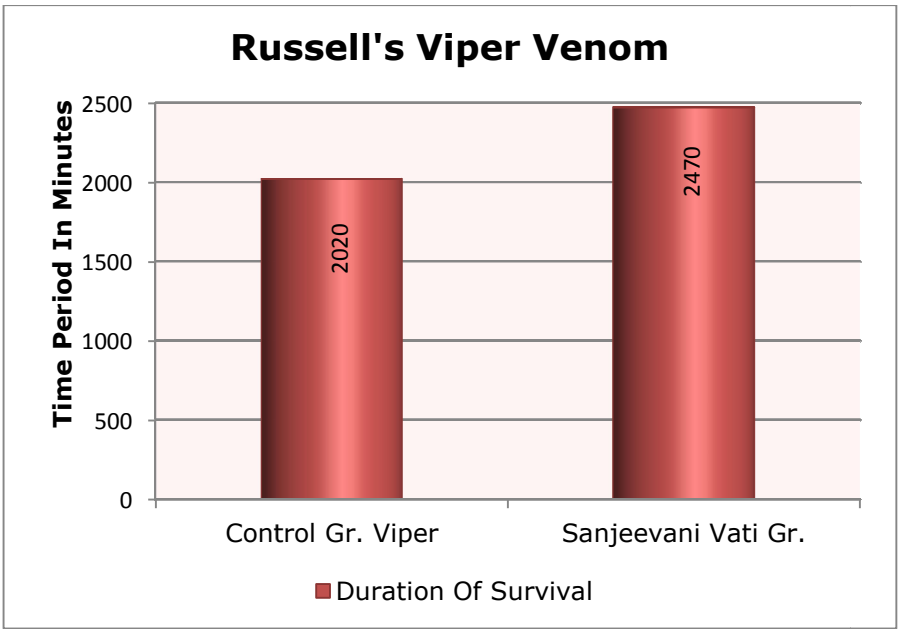

Fig. 2. Graph showed analysis of russell's viper venom

Dose Calculation: Initially it was decided that 80% fatal dose of venom should be used in cobra group & 100 % fatal dose of venom should be used in Russell's viper group. According to previous studies done at Tilak Ayurved Mahavidyalaya, Pune, they suggest that in future experiments of this kind 100% fatal dose of Common cobra 110-120% fatal dose of Russell's viper venom should be injected.To resolve this experimentally, we conducted the pilot study using two animals per dosage group. In the first stage these animals were given 31, 41 & 51 µgm (in cobra group) & 50, 60 & 70 µgm (in Russell's viper) dose levels of venom respectively. At this level in cobra group 100% animal died in 140 minutes (average) at minimum dose i.e. 31µgm. But in Russell's viper group no fatality or observable morbidity was seen. The reason for this discrepancy may be loss of potency of venom during storage. Therefore in Russell's viper group two more stages by increasing venom doses are carried, & at 345 µgm fatality were seen. Therefore 31µgm of cobra venom & 345 µgm of Russell's viper venom were used.

Dosing: For the ease of observation albino mice were chosen for the experiment, while oral and IM dosing was not a problem, but IV dosing PVASVS proved a big hazard. As the required amount of PVASVS was very small, its IV delivery is a very skillful and difficult procedure.

During Observations: It was very difficult to observe and distinguish the pre-paralytic and paralytic signs of the Common cobra venom. It was impossible to record the pre-paralytic signs. In paralytic signs tremors was not observed. Other signs i.e. paralysis, convulsion in Common cobra venom i.e. control group [I] were observed. As Russell's viper venom is haemostatic, external bleeding from mouth, nose, ear and necrosis at the bite site are the common symptoms in humans. But these symptoms were not observed in Russell's viper venom group i.e. control group [V].

In Common cobra control group (Gr. I) appearance of paralysis was observed after 130 min (average) and that of drug group (Gr. II) it was after 111.67 min (average) i.e. time duration of appearance of paralysis was decreased by 18.33 min in Sanjeevani Vati group, which is statistically not significant. P value is 0.2237 (Unpaired t-test; Two tail).

In Common cobra control group (Gr. I) appearance of convulsions was after 148.33 min (average) and in drug group (Gr. II) it was after 145 min (average) i.e. time duration of appearance of convulsions was decreased by 3.33 min in *Sanjeevani Vati* group, but P value is notstatistically significant. P value is 0.7878 (Unpaired t-test; Two tail).

In Common cobra control group (Gr. I) duration of survival was 151.33 min (average) & that of drug group (Gr. II) duration of survival was 148.66 min (average) i.e. duration of survival was decreased by ~3 min in *Sanjeevani Vati* group, but P value is notstatistically significant. P value is 0.8375 (Unpaired t-test; Two tail).

In Common cobra venom + PVASVS (standard group III) all mice survived completely without showing any signs.

In Common cobra venom + *Sanjeevani Vati*+ PVASVS group (Gr. IV) all mice survived completely without showing any signs. No adverse interaction between *Sanjeevani Vati* and PVASVS was seen.

In Russell's viper venom group (Gr. V) duration of survival was 2020 min (average) & in drug group (Gr. VI) duration of survival was 2470 min (average) i.e. duration of survival was delayed by 450 min in *Sanjeevani Vati* group, which is statistically significant. P value is 0.0055 (Unpaired t-test; Two tails).

In Russell's viper venom + PVASVS group (Gr. VII), all mice survived without showing any signs.In Russell's viper venom + *Sanjeevani Vati*+ PVASVS group (Gr. VIII), all mice survived without showing any signs. No adverse interaction between *Sanjeevani Vati* and PVASVS was seen.

5. CONCLUSION

The Present study partially proves the '*Vishaghna'* property i.e. Ant ophidian property of the *Sanjeevani Vati*. Itis useful as a first aid measure in Russell's viper venom because, Itincreases the survival period in Russell's viper venom. It does not interact with Poly Valent Anti Snake Venom Serum (PVASVS).

RESEARCH SIGNIFICANCE

The study highlights the efficacy of "Ayurveda" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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