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IN VIVO STUDY OF THE EFFICACY OF SINDUVAARAKA MOOLA AGADA (ROOT OF VITEX TRIFOLIA) AS A FIRST AID MEASURE IN SNAKE VENOM POISONING

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ABSTRACT

Snakebites are a major health threat in India. If patient need any medical or any other treatment they have to go to Higher Medical Centre. Hence we require the primary substitution or first aid measure before serotherapy, i.e. Poly-Valent Anti-Snake Venom Serum (PVASVS), which will increase the survival period and decrease the mortality and morbidity. We have selected *"Sinduvaaraka Moola Agada"* mentioned in *Asht'aamga Samgraha* in snake venom poisoning for animal experiment. The root of *Vitex trifolia* is triturated in swarasa of the same and mixed with honey. [1] This agada given by oral route is beneficial in elapidae snake venom poisoning. (A.S.U.Sarpavishapratished adhyaya 42/21).*Sinduvaaraka moola agada* is useful as a first aid measure in snake venom because, it delays the onset of symptoms in Common cobra and Russell's viper venom. It increases the survival period in Common cobra and Russell's viper venom. It does not interact with Poly Valent Anti Snake Venom Serum (PVASVS).

KEY WORDS: *first aid, Snakebite*, *Sinduvaaraka Moola Agada.*

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INTRODUCTION

About 2 million people are bitten by snakes annually of which 15,000 to 30,000 cases prove fatal. Snake bite cases are more common in the states of Maharashtra, west Bengal, Uttar Pradesh, Assam & Kerala. Currently the only scientifically validated treatment for snake venom envenomation is serotherapy i.e. Poly-Valent Anti-Snake Venom Serum (PVASVS), it is available at Rural Hospital., Public Health Centre, Government Hospital.[2]When a snake bites the patient, it takes time to raise alarm. First aid help will arrive within 10 min.Then they arrange a Van, Bullock-cart or any other vehicle to reach to PHC or RH. This traveling will take average 2-2.5 hrs. When patient reaches to PHC or RH it may not be OPD timing, doctors may not be available at that time, since snakebite, cases are more common at night. After arrival of doctors serotherapy will start, which will require minimum 30 min.Therefore, the average time lapse will be 3 hrs 10 min. Average fatal period of Common cobra is 2-3 hrs, Krait 6-12 hrs, Viper is 24 hrs. After irreversible damage by venom poisoning, serotherapy will not be able to rescue the patient, delay in treatment may turn to be fatal. Hence, we require the primary substitution or first aid measure before serotherapy, which will increase the survival period and decrease

the mortality and morbidity. In snakebite cases available first aid measures are, physical and external i.e. pressure bandage, application of tourniquet, incision and suction, hence there effectiveness is limited.[3] So we require a first aid measure for snake bite, that will be

a) A first aid measure should be safe i.e. nontoxic.

b) Its route of administration should be oral, because nasal or collyrium application will require trained person and it will time consuming.

c) It should be easy to available, easy to prepare, easy to administer.

d) It should prolong the appearance of symptoms or prolong the survival time, so that patient can get serotherapy before irreversible changes should happen.

e) The most important factor in first aid measure was that it should not interact with PVASVS.

Snake are classified into neurotoxic, vasculotoxic and myotoxic. Myotoxic snakes are sea snakes, these are less common. Out of neurotoxic and vasculotoxic Common cobra and Russells viper poisoning is more common.[4] So we wanted to see the efficacy of drug on

Common cobra venom and Russell's viper venom poisoning.

We have selected *"Sinduvaaraka Moola Agada"* mentioned in *Asht'aamga Samgraha* in snake venom poisoning.The root of *Vitex trifolia* is triturated in swarasa of the same and mixed with honey. This agada is given by oral route is beneficial in elapidae snake venom poisoning.Route of administration of *Sinduvaaraka Moola Agada* is oral. *Asht'aamga Samgraha* states that this remedy is effective in *Darveekara* (elapidae) type of snake venom poisoning.[5] In India Cobra and Russell's viper are more common. Therefore, it is necessary to check the efficacy of said remedy in both types of poisoning.

AIMS AND OBJECTIVES:

- To study the efficacy of *'Sinduvaaraka moola agada'* in Common cobra venom poisoning as a first aid measure.
- To study the efficacy of *'Sinduvaaraka moola agada'* in Russell's viper venom poisoning as a first aid measure.
- To study whether there is any adverse drug reaction between *'Sinduvaaraka moola agada'* and Poly Valent Anti Snake Venom Serum (PVASVS).

MATERIAL AND METHODS:

Collection of *Sinduvaaraka moola* –

Raw sample of *Sinduvaaraka moola* was collected from Sangli. Authentification of Sinduvaaraka was done at Botanical Survey of India Pune.

Preparation of *Sinduvaaraka moola Agada-*[6]

In a clean washed Morter & Pestle 100gm Powder of sinduvaaraka moola was taken,then the juice of sinduvaaraka patra was added to it. The trituration process was continued for day and night until it became a thin slurry. became a dry powder,then only it was considered as one Bhaavanaa was completed.Like this total 7 Bhaavanaas were given to that powder. For each Bhaavanaa process freshly prepared juice of sinduvaaraka was used. After completion of 7 Bhaavanaa Greenish coloured Sinduvaaraka moola agada was obtained.

Standardizations of Drug –

This study was done at Indian Drug Research Association Laboratory, Pune.

Collection of AGMARK standard Madhu -

AGMARK standard honey was collected from Mankarnika aushdhaalaya,pune.

Collection of Snake Venom -

Dried lyophilized form of 110 mg of Common cobra venom and 90 mg of Russell's viper venom was collected from

Snake farm, Haffkine Institute For Training Research and Testing, Mumbai.

Details are as per following:

Collection of Poly Valent Anti Snake Venom Serum (PVASVS)-

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

Experimental study

Conversion factor from man to mice was 0.0026 so according to this venom dose, drug (*Sinduvaaraka moola agada*) dose and PVASVS dose was calculated.

Dose calculation of Venom:

Human fatal dose for Common cobra is 12 mg.[7] According to conversion factor mice fatal dose for Common cobra venom was 0.0312 mg i.e. 31.2 ugm. Previously total fatal dose was taken but at this dose no death occurs in Albino mice. So After doing pilot Study following dose was taken.

Dose of Common cobra venom was =60 ugm.

Human fatal dose for Russell's viper is 20 mg.[8] According to conversion factor mice fatal dose for Russell's viper venom was 0.0520 mg i.e. 52 ugm. Previously total fatal dose was taken but at this dose no death occurs in Albino mice till 7 days. After doing pilot study following dose was taken.

Dose of Russell's viper venom was = 750ugm**.**

Dilution criteria for venom :

Dried lyophilized form of venom was diluted to get appropriate fatal dose to inject mice.

In a vial of 110 mg cobra venom 11 ml distilled water was added, from that 0.125 ml was removed and added to 5 ml distilled water containing glass bottle. Then it was sealed with rubber cork. From this dilution 0.24 ml was taken out and injected to mice.

100 mg ≈ 10ml ∴ 1 ml = 10 mg

1250 ugm. ≈ 5ml ∴ 60 ugm.= 0.24ml

In a vial of 90 mg Russell's viper venom 9 ml distilled water was added, form that 5 ml

was removed and added to 2.5 ml distilled water containing glass bottle.It was sealed with rubbre cork. From this dilution 0.38 ml was taken out and injected to mice.

90 mg ≈9 ml ∴ 1 ml = 10 mg

5000 ugm. ≈ 2.5ml ∴ 750 ugm.= 0.38ml

Dose calculation of *Sinduvaaraka moola agada:*

Dose of *Churńa* in *Śhaarangadhar samhitaa* was given as 1 *Karsha*[9] i.e.10gm.But for

todays time period it is too high. So ideal dose of *Churńa* was taken as 1gm. Human dose of *Sinduvaaraka moola agada* was 1gm.

According to conversion factor mice dose was 0.0026gm i.e.2.6mg.

For that purpose 130mg of *Sinduvaaraka moola agada* was weighed on balance and 130mg of *Madhu* was mixed then15ml distilled water was added to it .This mixture was stirred for 15 min and good suspension was made. Out of this 0.3 ml was given for oral dose.

Dose Calculation of PVASVS:

1 ml of reconstitute PVASVS neutralizes 0.6 mg of Common cobra venom.[10] For our animal experiment, we gave 60 ugm.of Common cobra venom.

So required dose of PVASVS was 0.1 ml

Dose of PVASVS for Common cobra Group = 0.1 ml

1 ml of reconstitute PVASVS neutralizes 0.6 mg of Russell's viper venom.[11]For our animal experiment we gave 750 ugm.of Russell's viper venom.

So required dose of PVASVS was 1.25 ml

Dose of PVASVS for Russell's Viper Group = 1.25 ml

Animal Experiment

Animal species	Albino Mice
Source of Animals	NTC, Pune
Avg. Wt. of mice	22 gm.
No. of Animals	3 mice in each group
Age of Animals	6 – 8 wks.
Sex of Animals	Female in each group
No. of groups.	8
Dosing	Snake venom was given by **Intramuscular** route, Sinduvaaraka moola agada was given by **Oral** route and PVASVS was given by **Intravenous** route.

Table no 1- Protocol used

Table no 2- Groups for Animal Experiments

Group I (Control Group)	Only Common cobra venom.
Group II	Common cobra venom + *Sinduvaaraka moola agada*
Group III (Control Group)	Only Russell's viper venom
Group IV	Russell's viper venom + *Sinduvaaraka moola agada*
Group V (Standard Group)	Common cobra venom + PVASVS
Group VI	Common cobra venom + *Sinduvaaraka moola agada* + PVASVS
GroupVII(Standard Group)	Russell's viper venom + PVASVS
Group VIII	Russell's viper venom + *Sinduvaaraka moola agada* + PVASVS

- **Method :**

a) Samples were converted into Suspension by through mixing with water
b) Samples were freshly prepared for each group and then administered.
c) Doses given to Animals according to their body weight.
d) After dosing all animals were observed for 24 hours for toxic signs and symptoms or mortality up to 7 days.

- **Procedure:**

a) First preliminary drug toxicity study was done.
b) In each group, weight of animal was taken first, accordingly route venom dose was given by IM route, after 5 min, drug dose was given by orally route and then PVASVS was given after 5 min by IV route.
c) After dosing animals were observed for 24 hrs up to 7 days.
d) Comparative observations were tabulated.

- **OBSERVATIONS OF 'IN - VIVO STUDY' :**

1. Appearance of tremors were delayed by 10 min in common cobra venom +*Sinduvaaraka moola agad* group.

2.Appearance of paralysis was delayed by 15 min in common cobra venom + *Sinduvaaraka moola agada* group.

3.Appearance of convulsions was delayed by 19min in common cobra venom + *Sinduvaaraka moola agada* group.

4. Duration of Survival was delayed by 29min in common cobra venom +*Sinduvaaraka moola agada* group.

5. Duration of Survival was delayed by 39min in Russell's viper venom + *Sinduvaaraka moola agada* group.

6.Group V (Standard Common cobra group) :- One mice died after 197 min., this may be due to serum sickness reaction of PVASVS. Remaining two mice survived completely.

7.Group VI -All three mice survived completely without showing any signs.

8.Group VII (Standard Russell's viper group)-All three mice survived completely without showing any signs.

9.Group VIII :- All three mice survived completely without showing any signs.

RESULTS: *Sinduvaaraka moola agada* is useful as a first aid measure in snake venom because,

It delays the onset of symptoms in Common cobra-Tremors- P Value(One tail) = 0.048170538,Convulsion-P Value(One tail)= 0.039824615, Paralysis-P Value(One tail)= 0.046171657

It delays the survival period in Common cobra- P Value(One tail)= 0.015518475

It delays the survival period in Russell's viper- P Value(One tail)= 0.014562386

It dose not interact with Poly Valent Anti Snake Venom Serum (PVASVS)

DISCUSSION

Dose calculation

we conducted the pilot study using two animal per dosage group. In the first stage these animals were given 25,50&100 ugm (in Russell's viper) & 12.5,25&50 ugm (in cobra group) dose levels of venom respectively.At this level in cobra group 50% animal died in 50 ugm.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 750 ugm fatality was seen. Therefore to achive good observation period 60 ugm of cobra venom &750 ugm of Russell's viper venom was used.

Observations

In Common cobra control group (Gr. I) appearance of tremors was observed after 36 min (average) and that of drug group (Gr. II) was after 46 min (average) i.e. appearance of tremors was delayed by 10 min in *Sinduvaaraka moola agada* group,which is statistically significant. P value is 0.04(one tail).

In Common cobra control group (Gr. I) appearance of paralysis was observed after 43min (average) and that of drug group (Gr. II) it was after 58 min (average) i.e. appearance of paralysis was delayed by 15 min in *Sinduvaaraka moola agada* group,which is statistically significant. P value is 0.04(one tail).

In Common cobra control group (Gr. I) appearance of convulsions was after 48 min (average) and in drug group (Gr. II) it was observed after 67 min (average) i.e. appearance of convulsions was delayed by 19 min in *Sinduvaaraka moola agada* group,which is statistically significant. P value is 0.03(one tail).

In Common cobra control group (Gr. I) duration of survival was 59 min (average) & that of drug group (Gr. II) duration of survival was 88 min (average) i.e. duration of survival was delayed by 29 min in *Sinduvaaraka moola agada* group,which is statistically significant. P value is 0.01(one tail).

In Common cobra venom + PVASVS (standard group) one mouse died after197min. This may be due to serum sickness reaction of PVASVS. Remaining two mice survived completely.

In Common cobra venom + *Sinduvaaraka moola agada* + PVASVS group (Gr. VI) all mice survived completely without showing any signs.

In Russell's viper venom group (Gr. III) duration of survival was 121min (average) & that of drug group (Gr. II) duration of survival was 160 min (average) i.e. duration of survival was delayed by 39 min in *Sinduvaaraka moola agada* group,Which is statistically significant. P value is 0.01(one tail).

In Russell's viper venom + PVASVS group (Gr. VII), all mice survived without showing any symptoms.

In Russell's viper venom + *Sinduvaaraka moola agada* + PVASVS group (Gr. VIII), all mice survived without showing any symptoms. No adverse interaction between *Sinduvaaraka moola agada* and PVASVS was seen.

Significant Results

Asht'aamga Samgraha states that this remedy is effective in *Darveekara* (elapidae) type of snake venom poisoning. But It was proved efficient in Russell's viper venom poisoning also.The results of survival period in Russell's viper venom group was proved to be statistically significant. P value is 0.01(one tail)The results of survival period in Common cobra venom group was proved to be statistically significant. P value is 0.01(one tail)

CONCLUSION

The Present study confirms the *'Vishaghna'* property i.e.Antiophidian property of the *Sinduvaaraka moola agada.Sinduvaaraka moola agada* is useful as a first aid measure in snake venom because, It delays the onset of symptoms in Common cobra and Russell's viper venom. It increases the survival period in Common cobra and Russell's viper venom. It does not interact with Poly Valent Anti Snake Venom Serum (PVASVS). Thus the null hypothesis is rejected and the hypothesis that *Sinduvaaraka moola agada* is efficient as a first aid measure in Common cobra and Russell's viper venom is accepted.

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