



“STUDY OF EFFICACY OF GOGHRUTA (COW’S GHEE) AS A FIRST AID MEASURE IN SNAKE VENOM POISONING – AN IN-VIVO EXPERIMENT”

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ABSTRACT

The present ‘*in vivo*’ study has been designed regarding efficacy of *Goghruta* as a first aid measure in Common cobra and Russell’s viper snake venom poisoning. The *Goghruta* for experimentation was of **AGMARK** standards of **Special** Grade and snake venom was procured from Haffkine Serum Institute, Mumbai.

In animal experimentation study, both the control groups injected respective venom and *Goghruta* given orally to trial groups. Each group having six albino mice. Mice were observed upto 7 days and observations recorded.

In trial group, delay in the duration of appearance of symptoms i.e. tremors, paralysis, convulsions, bleeding and increase in the duration of survival period in comparison with control group is noted. The results of survival period in Russell’s viper venom group was proved statistically significant whereas survival period in Common cobra venom group was nearly significant. The interaction with PVASVS is not noted. Thus, the Antiophidian property of the *Goghruta* as first aid measure is confirmed by ‘*in vivo*’ study.

Key words – Cobra Snake venom, Russel’s Viper snake venom, *Goghruta*, in-vivo study, first aid measure

1. INTRODUCTION

Snakebites are a major health threat in India. 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 and Kerala.*¹

Snakebite cases are more common in rural area and hilly regions which are covered by dense evergreen forests. They have to arrange some vehicles to come at Rural Hospital or Primary Health Centre for treatment. This all traveling process is time consuming and 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 doctor, serotherapy i.e. Poly-Valent Anti-Snake Venom Serum (PVASVS) will be started which is only scientifically validated treatment for snake venom envenomation is, it is available at Rural Hospitals, Primary Health Centres and Government Hospitals.^{*2} For serotherapy minimum 30 min will be required. Therefore, average time lapse will be 3hrs 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 and delay in treatment may turn to be detrimental.

Sensitivity test should be done prior to the administration of PVASVS, so it requires trained person. Irreversible changes may occur due to delay in getting PVASVS. Adverse reaction of PVASVS may turn as a fatal, so emergency Medical facility should be available at that time. There is limited effectiveness of serotherapy against venom components, so rapid local tissue damage can occur. 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 their effectiveness is limited.^{*3} So we require a first aid measure for snake bite. That will be safe i.e. nontoxic. Its route of administration should be oral, because nasal or collyrium application will require trained person and it will time consuming. A first aid measure should be easy to available, easy to prepare, easy to administer. It should prolong the appearance of sign-symptoms or prolong the survival time, so that patient can get serotherapy before irreversible changes should happen. The most important factor in first aid measure was that it should not interact with PVASVS.

For in vivo study snake venom, experimental drug and PVASVS are given accordingly, so that actual situation of snake bite will be developed. So ‘in vivo’ study have been planned for screening of remedy mentioned in Ayurved as a first aid measure.

Goghruta (Cow’s Ghee) treatment is mentioned in *Ashtang-Samgraha* as well as *Ashtang-Hrudya* in snake venom poisoning is.(A.S.Su.40/119, A.H.U.35/69, 70) ^{*4,5}

Route of administration of *Goghruta* is oral. *Samgrahkar Vagbhata* does not mention specifically the type of venom to which the said remedy is effective. In India Cobra and Russell’s viper Bites are more common.^{*6} Therefore, it is necessary to check the efficacy of said remedy in both types of poisoning.

A good first aid measure in snakebite should -

1. Delay the action of snake venom
2. Keep the patients vital functions within reasonable limits
3. Keep the patients ambulatory

4. Increase survival time of patient

Goghruta is easily available, easy to carry, does not require trained persons for administration. As compared to PVASVS, it is cheap and easily made available. It will reduce the fatality and morbidity if it will not interact with PVASVS.

2. OBJECTIVES OF STUDY

- To study the efficacy of ‘*Goghruta*’ in Common cobra venom poisoning as a first aid measure.
- To study the efficacy of ‘*Goghruta*’ in Russell’s viper venom poisoning as a first aid measure.
- To study whether there are any adverse drug reactions between ‘*Goghruta*’ and Poly Valent Anti Snake Venom Serum (PVASVS).

3. MATERIALS

3.1 For ‘in – vivo’ study:

A. Instrument and Apparatus

- 1) Single Pan Electronic Digital Balance
- 2) Beakers and Stirrer
- 3) Test tubes
- 4) 1cc syringe with 24 no. needle
- 5) Tuberculin syringe (0.1 x 1ml) with 14 no. needle curved at 130⁰ angle

B. Chemicals:

- 1) Dried lyophilized Common cobra venom
- 2) Dried lyophilized Russell’s viper venom.
- 3) Poly Valent Anti Snake Venom Serum (PVASVS)
- 4) *Goghruta*
- 5) Distilled water

4. METHODS

4.1 Procurement of *Goghruta*

The *Goghruta* sample of **AGMARK special** gradation standards was procured from Local grocery at Pune.

4.2 Standardization of Drug

Standardization of *Goghruta* was done at Akanksha Laboratory, Pune.

The *Goghruta* for experimentation as first aid measure in Snake Venom Poisoning was of **AGMARK** standards of **Special** Grade designation fulfilling all recommended norms. The

various tests done pertaining to standardization of Goghruata were as per standard Protocol (www.rosbrandghee.com/quality.htm)

Grade designation - **AGMARK GHEE SPECIAL GRADE**

4.3 Procurement of Venom

Dried lyophilized form of 100 mg of Common cobra venom and 100 mg of Russell’s viper venom was procured from Snake farm, Haffkine Institute for Training Research and Testing, Mumbai. The details are as per following:

1)	Common cobra venom	Vial No. 833 E	0.1 gm
2)	Russell’s viper venom	1)Vial No. 828 B	0.05 gm
		2)Vial No. 828 C	0.05m

4.4 Procurement of Poly Valent Anti Snake Venom Serum (PVASVS)

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

4.5 EXPERIMENTAL STUDY

4.5.1 Dose calculation for mice - Conversion factor from man to mouse was 0.0026 so according to this venom dose, drug (Goghruata) dose and PVASVS dose was calculated. (Ref: Experimental Pharmacology by Dr. Ghosh - Surface area ratio of some common laboratory species and man)

4.5.2 Dose Calculation of venom

Human fatal dose for Common cobra is 12 mg. According to conversion factor mice fatal dose for Common cobra venom was 0.0312 mg i.e. 31.2 µgm. Fatal period of Common cobra is short, so we have taken 80% of the total fatal dose to observe the effect of venom for a longer time.

80 % dose of Common cobra venom was = 24.96 µgm. i.e. ≈ 25 µgm

Human fatal dose for Russell’s viper is 20 mg. According to conversion factor for mice fatal dose for Russell’s viper venom was 0.0520 mg i.e. 52 µgm. Russell’s viper fatal period was sufficient to observe the effect of venom, so 100% fatal dose was taken.

100% dose of Russell’s viper venom was = 52 µgm.

4.5.3 Dilution of venom

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

In a vial of 100 mg cobra venom 10 ml distilled water was added, from that 1 ml was removed and added to 100 ml distilled water containing glass bottle. Then it was sealed with rubber cork. From this dilution 0.25 ml was taken out and injected to mice.

$$\begin{aligned} 100 \text{ mg} &\cong 10 \text{ ml} && \text{i.e. } 1 \text{ ml} = 10 \text{ mg} \\ 10 \text{ mg} &\cong 100 \text{ ml} && \text{i.e. } 1 \text{ ml} = 0.1 \text{ mg} = 100 \text{ } \mu\text{gm.} \\ &&& \text{i.e. } 0.5 \text{ ml} = 0.05 \text{ mg} = 50 \text{ } \mu\text{gm.} \\ &&& \text{i.e. } 0.25 \text{ ml} = 25 \text{ } \mu\text{gm.} \end{aligned}$$

In a vial of 50 mg Russell’s viper venom 5 ml distilled water was added, from that 1 ml was removed and added to 50 ml distilled water containing glass bottle. It was sealed with rubble cork. From this dilution 0.25 ml was taken out and injected to mice.

$$\begin{aligned} 50 \text{ mg} &\cong 5 \text{ ml} && \text{i.e. } 1 \text{ ml} = 10 \\ 10 \text{ mg} &\cong 50 \text{ ml} && \text{i.e. } 1 \text{ ml} = 0.2 \text{ mg} = 200 \text{ } \mu\text{gm.} \\ &&& \text{i.e. } 0.5 \text{ ml} = 100 \text{ } \mu\text{gm.} \\ &&& \text{i.e. } 0.25 \text{ ml} = 50 \text{ } \mu\text{gm.} \end{aligned}$$

4.5.4 Dose calculation of Goghruta

$$\begin{aligned} 1. \text{ Human therapeutic dose} &= 40 \text{ ml} \times 0.0026 \text{ (Conversion factor for mice)} \\ &= 0.104 \text{ ml} \\ &= 3.46 \text{ ml / kg} \end{aligned}$$

Therefore, dose of ‘Goghruta’ = 3.46 ml / kg. **The dose of Goghruta in mice was 3.46ml / kg.**

4.5.5 Dose Calculation of PVASVS

1 ml of reconstitute PVASVS neutralizes 0.6 mg of Common cobra venom. For my animal experiment, i gave 25 μ gm of Common cobra venom. So the required dose of PVASVS was 0.0416 ml.

Dose of PVASVS for Common Cobra Group = 0.04 ml.

1 ml of reconstitute PVASVS neutralizes 0.6 mg of Russell’s viper venom. For my animal experiment, i gave 52 μ gm of Russell’s viper venom. So the required dose of PVASVS was 0.086 ml.

Dose of PVASVS for Russell’s Viper Group = 0.08 ml.

4.6 Animal Experiment Protocol

Animal experiment for efficacy of *Goghruta* as a first aid measure on Common cobra venom and Russell’s viper venom was carried out in **National Toxicology Center (NTC), Sinhgad Road, Pune** after the approval from IEAC. Following was the protocol used.

Animal species	Albino Mice
Strain	Swiss albino
Source of Animals	National Toxicology Center (NTC), Pune.
Avg. Wt. of mice	20 gm
No. of Animals	6 mice in each group
Age of Animals	6 – 8 wks
Sex of Animals	50 % male and 50 % Female in each group
Diet	Pelleted feed supplied by Nav Maharashtra Chakan oil mills ltd. Pune.
Water	Community tap water ad libitum
Room Temperature	20 – 24 ⁰ C
Relative Humidity	40 % to 60 %
Light Cycle	12 hrs light and 12 hrs dark
Vehicle used	Water
No. of groups.	8
Period of Acclimatization	7 days
Period of fasting	Overnight
Dosing	Snake venom was given by Intramuscular route, <i>Goghruta</i> was given by Oral route and PVASVS was given by Intravenous route.

4.6.1 Groups for Animal Experiments

Group I (Control Group)	Only Common cobra venom
Group II	Common cobra venom + <i>Goghruta</i>
Group III (Control Group)	Only Russell’s viper venom
Group IV	Russell’s viper venom + <i>Goghruta</i>
Group V (Standard Group)	Common cobra venom + PVASVS
Group VI	Common cobra venom + <i>Goghruta</i> + PVASVS
Group VII (Standard Group)	Russell’s viper venom + PVASVS
Group VIII	Russell’s viper venom + <i>Goghruta</i> + PVASVS

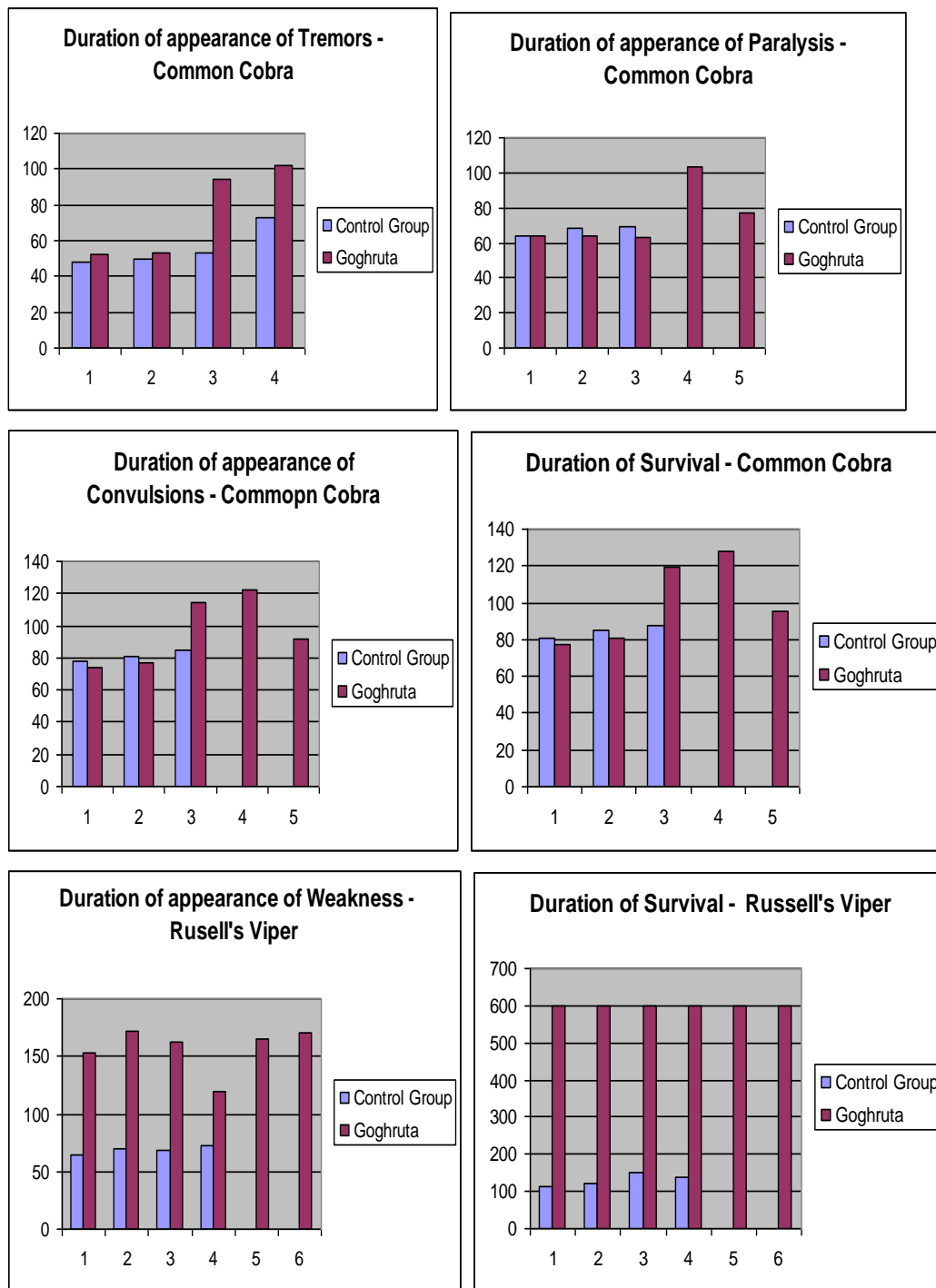
4.6.2 Method:

- Samples were freshly prepared for each group and then administered.
- Doses given to mice according to their body weight.
- After dosing all mice were observed for 24 hours for toxic signs and symptoms or mortality up to 7 days.

4.6.3 Procedure:

- First preliminary drug toxicity study was done.
- In each group, weight of mouse was taken first, accordingly adjusted venom dose was given by IM route, after 5 min, drug dose was given orally and then PVASVS was given after 5 min by IV route.
- After dosing mice were observed for 24 hrs up to 7 days / fatality.
- Comparative observations were tabulated.

5. GRAPHS OF IN-VIVO STUDY OBSERVATIONS



6. OBSERVATIONS WITH DISCUSSION –

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 e.g. tremors, paralysis, convulsion in Common cobra venom i.e. control group I were observed. As Russell’s viper

venom is haemotoxic, 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 II.

- In Common cobra control group (Gr. I) appearance of tremors was observed after 56 min (average) and that of drug group (Gr. II) was after 75 min (average) i.e. appearance of tremors was delayed by 33.92% in *Goghruta* group.
- In Common cobra control group (Gr. I) appearance of paralysis was observed after 67 min (average) and that of drug group (Gr. II) it was after 64 min (average) i.e. appearance of paralysis was not delayed by *Goghruta*.
- In Common cobra control group (Gr. I) appearance of convulsions was after 82 min (average) and that of in drug group (Gr. II) it was observed after 89 min (average) i.e. appearance of convulsions was delayed by 8.53% in *Goghruta* group.
- In Common cobra control group (Gr. I) average duration of survival was 85 min & all the six animals died within 1.5hr after the snake venom dosing. The histopathological reports of these animals showed congestion in liver and kidney.
- Five mice in the group treated with *Goghruta* (Gr. II) died within 2 to 2.5 hr. The died mice of this group showed paralytic symptoms. One mouse from this group survived for more than 5 hr.
- In Common cobra venom + PVASVS (standard group) one mouse died within 0.5 hr. This may be due to serum sickness reaction of PVASVS. Remaining five mice survived completely. Histopathological reports of these mice were **within normal limits**.
- In Common cobra venom + *Goghruta*+ PVASVS group (Gr. VI) all mice survived completely without showing any signs.
- In Russell’s viper venom group (Gr. III) average duration of survival period was 2 hr. All the mice of this group died within 2-2.5 hr. But three mice of drug group (Gr. IV) died within 4-5 hr. without any symptoms. Three mice from *Goghruta* group (Gr. IV) survived completely.
- In Russell’s viper venom + PVASVS group (Gr. VII), all mice survived without showing any symptoms.
- In Russell’s viper venom + *Goghruta* + PVASVS group (Gr. VIII), all mice survived without showing any symptoms. No adverse interaction between *Goghruta* and PVASVS was seen.

7. ANALYSIS RELATED DISCUSSION

The sample size of each group was small (6 in each group). As after the dosing of venom, the mice in control group died, while in *Goghruta* treated group four and three mice died respectively in Common cobra and Russell’s viper venom. Therefore, the big range of variation

was observed resulting in some of statistical tests significant and some non-significant or nearly significant. For the statistical analysis t-test was applied.

7.1 t-Test: Two-Sample Assuming Equal Variances – Goghruta

P(T<=t) one-tail , P(T<=t) two-tail

	P- Tests	Improvement
Duration of appearance of Tremors - Common Cobra	0.115382209 0.230764418	33.92%
Duration of appearance of Paralysis - Common Cobra	0.153265909 0.306531818	--- -
Duration of appearance of Convulsions - Common Cobra	0.153265909 0.306531818	8.53%
Survival Period - Common Cobra	0.081376792 0.162753583	17.64%
Duration of appearance of Weakness - Russell's Viper	1.2352E-05 2.4704E-05	127.53%
Survival Period - Russell's Viper	0.009118412 0.018236825	223%

8. SIGNIFICANT RESULTS RELATED DISCUSSION

The results of survival period in Russell’s viper venom group was proved to be statistically significant. P value is 0.01(one tail) and 0.02 (two tail).

Survival period in Common cobra venom group was nearly significant. P value is 0.08 (one tail). As we can see that all mice from control group died within observation period the survival of single mouse from drug group should be considered significant though it is not significant statistically at α level of 0.05.

Some results were statistically insignificant but they become significant if ‘ $\alpha=0.10$ ’ was taken. Considering the same in Common cobra venom group; appearance of tremors, appearance of paralysis, appearance of convulsions, and these results will become statistically significant. As we are dealing with highly toxic substance like cobra venom such relaxation of α value seems tolerable.

9. CONCLUSION

The Present study confirms the ‘*Vishaghna*’ property i.e. Antiophidian property of the *Goghruta*. *Goghruta* 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).

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