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IN VIVO STUDY OF EFFICACY OF *BAKUCHI (Cullen Corylifolia (L) Medik)BEEJCHURNA* IN SNAKE VENOM POISONING IN ALBINO MICE

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ABSTRACT

A majority of snakebites occur in rural parts of the country where good roads and motor vehicles are a rarity. Snakebite is still depending on the usage of antisera as the one and only source of treatment, which has its own limitations. Hence we have to increase the lifespan of patient till get serotherapy. This is done by proper first line of treatment in time that's only the purpose of the study. In *SushrutaSamhita, Kalpasthana*(chapter.5) *Eksaragana* is mentioned which include herbs effective in snake venom poisoning. Present study done by the use of *Bakuchi beej*. **Aim & Objectives** – 1.To study the efficacy of *'Bakuchi beej churna'* in Common Cobra & Russell's viper venom poisoning. 2. To study whether there is any interaction between *Bakuchi beej churna'* and Poly Valent Anti Snake Venom Serum (PVASVS). In Vivo study conducted on Cobra & Russell Viper venom in albino mice. Administration of both PVASVS & *Bakuchi beej churna* was done. Observations based on appearance of tremors, paralysis, convulsions, bleeding disorder & survival period in mice. Statistically significant results found in case of survival period in Common cobra venom group. P value – 0.0008 (one tail). In case of survival period in Russell's viper venom group, statistically insignificant results found. P value – 0.08 (one tail). **Conclusion**- It was found that there is no any interaction of *Bakuchi beej* with PVASVS. Also it delays symptoms caused due to Cobra venom poisoning & increases survival period of patient. `

Keywords: Snakebite, Cobra venom, Russell Viper venom, PVASVS, *Bakuchi beej*.

INTRODUCTION

The outcome of snake bites depends on numerous factors, including the species of snake, the area of the body bitten, the amount of venom injected, and the health conditions of the victim.[1] Although deaths are relatively rare in Australia, Europe and North America, the morbidity and mortality associated with snake bites is a serious public health problem in many regions of the world, particularly in rural areas lacking medical facilities.[2] This picture is more critical in India. A majority of snakebites occur in rural parts of the country where good roads and motor vehicles are a rarity. Transporting a snakebite victim in time to the nearest medical clinic or hospital is therefore difficult. Even though there are Govt. Primary Health Centers (PHCs) in many villages, most of them are under-staffed, & under-equipped. Antiserum is the only therapeutic agent available throughout the world.[3] Antiserum sometimes does not provide enough protection against venom- induced hemorrhage, necrosis, nephro toxicity and often produce hyper sensitive reactions.[4] Also Antisnake venom(ASV) is expensive, it is not easily available in Govt. hospitals, while at primary hospitals, many doctors avoid admitting snakebite cases due to fear of anaphy-

laxis. Herbal compounds that possess snake venom neutralization properties in experimental animal models (in vivo and in vitro) usually follows three protocols – 1) venom-herbal compounds mixed together, 2) herbal compounds followed by venom, and 3) venom followed by herbal compounds. Among these, the third technique is similar to clinical conditions. Such clinical condition develops in 'In vivo' study i.e. animal experiment. . For 'In vivo' study snake venom, experimental drug & PVASVS are given according to clinical scenario, so that actual situation of snake bite will be developed. In vivo studies allow looking at scientific & medical questions within the context of a living organism. I chose 'in vivo' study for proving efficacy of plant (herbal drug) in snake venom poisoning as a first line of treatment. In India snake bite cases of Common cobra & Russell's viper are most common. That's why I decided to prove antiophidian property of drug on Common cobra and Russell's viper as venoms of these snakes are available at Haffkin's institute, Mumbai. The advantages of herbal compounds are that, they are cheap, easily available, and stable at room temperature and could neutralize a wide range of venom antigen. In Ayurveda texts, 96 antiophidian formulations are mentioned which are useful against snake bite, Especially in *Sushrutasamhita, Kalpasthana 'Eksaragana'* is mentioned which contains drugs effective snake bite.[5] I selected **'*Somrajiphala*' i.e. *Bakuchi beej*** which is the starting drug of this *gana*, also in other texts *Bakuchi* is mentioned as antiophidian. This review is an attempt to focus on treatment of snake bite and use of drug like *Bakuchi* (***Cullen corylifolia (L)Medik.)*** *beejchurna* which can increase lifespan & health of patient in several pathophysiologiocal conditions including snake bite in the near future.

MATERIALS & METHODOLOGY:

❖ **PHASE I**

A) **Venom: Collection of cobra venom & Russell's viper venom:** The Lyophilized snake venom collected from Haffkin's Institute, Mumbai.

Dose calculation of venom: Cobra Venom dose: Fatal period of cobra was short duration so we took 80 % of the total fatal dose to observe the effect of venom for longer time. Fatal dose of cobra venom = 31.2 µ gm. ∴ 80 % dose of cobra venom = 24.96 µ gm.

Russell's viper venom dose: According to previous study done in Dept. of Agadtantra in Tilak Ayurved Mahavidyalaya, Pune, 120% fatal dose of Russell's viper venom should be injected[6]

Fatal dose of Russell's viper venom = 52 µ gm. ∴ 120 % dose of Russell's viper venom = 62.4µ gm.

Final Dose confirmation of venom:

After the advice & also suggestions of my guide & NTC expert, to confirm the dosage, it was decided to conduct pilot studies which will experimentally confirm the dosage. As earlier mentioned, we had chosen albino mice for the experiment. I performed the pilot study using two mice per dosage group. Our seniors who worked on same protocol, they confirmed fatal dose of 60 µgm in Common cobra group & 750 µgm in Russell's viper venom group in their studies. After reading these doses; I decided to start with this dosage in pilot study.

6 mice were taken for pilot study in Common cobra group. In first step, 2 mice were given 60 µgm, other 2 mice 90 µgm and remaining 2 mice were given 120 µgm of Common cobra venom. All mice were observe for signs & symptoms &

survival period. Among them, 50% mice died in 60 µgm with long survival period. 100% mortality was observed in 90µgm with long survival period but this is not significant for our study. In last dose i.e. in 120 µgm, 100% mortality found but there was significant survival period, which I wanted for our study;

Thus I confirmed the dose of 120 µgm Common cobra venom as a fatal dose. Same as done before, I chose 6 mice for pilot study in Russell's viper venom group. Now 2 mice were given 750 µgm, other 2 mice 800 µgm and remaining 2 mice were given 900 µgm of Russell's viper venom. I observed for survival period. At this stage, 50% mice died who were given 750 µgm and 800 µgm viper venom. Mice who were given 900 µgm viper venom in that 100% mortality was seen but with significant survival period which I wanted for our study. Thus I confirmed the 900 µgm dose of Russell's viper venom as a fatal dose.

B) Drug:

1) **Preparation of drug:** The seed of *BAKUCHI (Cullen corylifolia (L)Medik.)* in *CHURNA*(80 No. mesh) form. Suspention of *churna* made in water.

Standardization of drug: It was done at TilakAyurvedMahavidyalaya, Pune.

As per API Guideline.[7]

1) Description - Small, Color - Blackish, Odor - Characteristic, Taste - Characteristic

2) Foreign Matter – 1.5 %

3) Total Ash – 5.42 %

4) Acid insoluble ash – 1.2 %

5) Alcohol soluble extract – 21.84 %

6) Water soluble extract – 21.04 %

3) dose calculation of *Bakuchi beej (cullen corylifolia (l) medik.*) *Churna*

Dose of *CHURNA* in *Śhaarangadharasamhitaa* was given as 1 *karsha* i.e. 10 gm[8]

Human dose of *BAKUCHI BEEJ CHURNA* = 10 gm. But I found dose of *Bakuchi beej* in literature review = 1-3 gm So I had taken dose of *churna* as 1 gm in human.

According to conversion factor of mice (0.0026)[9],

Dose was 0.0026gm i.e.2.6mg for 20 gm mice. i.e. 130 mg/kg of mice.

Required dilution of drug is 13mg/ml.

C) Poly Valent Anti Snake Venom Serum (PVASVS)

Lyophilized PVASVS obtained from Halfkin's Institute, Mumbai.

D) Statistical test used in study:

T- Test. PHASE II - EXPERIMENTAL STUDY. A) IN VIVO EFFICACY OF *BAKUCHI BEEJ*:-

Animal Species used	**Albino-mice**
Place of Experiment	National Toxicology Centre, Pune.
Source of Animals	National Toxicology Centre, Pune.
Sex of Animals	50 % males and 50 % females in each group will be taken.
Avg. wt of Animals	20 gms.
No. of Animals	6 mice for each group
No. of Groups	8
Period of Acclimatization	7 days
Period of Fasting	Overnight
Feeding	Wheat bran and water ad libitum
Dosing	Snake venom by intramuscular route. PVASVS by IV, *Cullen corylifolia (L)Medik.BEEJ CHURNA* by oral route.

Note: Permission from National Toxicology Center (NTC) & approval of Institutional Animal Ethical Committee was procured before initiation of study.

Group I.	**Only common cobra venom**
Group II.	Common cobra Venom + '*Cullen corylifolia(L)Medik.BEEJ CHURNA*
Group III.	Only Russell's viper Venom
Group IV.	Russell's Viper Venom + .*Cullen corylifolia (L)Medik. BEEJ CHURNA*
Group V.	Common Cobra Venom + 'PVASVS'.
Group VI.	Common Cobra Venom + *Cullen corylifolia(L)Medik.BEEJ CHURNA*+ PVASVS.
Group VII.	Russell's Viper Venom + PVASVS.
Group VIII.	Russell's Viper Venom + *Cullen corylifoli (L)Medik.aBEEJ CHURNA* + PVASVS.

Group II & Group IV – Experimental Group.

Group I & Group III – Control Group.

Group V, VI, VII & VIII- Standard Group[10]

OBSERVATIONS:

Process of observations:

After dosing each group on day one on male mice & on next, on female mice, that made easier to observe the signs & symptoms & also to record time of each symptom and death. In Common cobra venom group i.e. control group-I, paralytic signs like tremors, paralysis, convulsions were observed. Russell's viper venom is haemotolxic but there were no signs of external bleeding from mouth, nose, ear and necrosis at injection site in Russell's viper venom group i.e. control group II, which are the symptoms of Russell's viper bite in humans.

- **Appearance of tremors: –**
  - In Common cobra control group (Gr.I) : after 42 min (average)
  - In Drug (*Bakuchi beej churna*) group (Gr.II) : after 55 min (average)

  In *Bakuchi beej churna* group, appearance of tremors delayed by 13 min – Statistically significant. P value ≤ 0.02.
- **Appearance of paralysis :**
  - In Common cobra control group (Gr.I) – after 68 min (average)
  - In Drug (*Bakuchi beej churna*) group (Gr.II) – after 90 min (average)

  In *Bakuchi beej churna* group, appearance of paralysis delayed by 22 min – Statistically significant. P value ≤ 0.01.
- **Appearance of convulsions :**
  - In Common cobra control group (Gr.I) – after 84 min (average)
  - In Drug (*Bakuchi beej churna*) group (Gr.II) – after 114 min (average)

  In *Bakuchi beej churna* group, appearance of convulsions delayed by 29 min – Statistically significant. P value ≤ 0.006.
- **Duration of survival :**
  - In Common cobra control group (Gr.I) – 94 min (average)
  - In Drug (*Bakuchi beej churna*) group (Gr.II) – 144 min (average)

  In *Bakuchi beej churna* group, duration of survival increased by 50 min – Statistically very highly significant. P value ≤ 0.0008.
- **In standard group (Common cobra venom + PVASVS)** : whatever mice we used that all completely survived & didn't show any signs.
- **In Gr.VI (Common cobra venom+ *Bakuchi beej churna*+ PVASVS) :** without showing any signs all mice we used completely survived. After studying

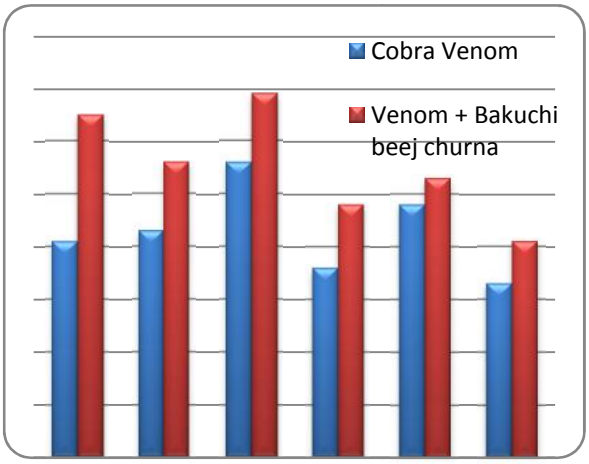
Common cobra venom group, I found significant results. Then I started Russell's viper venom group. First I did experiment with Russell's viper venom on male albino mice but I found insignificant results in that as follows:

- **Duration of survival :**
  - In Russell's viper venom group (Gr.III) – 46 min (average)
  - In Drug (*Bakuchi beej churna*) group (Gr.II) – 59 min (average)

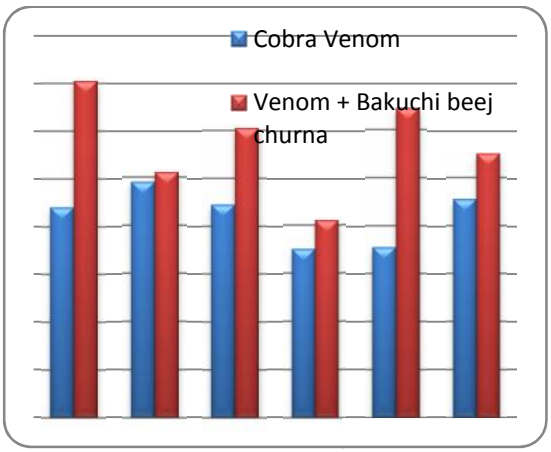
  In *Bakuchi beej churna* group, duration of survival increased by 13 min – Statistically insignificant. P value > 0.05 (P = 0.08).

  As I found insignificant results in male mice, NTC experts in consultation with Animal ethical committee advised not to perform same study in female mice. That's why I did not perform the study in female albino mice.
- **In Gr.VII- standard group (Russell's viper venom + PVASVS) :** Two mice completely survived but there may be serum sickness reaction of PVASVS in remaining one mouse, due to which that mouse died after 20 min.
- **In Gr.VIII (Russell's viper venom + *Bakuchi beej churna* + PVASVS):** There was no any adverse interaction between *Bakuchi beej churna* and PVASVS. So, whatever mice we used survived completely and didn't show any signs.

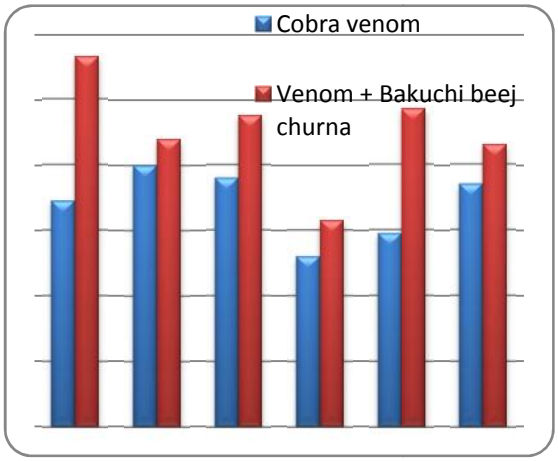
Graphs of Observations:



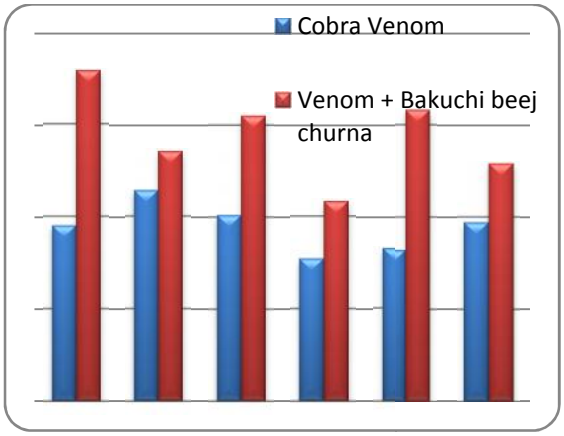
GRAPH 1:Appearance of Tremors



GRAPH 2:Appearance of Convulsions



GRAPH 3: Appearance of Paralysis



GRAPH 4: Survival Period (Cobra venom group)

Statistically significant results found in case of survival period in Common cobra venom group. P value – 0.0008 (one tail). In case of survival period in Russell's viper venom group, statistically insignificant results found. P value – 0.08 (one tail).

RESULT:

The present study proves the efficacy of *Bakuchi beej churna* in Common cobra venom as a first aid measure.*Bakuchi beej churna* neutralizes venom without any adverse interaction with Poly Valent Anti Snake Venom Serum (PVASVS).

Due to its action on Common cobra venom, it delays onset of symptoms:

- Appearance of tremors delayed by 13 min. P value (one tail) = 0.02
- Appearance of paralysis delayed by 22 min. P value (one tail) = 0.01
- Appearance of convulsions delayed by 29 min. P value (one tail) = 0.006

Thus *Bakuchi beej churna* increases survival period in Common cobra by 50 min.

P value (one tail) = 0.0008 (Statistically significant)

In case of Russell viper venom it shows insignificant results.

The null hypothesis is rejected and the hypothesis that *Bakuchi beej churna* is efficient as a first aid measure in Common cobra is accepted.

DISCUSSION

This study proves the efficacy of *Bakuchi* (antiophidian drug mentioned in literature) as a first line of treatment. For this particular protocol study was conducted 'in vivo' or in animals. 'In vivo' studies allow scientists to look at scientific & medical questions within the context of a living organism. In the study of snake venom with 'in vitro' studies, whatever drug we are screening it interacts chemically with the components present in venom either neutralizes it or after binding with components make them pharmacodynamically inactive. But if any drug acts by making modifications in immune response or changing physical properties, then that drug screening is not possible with 'in vitro' studies. Also it is not possible in case of drugs act by blocking receptor sites. In this situation screening of drug becomes effective if we create clinical scenario which is possible with 'in vivo' studies. For 'in vivo' study, snakevenom, experimental drug & PVASVS are given according to clinical scenario, so that actual situation will be developed. Thus for the screening of antiophidian drug, 'in vivo' study plays an important role.

In this study, lyophilized form of venom was taken and dilution made with distilled water. Doses of venom confirmed as Commom Cobra 120 µgm& Russell Viper venom 900 µgm after doing pilot studies. Study performed making 8 groups & using albino mice. After experiment it shows that appearance of tremors, convulsions, paralysis delayed with significant survival period in Common cobra venom by the use of *Bakuchi beej churna*.

Statistically significant results found in case of survival period in Common cobra venom group. **P value – 0.0008 (one tail).** In case of survival period in Russell's viper venom group, statistically insignificant results found. **P value – 0.08 (one tail).** Thus the study shows that *Bakuchi beej churna* increases survival period in common cobra venom poisoning. *Bakuchi* contains Psoralen ,psoralidin, isopsoralen and *Bakuchi*ol as active ingredients, hence single or all ingredients may responsible for anti cobra venom property of *Bakuchi*. Study of components present in *Bakuchi beej* and snake venom should be studied by HPTLC so that we will come

to know which components neutralization take place. This can be done by 'Pre-incubation' study and has wide scope for research.

CONCLUSION

Bakuchi beej churna delays the duration of appearance of symptoms and it increases the duration of survival period i.e. life span is increased till patient gets serotherapy. This experiment also shows that there is no any interaction of *Bakuchi beej churna* with PVASVS. In case of Russell viper venom it shows insignificant results. Thus null hypothesis is rejected & the hypothesis, *Bakuchi beej churna* is efficient as a first aid measure in Common cobra poisoning is accepted. It is our responsibility to identify, cultivate and culture these eco-friendly herbs for the alleviation of human suffering and death against snake bite.

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