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IN VIVO STUDY OF EFFICACY OF VARUNA (Crataeva nurvala Buch-ham) TWAK CHURNA IN SNAKE VENOM POISONING IN ALBINO MICE

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ABSTRACT

As snakebite is, threaten emergency, which required treatment within a time. Mortality among rural population is high due deficient treatment at that level. Therefore, increase in survival period with proper first aid measures before serotherapy is essential. I have chosen *Varuna* (Crataeva nurvala) for study, as Sushruta mentioned in *kalpasthana* (chapter 5) about its antiophidian property. In vivo study is carried out at NTC, Pune. After dosing the albino mice with Cobra & Russel viper venom, they were observed for paralytic signs for 24 hours & mortality up to 7 days in cobra venom group & mice from Russell's viper venom were observed for signs like external bleeding from natural openings, local signs & survival period. Observations were tabulated. Data is analyzed with the help of descriptive statistics along with unpaired't - Test' for statistical significance at alpha value of 0.05. Standard groups show there is no interaction between PVASVS & *Varun twaka* (Crataeva nurvala bark) *churna*.Conclusion is drawn based on analyzed data. *Varuna* (Crataeva nurvala) *twaka churna* is effective against common cobra venom poisoning as it delays the onset of signs & increases the survival period, P value 0.002 (one tailed) very highly significant. *Varuna twaka churna* does not interact with PVASVS (Polyvalent Antisnake Venom).

Keywords: Varuna twaka churna, PVASVS, cobra, Russell's viper

INTRODUCTION

In Asia, it has been estimated that a million snakebites occur each year, of which approximately 50% are envenomed, resulting in 1, 00,000 annual deaths (1). World Health organization has declared about 2, 00,000 snakebite cases occurred in India per year, among them 35,000-50,000 cases become the victims of snakebite. In India, Maharashtra, Tamil Nadu, west Bengal, Uttar Pradesh, Assam, Andhra Pradesh & Kerala shows high incidences of snakebites. Among them Maharashtra is on the top level by showing 70 snakebites per lakh population & more than 2000 deaths per annum(2). Nowadays, only ASV is an available tool to combat snake venom poisoning.

Snake venom-neutralizing effects of different herbs and herbal constituents have studied in India & in other countries (3). Many Indian medicinal plants are mentioned

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for their Antiophidian property in ayurvedic samhita , which are commonly used by vaidya's in south India still today, to treat majority of snake bite cases(4) . We can use those drugs with first aid to reduce the effect of venom if they are proved efficient by scientific study.

I have chosen *Varuna* (Crataeva nurvula Buch.-Ham.) from *Eksara gana* mentioned in Sushruta samhita *kalpasthana* to study its efficacy in Common cobra and Russell's viper venom poisoning in albino mice by in vivo study(5). *Eksara gana* is mentioned in Sarpadashtavishchikitstitkalpadhyay /kalpasthan of sushrut samhitaa. This *gana* contains group of drugs which in combination or individually effective against snake venom.

In this study, survival period with paralytic signs like tremors, paralysis and convulsion were observed for common cobra venom poisoning & survival period with signs like external bleeding, necrosis were observed for Russell's viper venom poisoning. Any interaction between ASV and drug also checked along with efficacy of drug.

Setting Hypothesis –

Null Hypothesis (H_0) –There is no significant difference in the survival period before & after administration of *Varuna twaka churna.*

Alternative Hypothesis (H_1) - There is significant increase in the survival period after administration of a *Varuna twaka churna*.

Level of significance – I selected level of significance 5% i.e. $P<0.05$.

Test criteria –

Unpaired't-test' is applied as data is quantitative.

Unpaired 't- Test' is applied to the related groups observations. All results were analyzed for statistical significance at alpha value 0.05.

Materials and Methodology:

Procurement of *Varuna twaka* (Bark of crataeva nurvala):

Bark of Crataeva nurvala (raw material) procured from karve road, Pune.

Authentication:

Authentication of drug has done at National Research Institute of Basic Ayurvedic science, Nehru Garden, Kothrud, Pune-38.

Standardization:

This study was conducted at Laboratory of Centre for Post Graduate Studies and Research in Ayurveda (CPGS&RA), Tilak Ayurved Mahavidyalaya, Pune.

Standardization report of Varuna twaka churna:

Organoleptic Test –

Color- Outer surface-

Grayish brown

Inner surface- Cream white

Odor - Indistinct
Taste - Slightly bitter

1) **Foreign matter** - Nil
2) **Total ash** - 8.54%
3) **Acid insoluble ash** - 0.27%
4) **Alcohol soluble extractive** - 2.47%
5) **Water soluble extractive** - 10 %
6) **Moisture - 5.2%**
7) **TLC Studies** - Four Spots were observed.

Preparation of fine *Varuna twaka churna* for experiment:

As per reference about preparation of *churna* in Sharangadhar Samhitaa, *varuna twaka churna* was prepared (6).

30 gm of dried *Varuna twaka* was taken from authenticated standardized sample & broken into small pieces. It grounded into fine powder. It is sieved through 100 no. mesh.

Procurement of cobra venom & Russell's viper venom:

Common cobra venom (100 mg) and Russell's viper venom (110 mg) in dried lyophilized form procured from Haffkine's Institute for Training Research and Testing, Mumbai.

1) Common cobra venom - Vial No.702 B - 0.10gm - Sealed 2001
2) Russell's viper venom - Vial No.794 B - 0.11gm - Sealed 1997

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

PVASVS was procured from National Medical Store, Parel, Mumbai.

Calculation of dosage:

A) **Dose calculation of Venom:**

According to OECD guideline No.425, LD50 of common cobra venom & LD50 of Russell's viper venom is 0.45mg/kg & 0.75 mg/kg respectively (7). Therefore, dose of common cobra venom is 9µgm/20 gm body wt of mouse & that of the Russell's viper venom is 15µgm/20 gm body wt of mouse.

Pilot study for common cobra venom dose –

We took 6 mice for the study and were divided into three groups each containing 2 mice. For first group 60µgm venom was injected. For second & third group 90µgm & 120 µgm of venom are injected respectively. These groups are properly observed. 50% of mice died in first group with long survival period. 100% mortality occurred with long survival period is found. We confirmed 120µgm dose for common cobra venom according to pilot study.

Pilot study for Russell's viper venom dose –

We divided 6 mice into 3 groups. In first group, 750µgm of venom is injected to each mouse. For the second & third group 800µgm & 900µgm venom is injected respectively to each mouse. We observed them for survival period. In first & second group 50% mortality occurred in significant survival period & 50% occurred after long survival period. In third group 100% mortality is seen with significant survival period. Therefore, we confirmed 900µgm Russell's viper venom dose.

Dilution procedure for venom:

Distilled water is used for the dilution of the venom.

1) Dilution of common cobra venom –

First dilution = 5 ml distilled water was added in a 100 mg cobra venom vial.

100 mg venom in 5 ml i.e. 20 mg or 20,000 µgm in 1 ml.

For easy & accurate calculation of dose, required dilution is 60µgm/0.1ml. For which 0.3 ml of first dilution is taken and diluted with 10 ml of distilled water in glass bulb.

0.2 ml of this preparation is used to inject in mouse of 20gms for envenomation.

2) Dilution of Russell's viper venom –

First dilution = 5.5 ml distilled water added in 110 mg Russell's viper venom vial

110 mg venom in 5.5 ml i.e. 20 mg or 20,000 µgm in 1 ml

Required dilution is 750µgm/0.1 ml. 0.5ml of first dilution is diluted with 1.33 ml of distilled water.

I used 0.12 ml of this preparation to induced poisoning in albino mice of 20 gms & like that doses were calculated accordingly weights of mice.

B) Dose calculation of PVASVS:

1) For common cobra venom:

1 ml of PVASVS neutralizes 0.6 mg of common Cobra venom. In experiment, we used 120 µgm of Common cobra venom to induced poisoning in mice; therefore required PVASVS dose was 0.2 ml. This dose was found to be insufficient. After pilot study, the dose of PVASVS is decided o.5 ml to each mouse.

Dose of PVASVS for Common cobra Group = 0.5 ml

2) For Russell's viper venom:

1 ml of PVASVS neutralizes 0.6 mg of Russell's viper venom.

I gave 900 µgm of Russell's viper venom to mice to induced poisoning in them. So dose of PVASVS was become 1.5 ml/mouse of 20gm, which was more than sufficient. After pilot study, the dose of PVASVS is decided 0.6ml/per 20 gm mouse.

Dose of PVASVS for Russell's viper Group = 0.6 ml

After pilot study, dose of PVASVS for Common cobra Group is 0.5 ml & that of the Russell's viper Group is 0.6 ml.

C) Dose calculation of *Varuna twaka churna*:

Surface area ratio of some common laboratory species and man:

Table-1

	20 gm mouse	**200 Gm Rat**	**400gm Guinea Pig**	**1.5kg Rabbit**	**2 kg Cat**	**4 kg Monkey**	**12kg Dog**	**70kg Man**
20 gm Mouse	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
200gm RAT	0.14	1.0	1.74	3.9	4.2	4.2	4.2	56.0
400gm Guinea Pig	0.08	0.57	1.0	2.25	2.4	5.2	10.2	31.5
1.5 kg Rabbit	0.04	0.25	0.44	1.0	1.08	2.4	4.5	14.2
2 kg Cat	0.03	0.23	0.41	0.92	1.0	2.2	4.1	13.0
4 kg. Monkey	0.016	0.11	0.19	0.42	0.45	1.0	1.9	6.1
12 kg. Dog	0.08	0.06	0.10	0.22	0.24	0.52	1.0	3.1
70 Kg. Man	**0.0026**	0.018	0.031	0.07	0.076	0.16	0.32	1.0

Conversion factor from man to mice is 0.0026. So according to this drug (*Varuna twaka churna*) dose calculated.

As per Śhaarangadhar samhitaa dose of the *churna* is 1 *Karsha* i.e. 12 gm. However, this dose is according to the physical status

of the human body at that time. Nowadays this dose is found to be more than requirement. By studying literature, we come to conclude that 1-4 gm dose *of varuna twaka churna* is sufficient for human being.

LD50 of Crataeva nurvala stem bark:

- The LD50 of 50% ethanolic extract of stem bark was found to be more than 1000mg/kg administered IP (intraperitonial) to adult rats.

Conversion factor for mice is 0.0026,

Dose of *VARUNA TWAK CHURNA* in mice = 1 x 0.0026 gms = 0.0026 gms. i.e. 2.6 mg for 20 gm of mouse and 130mg/kg of mouse

Required dilution of drug is 13 mg/ml. Therefore, 130 mg of drug was diluted by 10 ml of distilled water. This mixture stirred for 15 min and uniform suspension is made. 0.2 ml of this solution was given orally to the mouse of 20 gms and other mice accordingly their weights.

Animal Experiment

Animal experiment conducted at National Toxicology Center (NTC), Pune.

We have selected 'in vivo' study for the experiment, because it is short term acute study. Acute study in animals can determine toxicity, time of onset of toxic signs-symptoms and effect of screening drug on them. Occasionally, acute toxicity studies are used to establish antidotes to a given toxicant (8).

While in vitro test, the mechanisms like homeostatic and pathways found in animals are not present. The neutralization action of drug on venom other than chemical neutralization like modification in immune response or changes in physical properties cannot be studied by in vitro test (9). Thus, the creation of completely clinical scenario of poisoning in living organism & screening the drug with effective criteria is possible only with in vivo study.

Albino mice (Mus musculus) chosen for the experiment as they are Small, cheap, easy to handle, sensitive to small dose, genetic similarity to humans (at least 80% of DNA in mice is identical to that of humans).

Information about animal used for experiment: Table: 2

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 was 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	Standard pelleted diet
Water	Community tap water ad libitum
Temperature	20˚-24˚C
Humidity	40%-60%

Vehicle	Water
Dosing	Snake venom was given by intramuscular route, after 5 mins Varuna (Crataeva nurvala.) twaka churna was given by oral route. After 5 mins PVASVS was given intravascular.

Groups of animals for experiment: Table: 3

Group I.	Only common cobra venom
Group II.	Common cobra Venom + *VARUNA(*Crataeva nurvala.) *TWAK CHURNA.*
Group III.	Only Russell's viper Venom
Group IV.	Russell's Viper Venom + *VARUNA*(Crataeva nurvala.) *TWAK CHURNA*
Group V.	Common Cobra Venom + 'PVASVS'.
Group VI.	Common Cobra Venom + *VARUNA*(Crataeva nurvala.) *TWAK CHURNA*+ PVASVS.
Group VII	Russell's Viper Venom + PVASVS.
Group VIII	Russell's Viper Venom + *VARUNA(Crataeva nurvala.) TWAK CHURNA* + PVASVS.

(Ref: Experimental Pharmacology by Ghosh)

- Group II & Group IV – Experimental Groups.
- Group I & Group III – Control Groups.
- Group V, VI, VII & VIII – Standard Groups.

Procedure:

1. Every animal of group is marked with identification mark.
2. Each animal is weighted with digital weight machine.
3. Effective doses of venom & PVASVS were estimated by preliminary study.
4. Distilled water is used to made suspension of *Varuna twaka churna*.
5. Dose of the venom, *Varuna twaka churna* suspension, PVASVS was calculated according to weight of each animal.
6. According to group venom, *Varuna twaka churna* suspension, PVASVS was administered.
7. Venom is injected intramuscularly, *Varuna twaka churna* suspension is given orally & PVASVA is administered intravenously as per group.
8. Time interval between the administration of venom, drug suspension & PVASVS was kept 5 minutes.
9. All mice observed for the signs & symptoms like tremors, paralysis & convulsions for 24 hours.

10) Survival period of each mouse was observed up to the 7 days.

11) All observations of each group tabulated & compared within respective groups.

Observations:

It was found difficult to observe 12 mice from control group & experimental group or 12 mice of comparative standards groups at a time. To avoid this difficulty, we conducted experiment on male mice on one day & on female mice on next day. First common cobra venom was given to screened efficacy of *varuna.* In these groups, paralytic signs like tremors, paralysis, and convulsion were observed.

Observations were tabulated. Data is analyzed with the help of descriptive statistics along with unpaired't- Test' for statistical significance at alpha value of 0.05.

Tables and Graphs of observations: Table: 4

t-Test: Two-Sample Assuming Equal Variances		
Duration of tremors	**Common cobra venom Gr. I**	**Varuna twaka Churna Gr.II**
Mean	42.8333	57.3333
Variance	69.3666	58.2666
Observations	6	6
Pooled Variance	63.81666	
Hypothesized Mean Difference	0	
Df	10	
t Stat	-3.14384	
P(T<=t) one-tail	**0.0052**	
t Critical one-tail	1.81246	
P(T<=t) two-tail	0.01044	
t Critical two-tail	2.22813	

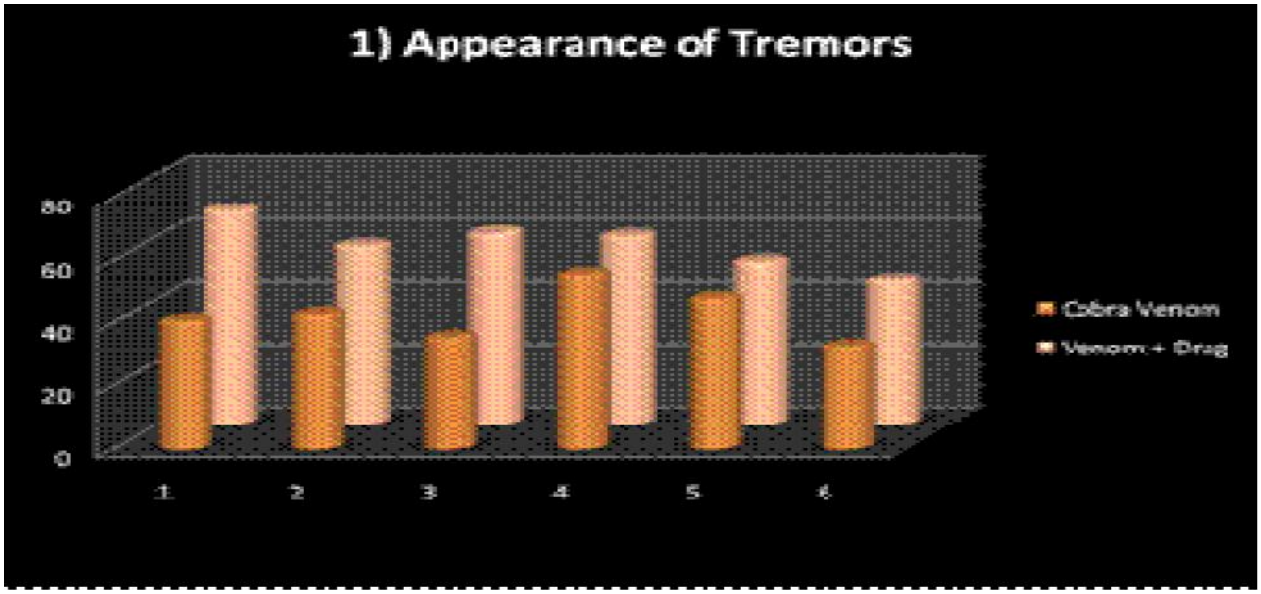

Table: 5

t-Test: Two-Sample Assuming Equal Variances		
Duration of paralysis	**Common cobra venom Gr. I**	**Varuna twaka Churna Gr.II**
Mean	68.3333	87.8333
Variance	116.2666	154.9666
Observations	6	6

Pooled Variance	135.6166	
Hypothesized Mean Difference	0	
Df	10	
t Stat	-2.90027	
P(T<=t) one-tail	**0.00791**	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.015826	
t Critical two-tail	2.228138	

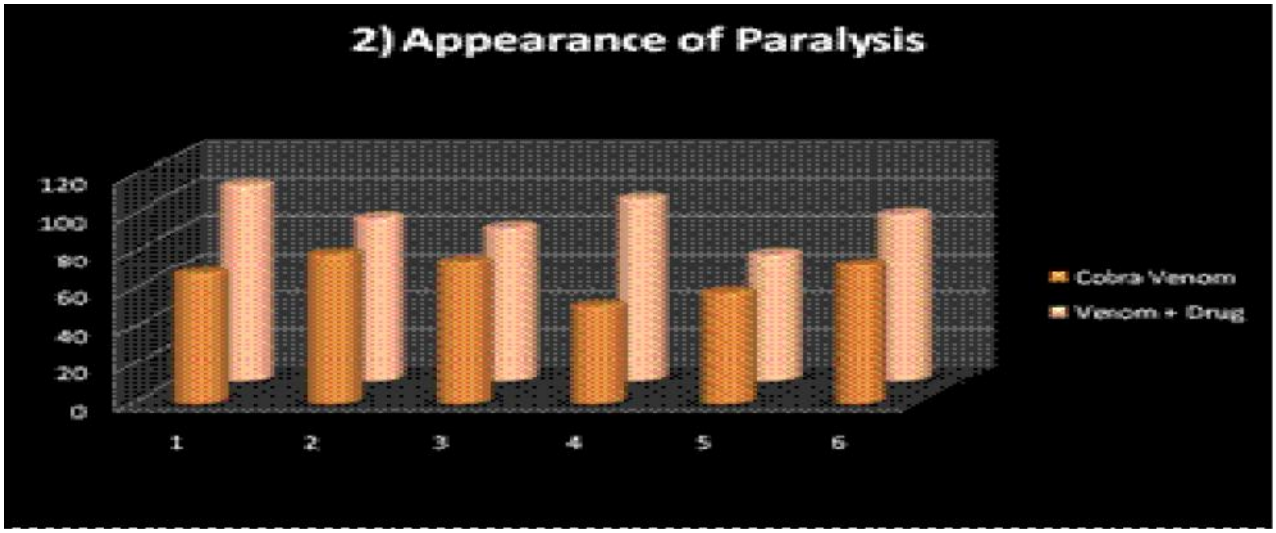

Table: 6

t-Test: Two-Sample Assuming Equal Variances		
Duration of convulsion	**Common cobra venom Gr. I**	**Varuna twaka Churna Gr.II**
Mean	84.5	109.1666
Variance	129.9	209.7666
Observations	6	6
Pooled Variance	169.8333	
Hypothesized Mean Difference	0	
Df	10	
t Stat	-3.2783	
P(T<=t) one-tail	**0.00415**	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.008311	
t Critical two-tail	2.228138	

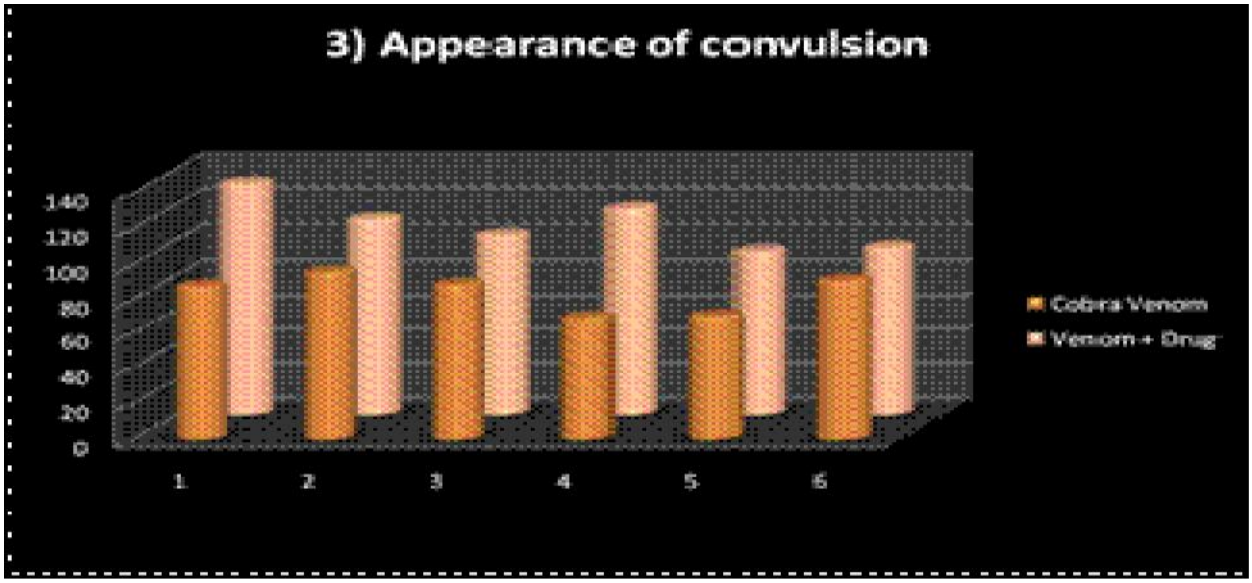

Table: 7

t-Test: Two-Sample Assuming Equal Variances		
Survival Period	**Russell's Viper Venom Gr. III**	**Varuna twaka churna Gr. IV**
Mean	46.3333	46.6666
Variance	60.3333	212.3333
Observations	3	3
Pooled Variance	136.3333	
Hypothesized Mean Difference	0.05	
Df	4	
t Stat	-0.04020	
P(T<=t) one-tail	**0.48492**	
t Critical one-tail	2.13184	
P(T<=t) two-tail	0.969853548	
t Critical two-tail	2.776445105	

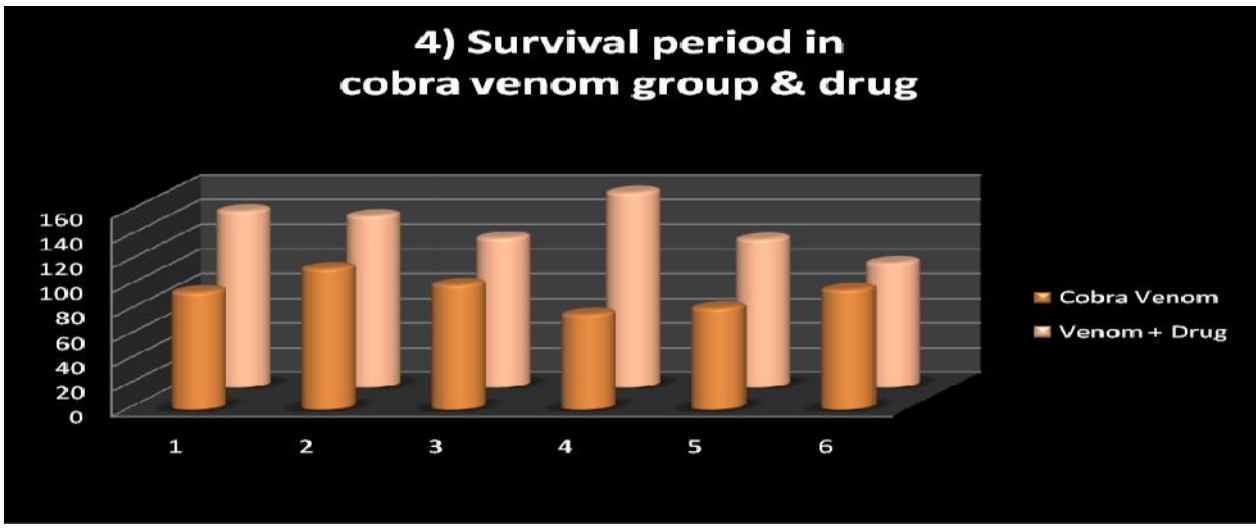

Table: 8

t-Test: Two-Sample Assuming Equal Variances		
Survival period	**Common cobra venom Gr. I**	**Varuna twaka Churna Gr.II**
Mean	94.3333	130.3333
Variance	178.2666	411.0666
Observations	6	6
Pooled Variance	294.6666	
Hypothesized Mean Difference	0.05	
t Stat	-3.6374	
P(T<=t) one-tail	**0.0022**	
t Critical one-tail	1.8124	
P(T<=t) two-tail	0.0045	
t Critical two-tail	2.2281	

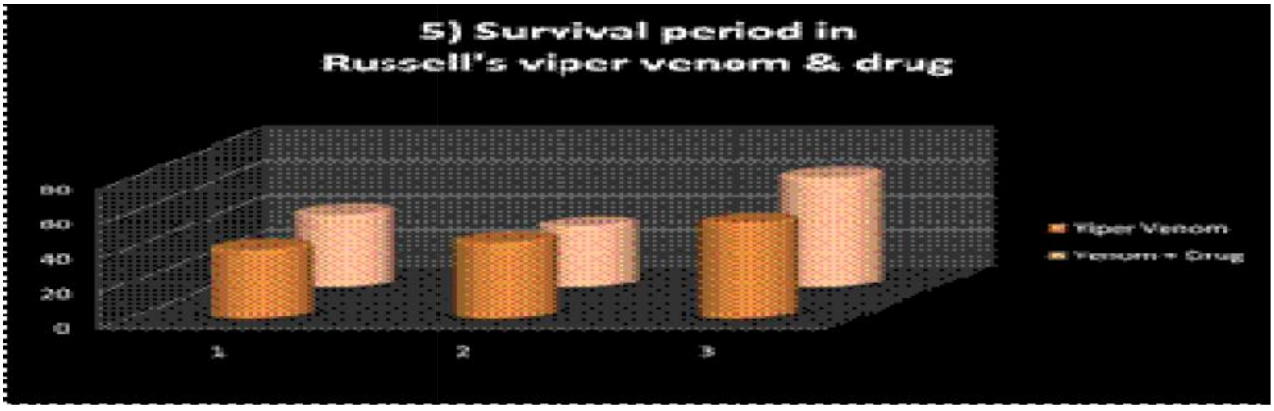

Table: 9

Onset of tremors delayed by (Gr.II)	14.5 minutes	P value 0.005 (one tailed)
Onset of paralysis delayed by (Gr.II)	19.5 minutes	P value 0.007 (one tailed)
Onset of convulsion delayed by (Gr.II)	24.66 minutes	P value 0.004 (one tailed)
Survival period is increased by (Gr.II)	36 minutes	**P value 0.002 (one tailed)**
Survival period is increased by (Gr.IV)	o.33 minutes	P value 0.48 (one tailed)
Onset of tremors delayed by (Gr.II)	14.5 minutes	P value 0.005 (one tailed)
Onset of paralysis delayed by (Gr.II)	19.5 minutes	P value 0.007 (one tailed)
Onset of convulsion delayed by (Gr.II)	24.66 minutes	P value 0.004 (one tailed)
Survival period is increased by (Gr.II)	36 minutes	**P value 0.002 (one tailed)**
Survival period is increased by (Gr.IV)	0.33 minutes	P value 0.48 (one tailed)

DISCUSSION

I have selected *Varuna* (Crataeva nurvula) for the study as its antiophidian property is mentioned in *sushrut samhitaa* & due to its easy availability. We have selected 'in vivo' study for the experiment. Because short term, acute study is required for this experiment. Acute study in animals can determine toxicity, time of onset of toxic signs-symptoms and effect of screening drug on them. Occasionally, acute toxicity studies are used to establish antidotes to a given toxicant.

First, I screened efficacy of *Varuna* in common cobra venom groups. In these groups, paralytic signs like tremors, paralysis, and convulsion were observed.

1) Appearance of tremors –

Group I (cobra venom) – after 42.83 min

Group II (cobra venom + drug) - after 57.33 min

Group II, appearance of tremors delayed by 14.5 minutes, P value 0.005 (one tailed), highly significant.

2) Appearance of paralysis –

Group I (cobra venom) – after 68.33 min

Group II (cobra venom + drug) - after 87.83min

Group II, appearance of paralysis delayed by 19.5 minutes, P value (one tailed) 0.007 (one tailed), highly significant.

3) Appearance of convulsion –

Group I (cobra venom) – after 84.5min

Group II (cobra venom + drug) - after 109.16min

Group II, appearance of convulsion delayed by 24.66 minutes, P value 0.004 (one tailed), highly significant.

4) Survival period –

Group I (cobra venom) – 94.33min

Group II (cobra venom + drug) - 130.33 min

Group II, survival period increased by 36 minutes, P value 0.002 (one tailed), highly significant.

5) Standard groups -

In standard group V (common cobra venom & PVASVS) – all mice survived without any toxic signs. In standard group VI (com-

mon cobra venom + *Varuna twaka churna* + PVASVS) – all mice survived without any toxic signs. This study shows efficacy of *Varuna twaka churna* in common cobra venom poisoning & there is no interaction between ASV & *varune twaka churna*.

Then experiment was conducted on male mice for Russell's viper venom group (Gr. III) & Russell's viper venom + *Varuna* (Gr. IV).

A. **Survival period -**

Russell's viper venom group (Gr. III) -46.33 min

Russell's viper venom + *Varuna* (Gr. IV) - 46.66 min

Survival period in *Varuna twaka churna* gr. IV is increased by o.33 minutes, P value 0.48 (one tailed), which is insignificant.

Due to this insignificant result on male mice, we didn't perform experiment on female albino mice.

B. **Standard groups –**

- In standard group VII (Russell's viper venom & PVASVS) – one mouse died after 20 minutes may be due to serum sickness & other survived without any toxic sign.
- In standard group VIII (Russell's viper venom + PVASVS+ *varuna twaka churna*) – one mouse died after 32 minutes, this is may be due to serum sickness & other well survived without any toxic sign.

This study shows there no interaction between PVASVS & Varuna twaka churna.

Result:

1) Survival period in-group II (common cobra venom + *Varuna*) is increased by 36 minutes, P value 0.002 (one tail), very highly significant.

2) Survival period in Gr. IV (Russell's viper venom + *Varuna*) is increased by 0.33 minutes, P value 0.48 (one tailed), which is insignificant.

CONCLUSION

I conclude that probably due to presence of various phytochemicals plays an important role in the anti-venom potential of *Varuna* (Crataeva nurvala) against common cobra venom. The above observations confirmed that,

1. *Varuna twaka churna* delays the action of common cobra venom as it delays the onset of signs (Gr. II).

- Appearance of tremors delayed by 14.5 minutes, **P value 0.005 (one tailed)**, highly significant.

- Appearance of paralysis delayed by 19.5 minutes, **P value (one tailed) 0.007 (one tailed)**, highly significant.

- Appearance of convulsion delayed by 24.66 minutes, **P value 0.004 (one tailed)**, highly significant.

2. *Varuna twaka churna* increases the survival period in common cobra venom + drug group (Gr. II).

- Survival period increased by 36 minutes, **P value 0.002 (one tailed)**, highly significant

3. *Varuna twaka churna* does not interact with poly valent anti-snake venom (From standard groups V, VI, VII, VIII).

Therefore, a null Hypothesis is rejected & *Varuna twaka churna* is efficient in common cobra poisoning is accepted.

According to observations related to Russell's viper venom groups,

- Survival period in *Varuna twaka churna* gr. IV is increased by o.33 minutes, which is insignificant.
- *Varuna twaka churna* does not interact with poly valent anti-snake venom.

Thus, *Varuna* possess snake venom neutralizing capacity and could be used with first aid measure in case of snakebite envenomation, especially against the cobra venom poisoning.

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