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Toxicological Report Of In Vivo Efficacy Of Acorus Calamus Linn And Piper Nigrum Linn Paste In Common Krait Venom Poisoning In Albino Mice

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Abstract
Snakebite, mortality and morbidity contribute a lot in national statistics. It is an important medical emergency and an important health problem in rural areas. In India, the four medically important poisonous land snakes are The Indian Krait (*Bungarus caeruleus*) The Common Cobra (*Naja Naja*), The Saw Scaled Viper (*Echis carinatus)*, The Russell's Viper (*Viper Russell*). Kraits are one of the common kinds of snake found on land, only dangerously poisonous land snake. Common kraits are not large in size the poisons fangs are small and short compared with those of another poisonous snake. Hence the poison injected by the krait is not large in amount though enough to kill the man, Anti snake venom (ASV) along with supportive care is the only specific treatment around the world.
Due to ignorance of people & lack of trained medical and paramedical staff, shortage and difficulties in transportation and storage of ASV (anti snake venom) in rural areas due to this a lot of valuable time is wasted resulting in considerable morbidity Hence, there is urgent need to find & prove remedy. In Ayurveda many Medicinal plants are recommended for the treatment of krait. Kalka yoga's as an antidote for krait venom poisoning. Krait bite induces **Kaphaja** diseases and the **Acorus calamus & piper nigrum is Kaphashamaka.** After reviewing these remedies **Acorus calamus & piper nigrum** seems to be easily available, Easy to carry, Easy to administer.
Key words: Common Krait Venom Poisoning, Vacha & Maricha paste, and standard treatment ASV (Anti snake venom).

INTRODUCTION

Snake venom poisoning is a global problem. It is particularly important public health problem in rural tropical areas of Africa, Asia, America and New Guinea. Epidemiological assessment of the true incidence of global mortality and morbidity from snake bite envenoming has been hindered by several well recognized problems. Snake bite is a common occupational hazard among the plantation workers, farmers, snake handlers and others resulting in deaths & many cases of chronic physical handicap in the active younger people.
World Health Organization has declared about 200000 snakebite cases occurred in India per year; among them 35,000-50,000 cases become the victims of snakebite. Kraits (*Bungarus caeruleus*) are one of the common kinds of snake found on land, only dangerously poisonous land snake. Kraits not large in size the poison fangs are small and short compared with those of another poisonous snake. Hence the poison injected by the krait is not large in amount though enough to kill the man, Anti snake venom (ASV) along with supportive care is the only specific treatment around the world. Due to ignorance of people & lack of trained medical and paramedical staff, shortage and difficulties in transportation and storage of ASV (anti snake venom) in rural areas due to this a lot of valuable time is wasted resulting in considerable morbidity Hence, there urgent need to find & prove remedy. In Ayurveda many Medicinal plants are recommended for the treatment of krait venom poisoning. Kalka yoga's

as an antidote for krait venom poisoning. Krait bite induces **Kaphaja** diseases and the **Acorus calamus & Piper nigrum** is **Kaphashamaka.** After reviewing these remedies **Acorus calamus & Piper nigrum** seems to be easily available, Easy to carry, Easy to administer.

MATERIAL AND METHOD

Literature data: IDENTITY (General Feature) OF COMMON KRAIT (B. caeruleus)

Head is covered with shields, only 4 shields along either side of lower lips. Body with smooth shines scales usually glistening black or steel, black in colour and has white linier arches across the back these are arranged singly or in pairs, Tail is round, Common krait when fully grow is 3-4 feetlong but may grow to over 5 feet, it is very timid snake usually inoffensive it is quit disposition, and is not easily irritated, it does not bite unless trodden, annoyed or injured. Following 2 points are sufficient to distinguish krait, The central row of scale down the back is distinctly enlarged is more or less hexagonal, the plates under the tail are entire & not divided.

Venom side effect: - 1. Neurotoxicity 2. Myocardial damage

EXPERIMENTAL SECTION:

Plant material: (*Acorus calamus*) AND (*Piper nigrum*) obtains from Mankarnika Aushdhalay Pune (Maharashtra state India) with its Authentication and standardization certificate from university of Pune. ((Maharashtra state India) and this was used as test drug.

Animal: Both male and female of Swiss Albino mice about age 6 weeks were procured from APT Testing and Research Pvt Ltd, Pune, India. they were housed under standard condition of temperature maintained between 22 ± 3^0C, relative humidity 50-60 % and illumination cycle setto 12 hours light and 12 hours dark. The animals were fed on standard Pelleted diet & water adlibitum. 12 animals were used for dose standardization and 30 animals for Efficacy study.

Chemicals: The Dried lyophilized Krait venom was obtained from Haffkine Institute, Parel, and Mumbai Maharashtra India. Poly Valente Anti Snake Venom Serum (PVASV) was obtained from Sassoon hospital Pune Maharashtra India. **Acorus calamus & Piper nigrum** obtained from Mankarnika aushadhalaya, Pune, Maharashtra India

Estimation: Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours. However, the duration of observation was not being fixed rigidly. All observations were systematically recorded with individual records being maintained for each animal in the daily observation record format. Survival time estimations were carried out using Kaplan - Meier Analysis.

Efficacy study: Total 42 animal were taken and divided in to 5 groups. Group 1: normal control. group 2: disease control (krait venom) group 3: krait venom (i.m.) + pvasvs (i.v.) group 4: krait venom (i.m.) + **Acorus calamus & Piper nigrum** paste. group 5: krait venom (i.m.) + pvasvs (i.v.) + **Acorus calamus & Piper nigrum** (1560 mg/kg) in each group, weight of animal was taken first, accordingly route-venom dose was given by i.m route, after 5 min, drug dose was given by orally route and thenpvasvs was given after 5 min by iv route. After dosing animals were observed for 24 hrs. Up to7 days

Statistical analysis

Statistical analysis was performed by **Kaplan -Meier Analysis** and prism card 5 graph pads. If every animal is followed until death, the curve may be estimated simply by computing the fraction surviving at each time. A Kaplan-Meier analysis allows estimation of survival over time, even when animal drop out or are studied for different lengths of time. For each interval survival probability is calculated as no. of animal surviving divided by Number of animals at risk. Animal who has died, dropped out or not reached the time yet are not counted as at a risk animal who lost are considered censored & not counted in the denominator. Probability of surviving to any point is estimated from cumulating probability of surviving each the preceding time interval.

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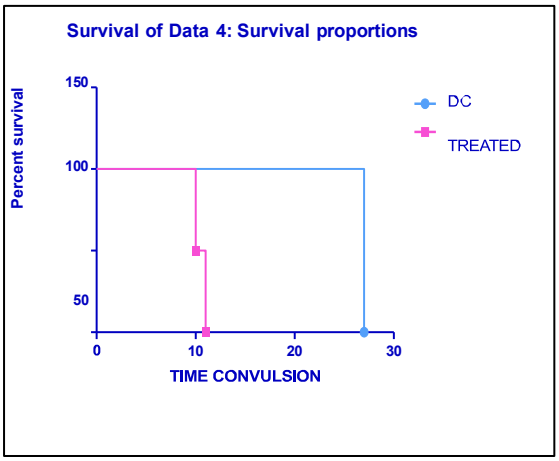
RESULT:

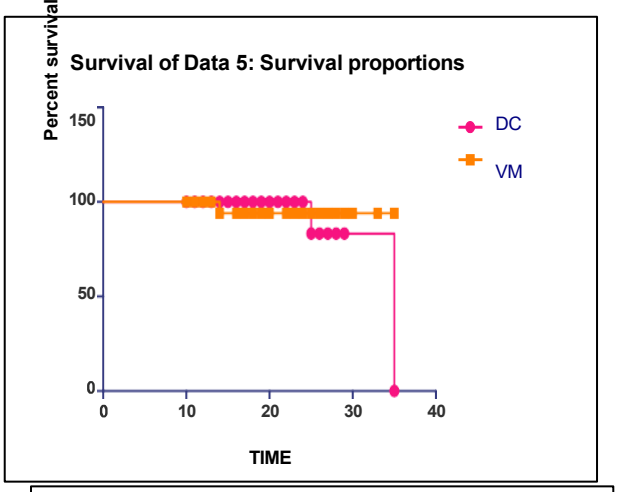
Group I In first group which was given with only Krait venom, the animals were survived forapproximately 30 min.

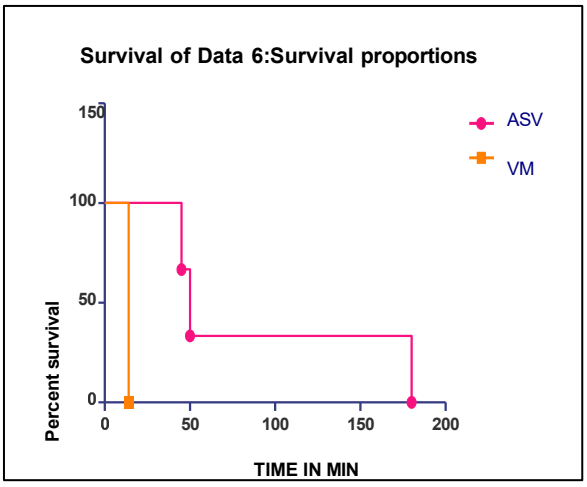
Group II Whereas, the animals which received ASV after Krait venom were survived for approximately 63 min.

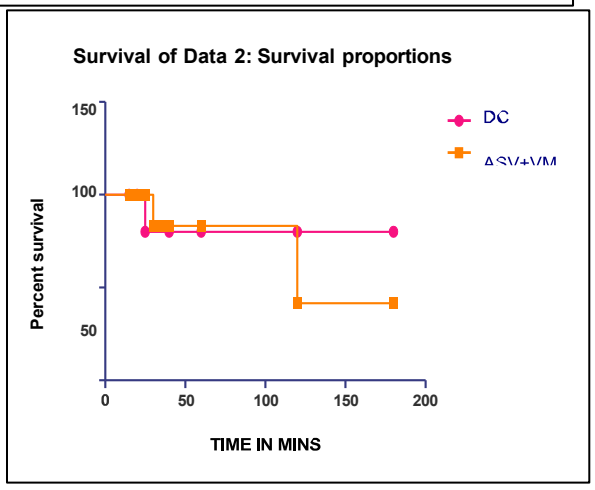
Group III In case of animals that were received Vacha and Marich after Krait venom, were survived forapproximately 16 min

Group Iv In the last group where animals received Vacha and Marich as well as ASV after Krait venom only 33.33% mortality was observed. Along with this, animals were also observed for differentsigns and symptoms in all groups as shown in the table of clinical observation data









DISCUSSION:

As revealed in literature review very few *in vivo* studies have been published for antiophidian drugs. Usual method for antiophidian drugs seems to be *in vitro* study or *pre incubation* study. The *in vitro* study may not be applicable to a living organism; a pharmacodynamics may change in different animals & for snake venom *in vitro* studies the assumption can be stated as "The drug interacts chemically with the venom compounds & neutralize them or binds with the components making them pharmacodynamically inactive." Preincubation study also assumes the first part of the above assumption namely chemical neutralization of venom components.

Any drug which acts by any other mode of action i.e., other than chemical neutralization cannotbe 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 paramount importance in proving the efficacy of antiophidian drugs. Many minute points of these studies were decided by conducting pilot experiments by senior researchers An experiment was carried out at well reputed Toxicology Centre.

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)

Antiophidian property of some drugs have also screened by my seniors of our institute. During this study, my senior's experiences, advices helped me a lot to avoid difficulties.

Therefore, selection of drug was **Acorus calamus & Piper nigrum***'* mentioned in "Vish Vaidya Jyotsnika" for

in vivo study of its efficacy in Krait (*Bungarus caeruleus)* venom poisoning. The previous in vivo studies have been very useful for this experiment which is basically for the extension of screening process by using a different drug on common krait as there is no work has been done on *Bungarus caeruleus. Krait correlated to* Rajeeman *snake* In Ayurveda many Medicinal plants are recommended for the treatment of Rajeeman snake Kalka Yogas as an antidote for krait (**Rajeeman**) venom poisoning. Krait (Rajeeman) bite induces **Kaphaja** diseases and the **Acorus calamus & Piper nigrum** is **Kaphashamaka.**

CONCLUSION

For determination of dose of Krait venom, different doses of Krait venom (100 µg, 200 µg, 400 µg, 600 µg) were administered to mice as shown in results. For 100 µg dose, all mice were survived. At 200 µg dose of Krait venom the average time of survival for mice was approximately 28 min. At 400 µg dose of Krait venom the average time of survival for mice approximately was 21 min. Whereas, at 600 µg dose of Krait venom the average time of survival for mice was approximately 22 min Therefore, the lower dose of Krait venom i.e. 400 µg was selected for efficacy study.

For efficacy study animals were first injected intramuscularly with Krait venom at 400 µg dose. After10 min test drugs (orally) and ASV (intravenously) were given to respective groups. In first group which was given with only Krait venom, the animals were survived for approximately 30 min. Whereas, the animals which received ASV after Krait venom were survived for approximately 63 min. In case of animals that were received Vacha and Marich after Krait venom, were survived for approximately 16 min whereas, in the last group where animals received Vacha and Marich as well as ASVafter Krait venom only 33.33% mortality was observed. Along with this, animals were also observed for different signs and symptoms in all groups as shown in the table of clinical observation data.

In this study the efficacy of Acorus calamus & Piper nigrum against Krait (Bungarus caeruleus) venom.

From the above study it can be observed that Acorus calamus & Piper nigrum along with ASV has prolonged survival time as well as reduced the clinical signs of toxicity in mice after giving the Krait venom.

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