IN VIVO STUDY OF ROLE OF ASHWAGANDHA (*Withania Somnifera*) ROOT CHURNA AS FIRST AID MEASURE IN SNAKE VENOM POISONING.

A Thesis

SUBMITTED TO THE

TILAK MAHARASHTRA VIDYAPEETH, PUNE

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BY

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Undertaking

I Vd. PRADEEP UTTAM JADHAV is the Ph. D Scholar of the Tilak Maharashtra Vidyapeeth in AYURVEDA (AGADATANTRA & VYAVAHAR AYURVEDA) subject. Thesis entitled IN VIVO STUDY OF ROLE OF ASHWAGANDHA (*Withania Somnifera*) ROOT CHURNA AS FIRST AID MEASURE IN SNAKE VENOM POISONING under the supervision of Dr V.P. JOGLEKAR, solemnly affirm that the thesis submitted by me is my own work. I have not copied it from any source. I have gone through extensive review of literature of the related published / unpublished research works and the use of such references made has been acknowledged in my thesis. The title and the content of research is original. I understand that, in case of any complaint especially plagiarism, regarding my Ph.D. research from any party, I have to go through the enquiry procedure as decided by the Vidyapeeth at any point of time. I understand that, if my Ph.D. thesis (or part of it) is found duplicate at any point of time, my research degree will be withdrawn and in such circumstances, I will be solely responsible and liable for any consequences arises thereby. I will not hold the TMV, Pune responsible and liable in any case.

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It is certified that work entitled IN VIVO STUDY OF ROLE OF ASHWAGANDHA (*Withania Somnifera*) ROOT CHURNA AS FIRST AID MEASURE IN SNAKE VENOM POISONING is an original research work done by Vd. PRADEEP UTTAM JADHAV under my supervision for the degree of Doctor of Philosophy in AYURVEDA (AGADATANTRA & VYAVAHAR AYURVEDA) to be awarded by Tilak Maharashtra Vidyapeeth, Pune. To best of my knowledge this thesis embodies the work of candidate himself has duly been completed fulfils the requirement of the ordinance related to Ph. D. degree of the TMV up to the standard in respect of both content and language for being referred to the examiner.

Signature of the Supervisor

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ABBREVIATIONS

Ashťaamga Hŕdaya	A.H.
Ashťaamga Samgraha	A.S.
Bhaishajya Ratnaavali	Bh.R.
Bhaarata Bhaishajya Ratnaakara	B.B.R.
Bhaavaprakaaśha	B.P.
Bhavaprakaaśha Nighańťu	Bh.N
Charaka Samhitaa	C.S.
Chikitsaa Sthaana	Chi.
Dhanvantaree Nighańťu	D.N.
Dravyaguńa Vidnyaana	D.V.
Kaiyadeva Nighańťu	K.N.
Kalpasthaana	Ka.
Madanapaala Nighańťu	M.N.
Madhyama Khanďa	Ma.Kha.
Nidaana Sthaana	Ni.
Nighańťu Aadarsha	N.A.
Nighańťu Ratnaakara	N.R.
Poorva Khanďa	Po.
Priya Nighańťu	P.N.
Raaja Nighańťu	R.N.

Rasatarangiṅi	R.T.
Śhaligraama Nighaṅťu	Śh.N.
Śhaarangadhara Samhitaa	Śh.S.
Śhodhal Nighaṅťu	Sho.N.
Sootra Sthaana	Su.
Sushruta Samhitaa	S.S.
Uttarasthaana	Ut..
Uttarkhanda	U.
Vanagasena	V.S.
Vimaana Sthaana	Vi.
Yoga Ratnaakara	Y.R.

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INTRODUCTION

India is the country having dense population in rural area and is situated in Asia. On the basis of area, India stands seventh in the world. And on the basis of most populated countries it stands second in the world. So incidences of Snakebites are also more. According to Internal Media centre of WHO updated in September 2017, 2 millions snakebites are observed in India.*1 According to "Snakebites Mortality in India: A Nationally Representative Mortality Survey" done by Mohapatra B in 2 April 2011, about 40900 to 50900 deaths are observed yearly in India.*2

Chougule Sarita in her article on IAMJ of Oct 2014 states that in Maharashtra, Kerala, West Bengal, Assam and Uttar Pradesh snakebite cases are observed more in number. She also states that as more population of India depends upon Farming, they have to live in rural areas. This rural area is full of dense forest. So there is more number of snakebites.*3 Tushar Khairnar in his article on WCT's starperson publishing corporation in 2016, described about the tendency of patient for not going to higher centre for the treatment for emergency medical conditions. Instead patient first goes to Primary centers for primary treatment and after increased severity of the patient's condition they will try to go for higher centers. As almost all patients in rural area have less economy they don't have their own vehicles. Patients have to take it on rent. Hence to arrange the vehicles and to do necessary things, more time is consumed.*4

In Harrisons Principles of Internal Medicine Vol. II, 14th edition in 1998, it is clearly stated that Snake venom poisoning has only one scientifically proven treatment which is Anti Snake Venom. In India Anti Snake Venom are available at all government hospitals and centers.*5

After incidence of Snakebite happen to patient, certain time goes to inform the relatives. After their decision of hospitalization, they move patient to nearest medical center which may be close or open at that time. Adjustment of vehicle for transportation of patient is also major problem in the hospitalization of patient. Averagely all this procedure of transportation takes around 3:30 to 4:00 hours to admit the patient in hospital. Four major snakebite cases observed in India are from following four snakes: Common Cobra, Common Krait, Russell's viper and Saw

scaled viper. Fatal period of these four ranges from 2:00 hours to 24:00 hours. There would be irreversible damage by snake venom if time was spent without treatment. So if patient was unable to admit within time, then it will be very difficult to treat these patients with Poly valent Anti Snake Venom. So it is very dangerous to the life of patient.

As Poly valent Anti Snake Venom is the only treatment on snakebite cases, there is more requirement than its production. So there is always shortage of Poly valent Anti Snake Venom in Government hospitals. Though it is available at the Government hospitals, before giving it to the patient sensitivity test is must. But if Doctor or nursing staff is absent then it will be life threatening to start Poly valent Anti Snake Venom.

These are some of the drawbacks of Poly valent Anti Snake Venom. To compensate these things about Poly valent Anti Snake Venom, there should be one another form of treatment which will definitely helps snakebite patients to come over the condition. Another form of treatment should be used as first aid treatment which should decrease transience and cognitive state of the patient. This treatment should have following characteristics.

1. This treatment should be accessible to all the peoples.
2. This treatment should not have its own toxic property.
3. Common man can administrate it properly.
4. This treatment should elongate signs and symptoms observed in snakebite cases.
5. And most important, this treatment should not interfere in the action of Poly valent Anti Snake Venom and should not form any other complication if given before Poly valent Anti Snake Venom.

According to the Article “Ethnobotanical survey of folk plants for the treatment in Southern part of Tamilnadu”, published in Journal of Ethnopharmacology, dated 17 Jan 2008, Vol. No. 115, issue2, pages 302-312 by Author Ramar Permual Samy, there are 850 species from 138 families of plants are antiophidian.*6 As well as 96 ayurvedic combinations are very useful in snake venom poisoning. So to prove the

usefulness of these ayurvedic drugs, the method known as 'in vivo study' have selected. In this study to develop the same condition as in snake venom in human, first of all snake venom will be given then experimental drug is given and at last Poly valent Anti Snake Venom will be given.

Colonel K.G. Gharpurey in his book "The Snakes of India" 4th edition published in 1954 by 'The Popular Book Depot', Mumbai on page no. 2 states that there are three types of Snakes viz. Neurotoxic, Vasculotoxic and Myotoxic. Out of which Myotoxic snakes are found in Sea and are very rare. But in Neurotoxic snakes, common cobra is more common. As well as in Vasculotoxic Russell's viper is more common.*7 Due to this reason Common cobra and Russell's viper are selected to study the usefulness of drug.

"*Vishavaidya Jyotsanika*" is one of the most popular books in Kerala and widely practiced for the snakebite treatment. In an English translation of it by Dr C.M. Sreekrishnan in first edition published in Nov. 2009 page no. 39, it is stated that, *churna* of *Ashwagandha (Withania somnifera*) root when given with pure water is very useful in all types of snake venom poisoning.*8

अश्वगन्धमरच्चिट्टु शुध्दतोये पिबेद् द्रुतम् ।

नन्द्यावर्त्तमतिन्मूलं मुळकुं कूट्टियुं तथा ॥

-विषवैद्य ज्योत्सनिका ०५ /०८

According to this book, it should be given by oral route. As *Ashwagandha* has less drawbacks compared to Poly valent Anti Snake Venom mentioned above and also posses all characteristics of ideal first aid treatment, it was selected for the experiment.

Thus this experiment aims to study the efficacy of *Ashwagandha* root *churna* as a first aid measure in snake venom poisoning. If this experiment proves the usefulness of *Ashwagandha*, it will be a gift for the human.

AIM AND OBJECTIVES

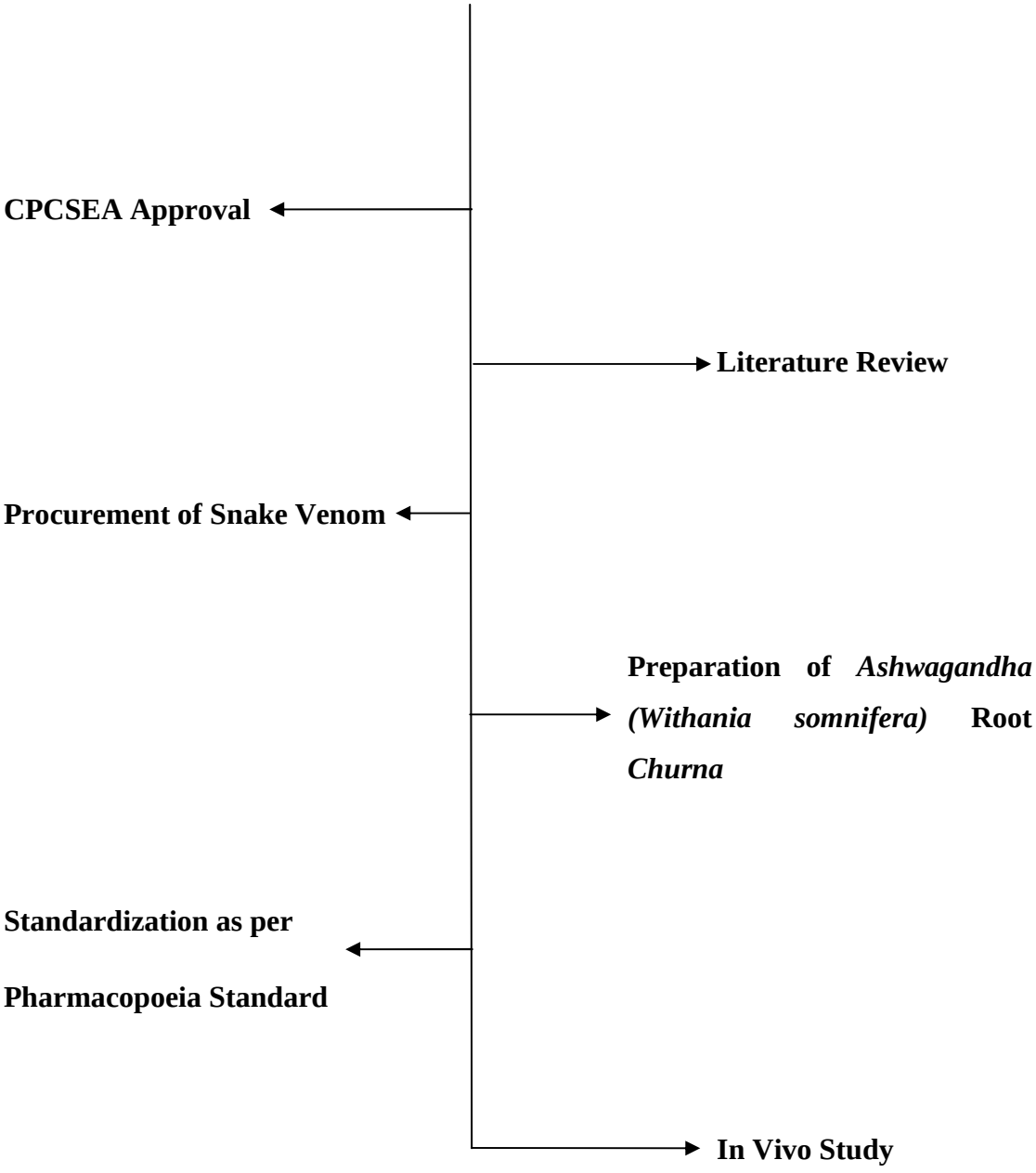
AIM:

To study the Role of *Ashwagandha (Withania somnifera)* Root *Churna* as a first aid measure in Snake Venom poisoning.

OBJECTIVES:

1) To Study the Role of *Ashwagandha (Withania somnifera)* Root *Churna* in Common Cobra Venom poisoning as a first aid measure.
2) To Study the Role of *Ashwagandha (Withania somnifera)* Root *Churna* in Russell's viper Venom poisoning as a first aid measure.
3) To Study Whether there is any adverse drug reaction between *Ashwagandha (Withania somnifera)* Root *Churna* and Polyvalent Anti Snake Venom (PVASV).

PLAN OF WORK


CPCSEA Approval
Literature Review
Procurement of Snake Venom
Preparation of Ashwagandha (Withania somnifera) Root Churna
Standardization as per Pharmacopoeia Standard
In Vivo Study

REVIEW OF SNAKES AND SNAKE BITE

According to Ayurved

- **Definition of Poison (*Visha*):**

1. जगव्दिषण्णं तं दृष्ट्वा तेनासौ विषसंज्ञितः ।

   विषादजननत्वाच्च विषमित्यभिधीयते ॥ - C.S.Chi.23/5

   A poison is commonly defined as a substance which when administered, inhaled or swallowed is capable of acting deleteriously on the body.

2. व्यवायी च विकाशी स्यात् सूक्ष्मं छेदि मदावहम् ।

   आग्नेयं जीवितहरं योगवाहि स्मृतं विषम् ॥ - Sha.S.Pu.Kh.4/22,23

   A substance having properties like *Vyavayi, Vikasi, Sukshmam, Chedi, Madkari, Aagneya, Jivitaharam* and *Yogvahi* is called as *Visha.*

3. A substance having 10 specific properties, producing 8 stages or *vegas,* having its origin from *Jala mahabhoota,* with effect like *Teja mahabhoota,* which can be found in both animate and inanimate substances and should be treated with 24 modalities can be called as *Visha.* *9
4. A substance which produces vitiation of *Dhaatu* (Bodily tissue) can be called as *Visha.**10

- **Classification of Poison:**

स्थावरं जङ्गमं चैव व्दिविधं विषमुच्यते ।

दशाधिष्ठानमाद्यं तु व्दितीयं षोडशाश्रयम् ॥ - S.S.Ka.2/3

Poison is said as of 2 types, one obtained from immobile source (*Sthavara*) and other from mobile (*Jangama*) one.

Charaka has given 21 examples of *Sthavara visha* and 18 examples of *Jangama visha*. *Charaka* does not describe *Adhisthana* (Poisonous parts). *11

The sixteen locations of the poison from mobile sources are mentioned in *Sushruta Samhitaa*- Sight, breath, teeth (Fangs), nails, urine, feces, semen, saliva, menstrual blood, suckers, bile, flatus, beak, bone, bristles and dead body.

तत्रदृष्टिनिःश्वासदंष्ट्रानखमूत्रपुरीषशुक्रलालार्तवमुखसंदंशविशर्धिततुण्डास्थिपित्तशूकशवानीति।

-S.S.Ka.3/4

तत्रदृष्टिनिःश्वासविषादिव्याःसर्पाःभौमास्तुदंष्ट्राविषाः। - S.S.Ka.3/5

Celestial snakes have venom in sight and breath; earthly snakes have venom in fangs.

❖ **Characteristics of Poison:**

लघुरुक्षमाशुविशदं व्यवायी तीक्ष्णं विकासि सूक्ष्मं च।

उष्णमनिर्देश्यरसं दशगुणमुक्तं विषं तज्ज्ञैः॥ -C.S.Chi. 23/24

रूक्षमुष्णं तथा तीक्ष्णं सूक्ष्ममाशु व्यवायी च।

विकाशी विशदं चैव लघ्वपाकि च तत् स्मृतम्॥ -S.S.Ka. 2/19

व्यवायी च विकाशी स्यात्सूक्ष्मं छेदि मदावहम्।

आग्नेयं जीवितहरं योगवाहि स्मृतं विषम्॥ -Sh.S.P.K. 4/22

Poison has ten properties. Nine of them are common in all *Samhitaas*. They are *Laghu, Ruksha, Aashu, Vishada, Vyavayi, Tikshna, Vikasi, Sukshma, Ushna*. 10th property is described differently in *Charaka, Sushruta* and *Vagbhata*. *Charaka* mentions *Anirdesshya Rasa*, where *Sushruta* mentions *Apaaki*. *Vagbhata* mentions both *Anirdesshya Rasa Apaaki* making total eleven attributes, *Sharangadhara* describes eight attributes where *Vyavayi, Vikasi* and *Sukshma* are common to *Samhitaa.*

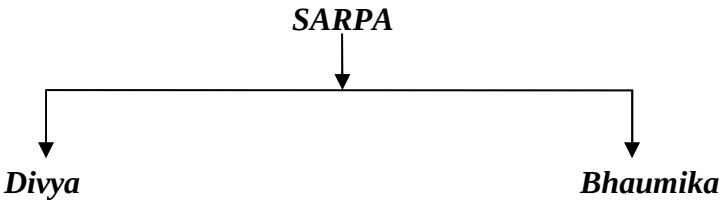
SNAKES (*SARPA*):

Snakes are the most despised animals in the world. Most of the people get chilled even on the sight of snake itself. Factors like magnitude of the complications, unexpectedness and immediate death warrant instantaneous emergency measures. Worshiping of snakes is still prevalent in India. *Nagpanchami*, *Anyilyam* day in Kerala are best examples.

Ample narrations about snakes are available in *Brihattrayee*. These references are the most ancient authentic literature, influencing contemporary Ayurved.*12

❖ **Classification of Snakes:**

➢ **According to *Sushrut Samhita:***



According to *Sushruta Samhitaa*, *Sarpas* are divided into *Divya* (Celestial) and *Bhoumika* (Terrestrial) types. *Vasuki, Ananta, Takshaka, Gulika, Karkataka, Shamkhapaala, Padma* and *Mahapadma* are the eight *Divya Sarpas* and they are entrusted with the duty of looking after the universe.*13

Bhoumika Sarpas are divided into 5 groups and are 80 in numbers.

अशीतिस्त्वेव सर्पाणां भिद्यते पञ्चधा तु सा ॥

दर्वीकरा मण्डलिनो राजिमन्तस्तथैव च ।

निर्विषा वैकरञ्जाश्च त्रिविधास्ते पुनः स्मृताः ।

दर्वीकरा मण्डलिनो राजिमन्तश्च पन्नगाः ॥ -S.S.Ka.4/9-12

Darveekara-26 *Nirvisha*-12

Mandalee-22 *Vaikaranja*-3

Rajimanta- 10 Progeny of *Vaikaranja*-7

❖ **Table no. 1: External features of Poisonous Snakes*14**

	Darveekara	*Mandalee*	*Raajimantaa*
External Identification Marks	Hooded, fast moving, having marks of wheel, plough, umbrella, Swastika on body	Coiled hood, Large body, slowly moving, circular marks and lustrous like fire and sun	Body variegated with spots and streaks oblique and straight
Poison	Rough & Pungent	Sour & Hot	Sweet & Cold
Aggravated *Dosha*	*Vaata*	*Pitta*	*Kapha*
Bite	Minute marks of fangs, black, congested with blood, tortoise like produces *Vatika* disorders	Big marks of fangs, is swollen, yellowish or yellow-red and causes *Paittika* disorders	Slimy, with stable swelling, unctuous, pale having viscous blood and causes disorders of *Kaphaa*
Stage of Life in which most poisonous	Young	Old	Middle
Roaming	In day	First 3 quarter of night	Last quarter of night

Male Snake- Large eyes, tongue, mouth and head.

Female Snake- Small eyes, tongue, mouth and head.

Hermaphrodite- Snakes having characteristics of both. They have Mild poison and less anger.

- **Classification on another Basis:*15**

a) ***Braahmanas*:** Snakes which have brilliance like that of pearl and silver or with golden hue, brownish and with pleasant aroma should be known as *Braahman.*

b) ***Kshatriyas*:** Are of glossy complexion, too wrathful and having marks of the sun, the moon, umbrella and conch-shell.

c) ***Vaishya:*** Those black, diamond like red in color, smoky and resembling dove are known as *Vaishya.*

d) ***Shudra:*** Those having color of buffalo and leopard, rough skin and colors different from those mentioned above are defined as *Shudra.*

- **Classification on another basis of gender:*16**

a) ***Puman:*** The snake that makes a round coil, has a huge body, that hisses and looks upward and has large head with all even body is a male.

b) ***Stree:*** The snake just possessing the contrary characteristic is female one. The person bitten by the female serpent has drooping looks, shivers and suffers by loss voice.

c) ***Kleebya (Napunsaka):*** The snake that is timid is sexless.

- **According to *Charak Samhita:***

इह दर्वीकरः सर्पो मण्डली राजिमानिति।

त्रयो यथाक्रमम् वातपित्तश्लेष्मप्रकोपणाः॥ -C.S.Chi.23/124

Gaudheyaka: Charaka describes this snake in addition to above categories. It is a quadruped serpent born by *Godha* and is known as *'Gaudheyaka'*. This is similar to black snake. Black snakes in young age, *Ghonasa* in old age and *Raajimanta* in middle age are like *Asivisha.*

- **According to *Ashtangahrudaya:***

दर्वीकरा मण्डलिनो राजिमन्तश्च पन्नगाः।

त्रिधा समासतो भौमाः भिद्यन्ते त्वनेकधा॥ -A.H.Uttar.36/1

Vagbhata describes *Bhouma* Snakes of three types as *Darveekara, Mandali* and *Rajimanta*. Further he said there are various types of these three main types.

❖ **Causes of Snake Bite:**

आहारार्थ भयात् पादस्पर्शादतिविषात् क्रुधः ।

पापृत्तया वैराद्देवर्षियमचोदनात् ॥

दशन्ति सर्पास्तेषूक्तं विषाधिक्यं यथोत्तरम् । -A.H.Uttar.36/8-9

According to *Vagbhata*, snake will bite the human when snake is in search of food, if snake is in fear, if feet of the human touch the snake, if there is increase in poison level in his body, if he is angry, with culprit mind, to take revenge and after the inspiration from *Dev, Rishi, Yama*.

❖ **Types of Snake Bite:**

➢ **According to *Sushruta Samhitaa*:**

सर्पितं रदितं चापि तृतीयमथ निर्विषम् ।

सर्पागाभिहतं केचिदिच्छन्ति खलु तद्विदः ॥ -S.S.Ka.4/14

1) *Sarpita* Bite:

When marks of bite are one or two or many, deep with little blood which is inflicted by twisting, covered with beak like projections, causing abnormal look, contracted or swollen, it should be known as *Sarpita* (deep) type of bite.

2) *Radita* Bite:

When there are reddish, blue, yellow and white streaks, it should be known as *Radita* (Superficial) type of bite which causes mild poisoning.

3) ***Nirvisha/ Avisha*** **Bite**:

If the mark of bite is free from swelling, has little vitiated blood and the patient is normal, it is known as *Nirvisha/ Avisha* bite.

4) Sarpaangaabhihata:

Sometimes, by the touch of snakes in timid person, *Vaayu* being vitiated by fear causes swelling, it is known as *Sarpaangabhihata.*

- **According to *Ashtaamga Hridaya:* *17**

1) ***Tundahata***: No bite marks, only saliva is present at the site.
2) ***Vyaaleedha***: One or two teeth marks present but no bleeding.
3) ***Vyaalupta:*** Two teeth marks & there is active bleeding from bitten site.
4) ***Damshtraka***: Three teeth are going up to muscles & there is continuous bleeding.
5) ***Damshtranishpeedita:*** Four teeth marks are present & there is active bleeding. First two are not poisoning but last one is fatal.

❖ SIGNS AND SYMPTOMS OF SNAKE- BITE:

सर्पदंष्ट्राश्चतस्त्रस्तु तासां वामाधराऽसिता।

पीता वामोत्तरा दंष्ट्रा रक्तश्यावाऽधरोत्तरा॥ -C.S.Chi.23/137

Darveekara:

- Black, small wound
- No bleeding
- Tortoise like swelling
- Symptoms of *Vaatavyaadhi*

Mandalee:

- Broad, deep, edematous wound
- Skin color yellowish or yellowish red

- Symptoms like *Pittaja Vyadhi*

Raajimanta:

- Sticky, non- movable swelling
- Shining yellow colored wound
- Sticky hemorrhage from bitten area
- Symptoms like *Kaphaja vyaadhi*

❖ **Phases of Poisoning:**

There are seven phases of poisoning by all types of snakes.

धात्वन्तरेषु याः सप्त क्लाः संपरिकीर्तिताः ।

तासामैकैक्रमतिक्रम्य वेगं प्रकुरुते विषम् ॥ -S.S.Ka.4/40

Poison crossing over each of the seven *Kalaas* successively situated in the intermediary region between one and the other *Dhatu* produces different phases of effects.

The interval which takes place while poison crosses one *Kalaa* to the other is known as *Vegantara*. The poison is driven by *Vaayu*.

❖ **Table no. 2: Signs and Symptoms of *Vishavegas* according to Ayurved *18**

Darveekara	***Mandalee***	***Raajimanta***
Poison vitiates blood due to which it becomes black, gives rise to blackness and feeling like crawling of ant on the body.	Poison vitiates blood which acquires yellowishness & gives rise to generalized burning sensation & yellowishness on body.	Vitiates blood which becomes pale, causing horripilation & whitish appearance.
It vitiates muscles which gives rise to marked blackness, inflammation and cysts in body.	It vitiates muscles which causes marked yellowishness, generalized burning sensation & swelling at the site of bite.	It vitiates muscles producing severe pallor, stiffness of body and swelling in head.
It vitiates fats which causes moistening at the site of bite. Heaviness of head & stiffness of eyes.	It vitiates fats which gives rise to stiffness of eyes.	It vitiates fats causing disorders of vision, moistening at the site of bite, sweating & discharge from nose & eyes.
Poison enter into Thoraco-abdominal cavity and vitiates *doshas* prominently *Kapha* which produces drowsiness & salivation.	Thirst, moistening at the site of bite and sweating.	Entering into thoraco-abdominal cavity it produces heaviness in head.
It further penetrates into bones & vitiates *prana* & *agni* which leads to pain in joints, hiccough & burning sensation.	Enters into thoraco-abdominal cavity and produces fever.	It causes loss of speech and fever with rigor.

It reaches marrow and highly vitiates *grahani* which gives rise to heaviness in body, diarrhea, cardiac pain and fainting.	Same as *Darveekara*	It causes loss of speech and fever with rigor.
It enters into semen highly vitiates *Vyan vayu* and causes discharge of *Kaphaa* from minute channels by which there are appearance of mucous wick, breaking pain in back waist.	Same as *Dareekara*	Same as *Darveekara*

❖ TREATMENT OF SNAKE BITE:

One should start the treatment after considering well *Dosha*, constitution, suitability, season, velocity of poison and patients strength.

मन्त्रारिष्टोत्कर्त्तननिष्पीडनचूषणाग्निपरिषेकाः ।

अवगाहरक्तमोक्षणवमनविरेकोपधानानि ॥

हृदयावरणमञ्जननस्यधूमलेहौषधप्रशमनानि ।

प्रतिसारणं प्रतिविषं संज्ञास्थापनं लेपः ।

मृतसंजीवनमेव च विंशतिरेते चतुभिरधिकाः । -C.S.Chi.23/35-37

If bitten in extremity by a snake of any type of bite, tourniquet should be applied four fingers above the site of bite. Tourniquet is of soft material like cotton cloth, leather piece or tree barks. Restrained by tourniquet the poison does not spread in body.

- Where binding is not applicable, site should be excised and then cauterized.
- Sucking, excision and cauterization are recommended everywhere. But the bite by *Mandalee* snake should never be cauterized because the poison being predominant in *Pitta*, the bite spreads out by cauterization.

Mantra & Tantra Chikitsaa:

Mantras of the nature and penance delivered by *Devarshi* and *Brahmarshi* cannot fail and as such destroy even the terrible poison immediately.

Mantras should be acquired by one abstaining from women, meat and wine, taking little food, pure and clean and sleeping on beds of *Kushtha* grass.

Tourniquet too should be bound, with *mantras* by the expert in them.

The expert clinician should puncture veins around the site of bite and in case the poison has spread veins at the end of extremities or in forehead should be punctured.

After scarifying the site, paste of anti-poisonous formulation should be applied around it. The site should also irrigate with decoction of *Chandana* and *Usheera*.

The Patient should be given to drink anti poisonous formulations with milk, honey, ghee etc. The patient should not drink oil, soups of horse gram, wine and *Sauveeraka*.

A poison is stable at bitten area only for 100 *Maatra Kaala* & then goes into *Raktaadidhaatu*. That's why; in this short period emergency treatment like *Utkartana* etc. should be done to stop the entry of poison in body.

Treatment of *Sarpaangabhihata*:*19

- Main treatment is to give assurance to the patient
- *Harshana Chikitsaa*
- *Mantrayukta* Water consumption
- Some Aayurvedic drugs like Sugar, *Draksha, Vidarikanda, Yashtimadhu* and Honey.

Table no. 3: *Ayurvedic Chikitsaa* of Snake Bite *20

Phase	*Darveekara*	*Mandalee*	*Raajimanta*
I	Blood-Letting	Same as *Darveekara*	Bloodletting should be performed with gourd and anti poisonous formulation mixed with honey & ghee should be given to drink
II	Anti-poisonous formulation with honey & ghee should be given to drink	Anti-poisonous formulation should be given to drink with honey & ghee. Then emesis followed by gruel.	Emesis should be applied, followed by intake of anti-poisonous formulation.
III	Anti-poisonous snuff and collyrium should be applied.	After purgation, he should be given wholesome gruel.	Same as *Darveekara*
IV	After emesis, gruel mentioned before should be given.	Same as *Darveekara*	Same as *Darveekara*
V	Physician should administer drastic evacuation followed by gruel.	Same as *Darveekara*	Same as *Darveekara*
VI	Physician should administer drastic evacuation followed by gruel.	Intake of *Kaakolyaadi* group & sweet anti-poisonous recipe.	Irritant Collyrium
VII	Head should be evacuated with irritant pressed snuff; irritant collyrium should be applied and making incision like crows foot with instrument on scalp.	Anti-poisonous formulations should be used as pressed snuff.	One pressed snuff should be applied.

Table no. 4: *SARPA VISHANAASHAKA YOGA*

Below mentioned 96 Ayurvedic formulations are mentioned as antiophidian

1	*Shireeshapushpadi Yoga*	*C.S.Chi.23/193*
2	*Takshaka Vishaghna Yoga*	*C.S.Chi.23/194*
3	*Darveekara Vishaghna Yoga*	*C.S.Chi.23/195*
4	*Mandalee Sarpa Vishaghna Yoga*	*C.S.Chi.23/197*
5	*Sarpa Vishanashak Yoga*	*C.S.Chi.23/198*
6	*Pancha Shireesha Agada*	*C.S.Chi.23/218*
7	*Nagdantyadi Ghruta*	*C.S.Chi.23/241*
8	*Mahaagada*	*S.S.Ka.5/61*
9	*Ajita Agada*	*S.S.Ka.5/63*
10	*Tarkshya Agada*	*S.S.Ka.5/66*
11	*Rushabha Agada*	*S.S.Ka.5/70*
12	*Samjeevana Agada*	*S.S.Ka.5/73*
13	*Shleshmantakaadi Agada*	*S.S.Ka5/75*
14	*Draksha Sarpagandhadi Agada*	*S.S.Ka.5/76*
15	*Vamshatvagaadi Agada*	*S.S.Ka.5/80*
16	*Shireeshamulaadi Agada*	*S.S.Ka.5/81*
17	*Kushthaadi Agada*	*S.S.Ka.5/83*
18	*Baakuchi Pushpa*	*S.S.Ka.5/84*
19	*Nirgundi*	*S.S.Ka.5/84*
20	*Punarnavaa*	*S.S.Ka.5/85*

21	*Shireesha Pushpa*	*S.S.Ka.5/85*
22	*Arka Pushpa*	*S.S.Ka.5/86*
23	*Nishottara*	*S.S.Ka.5/86*
24	*Kushtha*	*S.S.Ka.5/86*
25	*Ksharaagada*	*S.S.Ka.6/3*
26	*Kalyaanaka Ghrita*	*S.S.Ka.6/8*
27	*Apaaamargaadi Agada*	*S.S.Ka.6/12*
28	*Mahaasugandhi Agada*	*S.S.Ka.6/14*
29	*Himavaana Agada*	*A.S.Ut.42/27*
30	*Ashtaamga Agada*	*A.S.Ut.42/27*
31	*Shireeshapushpadi Yoga*	*A.S.Ut.42/53*
32	*Gaarudi Amjana*	*A.S.Ut.42/40*
33	*Churnaanjana*	*A.S.Ut.42/41*
34	*Himavaana Agada*	*A.H.Ut.42/36*
35	*Nirgumdi Kwatha*	*A.H.Ut.36/57*
36	*Kaakadaanimool Yoga*	*B.B.R.*
37	*Neelinimoola Kalka*	*B.B.R.*
38	*Punarnavaa Yoga*	*B.B.R.*
39	*Pimditagaramool Yoga*	*B.B.R.*
40	*Sarpavishahara Rasa*	*B.B.R.*
41	*Dashaamga Dhoopa*	*B.B.R.*
42	*Naktamaalaadya Amjanam*	*B.B.R.*

43	*Pindita Gaaramjanam*	*B.B.R.*
44	*Bilvaadi Yoga*	*B.B.R.*
45	*Bhimrudra Rasa*	*B.B.R.*
46	*Drakshyaadya Agada*	*B.B.R.*
47	*Marichaadi Churnam*	*B.B.R.*
48	*Lavamgadi Yoga*	*B.B.R.*
49	*Vatashrumgadi Yoga*	*B.B.R.*
50	*Mrutyupaashachedi ghritam*	*B.B.R.*
51	*Vishahaari Varti*	*B.B.R.*
52	*Vandhyaa Karkotaki*	*B.B.R.*
53	*Mahaamrutyanjaya*	*B.B.R.*
54	*Vishara jarapato Rasa*	*B.B.R.*
55	*Mahaagandhahasti Agada*	*B.B.R.*
56	*Lajjalumula Yoga*	*B.B.R.*
57	*Mritasanjeevanee Agada*	*B.B.R.*
58	*Shweta Punarnavaamoola Yoga*	*B.B.R.*
59	*Shikhari Ghritam*	*B.B.R.*
60	*Shireesharishta*	*B.B.R.*
61	*Shireeshaadi Lepa*	*B.B.R.*
62	*Somavalkalaadi Lepa*	*B.B.R.*
63	*Samdnya Prabodhan Rasa*	*B.B.R.*
64	*Sarpavishahara Amjanam*	*B.B.R.*

65	*Samjeevani Vati*	*B.B.R.*
66	*Krishna Sarpa Rasa Yoga*	*B.B.R.*
67	*Lamgaleemoola Nasya*	*Bh.R.*
68	*Masurnimba Patra Yoga*	*Bh.R.72/9*
69	*Dhavalaadi Yoga*	*Bh.R.72/10*
70	*Ghruhadhumaadi Yoga*	*Bh.R.72/11*
71	*Kulikamoola Nasya*	*Bh.R.72/12*
72	*Karnagutha Yoga*	*Bh.R.72/13*
73	*Shireeshapushpa Rasa*	*Bh.R.72/14*
74	*Tagaraadi Churna*	*Bh.R.72/15*
75	*Aparajitaa Mulaadi yoga*	*Bh.R.72/43*
76	*Ghritaadi Yoga*	*Bh.R.72/47*
77	*Naktamaaladi Yoga*	*Bh.R.72/48*
78	*Tankana Jala*	*Bh.R.72/49*
79	*Arkamoola Svarasa*	*Bh.R.72/49*
80	*Kulikadi Vatika*	*Bh.R.72/50*
81	*Tanduliyaka Ghritam*	*Bh.R.72/62*
82	*Vishavajra pato Rasa*	*R.S.*
83	*Tanduliya Root*	*Y.R.*
84	*Pippalyadi Churna*	*Y.R.*
85	*Shireeshamoola + tandul Water*	*Y.R.*
86	*Naktamaalaphalaadya Amjanam*	*Y.R.*

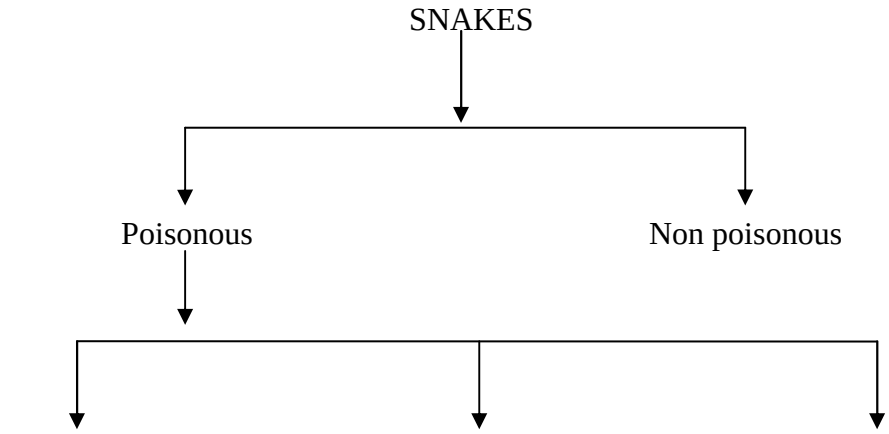
87	*Shweta moola*	*Y.R.*
88	*Shami moola*	*Y.R.*
89	*Ishwaree moola*	*Y.R.*
90	*Aadalika*	*Y.R.*
91	*Paatha*	*Y.R.*
92	*Kulikamoola Nasya*	*Y.R.*
93	*Jamgalikanda Nasya*	*Y.R.*
94	*Kapotadi Dhoopa Agada*	*Y.R.*
95	*Saatalaphala Phena Amjana*	*Y.R.*
96	*Kaalavajrashani Rasa*	*Y.R.*

REVIEW OF SNAKES AND SNAKE BITE

According to Modern

- **Classification:**

  There are more than 2000 species of snakes in the world & about 216 species in India, of which about 50 species are poisonous.



Neurotoxic	Vasculotoxic	Myotoxic
a) Common Cobra	a) Green Pit Viper	a) Sea Snakes
b) King Cobra	b) Russell's viper	
c) Common Krait	c) Saw Scaled viper	
d) Banded Krait		

❖ **Table no. 5: Venomous Snakes of the world *21**

Sr. No.	**Family**	**Subfamily**	**Representative Species**	**Remarks**
1	Typhlopidae (Worm Snakes)		*Typhlops braminus*	Blind Snakes look like worms found in the earth when it is Dug.
2	Leptotyphlopidae			Having same character of Typhlopidae.
3	Uropeltidae		*Uropeltis ausilatus*	Usually found in Mountains & Southern India.
4	Boidae		*Python morulus*	Found in whole India
5	Xenopeltidae		Xenopeltis unicolor	Found in the Burma & the Malay Peninsula.
6	Colubridae	Elapidae	Indian Krait, Banded Krait, Indian Cobra.	Australia, temperate and tropical new and old world.
		Hydrophidae	Sea snakes (*Hydrophis mamilaris*)	Pacific and Indian ocean.
7	Viperidae		Russell's viper, Echis carinatus	Europian, Asian African vipers.
8	Crotalidae		Pit viper	New world and Asia

➢ **Common Snakes of India*22**

- **Indian (Spectacled) Cobra (*Naja naja*):**

Distinctive features: Medium sized to large; smooth, shiny scales; wide head and neck; wide black band on underside of snake; distinctive hood marking on top of neck.

Average Length: 1m; at birth 25 cm; Maximum 2m (male).

Description: The Spectacled cobra is a smooth scaled snake with black eyes, wide neck and head and medium body. Coloring varies from black or dark brown to yellowish white. The underside is usually white or yellowish with a wide dark neckband. The body is generally covered with speckled white or yellow pattern, sometimes forming ragged bands. The famous hood marking of the classic design shows a connected pair of rings. Occasionally, it may not even resemble spectacles, or may be altogether absent. The cobras of northwest India are blackish and have a barely distinguishable hood marking. Cobras are often confused with the Indian rat snakes, which have a much thinner neck and head, and become 3 meters long, a meter more than the biggest Indian cobras.

The spectacled cobra is the most widely distributed of the generally accepted three sub species of cobras in India and is one of the Big four dangerous snakes. Six species of cobras occur in Asia and nine in Africa. The Jet black cobra of northwest India and Pakistan is another sub species or geographic race. Except for its color and absence of hood marking, it is very similar to the spectacled cobra.

Distribution: Throughout India, from sea level up to 4000 m (in the Himalayas)

Habitat: Cobras are common in rice growing areas, which have plenty of rats for food and holes to live in. However, they seem to be quite adaptable and can be found even in very dry parts of the country. Granaries, termite mounds, earth dams and rock piles are favorite haunts. Wherever rats multiply, so do cobras.

Habits: Cobras usually live in rat holes, often near villages, and shy existences, out of man's sight. Their warning mechanism consists of spreading the neck ribs,

startling the intruder with the bright ‘eyes’ on the hood. When more excited, they hiss by a sharp expulsion of air from the glottis. Some of the African species of cobras and parts of South – East Asia can ‘spit’ or propel their venom through the air for about 2 meters from its fangs in the way that person squirts liquid out of a syringe. It aims the venom at its enemy’s eyes to blind it while it makes its escape. Cobras in north eastern India shoot up their venom for a shorter distance. Evening hours are preferred for moving about and hunting. Like most snakes, cobras become very active in the rains and at dusk, hunting along the edges of paddy fields for rats and mice.

Young: Between May and July (depending upon the part of country), the female lays 12-30 eggs, usually in a rat hole or termite mound. She stays with them for 60 odd days still they hatch, feeding rarely. The young ones, which are perfect replicas of parents, usually disperse one or two weeks thereafter. Sometimes more than a single clutch is laid in one hole. Cobras, as well as many other snakes, may breed more than a year.

Food: Young snakes feed on insects, lizards, frogs, toads and small snakes. As they grow larger they take rodents, toads, frogs and birds in that order of preference. Observers have watched cobras swallowing monitor lizards as well. They normally maintain their grip until the prey is immobile.

Status: Cobras are hunted and killed for their skin throughout most of their range and this has made them shy and timid. Since 1973 the export of cobra skins has been controlled by Government of India; however, the market continues to flourish and some tanneries deal thousands of skin per day. As cobras favor farm lands, they probably benefit by the conversion of forests to agriculture lands.

Venom: The venom affects the nervous system leading to respiratory paralysis and cardiac failure. Usually less than fatal amounts are injected. However, all cobra bites should be treated immediately. Extensive research is being carried out on cobra venom. At the Tata Memorial Cancer Institute in Bombay it has been found that fractions of cobra venom destroy certain cancer cells in mice. Effective pain killers are made from cobra venom, such as ‘Cobroxin’ and ‘Nyloxin’ (Hynson, Wescott and Dunning, USA).

- **Russell's Viper (*Vipera russelli*)**

Distinctive Features: Medium size to large; strongly keeled scales; distinctive bright chain pattern, large triangular head.

Average length: 1 m; At Birth: 24 cm; Maximum: 1.8 m (male).

Description: Russell's vipers are heavy, rough scaled snakes with vertical eye pupils and generally a very bright pattern. The body color is usually brown or yellowish and the pattern is composed of dark, round spots edged with white and black. The underside is white in the western, partly speckled in the southeastern and heavily speckled in the northeastern races. Color variation is common, and the best recognition characters are the short, fat body, the triangular shaped or flat arrow shaped head and every regular chain like pattern. Russell's vipers resemble the fat, harmless common sand boas which however have shorter and blunter tails and irregular body patterns. The bright symmetrical spots on Russell's viper backs make them easy to differentiate. Its fangs are long and curved; all the better to kill rats and other prey with. Russell's viper is one of the Big four dangerous snakes of India. The other larger Indian viper is the Levantine Viper, a heavy brown snake found in the parts of Kashmir which grows to 1.5 m.

Distribution: Hills and plains throughout India up to 3000 m.

Habitat: Russell's vipers are equally at home in the open areas of the hilly country and the plains scrub jungle bordering farm lands. In very hot weather, they are found in termite mounds and rat holes but the more likely abodes are rock crevices, thick leaves, grass, thorns, bushes and cacti. Favorite dwelling include pandanus bushes (Kewda) and century plants (Agave).

Habits: Though Russell's vipers look sluggish, when provoked, they are capable of very fast movements in short spurts. They hiss loudly and bite in defense. They are timid and less likely to be commensal with man than the cobras and other streamlined snakes which are adept at quick escapes. Bites happen mostly to

plantation, estate and farm workers who incautiously put their hands or feet in dense bushes, or step on a viper in the dark.

Young: The female produces 20-40 living young mainly during May- July; these are exceedingly bright replica of the parents.

Food: The young are cannibalistic (in captivity) and will also eat other snakes, lizards and mice, land crabs and probably scorpions and other arthropods. Adults seem to be entirely rodent eaters, probably catching an occasional bird. In southern India, they mainly feed on the Jerbil (Tatera Indica).

Status: Russell's viper form a major resource of the skin industry in South India, and in some area has been completely exterminated through excessive all season collection.

Venom: The bite is considered one of the most dangerous of all Indian snakes and must be treated immediately with large amount of anti-venom. The very toxic venom affects the blood and is used in medicine to control bleeding and hematological studies.

- **Common Krait:**

The common krait is easy to recognize. Its bluish-black body has white cross bands and head is short and blunt. Kraits grow to 1.5 meters in length and are the most dangerous of the big four. Krait venom is ten times as powerful as that of the cobra. Of all Asian land snakes its venom is the most toxic.

Kraits are nocturnal. They are active at night and rest during the day. They are found throughout India and live mostly in sandy soil in rat burrows. Their favorite hiding places are piles of rubble and bricks which provide many nooks and crannies to shelter in. Kraits are cannibalistic, they eat snakes. They also eat rats, lizards and birds. The female lay about 10-15 eggs and like the cobra, stays with them until they hatch.

The fangs of the krait are short, and the venom is very toxic. Typical advanced symptoms (3 to 50 hours) of krait bite are the same as of the cobra with these exceptions-

1) Usually no local symptoms of pain or swelling (this is the misleading and dangerous part of krait bite).
2) Symptoms may not appear for several hours following the bite- their onset may be sudden and rapid.
3) There may be severe stomach and joint pains (6 to 12 hours). Krait bites happen usually at night. As the snake is rarely seen, it is always sensible to take nocturnal victims of snakebite to the nearest source of anti-venom treatment (doctor or hospital) without delay. There is a folk belief that, if bitten at night, the patient will die before daybreak. It was a serious krait bite and no anti-venom was given, it would be quite true.

- **Saw-Scaled Viper:**

The fangs of the saw-scaled viper are large for a little snake but are easily deflected by clothes or shoe leather. The venom has a powerful clotting action & is more toxic than that of the Russell's viper. Fortunately, the amount of venom injected is usually very small. Typical symptoms of severe saw-scaled viper bite are:

1) Burning pain (may be only slight); later pain in arm and leg joints.
2) Swelling within 2 hours, sometimes massive, especially in foot-bites if the victim has walked or run after bite.
3) Bleeding from gums; bleeding from cuts; blood in urine.
4) 12-24 hours later, evidence of internal bleeding.
5) Anemia, weakness from loss of blood, possible heart failure.

❖ **Table no. 6: OTHER POISONOUS SNAKES OF INDIA*23**

NAME OF SNAKE	DISTINCTIVE FEATURE	AVG. LENGTH	HABITS	PARITY	VENOM
Common Krait (Bungarus caeruleus)	Medium sized, glossy scales, Head is slightly wider than neck.	1 meter At birth- 25 cm Max. - 1.75 meter.	Mainly nocturnal Territory.	Oviparous	Neuroparalytic Fatal Dose- 6 mg
Banded Krait (Bungarus fasciatus)	Medium sized to large, smooth shiny scales, wide bright yellow & black bands on back.	1.5 meter At birth- 25-30 cm Max. 2.25 meter.	Nocturnal, Timid& mild tempered.	Oviparous	Venom is toxic but rarely bites.
Coral snake (Calliophis melanurus)	Small, slender, smooth shiny scales, blunt black head, tail black, scarlet & blue.	25-35 Diameter- 5 mm.	Nocturnal, good burrowers in sandy oils.	Oviparous	
King Cobra (Ophiophagus hannah)	Large, smooth shiny scales, distinct high cross bands on fore body.	3 meter At birth- 25-30 cm Max.- 2.25 meter	Timid, behave with intelligence and awareness.	Oviparous	Slightly less toxic cobra
Sea snake	Medium sized,	60 cm	Active	Oviparous	Rarely bites 4-

(Enhydrina schistose)	rough, dull scales, flattened tail flat and paddle shaped.	At birth- 15 cm Max.- 1.5 meter	both day and night.		8 times toxic than Cobra venom.
Saw scaled viper (Echis carinatus)	Small, strongly keeled scales, head wider than neck.	30-50 cm, At birth- 8 cm, Max.- 80 cm	Nocturnal, active on rainy night.	Viviparous	Bite is rarely fatal Fatal dose- 8 mg.
Bamboo pit viper (Trimeresurus gramineus)	Small, slightly keeled scales, Head triangular, Neck thin.	40 cm, at birth- 16 cm, Max- 80 cm.	Slow moving, active at night.	Viviparous	Venom is low in toxicity. So bites are rarely serious.

- **Snake Anatomy: *24**

Snakes have characteristically elongated body, a proportionately short tail and no limbs. An opening, known as vent, is present in the rear part of the body. This serves as a common orifice for the intestinal as well as genitourinary systems. The part behind the opening is called the tail, which is round in land snakes and flat in sea snakes.

The body is covered with scales. On the head there are 2 eyes, 2 nostrils and no external ear. The eye is covered by transparent scale, has a round or vertical pupil but no eyelid. The lower jaw consists of 2 bones in front joined by an elastic ligament. It is not properly articulated with the upper jaw so that the mouth of snake is widely distensible. This is an adoption for the mode of feeding because a snake may swallow creatures as a whole. The upper marginal teeth are modified to form fangs. When a fang is broken, its place is taken by a new one, which gets developed out of the fang buds in 3-6 weeks. The fangs are solid in non-poisonous snakes where as they bear a groove or channel in the poisonous ones. They are connected to the poison gland by means of a duct. The parotid salivary gland is modified in poisonous snakes to act as a venom gland. It is situated below and behind the eye and secretes toxic saliva, known as venom. The fang of snakes is forked at the outer end and often project out of mouth even when it is closed. It is primarily a sense organ to help the snake in its search for food, opposite sex and helping to detect the enemies.

The typical snake venom apparatus consists of paired venom glands- one on each side of head, below and behind the eyes – connected by ducts to hollow, anterior maxillary teeth. In vipers, these teeth are large, mobile fangs that retract against the roof of the mouth when the animal is at rest. In elapids and sea snakes, fangs are only slightly enlarged and are fixed in an erect position. For reasons that are unclear but may be related to venom apparatus itself, venomous snakes can bite without injecting any venom. Approximately, 20% of pit viper bites and an even higher percentage of bites inflicted by some other snake families (up to 75% sea families) are “dry”. Most colubrids have a different arrangement. Rather than use those modified salivary glands they use a larger gland known as the Duvernoy’s gland. This gland is situated right under the skin, above and near the angle of the jaw.

These glands open from a duct at the base of one or more posterior usually enlarged fangs that may or may not be grooved. These glands do not have a lumen (central storage chamber) so the snakes must give off a continuous stream of venom into their prey which means that they must continue to hold on to the animal to ensure envenomation.

True venom glands are made of thick connective tissue. They contain a lumen, a separate compressor muscle and a duct connecting them to a single fang on each side of the jaw. These glands dominate all elapids and viperids (along with some atractaspidids).

Immunodiagnostic techniques have been developed for species identification of the snakes involved in bites. An Enzyme Linked Immune Sorbet Assay (ELISA) can be used to identify a specific type of snake venom in a victim's blood, wound aspirate, or urine and this method is finding clinical application around the world.

- **Snake Venom*25:**

Venom is clear, amber-colored digestive juice of snake. The venom on drying forms fine needle like crystals, which are easily soluble in water.

Snake venoms are complex mixtures of enzymes, low molecular weight polypeptides, glycoprotein and metal ions. The enzymes and polypeptides affect the human body in a multi system fashion. Among the deleterious components are hemorrhages that render the vasculature leaky and thus cause both local and systemic bleeding. Various proteolytic enzymes that cause local tissue necrosis, affect the coagulation pathway at various steps or impair organ functions, myocardial depressant factor that reduce cardiac output and neurotoxins that act either pre or post-synoptically to inhibit peripheral nerve impulse. Most snake venoms can adversely affect multiple organs.

They may be in the form of enzymes like phospholipase RNAase, DNAase, hyalurenodase, acetycholine stearase, collagenase, proteases, erepsin and pulse-faction. Leuketrienes, kinins, histamins, serotonin may be present in the venom, imparting a number of functional abnormalities in the various systems in body.

Certain unidentified complex components may be coagulants, anticoagulant cardiotoxins, haemotoxins and neurotoxins.

Venom may cause damage to skin, blood vessels, blood cellular components, nerve transmission and myo neural junctions. The content and toxic potential of venom vary in various families of snakes and also in a given snakes with size, age, climate etc.

Source: It is the toxic saliva secreted by modified parotid gland of venomous snake.

Constituents:

Toxins: Mostly peptides & proteins of low molecular weight.

1) **Enzymes:** Proteinases, hydrolases, transaminase, hyalaronidase, cholinesterase, phospholipase, ATPase, ribonuclease, deoxyribonuclease, etc.
2) **Miscellaneous:** Agglutinins, proteolysins etc.
   Nature of venom: Neurotoxic, haemotoxic, Myotoxic.

- **Cobra Venom:**

When freshly ejected, it is a light amber colored liquid like clear varnish. Specific Gravity= 1046. It is weakly acidic.

It drives rapidly in the air into a thin yellowish film, which becomes scaly. It is water soluble & upon dissolution is highly toxic.

When it is dry, it has an acrid odor, is active for several years & retains potency upon being heated to 125˚C. The toxic element is contained in the non coagulation protein. Neurotoxins of cobra venom are rapidly absorbed into blood stream.

Postsynaptic neurotoxin such as α bangarotoxin & cobrotoxin contain about sixty short chain or 60-70 long chain amino acid residues & bind to acetylcholine receptors at the motor end plate.

Cobra snake venom reduces significantly tissue nucleic acid levels in human breast cancer. (J. Park Med. Assoc.2005.JokhioR, Ansari AF)

Cobra Venom contains a pool of cystein rich secretary proteins. (biochem Biophys. Res. Commum.2005).

- **Viper Venom:**

It contains a strong blood coagulant, which is responsible for fatality. Larger molecules of viper venom taken up more slowly through lymphatic.

- Snake venom may contain 20 or more components. More than 90% of dry weight is protein in the form of enzymes, non- enzymatic polypeptides toxins and non-toxic proteins.
- Phospholipase A2 (lecithinase) is the most spread enzyme which may contribute to myotoxicity, neurotoxicity, cardiotoxicity, haemolysis and increased vascular permeability.
- Hyaluronidase enzyme promotes spread of venom through tissues.
- L-amino acid oxidase is responsible for bright yellow color of some viper venoms.
- Toxic phospholipase A2 can block neuromuscular transmission by acting pre or post viper venoms.
- They include presynaptic (β) neurotoxins, β-bangarotoxin, crotoxin and taipoxin.
- Presynaptic neurotoxins block the release of acetylcholine at the neuromuscular junction.
- Swelling and bruising of bitten area result from increased vascular permeability induced by protease, phospholipases, membrane damaging polypeptide toxin and endogenous autocoids released by the venom such as histamine, 5 hydroxytryptamine and kinins.
- Tissue necrosis near to the site of bite is caused by myotoxic & autolytic factors.
- Snake venom can cause haemostatic defects through pro coagulants which can activate the blood clotting cascade at various sites. Some viperidae venom contains fibrinogenous which degrade fibrinogen directly and others activate endogenous plasminogen.
- Spontaneous systematic bleeding is caused by haemorrhagins which damage vascular endothelium. The combination of defibrination, thrombocytopenia & vessel wall damage can result in massive and continuous bleeding, a common cause of death after viper bite.

Although Cytotoxic, Neurotoxic and Haemolytic action of snake venom are well known, airborne inhaled snake venom of spitting cobra is known to induce asthma in snake handlers.

Aerolized snake venom is considered a new potential source of allergens that may result in anaphylaxis on subsequent exposure.

Development of specific Ig E sensitization following snakebites and risks of such sensitization should be conducted on snake handlers, particularly those who demonstrate the spitting species.

- **Practical application of snake venom toxins in haemostasis:**

a) Snake venom thrombin like enzyme (SVTLE) is used for fibrinogen breakdown product assay & for detection of fibrinogen dysfunction. SVTLE are not inhibited by heparin & can thus be used for assaying antithrombin III & other haemostatic variables in heparin containing samples.
b) Snake venom is reach source of prothrombin activators & there is utilized in prothrombin assay, for studying dysprothrombinaemias & for preparing meizothrombin & non-enzyme forms of prothrombin.
c) Russell's viper venom contains toxins which have been used to assay blood clotting factors V, VII, X, Platelet factor 3 & importantly, lupus anticoagulants (LA). (Toxins-2005, Jun- Marsh N. Williams V.)

- **Diagnostic Uses of Snake Venom (Haemostasis 2001 Marsh N.A.):**

a) Thrombin like enzymes (SVTLE) are used for fibrinogen and fibrinogen breakdown product assay as well as detecting disfibrinogenaermias.
b) Since SVTLE are not inhibited by heparin, they can be used for assaying antithrombin III in samples containing heparin.
c) Snake venom prothrombin activators are utilized in prothrombin assays.
d) Russell's viper venom (RVV) can be used to assay clotting factor V, VII, X and lupus anticoagulants.

e) Protein C and activated protein C resistance can be measured by means of RVV.

f) Snake venom C type lectins & metalloproteinase disintegrins are being used to study platelet glycoprotein receptors & show great potential for use in routine coagulation laboratory.

- **Muscarinic toxin like proteins from Cobra Venom (Eur & Biochem 2000):**

Three new polypeptides were isolated from venom of cobra (Thailand) and their amino acid sequences determined. They consist of 65 amino acid residues and have 4 disulphide bridges. A comparison of the amino acid sequences of new polypeptides with those of snake toxins show that 2 of them share a high degree of similarity (55-74% Sequence Identity) with muscarinic toxicity of mamba. The third polypeptides (MTLP-3) B similar to muscarinic toxins with respect to position of cysteine residues and the size of disulphide confined loops but less similarly to these toxins (30-34 % sequence identity).

- **Table no. 7: Biochemistry of Snake Venoms* 26**

Sr. No.	**Toxins**	**Family**	**Mechanism of injury/ death**
1)	Neurotoxins	Elapidae, hydrophidae, Palestine viper	Respiratory Paralysis
2)	Cardiotoxin	Elapidae	Cardiovascular depression
3)	Enzymes Phospholipase A 5-Nucleotidase, Adenosine Triphosphatse, Exopeptidase	Elapidae, hydrophidae, viperidae	Haemolysis
4)	Proteases	Viperidae	Hypotension

Types of snake Bites:*27

1) Two clear puncture wounds with or without small marks of other teeth.
2) Local skin may be scratched or lacerated because of deviation of fangs by clotting or movement of victim.
3) Edema or discoloration present around site of bite.

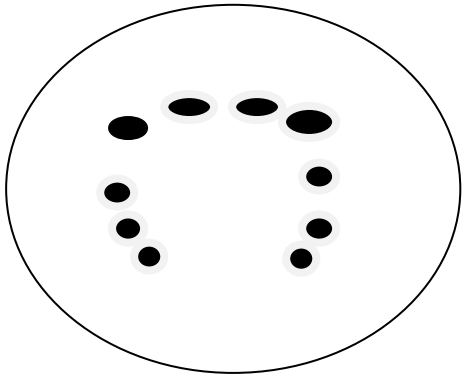


Fig.: Teeth marks of Non- Poisonous Snakes

- **EPIDEMIOLOGY OF SNAKE BITE:**
  1) **World:** About 5.4 million snake bites occur each year, resulting in 1.8 to 2.7 million cases envenoming (poisoning from snake bite). There are between 81,410 to 1,37,880 deaths and around three times as many amputations and other permanent disabilities per year.
  2) **Other Nations:**
     a) In a world survey, Swaroop & Grab recorded yearly death rates per 100000 populations as Burma 15.4, Pakistan 14.2, India 5.4 and Thailand 1.3.
     b) Large number of snake bites cases occurs in Burma & Brazil. In United States it is estimated at 8000 per year.
  3) **India:** Around 40900 to 50900 death occurs in India per year*2. And around three times amputations and other permanent disabilities are caused by snake bites annually.

4) **States:** In India most snake bites occur in Kerala, Bengal, U.P., Rajasthan, Maharashtra and Karnataka.
5) **Area:** Snake bite is a rural and occupational hazards.
6) **Sex:** The risk to male is more than twice that of female.
7) **Age:** There is increasing liability to snake bite with aging until the fifties.
8) **Diurnal variation:** Two thirds of the snake bites occurring in day light.

➢ SIGNS AND SYMPOTOMS OF SNAKE BITE:

The most important point to note is that the majority of snake bites involve non-venomous snakes. Even in venomous snake bites over half of the cases show only mild manifestation because of inadequate venom injection. However, because of fear associated with snakes, the bitten victim is usually convinced he has no chance of survival and goes into a state of profound shock. This neurogenic shock is the commonest manifestation of all snake bites and is characterized by semiconscious state, cold and clammy skin, feeble pulse and rapid, shallow breathing. All these will resolve dramatically in non-venomous bite if the physician administers a placebo injection.

❖ Sign and Symptoms of non-venomous Snake-bite:

- Fright- It is the most common symptom.
- Lack of pain and local swelling
- Fear of rapid and unpleasant death
- Semiconscious due to fear
- Cold, clammy skin
- Feeble pulse
- Rapid breathing
- Psychological shock and even death.

❖ Degree of Toxicity of Snake Bite Depends upon:

- Age and size of bitten person
- Potency of venom

- Main toxic principles of venom
- Amount of venom injected
- Nature, location, depth and number of bites
- Length of time the snake hold on
- Extent of anger or fear that motivates snake
- Species and size of snake
- Condition of fangs and venom glands
- Victims sensitivity to the venom
- First aid and medical care provided or gets to patient.

❖ SYMPTOMS OF COBRA(ELAPID) BITE:

- **Local features:**
  - Indistinct fang marks
  - Burning pain
  - Swelling
  - Discoloration of skin
  - Serosanguinous discharge

- **Systemic Features:**

Pre-paralytic stage	**Paralytic Stage**
Vomiting	Ptosis
Headache	Opthalmoplegia
Loss of consciousness	Drowsiness
	Dysarthria
	Dysphagia
	Convulsions
	Bulbar paralysis
	Respiratory failure

- **Symptoms appear after 30 minutes:**
  - Patient feel sleepy
  - Slightly intoxicated

- Weakness of legs
- Nausea, vomiting
- Weakness of muscles increase paralysis of lower limb
- Paralysis then spreads to the trunk and affects head
- Eye lids hang down (Ptosis)

- **After 30 min. – 1 hour:**
  - Excessive salivation and vomiting
  - Paralysis and swelling of tongue and larynx
  - Difficulty in speech and swallowing
  - Extra-ocular muscle weakness
  - Ptosis
  - Strabismus

- **After 2 hours:**
  - Complete Paralysis
  - Respiration slowed
  - Heart rate increased
  - Unable to speak though the patient is conscious
  - Coma
  - Convulsions may be present or not

➢ **SYMPTOMS OF RUSSELL'S VIPER BITE:**

❖ **Local Features:**

- Rapid swelling with discoloration
- Blister formation over bitten limb
- Swelling over trunk
- Frank bleeding from bite site
- Severe pain

❖ **Systemic Features:**

- Generalized bleeding tendency e.g. Epitasis, Hematuria, Hemoptysis, Malena, Bleeding gums, Purpuric spots on skin.
- Renal failure

- More than 50% of the victims have minimal or no poisoning
- About 25% will develop serious generalized poisoning
- Death is Rare.

❖ **Local Symptoms:**

- Within 8 min.
  - Spot develops several pain
  - area around bite becomes red and painful
- Within 15 min.
  - Onset of swelling starts
  - Blood stained discharge from wound

❖ **In Moderate Poisoning:**

-Marked feeling of intense pain.

-Pupils- Dilation and insensitive to light in about 1-2 hours.

-Skin- Temperature raised.

-Tingling and Numbness over tongue and mouth or scalp.

-Paraesthesia around wound

-Extravasation of blood.

-Swelling spreads over the trunk within 1 to 2 days.

-Tenderness Absent

- **Within 1 to 4 week:**

-Swelling and discoloration subsides

-Blisters heal without necrosis

❖ **In Severe Poisoning:**

-Persisting shock is the main feature

-In blood- early hemoconcentration occurs

-Red cells decreased

-B.T. / C.T. – Prolonged

-Platelets- decreased

-Urine contains- Blood, Sugar Proteins

-Hemorrhagic syndrome

-Blood stained sputum

-Hemorrhages from gums, rectum

-Bleeding from site of bite

-Intravascular Hemolysis lead to hemoglobinuria, Renal failure

-Petetchial hemorrhage is very common.

- **Systemic symptoms:**

-Respiratory depression increased

-Blurring of vision

-Headache

-Dizziness & Weakness

- **Towards End:**

-Extensive suppuration and sloughing

-Malignant edema of bitten area

-Paralysis does not occur.

Snake	**Usual Fatal Dose**	**Approximate yield of venom per bite**
1) Common cobra	12 mg	170-300 mg
2) Russell's viper	20 mg	130-250 mg

- **LABORATORY FINDINGS*28:**

❖ **Within the first few hours:**

- Drop in platelets due to local consumption
- Decrease in fibrinogen
- Increase in fibrin degradation products
- Increase in Prothrombin time
- Erythrocyte show a peculiar “burring” indicating membrane damage
- Drop in hematocrit and hemoglobin concentration.

❖ **In Viper envenomation baseline investigations are**

- Complete blood count with platelet count
- Prothrombin time & partial thromboplastin time
- Bleeding time
- Urine analysis
- Serum electrolytes
- Arterial blood gas analysis
- E.C.G.

Hematological studies should be repeated every four to six hours for the first day. ELISA test for diagnosis of snake species is now available.

➢ MANAGEMENT OF SNAKE BITE POISONING:

Most of snake bites occur in remote places from where it is difficult to come immediately at Rural Hospital for proper treatment. At such a time, we have to give first-aid treatment to snake bite patient as follows:

❖ Assurance and Reassurance:

Most of deaths in snake bites occur due to extreme frightening. Many times, it is non-poisonous snake bite but due to frightening patient may die, that's why assurance & reassurance plays an important role in first aid treatment of snake bite.

Reassure the patient by stating the facts that all snakes are not poisonous, that even poisonous snakes are not always fully charged with the poison and that even a poisonous snakes fully charged with the poison cannot always inject a lethal dose.

❖ Keep patient warm & at rest:

Activity may enhance the spread of venom. The patient is not to run, walk and even be removed to another place.

❖ Immobilization:

Snake venom mostly spread in body mostly by diffusion & lymph circulation. Therefore efforts to reduce lymph circulation are helpful and this can be achieved by immobilization of bitten limb. Immobilize the affected part in functional position.

If possible, kill the offending snake & preserve for identification. For pain, a mild, non sedating analgesic can be administered (e.g. Paracetamol). Do not give aspirin which can aggravate bleeding tendency.

❖ Tourniquet Application:

Tourniquet means a strip of belt, rope or dhoti that is tied tightly around the part of body where snake bite was occurred.

- Apply on proximal part i.e. towards heart, 2-4 inches above the bite.
- Do not apply on joint, head, neck, trunk.

- It should not be too tight or too loose. It should be capable of admitting a finger beneath it.
- Pulse should be checked and trained distal to it.
- Pressure of tourniquet should be light enough to occlude superior venom and lymphatic circulation without impending the arterial or deep venom blood flow.
- It should be released for 30 seconds after every 10 min.

Disadvantages of Tourniquet:

- It can cause ischemic gangrene if pressure is too tight.
- It leads to damage to superficial nerves.
- It increase swelling and bleeding from occluded limb.
- Due to application of tourniquet, local effect of venom is more.

Wash off venom on the surface of skin or in the wound with water. Wipe the bite site and cover loosely with a piece of clean cloth.

❖ **Incision & Suction:**

As far as possible avoid incision & suction since it increase the chance of wound infection, aggravates bleeding & may cause accidental severance of nerves, tendon etc. It may be done if.

- Medical help is more than 1 hour away.
- Done within a few minutes of the bite.
- Parallel incision must be made through fang marks about 1 cm long and 3 mm deep.
- Use of commercial suction device rather than suction by mouth, because any minute cut or wound in oral cavity can lead to poisoning.

Disadvantages of Incision & Suction:

- It produces ulcers and spread infections.
- Aggravates bleeding due to suction.
- May cause accidental severance of nerves, tendons etc.

- **Avoid Cauterization:** As it seals the poison within tissue.
- **Don't do cryotherapy:** It may cause necrosis of tissues.
- Do not give alcohol or apply Potassium Permanganate. Excitement, palpitation and quickening of circulation with consequent quickening of diffusion of venom must be stopped. Alcohol is withheld for the same reason.

❖ **Specific Therapy:**

Concentrated antisera, anti-venins are now available. Antivenin is indicated when there are signs of systemic bleeding (from nose, gums etc), prolonged clotting time, thrombocytopenia, signs of neurotoxicity, impaired consciousness. Antivenom is also indicated when there are definite signs of local envenomation, local swelling, and blistering signs of necrosis.

Give Tetanus toxoid to all previously immunized patients otherwise give ATS/ Tetanus toxoid.

Two Types of Anti-snake venom are:

i) Mono valent Anti-snake Venom.
ii) Poly Valent Anti- snake Venom serum (PVASVS).

- **Mono Valent Anti Snake Venom:**

It is prepared by hyper immunized horses against the venom of particular snake.

- **Poly Valent Anti-snake venom(PVASVS):**

Snake antivenin is of equine origin derived from the plasma of the horses, ponies, mules etc that have been hyper immunized against the venoms of the four most common venomous snakes of India

namely 1) Indian Cobra (*Naja naja*), 2) Common krait (*Bungarus caeruleus*), 3) Russell's viper (*Vipera russellii*) and 4) Saw scaled viper (*Echis carinatus*). Serum, obtained from the plasma contains purified, enzyme- refined and concentrated specific/ heterologus immunoglobulins. It is used as a passive immunizing agent and affords protection to the susceptible victims against the bite of the snake species mentioned above.

- **Strength of PVASVS 1 ml neutralizes:** 0.6 mg of dried cobra venom, 0.45 mg of dried krait venom, 0.6 mg of dried Russell's viper venom, and 0.45 mg of dried saw scaled viper venom. The moisture content in the Lyophilized Snake Antivenin does not exceed 1%. Potency of PVASVS is for ten years.

- **Indications:** The snake antivenin is indicated for all bites caused by Indian cobra, Common krait, Russell's viper and Saw scaled viper; where the patients presents with clinical signs and symptoms of envenomation.

- **Precaution to be observed before administration of Snake Antivenin:**
    1) Elicit history of familiar allergic disorders such as Asthma, Eczema, drug allergy from the patient
    2) Whether he had received earlier, injection of serum such as anti tetanus serum, anti diphtheria serum etc.
    3) Carry out the sensitivity test on the patient, inject subcutaneously 0.1 ml. Of the serum diluted 1:10. Observe the patient for 30 minutes for local or general reactions, if any. In the absence of adverse reaction administer the requisite dose by chosen root of injection.
    4) Keeps handy injection Adrenaline (Epinephrine) 1 ml of 1: 1000 along with antihistamines & steroids to meet any emergency arising out of sensitivity reaction.
    5) After administering the full dose of serum, the patient should be kept under observation for at least 30 minutes.

6) If patient is found sensitive to the equine antiserum, you may desensitize him by administering graded doses of the antiserum at regular & adequate intervals. Before desensitizing the patient, the doctor has to decide whether serotherapy is really needed.
7) In sensitive individuals where time factor is of paramount importance, it is advisable to administer Snake Anti venin under the cover of Inj. Adrenaline (Epinephrine) 1 ml. (1: 1000) IM and antihistaminic, without awaiting result of the test dose.

- **Contraindications:**

  There is no known contraindication for the administration of Snake Antivenin.

- **Adverse Drug Reactions:**

  This serum being heterologous is liable to cause sensitivity reactions in occasional patient. The immediate reactions is anaphylactic shock (immediate hypersensitivity) characterized by sweating, pallor, bronchospasm, laryngeal spasm, hypotension leading to shock, coma and death.

  Treatment contains prompt administration of injection epinephrine, steroids and anti-histamines along with endotracheal intubation, oxygen therapy and treatment of shock.

  A late serum sickness like syndrome (delayed hypersensitivity) occurring seven to eight days after serum administration consists of fever, rashes, lymphadenopathy and arthralgia. This is usually self limiting & does not required treatment.

  If the precaution mentioned above is followed, there is less likelihood of any of these reaction occurring.

- **Reconstitution of Lyophilized Antivenin**

Draw 10 ml of sterile water for injection in a clean, sterile syringe. Break open the central ring of the seal of the vial & transfer the contents of the syringe into the vial, shake well & allow the powder to dissolve completely.

Allow it to stand about a minute to get crystal clear solution.

The solution is now ready for administration. Froth & undisolved particles, if any, should be left in vial. For the second and subsequent injection, more time is available to dissolve the serum. For this, add 10 ml sterile water for injection to the serum vial & rotate it between the palms of your hands until the serum is fully dissolved & let the vial stand for serum to clear.

- **Dosage & Administration:**

Prior to administering the snake antivenin it is obligatory to observe precaution & carry out sensitivity test as mentioned above. The usual mode of administration of snake antivenin is either by intramuscular or intravenous route. The actual dose of snake antivenin, to be administered to the patient varies according to site of bite, severity of bite, age and physical status, degree of envenomation, involvement of systemic organs and time factor of initiation of treatment.

- **A suitable dosage schedule recommended is as follows:**
  - **Table no. 8: Dose Schedule of Antivenin Administration*29**

No. of hours after administration	**Clotting time (Lee-white method)**	**Lyophilized Polyvalent Antivenin 1 vial= 10 ml**
0 hour	Normal	No treatment
1 hour	More than 10 min.	2 vials of antivenin in 100 to 500 ml fluid infused in 2 hours
3 hours	More than 10 min.	2 vials of antivenin in 100 to 500 ml fluid infused in 3 hours
6 hours	More than 10 min.	2 vials of antivenin in 100 to 500 ml fluid infused in 3 hours

Special Note: In severely envenomed patient, initial dose of 10-20 ml of Antivenin may be administered in bolus, provided the patient is not sensitive to antivenin.

The above regimen is to be continued for 3 hours. There after till clotting time is less than 10 min. And the clot is firm. After completing the above regimen, 2 vials of antivenin are infused in 500 ml. fluid and given in 24 hours. In case of viper-bite, some of the antivenin may be infiltrated around the site of bite with extreme caution. In all cases of snake bites, antivenin is never discontinued till all the signs of envenomation disappear and clotting time is less than 10 min.

- **Storage:** Being lyophilized, the snake antivenin is stable at room temperature and does not require special storage facilities ideally, it should be stored in a cool and dark place.

❖ **Atropine- Neostigmine Therapy:**

Neurotoxin- Carbotoxin and alphabungarotoxin found in elapid venoms which produce neuro-muscular block causing neuro-muscular paralysis. This can be reserved using Neostigmine which is anti-cholinesterase (cholinesterase inhibition). Best results obtained as soon as neurological signs-symptoms appear and while continuing till there is complete, neurological recovery. Neostigmine is given in doses of 0.5 mg I. V. 11/2 vials for first 5 doses and thereafter the interval increased depending upon clinical response. Atropine 0.6 mg I.V. usually precedes Neostigmine to block the muscarinic side effects of latter on gland cells, smooth muscles and heart.

❖ **Heparin:**

The primary treatment of DIC is that of underlying cause. But when the primary disease can't be treated effectively- heparin will inhibit intravascular clotting and elevate clotting factor level.

❖ **Supporting Treatment:**

- 0.5 ml I.M., TT or ATS should be given.
-To recover the secondary infection, broad spectrum antibiotic should be given.
-Volume expanders including plasma and blood are recommended in shock.
-Mechanical ventilation is advocated in Respiratory failure.
-In case of local necrosis of bitten area, surgical debridement is necessary.

❖ **Acute Renal Failure:**

1) First try to correct the fluid and electrolyte imbalance.
2) Administration of I.V. Frusemide 1 mg/kg also as a preventive step after correction of local dehydration. Urine output below 600 ml/day and raised urea and creatinine values suggestive of renal failure that should be referred to dialysis.

REVIEW OF *ASHWAGANDHA (Withania somnifera)*

❖ HISTORICAL REVIEW:

➢ Samhitaa Kaala:

In this time period, the Ayurved was at its developing age. Basic and the most important ayurvedic texts were compiled in this period.

A) Charaka Samhitaa:

a) In third chapter of *Sutrasthana* i.e. *Aaragvadhiyaadhyaya, Ashwagandha churna* is included as *Kushthaghna.*

b) Along with this, *Ashwagandha* is included in the following 2 *Mahakashayas* of the 4th chapter of *Sutrasthana. 1) Bruhaniya 2) Balya.*

B) Sushruta Samhita:

In *Sushruta samhitaa, Ashwagandha* is the main content of the following *Gana.*

a) Urdhva bhagahar

b) Vranropana

c) Vranautsadana

d) Kaphashodhaghna

➢ *Samgraha Kaala:*

It is considered as the golden period of the Indian Medicine. Important works in this period are *Ashtaamga Saamgraha* and *Ashtaamga Hridaya*. Similarly, various *Nighantus* were also composed.

A) Ashtaamga Samgraha:

a) In 15th chapter of *Sutrasthana* i.e. *Mahakashayasamgraha Adhyaya, Ashwagandha* included in *Bruhaniya Gana* and *Balya Gana.*

b) In 14th chapter of *Sutrasthana* i.e. *Shodhanadiganasamgraha Adhyaya, Ashwagandha* is included in *Vamanopyogi gana.*

B) Ashaamga Hridaya:

Ashwagandha was not included in any *Gana* described in 33 *ganas* of *Shodhanadi Gana Samgraha Adhyaya*.

- ***Madhyama Kaala:***

A) Shaaramgadhara Samhitaa:

In *Shaaramgadhara* there are many medicinal preparations in which *Ashwagandha* was used as key ingredient.

a) Ashwagandhadi Churna- It is mentioned in *Madhyama Khanda Churna Kalpanaadhyaya* as *Vajikara Kalpa.*

b) *Kamdev Ghrita- Ashwagandha* is the main content of this *ghrita* and is useful in *Raktapitta, Kamala, Shukrakshaya*.

c) Narayan Tail- Narayan tail is used as *Abhyanga, Pan, Basti* in all *Vata roga.*

Other preparations like *Baladi taila, Shatavari tail, Madankamdev rasa* also contents *Ashwagandha.*

B) Yogaratnakar:

There are many references of *Ashwagandha* in *Yogratnaka* e.g. *Trayodashanga Guggula, Yograj Guggul, Vajigandhadi kwatha, Aabhadi Churna, Mahavishagarbha Tail, Narayan Tail, Shatavaryadi yoga* etc.

C) Nighantu:

In *Nighantus*, detailed information like Synonyms, Classification, Pharmacological actions etc are described of all available herbs. They are classified according to groups and they are termed as *Varga*. The list of various *Nighantus* along with various *Varga* in which *Ashwagandha* is included is listed below.

❖ **Table no. 9: *Nighaṅt'us* and *Varga* containing *Ashwagandha***

Name of *Nighantu*	***Varga***
Raja Nighantu	*Shatavahadi*
Nighantu Aadarsh	*Kantakaryadi*
Bhavaprakash	*Guduchyadi*
Shaligraama	*Guducyadi*
Kaiyyadev	*Aushadhi*
Priya	*Shatapushpadi*
Dhanvantari	*Guducyadi*

❖ **Modern Period:**

A lot of research has been conducted on *Ashwagandha* with respect to its pharmacological action. The Physical and chemical properties and other standardization criteria are also available. Important Books of Botany, Pharmacognosy, Journals and Websites and Research Papers were reviewed for the detail information regarding *Ashwagandha*.

After conducting a review of these sources, following information from the ancient time to the current date is available.

- **Vernacular Names:**

Ashwagandha has been mentioned by different names in different regions. Below are given regional names according to region.

❖ **Table no. 10: Vernacular names**

Region	Vernacular Name
English	Winter Cherry
Hindi	Asagandha, Asgandh
Marathi	Dhorgunja
Bengali	Ashwagandha
Gujarati	Asandh, Ghoda Aahan
Tamil	Chuvadigam, Aamkulang
Telugu	Piniru
Kannad	Angarveru
Malyaalam	Amukkura
Farasi	Behamnavari
Punjabi	Asgandh
Sanskrut	Ashwgandha
Urdu	Asgandanagaori
Arabic	Hajarat el dib

- **Synonyms:**

Various synonyms have been used in texts for *Ashwagandha* among which some signify its external features, some signify its properties and some signify its action.

❖ **Table no. 11: Synonyms**

Synonyms	Meaning
Ashwagandha	Plant smells quite like hoarse dung
Hayagandha	Plant smells quite like Hoarse dung
Vajigandha	Plant smells quite like Hoarse dung
Turagi	Useful to improve sexual performance
Vajikari	Can perform sex like hoarse
Balada	Improves strength
Varahakarni	Its leaf looks similar to ear of pigs
Vajinama	Named according the name of *Ashwa*

Some other synonyms are as follows-

Gandhanta, Varada, Vanaja, Vajini, Pushpada, Punya, Pivara, Palashaparni, Vataghni, Shamala, Kamrupini, Kalapriya, Gandhapatri, Hayapriya, Varahaputri, Vajikari, Kanchukya, Gokarni, Vrusha.

- **Classification- According to Modern Science (Botanical information):**

Taxonomy:

Kingdom- Plantae

Subkingdom- Angiosperms

Division- Eudicots

Class- Asterids

Order- Solanales

Family- Solanaceae

Genus- Withania

Species- somnifera

- **Geographical Distribution:**

Withania somnifera is cultivated in many of the drier regions of India such as Mandsaur District of M.P., Punjab, Sindha and Rajasthan. *Withania somnifera* plant can easily survive & grow in almost all parts of South India. Apart from India, it is also found in Sri Lanka, Afghanistan & Nepal.

- **Key Characters of Solanaceae Family:**

1. The Solanaceae family ranges from annual & perennials herbs to vines, lianas, shrubs & trees.
2. The Solanaceae family consists of about 98 genera & some 2700 species with great diversity of habitats and morphology.
3. The leaves are generally alternate to opposed (i.e. alternate at the base of the plant & opposed towards the inflorescence).
4. The flowers are generally hermaphrodites, although some are monoecious, andromonoecious or dioecious.
5. The calyx is gamosephalous (as the sepals are joined together forming a tube)

6. In the majority of species the flowers have a differentiated perianth with a calyx & corolla, an androecium with five stamens & 2 carpels forming a gynoecium with superior ovary.
7. Gynoecium is bicarpelar (rarely 3 or 5 locular) with superior ovary & two locules.
8. The fruits of the great majority of the Solanaceae are berries or capsules and less often drupes. The seeds are usually endospermic, oily and without obvious hairs.

- **External Morphology:**

In Ayurvedic texts very brief description about *Withania somnifera* is found. In Botanical texts following description are mentioned.

1. A short, tender perennial shrub.
2. Growing 35-75 cm(14-30 inch) tall
3. Tomentose branches extend radially from a central stem.
4. Leaves are dull green, elliptic, usually up to 10-12cm long.
5. Flowers are small, green, and bell shaped.
6. The ripe fruit is orange-red.

- **Chemical Constituents:**

1. The main active constituents are alkaloids and steroidal lactones. These include tropine and cuscohygrine.
2. The leaves contain the steroidal lactones, withanolides, notably withaferin A, which was the first withanolide to be isolated from Withania somnifera.
3. Tropine is a derivative of tropane containing a hydroxyl group at third carbon. It is a building block of atropine, an anticholinergic drug.
4. Cuscohygrine is a pyrrolidine alkaloid found in coca. It can also be extracted from plants of the family Solanaceae as well.

- ***Prayojya Amga*** **(Useful Part):**

Root, Leaves and *Kshar* of *Withania somnifera* are used in medicinal preparations.

- ***Rasa pamchaka*** **(Properties):**

Rasa- Madhur, Kshaya, Tikta.

Veerya- Ushna

Vipaka- Madhura.

Guna- Laghu, Snigdha.

Prabhava- no specific *prbhava*.

- **Karma: Action on Tridosha**

Vaata- Vaataghna (R.N., N.A., B.N., D.N., S.N., M.N., P.N.)

Kapha- Kaphaghna (N.A., B.N., D.N., S.N., M.N., K.N.)

- **Pharmacological Actions:**
  - **According to Ayurved:**

Krumighna (S.N.,K.N.)

Kushthaghna (B.N., S.N., M.N., K.N)

Shvasaghna (R.N.,S.N.,K.N.)

Shothaghna (B.N.,S.N.,M.N.)

Vatavyadhihar (R.N.,N.A.)

Galgandahar (S.N.)

Vishaghna (S.N., K.N., D.N.)

On the basis of above mentioned pharmacological action it is used in many medicinal preparations.

- **According to Modern Science:**

1. Anti Cancerous Activity- *Ashwagandha* is a proud herb of Ayurved having great anti-tumorogenic activity against various cancer cell due to the presence of Withaferin A, a withanalide derived from this medicinal plant.*30
2. Anti Stress Activity- Researchers using *Ashwagandha* discovered the animals given the herb an hour before the electric shock to foot experienced a significantly reduced level of stress. *31
3. Anti inflammatory Activity- This activity of *Ashwagandha* has been attributed to the naturally occurring steroids, of which withaferin A is a major component and is as hydrocortisone sodium succinate dose, anti inflammatory drug.*32
4. Antibiotic Activity- The antibiotic activity of the roots as well as leaves has recently been shown experimentally. Withaferin A inhibited Ranikhet virus. The shrubs extract is active against Vaccinia virus and Entamoeba histolytica. *33
5. Anti diabetic effect- *Ashwagandha* root and leaf extract show hypoglycemic and hypolipidemic effect on alloxan-induced diabetic rats.*34
6. Antioxidants- *Withania somnifera* acts as a powerful antioxidant by increasing the level of three naturally occurring antioxidant enzyme like superoxide dismutase, catalase and glutathione peroxide in the brain of rats.*35
7. Anti aging activity- *Ashwagandha* was tested for its anti aging properties in a double blind clinical trial. A group of 101 healthy males, 50-59 years old were given the herb at a dosage of 3 grams daily for one year. The subjects experienced significant improvement in hemoglobin, red blood cell count, hair melanin and seated stature. Serum cholesterol decreased and nail calcium was preserved. Seventy percent of the research subjects reported improvement in sexual performance.*36
8. Immunomodulatory effect- A series of studies conducted on animals showed that *Withania somnifera* has a profound effect on hematopoietic system by acting as an immune regulator and chemo protective agent.*37,38

- **INDICATION:**

In classical Ayurvedic texts, *Withania somnifera* is indicated in following diseases mentioned according to *Srotasa.*

❖ **Table no. 12: Indicated diseases according to *Srotasa***

Srotasa	**Diseases**
Praanavaha	*Kaasa, Shwaas*
Annavaha	*Parinamshool, Krimi, Agnimandya, Aruchi.*
Udakavaha	*Udar*
Rasa	*Hrudya, Hrudayottejaka*
Raktavaha	*Kushtha, Raktashodhaka, Shotha, Shwitra.*
Mamsavaha	*Karshya, Dourbalya.*
Shukravaha	*Shukrakshaya*
Mutravaha	*Mutraghna*
Aartavavaha	*Yonishool, Shwet pradar*

- ***MATRA*/ DOSE:**

Churna of roots- 3-5 gm

Kshar- 1-2 gm

- ***AAMAYIKA PRAYOGA-***

1) *Ashwagandha churna* if taken with milk increase the *Dhatus* in body.
2) In *Dhatukshaya* condition, *Ashwagandha churna* is taken with *ghrita* and honey.
3) For the *Stanyajanana* action, decoction of *Ashwagandha, Bhoi kohala* and *Yashtimadhu* should be taken with Cow's milk.
4) *Ashwagandha kalpa* is used in *Unmada, Rajyakshma, Nidranasha* and *Shukrakshaya.*
5) In *Sandhishula, Ashwagandha churna* is taken with *ghrita* and sugar.
6) In *Rajyakshma,* decoction of *Ashwagandha* is taken with *Ashwagandha churna.*
7) In *Anidra, Ashwagandha* is very important medicine.
8) *Ashwagandha lepa* is beneficial in *Vrana, Dushta Vrana* and *shotha*.
9) *Ashwagandha churna* and *Chopchini churna* in same quantity taken with *Ghrita* and honey to purify the blood.

MATERIALS AND METHODS

MATERIALS:

Instruments, chemicals and other items used in the experiment were described under materials. First of all, the list of materials that were used in the preparation of *Ashwagandha (withania somnifera)* root *churna* was given below.

a) Cutter
b) *Ashwagandha (Withania somnifera)* root
c) Plate
d) Blender
e) Filter
f) Electronic digital Balance
g) Glass Bottle

After preparation of *Ashwagandha (Withania somnifera)* root *churna*, standardization of that *churna* was done. For that following instruments were used.

a) Electronic Digital Balance
b) Microwave
c) Evaporating Dish
d) Drier
e) Petri dish
f) UV Pointer

In the procedure of standardization of *Ashwagandha (Withania somnifera)* root churna following chemicals were also used.

a) Methalone
b) 1N HCL
c) Distilled water
d) Solvent
e) Chloroform saturated water
f) Silica gel G 254

After standardization of *Ashwagandha (Withania somnifera)* root *churna*, in experiment of *'In vivo'* study following instruments and chemicals were used.

a) Test Tubes
b) Electronic digital balance
c) Beakers & Stirrer
d) Tuberculin syringe with no. 14 needle curved at 130^0 angel.
e) 1 cc Syringe

Chemicals:

a) Dried Lyophilized Common Cobra Venom
b) Dried Lyophilized Russell's Viper Venom
c) Poly Valent Anti Snake Venom
d) *Churna* of *Ashwagandha (Withanis somnifera)* root
e) Distilled water

METHODS:

A) Collection of *Ashwagandha (Withania somnifera)* root:

As experiment was totally depends upon the selected drug i.e. *Ashwagandha (Withania somnifera),* its collection was also too much important. Barshi was the city in the Solapur district of the Southern Maharashtra from where *Ashwagandha (Withania somnifera)* root was collected.

B) Authentication:

Savitribai Phule Pune University of Pune was one of the renowned universities of the Maharashtra. Department of Botany was also well equipped and working department of the university. Authentication of *Ashwagandha (Withania somnifera)* carried out by the same department of University of Pune.

C) Preparation of *Ashwagandha (Withania somnifera)* Root *Churna:*

In the sixth chapter of *Madhyam Khanda* of *Sharangadhar Samhitaa,* procedure of making *churna* was described.*39 But for this, one should have *Ashwagandha (Withania somnifera)* root. For making fine *churna* of it, root was dried totally not in sunrays but in shadow. After that, it was cut into small parts. By churning it in mixer,

small parts of *Ashwagandha (Withania somnifera)* root were converted into fine powder. After filtering it, *churna* was kept in clean glass bottle.

D) Standardization of *Ashwagandha (Withania somnifera)* root *churna:*

As this procedure confirms the quality of the drugs which we were using, it was one of the most important necessities of each experiment. Department of Botany of Savitribai Phule Pune University of Pune had carried the standardization of *Ashwagandha (Withania somnifera)* root *churna*. In standardization of the drug following tests were done in the department of Botany.

a) Total Ash value:

First of all, by using distilled water crucible was washed and dried in microwave at 110^0 C. After cooling in desiccator the weight of it was taken as W1. 2 gm quantity of dried *Ashwagandha (Withania somnifera)* root *churna* was taken and weighed as W2. Then that sample was charred by keeping it in furnace for 2 hours at 750^0 C. After 2 hours it was kept in desiccators for cooling. After cooling totally, accurate weight was taken and named as W3. Then Total Ash value was determined by

Total Ash Value = Wt. (W3-W1) X 100 / Wt. (W2-W1)

b) Acid insoluble Ash:

Empty crucible was weighed first. Then on that crucible accurately weighed Ash was taken. After that hot 1N HCL (5 ml) was added to this crucible and sucked away after 5 minutes. By using distilled water it was washed till filtrate was free of chloride. Then by keeping it in desiccators, it was cooled and after that it was weighed accurately. Finally percentage of acid insoluble ash was determined by following method.

$$\text{Percentage of Acid Insoluble Ash} = \frac{\text{Weight of insoluble Ash X 100}}{\text{Weight of Sample taken}}$$

c) Water Soluble Extractives:

In 100 ml chloroform saturated water, 10 gm of fine powder of *Ashwagandha (Withania somnifera)* root *churna* was submerged, then it was cooled and filtered. Then in a tare Petri dish only 10 ml of the filtrate was taken and dried by evaporating it at 100^0 C. After it dried residue in it was weighed. Finally percentage of water soluble extractives was determined by following method.

Percentage of Water soluble Extractives = Residue X 100

d) Alcohol Soluble Extractives:

First of all, 250 ml round bottom flask was taken. In that flask 10 gm of *Ashwagandha (Withania somnifera)* root *churna* was taken. To this *churna* 100 ml methanol was added. After adding methanol, round bottom flask was closed well and it was kept for 20 hours. After 20 hours that flask was connected to condenser and boiled for 120 minutes. After that it was cooled and filtered. This filtered content taken in tare Petri dish in 10 ml quantity. It was then dried by using boiling water bath method. After that it was cooled and dried in microwave at 100^0 C and the residue of this was weighed accurately. Finally Alcohol soluble extractive was determined by following method.

Percentage of Alcohol soluble Extractive = Residue X 100

Standardization Report of *Ashwagandha (Withania somnifera)* root *Churna:*

After doing above procedures of standardization of selected *Ashwagandha (Withania somnifera)* root *churna,* department of Botany of University of Pune had given the following report.

I. Description of *Churna:* Fine powder, Color- Buff to grey, Odor- Aromatic, Taste- Bitter and Acrid.
II. Foreign Matter: 0.06%
III. Total Ash value: 5.840 %

IV. Acid Insoluble Ash: 0.695 %

V. Alcohol Soluble Extractive: 15.527 %

VI. Water Soluble Extractives: 18.806 %

The values were in the same range as that was mentioned in the Pharmacopoeia of India. Hence the *Ashwagandha (Withania somnifera) root churna* was standardized.

Collection of Venom:

After long procedure of formalities, two types of venom i.e. Common cobra and Russell's viper venom were collected from Mumbai based Haffkine Institute. They both were in dried lyophilized form and also both were obtained in 100 mg packing. Common cobra vial had 782 H number while viper venom vial had 837 F numbers. Common cobra vial was sealed in Oct 1999 and Russell's viper vial was sealed in June 2001.

Collection of Poly Valent Anti Snake Venom:

Poly Valent Anti Snake Venom was also collected from the Haffkine Institute Mumbai.

EXPERIMENTAL STUDY:

Dose Calculation for Mice:

In textbook "Evaluation of Drug Activities: Pharmacometrics" edited by D.R. Laurence and A.L.Bacharch, Vol. I, published by Academic Press, London in 1964, on page no 161, table of surface area ratio of some common laboratory species and man was given. According to this table, conversion factor of dose from man to mice was 0.0026. By using this conversion factor dose of venom, *Ashwagandha (Withania somnifera)* root *churna* and Poly Valent Anti Snake Venom were calculated.

❖ **Table no. 13: Surface area ratio of some common laboratory species and Man*40**

	20 gm Mouse	200 gm Rat	400 gm Guinea Pig	1.5 kg Rabbit	2 kg Cat	4 Kg Monkey	12 kg Dog	70 kg Man
20 gm Mouse	1.0	7.0	12.25	27.8	29.7	64.1	124.2	387.9
200 gm Rat	0.14	1.0	1.74	3.9	4.2	9.2	17.8	56.0
400 gm 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
70kg Man	0.0026	0.018	0.031	0.07	0.076	0.16	0.32	1.0

Dose Calculation of Venom:

From the above table, it was clear that the dose of albino mice was calculated by multiplying to human dose by 0.0026. Fatal dose of Common cobra and Russell's viper venom were 12 mg and 20 mg respectively. Therefore dose of Common cobra venom for albino mice was 0.0312 mg i.e. 31.2 µgm and of

Russell's viper venom was 0.0520 mg i.e. 52 µgm. But when these doses were given to albino mice there were no death in mice because of decreased potency of the venom. So after doing pilot study dose of common cobra venom and Russell's viper venom for albino mice were finalized as 100 µgm and 850 µgm respectively.

Dilution criteria for Venom:

From above calculations doses of Common cobra venom and Russell's viper venom were finalized. As there was much more amount of lyophilized venom, it was diluted in vial for getting appropriate dose for injecting mice. So first of all Common cobra venom was diluted. For that 10 ml distilled water was added to 100 mg lyophilized common cobra venom. So now 1 ml was equivalent to 10 mg. To dilute more 0.125 ml from vial was taken and added it to other glass bottle containing 5 ml distilled water. As 1 ml was equivalent to 10 mg, then 0.125 ml was equal to 1250 µgm. So now 1250 µgm was equal to 5 ml and finalized dose was 100 µgm. So 0.4 ml of this dilution was equivalent to 100 µgm.

Same technique was used in Russell's viper venom dilution. First of all 10 ml distilled water was added to 100 mg lyophilized Russell's viper venom. That means 1 ml was equivalent to 10 mg. To dilute more 2 ml was taken from that and added to other glass bottle containing 5 ml distilled water. As 2 ml was equivalent to 2 mg i.e. 2000 µgm, so now 2000 µgm was equal to 5 ml. We wanted dose of 850 µgm, so we had taken 0.21 ml from this dilution.

Dose Calculation of *Ashwagandha (Withania somnifera)* root *Churna:*

To finalize dose of *Ashwagandha (Withania somnifera)* root *Churna* in albino mice, one should know the dose of *churna* in human. 1 *karsha* means 10 gm was the dose of *churna* for the humans which were described in *Sharangadhara Samhitaa*. Doses of venom were calculated from the conversion factor of mice i.e. 0.0026. Same rule was applied for calculation the dose of *Ashwagandha (Withania somnifera)* root *Churna* i.e. 10 gm was multiplied by 0.0026. Then dose for the mice was finalized as 26 mg. But it was for 20 gm mice. To obtain per kg dose, it was multiplied by 50. i.e. 50 X 26 = 1300 mg. i.e. 1.3 gm/ kg. So 1.3 gm

of *Ashwagandha (Withania somnifera)* root *Churna* was added to 10 ml water. After calculation according to body weight, dose for 20 gm mice was 0.2 ml.

Dose Calculation of PVASV:

According to standards of PVASV, 1 ml of PVASV can neutralize 0.6 ml venom of Common cobra as well as 0.6 ml venom of Russell's viper. By above calculations doses of Venoms finalized for our experiments were 100 µgm and 850 µgm for Common cobra venom and Russell's viper venom respectively. So after doing calculations, dose of PVASV was finalized as 0.17 ml and 1.41 ml for Common cobra venom and Russell's viper venom respectively.

ANIMAL EXPERIMENT:

APT Research Foundation, National Toxicology Centre, Pune was one of the famous institute in Pune for the Research related work. Hence the animal experiment for the "evaluation of efficacy of *Ashwagandha (Withania somnifera)* root *churna* as first aid measure in snake venom poisoning" was done at the same place. Before experiment CPCSEA approval was taken. The CPCSEA number was R 02/1516.

a) For this experiment animal species were selected as Mice and their strain was Albino mice.
b) APT research foundation was the source of animals used for the experiment
c) Average weight of the animals was 20-25 gm.
d) There were 8 groups for experiment and in each group there were 3 mice.
e) Room temperature kept during experiment was 19-25^0 C and humidity was 50-60%.
f) Light cycle of 12 hours light and 12 hours dark was maintained.
g) Age of each albino mice was in between 6-8 weeks.
h) In all groups of experiment, there were male and female mice.
i) Accommodation of mice was maintained well by keeping three mice per cage housed in Polypropylene cages with stainless steel grill top.
j) Before dosing in the experimental room, the mice were housed in their cages for 5 days prior to start dosing.

❖ **Table no. 14: Groups for Animal Experiment:**

Group I (Control Group)	Only Common cobra venom
Group II	Common cobra venom + *Ashwagandha (Withania somnifera)* root *Churna*
Group III (Control Group)	Only Russell's viper venom
Group IV	Russell's viper venom + *Ashwagandha (Withania somnifera)* root *Churna*
Group V (Standard Group)	Common cobra venom + PVASVS
Group VI	Common cobra venom + *Ashwagandha (Withania somnifera)* root *Churna* + PVASVS.
Group VII (Standard Group)	Russell's viper venom + PVASVS
Group VIII	Russell's viper venom + *Ashwagandha (Withania somnifera)* root *Churna* + PVASVS.

Procedure:

1. Before experiment, samples of venom, *Ashwagandha (Withania somnifera)* root *Churna* and PVASV were prepared freshly and then administrated.
2. During experiment below mentioned protocol was followed strictly
   - First of all weight of the animal was noted
   - After that calculation of all doses according to body weight were done.
   - Then Snake venom dose was given by Intramuscular route
   - After 5 minutes of that dose *Ashwagandha (Withania somnifera)* root *Churna* was given orally.
   - And at last, after 5 minute of *Ashwagandha (Withania somnifera)* root *Churna* dose, PVASV was given by Intravenous route.
3. Toxic signs and symptoms in animals were observed for 24 hours and for Mortality, observed for 7 days and all observations were tabulated.

OBSERVATIONS

Table no. 15: Common Cobra Groups Observations

Male Albino Mice

Group	Markings	Body Weight (Gm.)	Tremors (Min)	Paralysis (Min)	Convulsions (Min)	Survival (Min)	Clotting Time (Second)
I	H	29	28	39	47	52	60
	B	31	66	77	82	86	85
	T	32	28	45	50	67	-
II	HT	30	53	65	70	76	45
	B	32	68	76	83	88	61
	W	28	69	74	86	96	60
V	BT	33	-	-	-	Survived	66
	HT	24	-	-	-	Survived	57
	W	29	-	-	-	Survived	50
VI	RF	31	-	-	-	Survived	55
	LH	26	-	-	-	Survived	60
	T	32	-	-	-	120	65

Table no. 16: Common Cobra Groups Observations

Female Albino Mice

Group	Markings	Body Weight (Gm.)	Tremors (Min)	Paralysis (Min)	Convulsions (Min)	Survival (Min)	Clotting Time (Sec.)
I	H	28	41	49	59	64	30
	B	32	67	73	78	86	40
	T	29	66	74	80	89	60
II	HT	33	48	54	60	68	40
	HB	28	62	66	72	79	60
	H	33	67	74	79	87	50
V	HBT	29	-	-	-	Survived	70
	BT	32	-	-	-	Survived	66
	W	27	-	-	-	131	59
VI	B	28	-	-	-	Survived	45
	T	27	-	-	-	Survived	45
	W	32	-	-	-	129	50

Table no. 17: Common Cobra Groups Observations

Hematological Data

Group	Sex	Animal Number	WBC (10^3/µl)	RBC (10^6/µl)	HGB (g/L)	HCT (%)	PLT (10^3/µl)
I	M	1	98.3	8.77	14.7	41.8	540
	M	2	110.6	8.64	15.2	40.7	684
	M	3	155.7	9.25	18.3	44.5	522
	F	4	202.6	10.28	16.5	43.6	446
	F	5	78.6	9.45	14.3	42.6	384
	F	6	82.2	8.28	18.9	43.1	402
II	M	7	39.5	8.24	12.6	33.5	395
	M	8	45.6	8.11	11.4	40.8	491
	M	9	65.9	7.89	13.1	39.9	388
	F	10	43.2	7.14	12.9	42.7	382
	F	11	36.6	8.19	11.9	33.9	267
	F	12	35.2	9.12	14.3	36.7	135
V	M	13	28.1	7.63	11.98	49.1	275
	M	14	62.8	8.83	13.5	44.3	174
	M	15	35.7	4.09	10.9	22.4	168
	F	16	40.4	6.63	11.2	39.8	165
	F	17	29.5	7.22	12.2	36.9	353
	F	18	41.5	5.95	13.7	34.3	395
VI	M	19	33.9	7.19	13.7	36.5	365
	M	20	27.9	6.98	12.8	36.3	323
	M	21	32.1	6.41	12.5	34.7	368
	F	22	27.4	7.51	10.9	33.9	325
	F	23	29.5	8.63	10.6	34.8	361
	F	24	59.7	7.93	13.8	41.6	398

Table no. 18: Russell's viper Groups Observations

Survival Time and Clotting time data

Male Albino Mice

Group	Marking	Body Weight (Gm)	Survival time (Min)	Clotting Time (Sec)
III	H	34	87	48.12
	B	35	Survived	35.36
	T	38	126	159
IV	H	34	Survived	157
	B	35	Survived	145
	T	37	49	63
VII	H	28	113	-
	B	29	Survived	162
	T	30	96	35
VIII	H	35	Survived	133
	B	28	Survived	147
	T	30	Survived	143

Table no. 19: Russell's viper Groups Observations

Survival time and Clotting time data

Female Albino Mice

Group	Marking	Body Weight (Gm)	Survival time (Min)	Clotting Time (Sec)
III	H	29	Survived	134
	B	29	115	150
	T	29	90	60
IV	H	30	Survived	174
	B	30	142	162
	T	30	95	142
VII	H	29	157	-
	B	30	166	120
	T	26	144	-
VIII	H	29	120	119
	B	28	Survived	138
	T	28	135	60

Table no. 20: Russell's viper Groups Observations

Hematological data

Group	Sex	Animal number	WBC (10^3/µl)	RBC (10^6/µl)	HGB (g/L)	HCT (%)	PLT (10^3/µl)
III	M	1	62.3	9.07	13.7	40.7	401
	M	2	70.8	9.14	14.2	42	720
	M	3	253.8	10.25	19.3	44.5	661
	F	4	95.6	9.56	-	45.6	531
	F	5	235.2	8.45	15.2	39.6	470
	F	6	76.7	8.78	14.6	43.1	366
IV	M	7	47	7.74	11.8	34.4	457
	M	8	49.1	8.58	13.9	41.3	592
	M	9	75.9	9.19	15.1	45.6	448
	F	10	33.2	8.34	13.3	39.4	282
	F	11	37.6	7.19	12.2	34.7	257
	F	12	38.2	7.12	11.8	35.8	105
VII	M	13	38.1	8.43	13.8	44	280
	M	14	72.8	9.93	18	57.2	154
	M	15	15.7	3.57	5.9	19.3	188
	F	16	30.4	7.03	12.2	40.6	170
	F	17	30	7.84	13.2	39.5	463
	F	18	33.5	6.75	10.7	32.8	366
VIII	M	19	34.5	8.19	12.7	38	380
	M	20	28.7	7.01	11.8	37.3	313
	M	21	41	7.51	11.5	34.9	375
	F	22	31.4	7.81	9.9	35.2	315
	F	23	27.1	7.63	11.8	35.6	376
	F	24	63.4	8.93	14.9	44.5	488

ANALYSIS

Table no. 21: Analysis of Common Cobra Groups - Tremors

Log-rank (Mantel-Cox) test	
Chi-square	1.884
Df	1
P Value	0.1699
P value Summary	Ns
Are the survival curves sig different?	No
Gehan-Breslow-Wilcoxon test	
Chi square	1.891
Df	1
P value	0.1691
P value summary	Ns
Are the survival curves sig different?	No

Paralysis

Log-rank(Mantel-cox) test	
Chi square	0.0414
Df	1
P value	0.8388
P value summary	Ns
Are the survival curves sig different?	No
Gehan-Breslow-Wilcoxon test	
Chi sqare	0.6494
Df	1
P value	0.4203
P value survival	Ns
Are the survival curves sig different?	No

Table no. 22: Convulsions

Log-rank(Mantel-Cox) test	
Chi square	1.312
Df	1
P value	0.252
P value summary	Ns
Are the survival curves sig different?	No
Gehan-Breslow-Wilcoxon test	
Chi sqare	1.256
Df	1
P value	0.2623
P value summary	Ns
Are the survival curves sig different?	No

Survival

Log-rank (Mantel-Cox) test (recommended)	
Chi square	28.11
Df	3
P value	< 0.0001
P value summary	****
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	19.97
Df	1
P value	<0.0001
P value summary	****
Sig. trend?	Yes

Table no. 23: Clotting Time

Log-rank (Mantel-Cox) test (recommended)	
Chi square	2.823
Df	3
P value	0.4198
P value summary	Ns
Are the survival curves sig different?	No
Logrank test for trend (recommended)	
Chi square	0.2562
Df	1
P value	0.6127
P value summary	Ns
Sig. trend?	No

WBC

Log-rank (Mantel-Cox) test (recommended)	
Chi square	20.84
Df	3
P value	0.0001
P value summary	***
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	20.06
Df	1
P value	<0.0001
P value summary	****
Sig. trend?	Yes

Table no. 24: RBC

Log-rank (Mantel-Cox) test (recommended)	
Chi square	12.29
Df	3
P value	0.0065
P value summary	**
Are the survival curves sig. different?	Yes
Logrank test for trend (recommended)	
Chi square	11.41
Df	1
P value	0.0007
P value summary	***
Sig. trend?	Yes

HGB

Log-rank (Mantel-Cox) test (recommended)	
Chi square	15.86
Df	3
P value	0.0012
P value summary	**
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	11.58
Df	1
P value	0.0007
P value summary	***
Sig. trend?	Yes

Table no. 25: HCT

Log-rank (Mantel-Cox) test (recommended)	
Chi square	8.236
Df	3
P value	0.0414
P value summary	*
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	3.095
Df	1
P value	0.0785
P value summary	Ns
Sig trend?	No

PLT

Log-rank (Mantel-Cox) test (recommended)	
Chi square	14.38
Df	3
P value	0.0024
P value summary	**
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	9.562
Df	1
P value	0.002
P value summary	**
Sig. trend?	Yes

Analysis of Russell's viper Groups

Table no. 26: Survival Period

Log-rank (Mantel-Cox) test (recommended)	
Chi square	2.43
Df	3
P value	0.4881
P value summary	Ns
Are the survival curves sig trend?	No
Logrank test for trend (recommended)	
Chi square	1.217
Df	1
P value	0.2699
P value summary	Ns
Sig. trend?	No

Clotting Time

Log-rank (Mantel-Cox) test (recommended)	
Chi square	3.655
Df	3
P value	0.3012
P value summary	Ns
Are the survival curves sig different?	No
Logrank test for trend (recommended)	
Chi square	0.2604
Df	1
P value	0.6099
P value summary	Ns
Sig. trend?	No

Table no. 27: WBC

Log-rank(Mantel-Cox) test (recommended)	
Chi square	13.93
Df	3
P value	0.003
P value summary	**
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	12.8
Df	1
P value	0.0003
P value summary	***
Sig. trend?	Yes

RBC

Log-rank (Mantel-Cox) test (recommended)	
Chi square	6.209
Df	3
P value	0.1019
P value summary	Ns
Are the survival curves sig different?	No
Logrank test for trend (recommended)	
Chi square	5.572
Df	1
P value	0.0183
P value summary	*
Sig. trend?	Yes

Table no. 28: HGB

Log-rank (Mantel-Cox) test (recommended)	
Chi square	5.543
Df	3
P value	0.1361
P value summary	Ns
Are the survival curves sig different?	No
Logrank test for trend (recommended)	
Chi square	4.859
Df	1
P value	0.0275
P value summary	*
Sig. trend?	Yes

HCT

Log-rank (Mantel-Cox) test (recommended)	
Chi square	2.983
Df	3
P value	0.3943
P value summary	Ns
Are the survival curves sig different?	No
Logrank test for trend (recommended)	
Chi square	2.724
Df	1
P value	0.0988
P value summary	Ns
Sig. trend?	No

Table no. 29: PLT

Log-rank (Mantel-Cox) test (recommended)	
Chi square	9.087
Df	3
P value	0.0282
P value summary	*
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	4.637
Df	1
P value	0.0313
P value summary	*
Sig. trend?	Yes

Table no. 30: Common Cobra Analysis Chart

Sr. No.	Parameters	Log-rank(Mantel-Cox) test (recommended)		Logrank test for trend (recommended)		Gehan-Breslow-Wilcoxon test	
		P-value	Significant	P-value	significant	P-value	Significant
1	Clotting	0.4198	No	0.6127	No	0.5274	No
2	HCT	0.0414	Yes	0.0785	No	0.0677	No
3	HGB	0.0012	Yes	0.0007	Yes	0.0084	Yes
4	Platelet	0.0024	Yes	0.002	Yes	0.0062	Yes
5	RBC	0.0065	Yes	0.0007	Yes	0.0062	Yes
6	WBC	0.0001	Yes	<0.0001	Yes	0.0006	Yes
7	Tremors	0.1699	No	-	-	0.1691	No
8	Convulsions	0.252	No	-	-	0.2623	No
9	Paralysis	0.8388	No	-	-	0.4203	No
10	Survival	<0.0001	Yes	<0.0001	Yes	<0.0001	Yes

Russell's viper Analysis chart

Sr. No.	Parameters	Log-rank (Mantel-Cox)test (recommended)		Logrank test for trend (recommended)		Gehan-Breslow-Wilcoxon test	
		P- value	Significant	P- value	Significant	P-value	Significant
1	Clotting	0.3012	No	0.6099	No	0.3749	No
2	HCT	0.3943	No	0.0988	No	0.1975	No
3	HBG	0.1361	No	0.0275	Yes	0.0892	No
4	Platelet	0.0282	Yes	0.0313	Yes	0.0318	Yes
5	RBC	0.1019	No	0.0183	Yes	0.0605	No
6	WBC	0.003	Yes	0.0003	Yes	0.0061	Yes
7	Survival	0.4881	No	0.2699	No	0.4928	No

DISCUSSION

As per discussion with guide this experiments emphasis is on '*in vivo'* study. As observed in literature review, very few *'in vivo'* studies have been published for antiophidian drugs. Usually for the research on antiophidian drugs, researchers follow *'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". Pre incubation 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 cannot be studied by these methods. 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. Many of the difficulties in the previous studies were avoided in the current study. As there are 96 formulations mentioned in Ayurved as antiophidian drugs, there is a great scope for this type of study.

Drug Procurement:

The herb *Ashwagandha (Withania somnifera)* which is an important ingredient in this study is easily available. Raw sample of *Ashwagandha (Withania somnifera)* was collected from Barshi, Dist. Solapur. Authentication and Standardization of *Ashwagandha (Withania somnifera)* was done at Department of Botany, University of Pune, Pune.

Venom Procurement:

For the entire animal experiment phase in this study, needs only few grams of the Common cobra venom and Russell's viper venom. But due to very complicated procedure of formalities, it took 3 years for the procurement of venom. Due to which the period of experiment was extended.

Before 10 years, there were no that much formalities for the procurement of snake venom. At that time only application to Director, Haffkine Institute, Mumbai was sufficient for the procurement of snake venom. So it was very easy to do the research on snake venom. Procedure was as follows.

Application to Director, Haffkine Institute, Mumbai

↓

Payment of fees of Venom

↓

Delivery of Venom

But nowadays government had taken venom procurement under the regulation of Forest department, which is very critical decision for the researchers as it will definitely decrease the research related to venom. Nowadays following complicated procedure is followed.

Application to Chief Conservator of Forest, Pune.

↓

Then application to Principal Chief Conservator of Forest, Nagpur (with all documents like IBSC Certificate, IAEC, Synopsis with Letter of HOD)

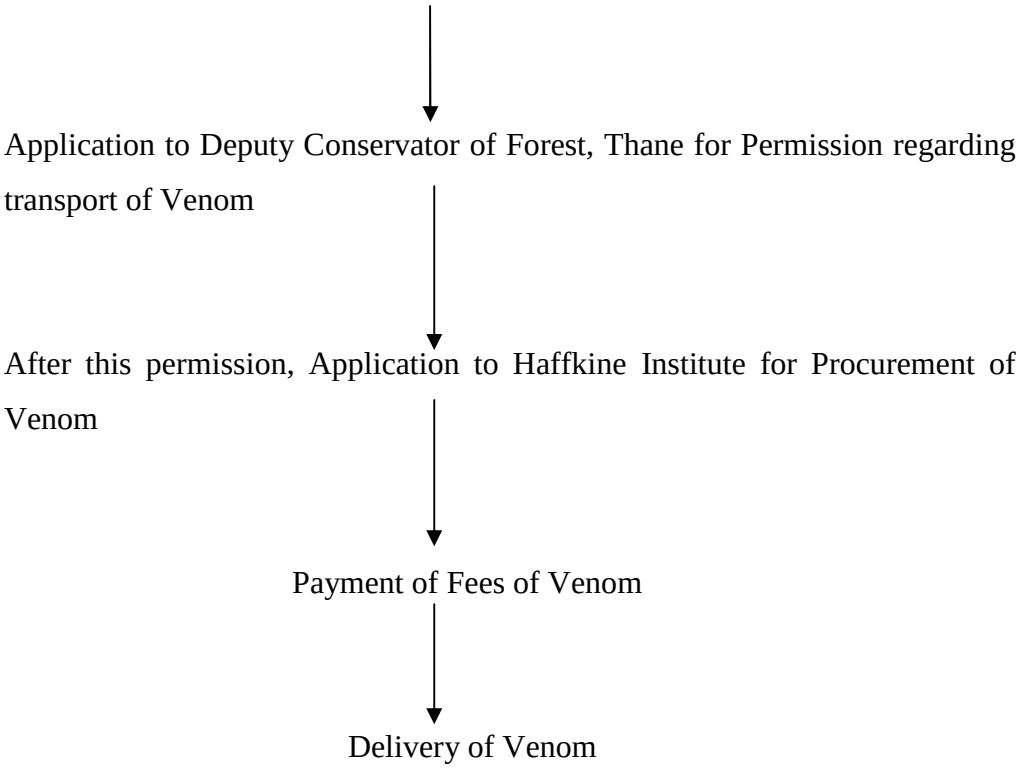
↓

After that they will confirm the issue by contacting 'The Ministry of Environment, Forest and climate change'

↓

Gives Permission to Procurement of Venom

↓



For all these formalities it will take valuable time of 3 years and other expenses for the follow up. There is also academic loss of Researchers. So I suggest in further experiments of venom, government should help researchers by giving easy permission for procurement of venom, neither research related to venom will stop and it will be very dangerous thing to all humanity.

After this long procedure, venom was acquired. Lyophilized Common Cobra Venom was in 100 mg packing and Lyophilized Russell's viper venom was also in 100 mg packing.

Dose Calculation:

Initially it was decided that 80% fatal dose of venom should be used in cobra group & 100% fatal dose of venom should be used in Russell's viper group. According to previous studies done by senior researchers, there was a suggestion that in future experiments of this kind 110-120% fatal dose of Russell's viper venom should be injected.

To resolve this experimentally, the pilot study was conducted by using two animals per dosage group. In the first stage these animals were given 25, 50 & 100 µgm (in Russell's viper) & 25, 50, 75 & 100 µgm (in Cobra group) dose levels of venom respectively. At this level in cobra group all animals died in 100 µgm. 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 850 µgm fatality were seen. Therefore to achieve good observation period 100 µgm of cobra venom & 850 µgm of Russell's viper venom was used.

Dosing:

For the ease of Observation albino mice were chosen for the experiment, while oral and IM dosing was not a problem, IV PVASVS proved a big hazard. As the required amount of PVASVS was very small, its IV delivery is a very skillful and difficult procedure.

During Observations:

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. Paralytic signs e.g. tremors, paralysis, convulsion in Common cobra venom i.e. control group I were observed. As Russell's viper venom is hemotoxic, 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 III.

- In Common cobra control group (Gr.I), appearance of tremors was observed after 49.33 min (average) and that of drug group (Gr.II) was after 61.16 min (average) i.e. appearance of tremors was delayed by 11.83 min in *Ashwagandha (Withania somnifera)* group, which is statistically not significant. P value is 0.1699.
- In Common cobra control group (Gr.I), appearance of paralysis was observed after 59.5 min (average) and that of drug group (Gr.II) it was observed after 68.17 min (average) i.e. appearance of paralysis was delayed by 8.67 min in *Ashwagandha (Withania somnifera)* which is statistically not significant. P value is 0.8388.
- In Common cobra control group (Gr.I), appearance of convulsions was observed after 66 min (average) and in drug group (Gr.II) , it was observed after 75 min (average) i.e. appearance of convulsions was delayed by 9 min in

Ashwagandha (Withania somnifera) group which is statistically not significant. P value is 0.252.

- In Common cobra group (Gr.I) duration of survival was 74 min (average) & that of drug group (Gr.II), duration of survival was 82.33 min (average) i.e. duration of survival was delayed by 8.33 min in *Ashwagandha (Withania somnifera)* group, which is statistically significant. P value is < 0.0001.
- In Common cobra venom + PVASVS (standard group) one mouse died after 131 min. This may be due to serum sickness reaction of PVASVS. Remaining two mice survived completely.
- In Common cobra venom + *Ashwagandha (Withania somnifera)* + PVASVS group (Gr.VI), two mice died at 120 & 129 min. This may be due to serum sickness reaction of PVASVS, as there are no any major changes in blood investigations.
- In Russell's viper venom group (Gr.III), two mice were survived and duration of survival was 104.5 (average) & that of drug group (Gr. IV), three mice were survived and duration of survival was 95.33 min (average) i.e. there is no any delay in survival in *Ashwagandha* group.

  Though some mice did survive in both groups, survival was equivalent in drug group and standard control group. Complete survival was observed only in the group receiving both the drug and ASV. Duration of the survival was comparable in drug and standard control group. Though the difference in survival duration was statistically not significant it was slightly more in the drug group. This indicates that the drug is definitely useful but requires further optimization.
- In Russell's viper venom + *Ashwagandha (Withania somnifera)* + PVASVS group (Gr. VIII), four mice survived without showing any symptoms and two mice died after 120 min and 135 min. This may be due to serum sickness reaction of PVASVS, as there are no any major changes in blood investigations.

RESULTS:

In Common Cobra Groups:

1. The results of survival period in Common cobra venom group was proved to be statistically significant. P value is <0.0001.
2. The results in tremors, paralysis, convulsions and clotting time were not statistically significant as their P values are

   Tremors- P value- 0.1699

   Paralysis- P value- 0.8388

   Convulsions- P value- 0.252

   Clotting time- P value- 0.4198

   Thus results of *Ashwagandha (Withania somnifera)* are not significant in Neurological symptoms and clotting time.

3. The results in WBC, RBC, HGB, HCT, Platelet are significant as their P values are

   WBC- P value- 0.0001

   RBC- P value- 0.0065

   HGB- P value- 0.0012

   HCT- P value- 0.0414

   Platelet- P value- 0.0024

   Thus results of *Ashwagandha (Withania somnifera)* are significant in hematological findings.

In Russell's viper Groups:

1. The results of survival period in Russell's viper venom group were proved to be statistically not significant. P value is 0.4881.
2. The results of clotting period in Russell's viper venom group were proved to be statistically not significant. P value is 0.3012.
3. According to Logrank test for trend (recommended), the results in WBC, RBC, HGB, Platelet are significant as their P value are

   WBC- P value- 0.0003

RBC- P value- 0.0183

HGB- P value- 0.0275

Platelet- P value- 0.0313

Thus results of *Ashwagandha (Withania somnifera)* are significant in hematological findings.

Future Roadmap:

1) In this study, the efficacy of *Ashwagandha (Withania somnifera)* against Common cobra venom and Russell's viper venom is studied. In future study of efficacy against Saw scaled viper and Banded krait, can also be done.
2) Once the efficacy of *Ashwagandha (Withania somnifera)* is proved in *'in vivo'* study, it is necessary to do the *'in vitro'* study for dose optimization of *Ashwagandha (Withania somnifera).*
3) HPTLC of the snake venom and *Ashwagandha (Withania somnifera)* should be done to know the various components present in both of them. 'Pre-incubation' study of the above experiment should be done to know which components of the snake venom are neutralized by which component of the drug.
4) In Ayurved, 96 formulations are mentioned as antiophidian drugs, but these are not proved scientifically or proved by *'in vitro'* study and 'Pre-incubation' study. There is need of extensive trials for such formulations to prove their efficacy.
5) Standardization of selected drug according to Pharmacokinetics and Dynamics should be done. Reverse pharmacological study for this drug is necessary for further development of the drug. To standardize the drug, it is impossible to compare all possible *Ashwagandha (Withania somnifera)* products available in the market.
6) *Sushruta* states that, there are seven *vishavegas* in human & four *vegas* in animals. Study of signs and symptoms during these *vegas* can be a topic of research.

SUMMARY

NEED:

Snakebites are a major health threat in India. About 2 million peoples are bitten by snakes annually of which around 40900 to 50900 deaths occur in India per year. In India majority of population is crowded in rural area. If patient need any medical or any other treatment they have to go to Higher Medical Centre. 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. Every snake bite patient cannot get its advantage. Hence requirement of the primary substitution or first aid measure before serotherapy, which will increase the survival period and decrease the mortality and morbidity. *"Ashwagandha (Withania somnifera)"* mentioned in *Vishavaidyajyotsanika* is selected for study in snake venom poisoning. It is easily available, easy to carry, does not require trained person for administration. As compared to PVASVS, it is cheap and easily available. It will reduce the fatality and morbidity as it will not interact with PVASVS.

PLAN OF WORK:

1. Collection of *Ashwagandha (Withania somnifera)*
2. Standardization of *Ashwagandha (Withannia somnifera)*
3. Preparation of *Ashwagandha (Withania somnifera)* root *churna*
4. Collection of snake venom and PVASVS
5. *'In Vivo'* efficacy study.

METHOD:

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

RESULTS:

1. The results of survival period in Common cobra venom group was proved to be statistically significant. P value is <0.0001.
2. The results in tremors, paralysis, convulsions and clotting time were not statistically significant as their P values are

   Tremors- P value- 0.1699

   Paralysis- P value- 0.8388

   Convulsions- P value- 0.252

   Clotting time- P value- 04198

   Thus results of *Ashwagandha (Withania somnifera)* are not significant in Neurological symptoms and clotting time.

3. The results in WBC, RBC, HGB, HCT, Platelet are significant as their P values are

   WBC- P value- 0.0001

   RBC- P value- 0.0065

   HGB- P value- 0.0012

   HCT- P value- 0.0414

   Platelet- P value- 0.0024

   Thus results of *Ashwagandha (Withania somnifera)* are significant in hematological findings.

In Russell's viper Groups:

1. The results of survival period in Russell's viper venom group were proved to be statistically not significant. P value is 0.4881.
2. The results of clotting period in Russell's viper venom group were proved to be statistically not significant. P value is 0.3012.
3. According to Logrank test for trend (recommended), the results in WBC, RBC, HGB, Platelet are significant as their P value are

   WBC- P value- 0.003

   RBC- P value- 0.0183

   HGB- P value- 0.0275

   Platelet- P value- 0.0313

   Thus results of *Ashwagandha (Withania somnifera)* are significant in hematological findings.

CONCLUSION

The present study confirms the *'Vishaghna'* property of *Ashwagandha (withania somnifera)* in Common cobra groups, but at the same time *Ashwagandha (Withania somnifera)* is not useful in Neurological symptoms like Tremors, Paralysis and Convulsions. In Russell's viper venom poisoning, *Ashwagandha (Withania somnifera)* is definitely useful to compensate the poisoning as well as useful in survival but requires further optimization.

- *Ashwagandha (Withania somnifera)* does not interact with Poly Valent Anti Snake Venom (PVASVS).

- **Thus *Ashwagandha (Withania somnifera)* has more action on cytotoxic properties of snake venom so it is useful as first aid measure in Common Cobra bites and Russell's viper bites.**

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