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Research Article

PRECLINICAL STUDY OF KUTAJADI YOGA IN RELATION WITH PRAZOSIN AGAINST INDIAN RED SCORPION VENOM POISONING IN ALBINO MICE

Patil Akshara Akash*

Assistant Professor, Department of Agadtantra Avum Vidhivaidyak Yashwant Ayurved college, P.G.T.& R.C., Kodoli, Kolhapur, Maharashtra, India

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*Corresponding Author: **Dr. Akshara A Patil,** M.D.(Ayu)
Asst. Professor, Department of Agadtantra Avum Vidhivaidyak Yashwant Ayurved College, PGT.& RC., Kodoli, Tal.- Panhala, Dist.- Kolhapur, Maharashtra, India
Email: dr.aksharapatil25@gmail.com, Mobile: +9970040451

ABSTRACT

Ayurveda is the God gifted very ancient and the first medical science which was memorized and composed by the originator *Brahma* (*Swayambhoo*) and considered it as the branch of *Atharva veda*. It is aimed to protect the health by giving the knowledge of preventive health principles and cure the diseases by explaining different types of treatment procedures and principles. The present study entitled "Preclinical study of efficacy of *Kutajadi yoga* in relation with Prazosin against Indian Red Scorpion venom poisoning in albino mice" was on attempt to study the *Kutajadi yoga.* From the detailed preclinical study I conclude that, the *Kutajadi yoga* individually and also in combination with Prazosin show significant result like Prazosin by statistically. So the experimental drug is useful against Indian Red Scorpion venom poisoning.

Keywords: Prazosin, Agadatantra, Ayurveda, Visha, Kutajadi Yoga, Vrishchika.

INTRODUCTION

Ayurveda is the God gifted very ancient and the first medical science which was memorized and composed by the originator *Brahma* (*Swayambhoo*) and considered it as the branch of *Atharva veda*. It is aimed to protect the health by giving the knowledge of preventive health principles and cure the diseases by explaining different types of treatment procedures and principles. *Ayurveda* has its own basic principles. Now days *Ayurveda* is also being universally accepted it is necessary therefore to prove it as one of the best medical science useful in day-to-day life. Its basic principles should be propagated over world with the help of today's modern parameters pharmacology and experimental studies. *Agadtantra* one among the eight branches of *ayurveda* special branch of *Ashtang ayurveda* and well recognized for the study of toxicity and management of poisonous bites, toxic combination of food and drugs etc. *Visha* (Poison) is a substance which after entering, the body disturbs natural and physiological functions of the body (i.e. *Dosh, Dhatu* and *Mala*) and it is also degrades the health of the human being or result in destructions of life.

"अगदतंत्रं नाम सर्पकीटलूतामूषकादिष्टविषव्यंजनार्थं
विविधविषसंयोगोपशमनार्थंच । "(सु. सू. १)

Agadtantra deals with the bites of snakes, insects, spiders, scorpions, rats etc., their characteristics, the signs and symptoms of accidental or purposeful ingestion of poison including *Kritrim Visha* concocted poisons (*Gara*) and denatured poisons (*Dooshi Visha*). In *Ayurveda* ***Visha*** is classified as

स्थावरं जंगम चैति विषं प्रोक्तमकृत्रिमम् ।
कृत्रिमं गरसंज्ञं तु क्रियते विध्यौषधैः ॥ (अ. ह्र. उ. ३५/५)

1. Akritrim visha (natural poison)
   - Sthavar visha (plant origin)
   - Jangam visha (animal origin)
2. Kritrim visha (artificial poison)
   - Dushivisha
   - Garavisha (cumulative poison)

Poisoning by insects bites are very common in clinical practice and present with a simple itching to life threatening problem, mimicking in the symptoms of snake bites. Today also, cases of mortality are reported due to poisonous bites because of non-availability of timely medical aid. Among the poisonous insects, scorpions are given prime importance due to the fact that world statistics shows that, the estimated annual number of scorpion stings is 1.2 million leading to 3250 deaths (0.27%). **[Ref.: e-medicine, David Cheng M.D.]** Furthermore scorpions can be found outside their normal range of distribution, that is when they crawl into luggage,

boxes, containers, or shoes and are unwittingly transported home via human travellers.

Experimental study:

In the present study as the Indian Red Scorpion venom poisoning is involved so I chose the preclinical study design as it is very difficult on the Human volunteers and patients. In the present Preclinical study the animal model was Albino mice as they are the most sensitive animals than humans. Mice are most commonly used vertebrate species because of their size, low cost, ease of handling and fast reproductive rate. Mice are widely considered to be the best model in toxicological studies and they share 99% of their genes with humans. In *Ayurveda,*

कुटजादी योग

कुटजस्य फलं पिडं तगरं जालमालिनी ।
तिक्तेश्वाकु श्च योगोऽयं पानप्रधमनादिभिः ॥
वृश्चिकोन्दुरुलूतानां सर्पाणां च विषं हरेत् ।
समानो ह्यमृतेनायं गराजीर्ण च नाशयेत् ॥ (च. चि. २३/२०६–२०७)

Charakacharya in his *Vishachikitsa Adhyaya* explained the details *Kutaja, Tagar, Devadali and Ekshwaku-* this formulation used as *pana; pradhamana* etc. alleviates poison of scorpion, rat, spider, and snake. Treatment of *Madhya visha* & *Maha visha* of *Vrishchika* is similar to snake bite poisoning. And this drug is easily available in market and easy to prepare. So I have chosen *Kutajadi Yoga*. Preclinical study was selected for study because the aim is to collect data in support of safety. It is required before clinical trials can be started. No scientific data is available along with *Kutajadi yoga*. There is limited study and treatment regarding of *Vrishchika* in *Ayurveda.* Among this limited treatment I selected *Kutajadi Yoga* for the treatment of scorpion poisoning.

In modern system of medicine, Prazosin therapy was scientifically established in India by ***Dr. H. S. Bawaskar***. Due to lack of medical ICU facility in rural areas, case fatality rate was up to 30% in pre *Prazosin* era. With the use of Prazosin mortality rate has come down to 1%. Now Prazosin is the line of treatment in the areas of scorpion poisoning as Prazosin is alpha-1 blocker. It reduces preload and left ventricular impedance without rise in heart rate. It enhances insulin secretion which is suppressed in scorpion sting victim due to catecholamine excess. Thus Prazosin may rightly be called a physiological and pharmacological antidote to scorpion venom and it is administered orally.

Scorpion is an ancient venomous and the terrestrial arthropod found in tropical and subtropical region belonging to the class of animals known as arachnid. The sting of a scorpion is sometimes fatal because certain species are dangerously poisonous. A scorpion has a pair of appendages extending from the sides of the mouth and also has a diabolic tail with a pair of powerful pincers. A scorpion attacks its predators and its prey with its tail. There are many species of scorpions found all over the world. There are a few species of scorpions whose sting is not harmful. The toxicity of the venom of the other type of scorpions is similar to that of the venomous snakes such as cobra and coral snakes. That is, the venom of these arthropods is neuro-toxic. Nearly 1000 species of scorpions belonging to six families have been mentioned, but only some species belonging to the family *Buthidae* produce neurotoxin venom that is potent, lethal and toxic to human victims and 86 species found in India. Depending on the toxicity of their venom, the scorpions are classified into two types. *Mesobuthus tamulus* (as *Indian Red scorpion*, called *Buthus tamulus*) and Black scorpion i.e. *Palmaneus garvimanus* are the two scorpion– species that are most commonly found in all over western Maharashtra, Anantpur and Karnool districts of Andhra Pradesh, Chennai, Pondicherry and Madurai in Tamil Nadu, Bellary in Karnataka, part of Gujarat, Patna area from Bihar. In Indian Red scorpion poisoning sign and symptoms like burning at the site of the sting, pain, sweating, vomiting, difficulty in breathing, thick feeling in tongue, convulsions, pulmonary oedema, dyspnoea etc. are all readily identified in *Ayurvedic samhitas* in an ancient times which is similar as like as *Daha, shool, shotha, sweda, moorchha, jivhashotha, chhardi, sarvang kampa, shwasavrodha*, etc. by *Aacharyas*.

Indian Red Scorpion sting is a common problem in villages of India. The life-threatening complication of myocarditis and pulmonary oedema is known in red scorpion in India. This condition requires urgent attention and ICU care from few hours to days. Delay in recognition and the hypoxemia increase the morbidity and mortality. Illiteracy, ignorance, poverty, traditional faith healers trying treatment in remote areas, lack of transport in difficult terrains and the non-availability of ventilation facility in nearby hospital, add to delay in appropriate treatment. Scorpion sting, commonly being less fatal than the snakebite, gets less attention in health setup in this region. Mostly being non-fatal, victims present with mild to moderate symptoms and are attended by the traditional healers in village. Scorpion sting in its severest presentation leads to respiratory distress and mortality is related to development of myocarditis and the pulmonary oedema in India.

Anatomy: The body of a scorpion is divided into three parts (tagmata): the head (cephalothorax), the abdomen (mesosoma) and the tail (metasoma).

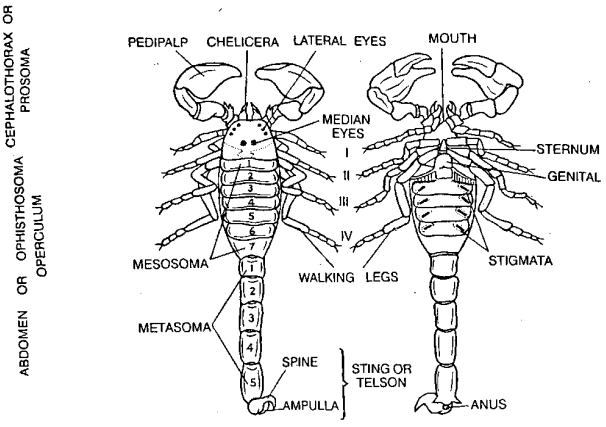


Fig. 2 : Scorpion : Body structure.

Signs and Symptoms of Indian Red Scorpion poisoning - Vomiting, Sweating, Priapism, Cyanosis, Unconsciousness, Muscular convulsions, Breathlessness, Pulmonary oedema, Cardiac dysrhythmia, Tachycardia or bradycardia, Hypotension or hypertension, Acute myocarditis, Shock. The venom mainly affects the cardiovascular and pulmonary system, eventually leading to a pulmonary oedema, which may cause death.

Scorpion venom

Delays opening of neuronal NA channels

⇩

Raised level of circulating Catecholamines
"Autonomic storm"

⇩

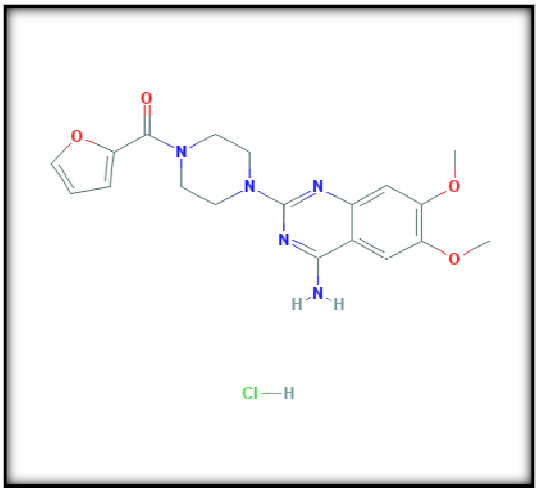
Transient Para-Sympathetic Stimulation
{Vomiting, Sweating, Salivation Priapism, Hypotension}

⇩

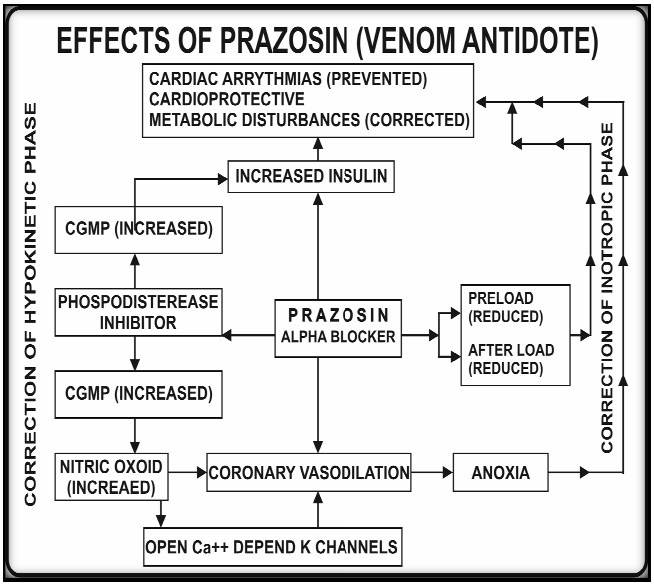
Prolonged Sympathetic-Stimulation
{Hypertension, Tachycardia, Cold Extremities, Pulmonary Edema}

Pharmacological action

When Scorpion venom injected it is produce local irritation due to stimulation of the terminations of the sensory nerves of the skin. The vasomotor and respiratory centres are stimulated leading to high blood pressure and an increase of the respiratory excursions. Excessive lachrymal, nasal and salivary secretions are due to stimulation of the facial nerve centres. The heart is definitely stimulated and continues to beat even after the paralysis of the respiratory centre. The nervous system is generally excited. Reflexes are increased as evidence by tremor and muscular twitching. Death in experimental animals is always due to direct paralytic action of the venom on the respiratory centre. Prazosin hydrochloride, a quinazoline derivative, is the first of a new chemical class of antihypertensive. It is the hydrochloride salt of 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl) piperazine and its structural formula is



Molecular Weight: 419.86208 [g/mol]
Molecular Formula: $C_{19}H_{22}ClN_5O_4$



MATERIALS AND METHODS

Materials

For Preparation of Kutajadi Yoga: *Kutaja phala, Tagar moola, Ikshwaku phala and Devadali phala*

For pre-clinical study

- **Instrument and apparatus:** Single pan electronic digital balance, Test tube, 1cc syringe with needle, Feeding syringe and Mixer
- **Chemicals:** Dried lyophilized Indian Red Scorpion venom, *Kutajadi Yoga and* Distilled water

Methods

- **Collection of Kutajadi Yoga ingredients:** Raw samples of *Kutajadi Yoga* were collected.

Kutaja Phala (Fruit of Holarrhena antidysenterica), *Tagar Moola* (Root of Valeriana wallichii), *Devadali Phala* (Fruit of Luffa echinata) and *Ekshwaku Phala* (Fruit of Lagenaria siceria)

Authentication: Authentication of *Kutaja phala, Tagar moola, Ikshwaku phala, Devadali phala* was done at college pharmacy.

Preparation of Kutajadi Yoga: For preparation of *Kutajadi Yoga* four main ingredients *Kutaja phala, Tagar moola, Ikshwaku phala, Devadali phala* were required. The '*Kutajadi Yoga'* was prepared in *Rasa Shastra* and *Bhaishajya Kalpana* Department of College. In the process of preparation *Kutajadi Yoga's* ingredients were collected in equal dose. This was converted into *churna* by using grinding mixer. In a clean washed Mortar and Pestle, powder of all ingredients was taken, and then triturating process was continued until it became mix totally. *Kutajadi yoga* was obtained. Then it was forwarded for standardization.

Standardization of drug: To ensure the quality of drug it is necessary to standardize that drug before using in experiment. The study was done at college pharmacy.

Determination of Total Ash Value

Procedure: Silicon crucible was washed with Distilled water and dried in oven at 110^0 C and put in desiccator and then

weighed as A. 2 gm. of dried powder is taken and weighed as B. then crucible with powder kept in furnace at 750^0 C for 2 hrs. Then it was transferred to a desiccator for cooling. Accurate weight was taken after cooling as C.

Total Ash Value = Wt. (C-A) x 100/Wt. (B-A)

Acid insoluble Ash:

Procedure: Weight of empty crucible was taken; accurately weighed ash was taken in it. Boil the ash for 5 minutes with 25 ml of dilute hydrochloric acid, collect the insoluble matter in a crucible or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid insoluble ash with reference to the air dried drug. Percentage of acid insoluble ash was obtained from following formula

$$\textit{Acid insoluble Ash} = \frac{\textit{Wt of insoluble Ash x 100}}{\textit{Wt of Sample taken}}$$

Water soluble extractive

Procedure: 10 gm. of fine powder of sample was soaked in 100 ml of chloroform saturated water refluxed for 4 hrs, cooled and filtered. 10 ml of the filtrate was taken in a petri dish, Evaporated for dryness at 100^O C and weigh the residue. Water soluble Extractives were obtained from the following formula:

Residue x 100 = Percentage of water soluble extractives

Alcohol soluble extractive

Procedure: Macerate 5 gm of the air dried drug, coarsely powdered with 100 ml of Alcohol of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^0 C, to constant weight and weigh. Calculate the percentage of Alcohol Soluble Extractives with reference to the air dried drug. Alcohol Soluble Extractives was obtained from the following formula

Residue x 100 = Percentage of Alcohol Soluble Extractives

Standardization report of Kutajadi Yoga: Identification Tests: Standard values (Ref.-API)

Table 1

Tests	*Kutaja*	*Tagara*	*Devadali*	*Ikshwaku*
Foreign matter in %	Not more than 2	Not more than 2	-	-
Total ash in %	Not more than 8	Not more than 12	12.20	Not more than 12
Acid insoluble ash %	Not more than 3	Not more than 10	1.50	Not more than 0.6
Alcohol soluble Extractive in %	Not less than 12	Not more than 30	19.90	Not less than 10
Water soluble Extractive in %	-	Not less than 19	20.20	Not less than 25

Table 2: (Obtained values)

Tests	*Kutaja*	*Tagara*	*Devadali*	*Ikshwaku*
Foreign matter in %	0.75	0.56	0.50	-
Total ash in %	7.46	11.5	9.88	6.96
Acid insoluble ash %	2.14	2.0	0.98	0.2
Alcohol soluble extractive in %	14.62	12.5	13.26	13.4
Water soluble extractives in%	18.12	22.12	18.34	32.41

Table 3: Result of final products (Kutajadi Yoga)

Tests	*Kutajadi Yoga*
Foreign matter in %	0.96
Total ash in %	11.5
Acid insoluble ash %	3.03
Alcohol soluble extractive in %	17.45
Water soluble extractives in%	18.0

Collection of Venom: Dried lyophilized form 2 vials of 300mg of Indian red scorpion venom was collected from Haffkine Institute for Training, Research and Testing (HITRT), Mumbai.

Table No.4 Organoleptic Test of Kutajadi Yoga

Test	Result
Colour	Brownish black
Odour	Characteristic
Taste	*Tikta-Katu*
Consistency	Liquid (*Kwath*)

Table No.5 (Details of Indian Red Scorpion venom vials)

Indian Red Scorpion Venom		
Vial No.842 D	0.29 gm	Sealed at 2006
Vial No.839 M	0.01 gm	Sealed at 2003

Collection of Prazosin: Prazosin Tablet collecting from market. Tablet Prazopress 1mg (Sun Pharmaceutical Industries Jammu) and their market preparations are Minipress XL, Prazopress (1mg, 2mg tablets)

Animal experimentation study:

Dilution criteria for venom

Dried lyophilized form of venom was diluted to get appropriate fatal dose to inject mice. In a vial of 0.29 gm of Indian Red Scorpion Venom added 1ml of PBS (Phosphate Buffer Solution). From that 0.01 ml was removed and added into 5 ml of PBS containing glass bottle. Then it was sealed with rubber cork. From this dilution 1.5 ml was taken out and injected to mice. (1.5 ml =0.9 mg concentration)

Dose calculation of venom

Human fatal dose for Indian Red Scorpion is 1.5 mg. According to conversion factor mice fatal dose for Indian Red Scorpion was 0.3 mg. Previously total fatal dose was taken but at this dose no death occurs in Albino mice. To resolve this experimentally, we conducted the pilot study using 5 animals.

Table No.6

Route of administration	Markings	Weight of mice (gm)	Dose of I.R.S. Venom given (ml)
Subcutaneously (SC) on dorsal aspect	H	32.5	0.2
	B	30.0	0.4
	T	34.5	0.6
	BT	30.0	0.8
	W	34.0	1.0

(*H=Head, B=Back, T=Tail, W=White, BT=Back-Tail*)

Table No.7

Route of administration	Markings	Weight of mice (gm)	Dose of I.R.S. Venom given (ml)
Intramuscularly (IM)	H	38.0	0.2
	HBT	32.0	0.4
	HT	40.0	0.6
	B	39.5	0.8
	W	40.0	1.0

In first stage above dose was given, at that time concentration was 0.3 mg. Indian Red Scorpion venom was injected subcutaneously and intra muscularly. When venom was injected subcutaneously there were no symptoms, except the irritation and pain at injecting site. Then the same dose 0.3 mg/ml was repeated intra muscularly. Symptoms were same but intensity was less when given by intramuscular route compared with subcutaneous route. So the subcutaneous route was finalized. The initial dose was given as 0.3mg/ml showed only itching in albino mice. So the new concentration dose was fixed 0.6 mg/ml with changing concentration of the dilution. Keeping this aim, the study was designed to conduct the preclinical study on albino mice, at authorized laboratory. The research protocol was approved by the Institutional Animal Ethical Committee, Research Project No. 65.

Table No.8

Markings	Weight of mice (gm)	Dose of I.R.S. Venom given (ml)	Death occurred
H	34.0	0.25	Survived
HB	37.0	0.5	Survived
W	36.0	0.75	Died at 4th day

Table No.9

Markings	Weight of mice (gm)	Dose of I.R.S. Venom given (ml)	Death occurred
H	36.5	0.8	Survived
W	35.5	0.9	Survived
HB	34.0	1.0	Survived
T	37.5	1.5	Died at 30th min

Still there were no deaths in animals mannerly at the dose of 1.0 ml. So we injected the 1.5 ml, death was occurred at 30th min. Then repeat of the dose 1.5 ml of the venom for the fixation of the study

Table No.10

Markings	Weight of mice (gm)	Dose of I.R.S. Venom given (ml)	Death occurred
H	31.5	1.5	Died at 23rd min.
HB	35.0	1.5	Died at 30th min.

So from above data, the dose of 1.5 ml was injected to the albino mice, and death was occurred at 23rd min, 30th min respectively. So finally it was decided that 1.5 ml having the concentration of the Indian Red Scorpion venom 0.9 mg/ml fatal dose was used in preclinical study for albino mice. Dose of Indian Red Scorpion Venom was = 1.5 ml (concentration 0.9 mg)

Dosing of Kutajadi Yoga: According to OECD guideline No. 423 the dose 2000 mg/kg body weight of *Kutajadi yoga* have been fixed and it was administered orally to 3 female albino mice to access the acute oral toxicity to check whether the experiment drug is toxic or not. For experiment purpose 400 mg of *Kutajadi Yoga* was weighed using weighing balance. Distilled water was added to it. This mixture was boiled and good suspension of *Kwatha* was made by filtration. The dose was given to mice according to body weight. It was given to the mice by oral route.

Table No.11

Markings	Weight of mice (gm)	Dose of *Kwath* given orally (ml)
H	31.0	0.31
B	23.0	0.23
HT	33.5	0.33

The acute toxicity study showed that *Kutajadi Yoga* is SAFE at 2000mg/kg body weight. For that, 3 female mice were used.

Dose calculation of Prazosin:

0.5 loading dose x 0.0026 = 0.0013 mg to 20 gm of mice per kg x 50

100

= 0.0013 x 50

= 0.0065 / kg for mice

1mg tablet of prazosin + 10ml Distilled water

⇩

0.1mg/ml mixed solution of prazosin + 9 ml Distilled water

⇩

0.01mg/ml solution of Prazosin + 9ml Distilled water

⇩

0.001 mg/ml

6.5 ml out of 10ml

Total Prazosin Solution 6.5 ml + Distilled water 3.5 ml= 10 ml

(Concentration = 0.0065)

Animal Experiment: Animal experiment for efficacy of *Kutajadi Yoga* in relation with Prazosin against Indian Red Scorpion venom poisoning in albino mice was carried out in authorized laboratory. Following protocol was used:

Table No.12

Animal species	Albino-mice
Strain	Swiss albino
Place of experiment	Authorized laboratory.
Source of animals	Authorized laboratory.
Sex of animals	50% males and 50% females in each group
Average weight of animals (Albino mice)	20-40 gms.
Age of Animals	6 – 8 wks.
No. of animals	6 mice for each group
No. of groups	4
Light Cycle	12 hrs light and 12 hrs dark
Vehicle used	Water
Relative Humidity	40 % to 60 %
Room Temperature	20 – 24 ^{0} C
Diet	Pelleted feed supplied
Dosing	Indian Red Scorpion venom was given by subcutaneous route, *Kutajadi Yoga* and Prazosin was given by oral route.

Groups for Animal Experiments

Table No.13

Group I (Control Group)	Only Indian red scorpion venom was injected by subcutaneous route in albino mice.
Group II (Experimental Group)	Indian red scorpion venom(subcutaneously) + *Kutajadi Yoga* (orally)
Group III (Experimental Group)	Indian red scorpion venom(subcutaneously) + *Kutajadi Yoga* (orally) + Prazosin (orally)
Group IV (Standard Group)	Indian red scorpion venom (subcutaneously) + Prazosin (orally)

Methods: Dried lyophilized form of venom was diluted into Phosphate Buffer Solution to get appropriate fatal dose to inject mice. *Kutajadi Yoga* and Prazosin were converted into suspension by through mixing with Distilled water. Samples were freshly prepared and then administered. Doses given to albino mice according to their body weight. After dosing all mice were observed for 24 hours for toxic signs and symptoms.

Procedure: First preliminary acute toxicity for drug was done. In each group, weight of albino mice was taken first. Accordingly, Indian Red Scorpion venom was given by SC route, after 5 min drug dose was given by oral route. After dosing albino mice were observed for 24 hrs. Comparative observations were tabulated.

RESULT AND DISCUSSION

Group I (Control Group)

Table No.14

Sex	Markings	Weight (gm)	Prolonged time	Death occurred
Male	HBT	33.0	27 min	6 out of 6
	HB	35.0	30 min	
	W	31.5	38 min	
Female	H	30.0	34 min	
	B	32.5	46 min	
	T	34.0	35 min	

(*H=Head, B=Back, T=Tail, W=White*)

Group II (Experimental Group)

Table No.15

Sex	Markings	Weight (gm)	Prolonged time	Death occurred
Male	H	35.5	41 min	3 out of 6
	T	37.0	Survived	
	B	36.5	49 min	
Female	HB	37.0	Survived	
	BT	35.0	44 min	
	W	33.0	Survived	

Group III (Experimental Group)

Table No.16

Sex	Markings	Weight (gm)	Prolonged time	Death occurred
Male	BT	33.0	Survived	3 out of 6
	W	34.0	120 min	
	T	37.5	Survived	
Female	H	36.0	45 min	
	B	38.0	48 min	
	W	34.5	Survived	

Group IV (Standard Group)

Table No.17

Sex	Markings	Weight (gm)	Prolonged time	Death occurred
Male	HBT	32.5	Survived	All survived
	W	35.0	Survived	
	HT	37.5	Survived	
Female	H	32.5	Survived	
	B	36.0	Survived	
	BT	34.5	Survived	

Wilcoxon Rank Sum Test (U test)

1) Control group and Experimental group

Table No.18

Group I	Group II	'T' value	'z' value	'P'
27	41	23	2.56205	< 0.01
30	1440			
38	49			
34	1440			
46	44			
35	1440			

The above two samples are significantly different (P < 0.01, two tailed test)

2) Control group and Experimental group

Table No.19

Group I	Group III	'T' value	'z' value	'P'
27	1440	22	2.72218	< 0.01
30	120			
38	1440			
34	45			
46	49			
35	1440			

The above two samples are significantly different (P < 0.01, two tailed test)

3) Control group and Standard group

Table No.20

Group I	Group IV	'T' value	'z' value	'P'
27	1440	21	2.88231	< 0.01
30	1440			
38	1440			
34	1440			
46	1440			
35	1440			

The above two samples are significantly different (P < 0.01, two tailed test)

4) Experimental group

Table No.21

Group II	Group III	'T' value	'z' value	P' value
41	1440	36.5	0.40032	>0.05
1440	120			
49	1440			
1440	45			
44	49			
1440	1440			

The above two samples are significantly different (P >= 0.05, two tailed test)

5) Experimental group and Standard group

Table No.22

Group II	Group IV	'T' value	'z' value	'P'
41	1440	30	1.44115	>0.05
1440	1440			
49	1440			
1440	1440			
44	1440			
1440	1440			

The above two samples are significantly different (P >= 0.05, two tailed test)

6) Experimental group and standard group

Table No.23

Group III	Group IV	'T' value	'z' value	'P'
1440	1440	30	1.44115	>0.05
120	1440			
1440	1440			
45	1440			
49	1440			
1440	1440			

The above two samples are significantly different (P >= 0.05, two tailed test)

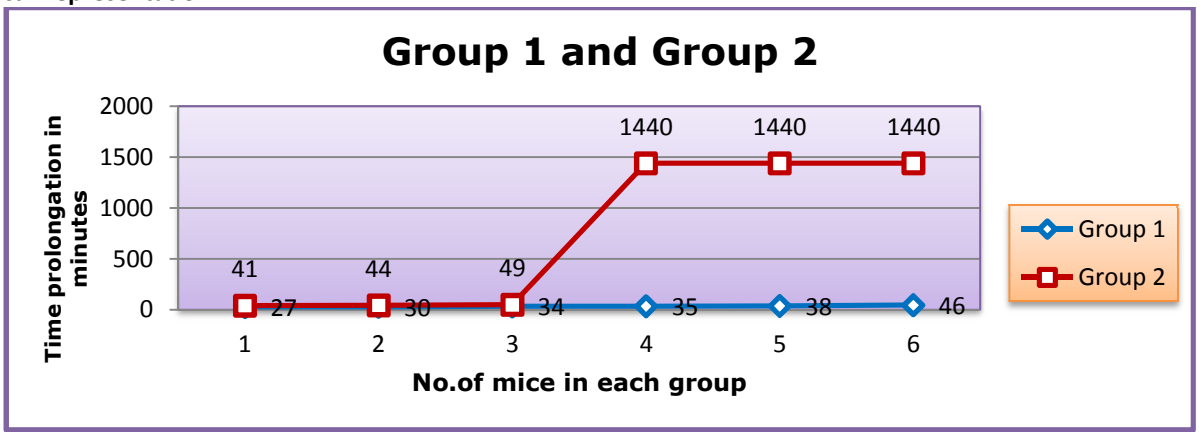
- **Clinical profile of the prognosis of all groups at the end of study**

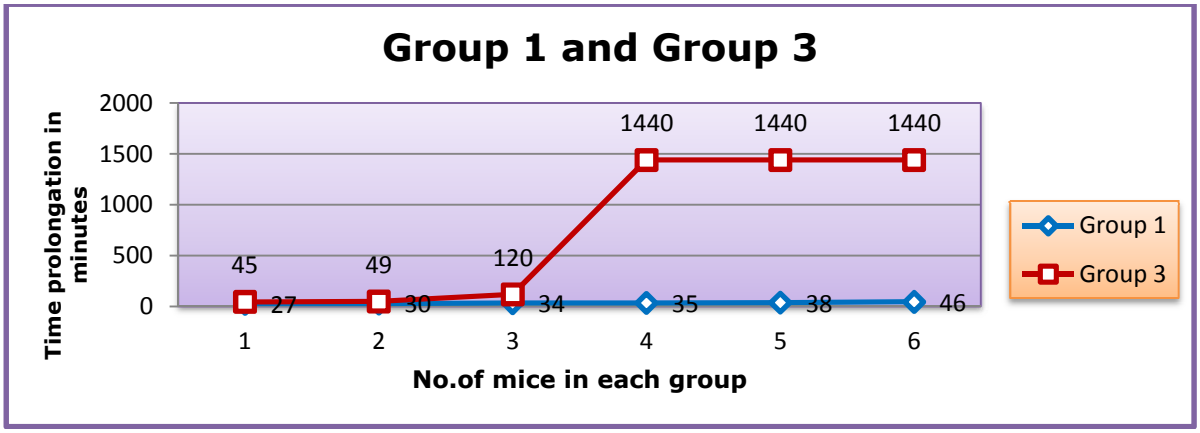
Table No.24

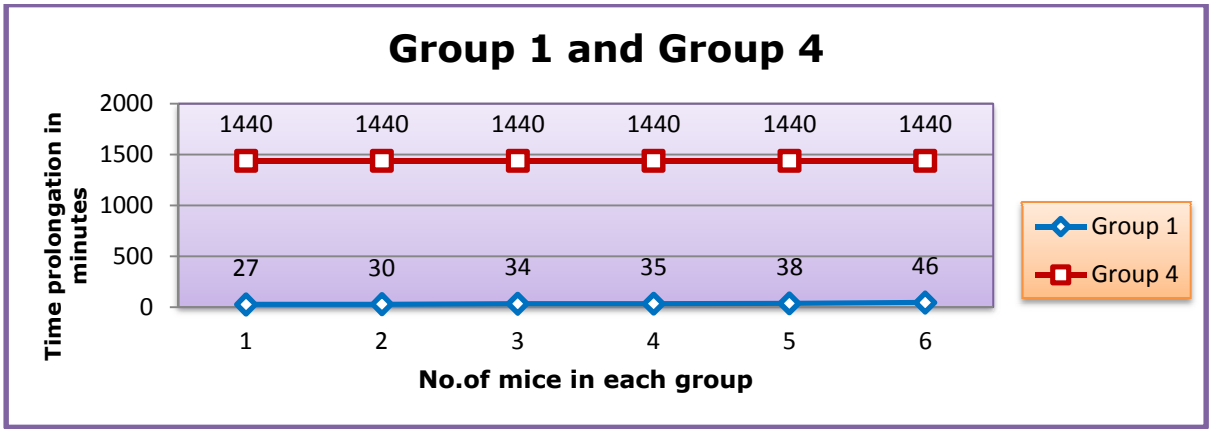
Groups	Survived/Dead Albino mice no						Total	%
	1	2	3	4	5	6		
Group I	-	-	-	-	-	-	0/6	0
Group II	-	+	-	+	-	+	3/6	50
Group III	+	-	+	-	-	+	3/6	50
Group IV	+	+	+	+	+	+	6/6	100

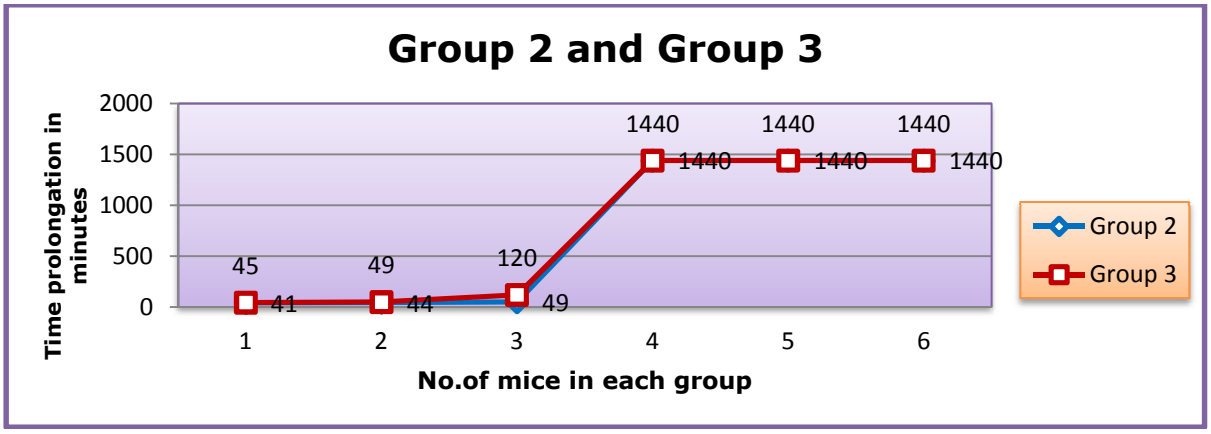
Group I: All albino mice were dead.
Group II: 2 male and 1 female were dead.
Group III: 1 male and 2 female were dead.
Group IV: All albino mice were survived.

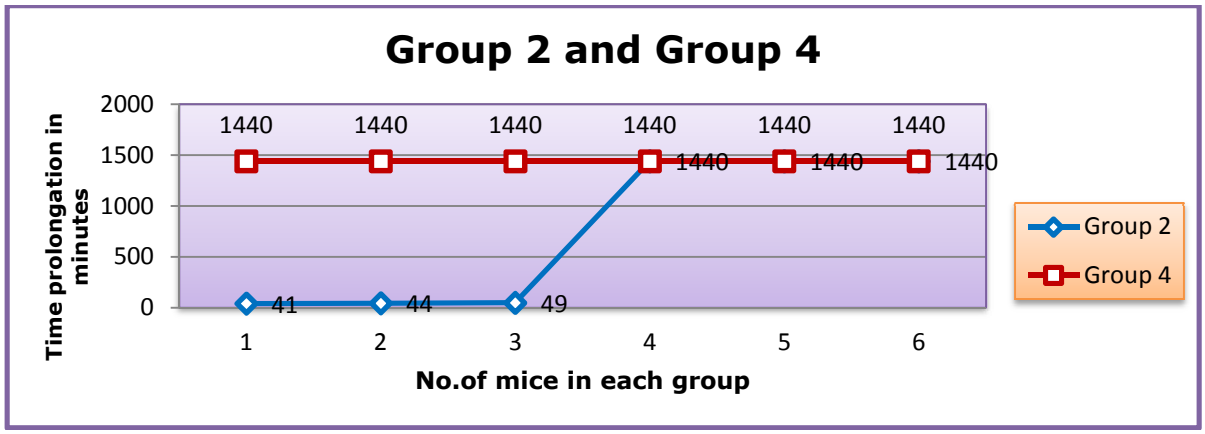
- **Graphical Representation:**

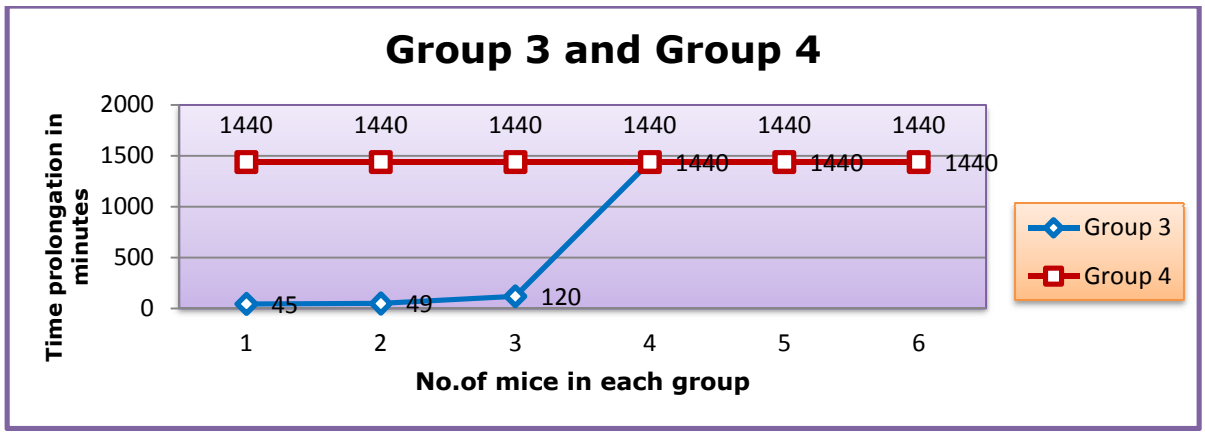


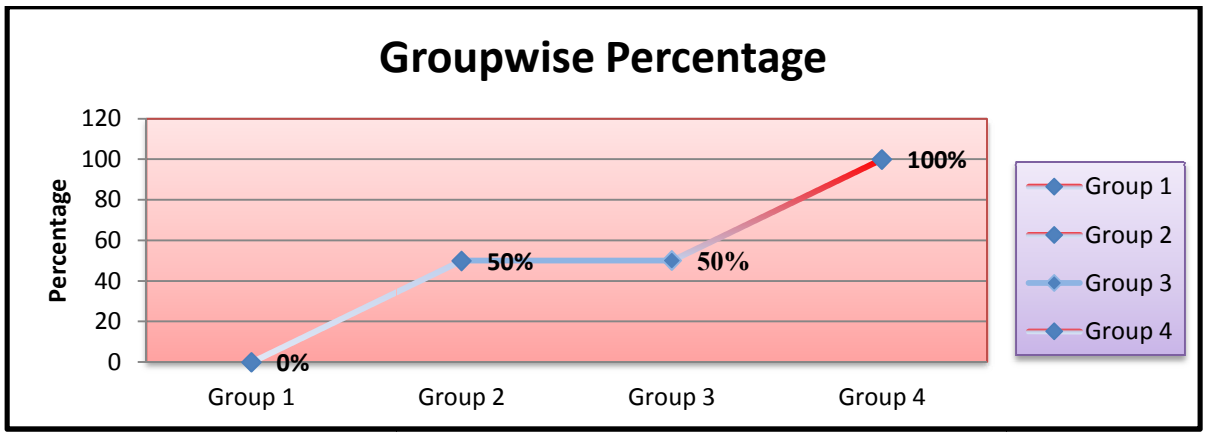





Group 2 and Group 3
Time prolongation in minutes
2000
1500
1000
500
0
1440
1440
1440
1440
1440
1440
45
49
120
41
44
49
1
2
3
4
5
6
No.of mice in each group
Group 2
Group 3


Group 2 and Group 4
Time prolongation in minutes
2000
1500
1000
500
0
1440
1440
1440
1440
1440
1440
41
44
49
1
2
3
4
5
6
No.of mice in each group
Group 2
Group 4


Group 3 and Group 4
Time prolongation in minutes
2000
1500
1000
500
0
1440
1440
1440
1440
1440
1440
45
49
120
1
2
3
4
5
6
No.of mice in each group
Group 3
Group 4


Groupwise Percentage
Percentage
120
100
80
60
40
20
0
100%
50%
50%
0%
Group 1
Group 2
Group 3
Group 4
Group 1
Group 2
Group 3
Group 4

DISCUSSION

Scorpions are one of the oldest known terrestrial arthropods. The Indian Red Scorpion is dangerous to humans due to their ability to give painful & sometimes life threatening stings. They cause severe manifestations in human being. Insect poisoning is described under the heading "*Keetavisha*" under *Jangama Visha*, in *Ayurvedic* science. Poisoning by them is of more importance because of their severe manifestation. *Vrishchika* is explained in almost all the *Ayurvedic* literature. The Classical texts like *Sushruta* and *Charak Samhita*, *Ashtanga Samgraha* and *Ashtanga Hridaya* explains about the details regarding the *Vrishchika damsha* and *chikitsa* except *Sharangadhara Samhita*.

The manifestation of *Vrishchika damsha* varies widely from mild pain to cardiovascular disturbances & even up to death. Common manifestation of Vrishchika damsha include severe cutting pain, burning sensation, oedema, erythema & pruritis, nausea, vomiting, fever, local rise in temperature, breathlessness, nasal secretion, salivation, abdominal pain, tingeing sensation of the tongue, muscular fasciculation etc. may be associated. According to these symptoms Acharyas classified Vrishchika into three categories; *Manda, Madhya* and *Maha visha*. Indian Red Scorpion venom symptoms just like *Maha visha.* In such treatment, I have selected *Kutajadi yoga*.

कुटजस्य फलं पिः तगरं जालमालिनी ।
तिक्तेश्वाकु श्च योगोऽयं पानप्रधमनादिभिः ॥
वृश्चिकोन्दुरुलूतानां सर्पाणां च विषं हरेत् ।
समानो ह्यमृतेनायं गराजीर्ण च नाशयेत् ॥
(च. चि. २३/२०६–२०७)

Charakacharya in his *Vishachikitsa Adhyaya* explained the details *Kutaja, Tagar, Devadali* and *Ekshwaku.* This formulation used as *pana* and it alleviates poison of snake also. Treatment of Madhya *visha* & *Maha visha* of *Vrishchika* is similar to snake bite poisoning. And this drug is easily available in market and easy to prepare. So I have chosen *Kutajadi Yoga*. Preclinical study was selected for study because the aim is to collect data in support of safety. And it is required before clinical trials can be started. No scientific data is available along with *Kutajadi yoga.*

Prazosin is simple, scientific pharmacological and physiological antidote to scorpion venom actions. It is already proved by Dr. H. S. Bawaskar to scorpion envenomation have real advantages in treatment of red scorpion envenomation, the cardiovascular complication of which often produce medical emergencies everywhere. The work done at Mahad region in Maharashtra state.

Drug procurement

The herbal drugs *Kutaja phala, Tagar moola, Ikshwaku phala* and *Devadali phala* which are important ingredients in study are easily available. Raw samples of *Kutajadi Yoga's* ingredients were collected from market. Authentication of *Kutajadi yoga* ingredients were separately done at Pharmacy College. Preparation of the *Kutajadi yoga* was done at the college pharmacy. And its standardization was done at Pharmacy College.

Venom procurement

For entire animal experiment phase in this study it was needed Indian red scorpion venom was obtained from the Haffkine institute, Mumbai which was available in 2 vials containing 300 mg of lyophilized venom of Indian Red Scorpion (Mesobuthus Tamulus) is procured by completing their paper formalities.

Dose calculation of venom

Before starting of trials the dose range finding study is carried out to fix the dose of the venom. Initially it was decided that 0.3 mg/ml fatal dose of Indian red scorpion venom was used in preclinical study in albino mice. To resolve this experimentally, we conducted the pilot study using 5 animals. In first stage these animals were given 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml at that time concentration was 0.3 mg/ml. Only itching and irritation was developed. So the new concentration dose 0.6 mg/ml was injected to each mouse. Even though this dose was not sufficient. Still there were no deaths in experimental animal. So the dose of 1.5 ml was injected to the albino mice, and death was occurred at 23rd min, 30th min respectively. So finally it was decided that 1.5 ml having the concentration of the Indian Red Scorpion venom 0.9 mg/ml fatal dose was used in preclinical study for albino mice.

Dose calculation of Kutajadi Yoga

According to OECD guideline No.: 423 the dose 2000 mg/kg body weight of *Kutajadi yoga* have been fixed and it was administered orally to 3 female albino mice to access the acute oral toxicity to check whether the experiment drug is toxic or not. For experiment purpose *churna* of *Kutajadi Yoga* was weighed using weighing balance. Distilled water was added to it. This mixture was boiled and good suspension of *kwatha* was made. The dose was given to mice according to body weight. For e.g. If weight of albino mice is 31 gm, then the dose of *kwath* was given 0.31 ml to albino mice. For the others the dose of *kwath* was given according to its weight.

Discussion on presenting Signs and Symptoms

The mice having symptoms like tachypnea, restlessness, salivation, increased nasal secretion, increased lacrimation, tachycardia, tremors, horripilation & convulsion and then death.

Associated Symptoms: All group of mice had restlessness, increased heart beats, rapid breathing immediately after the injecting venom. No other associated symptoms were seen.

The Effect of Kutajadi Yoga

Kutajadi yoga was administered to mice orally by using oral feeding syringe was given. The dose was given as per body weight. The time was prolonged up to 10 to 15 min. and in this group 3 mice were survived.

The Effects of Kutajadi yoga along with Prazosin

The *Kutajadi Yoga* was administered orally and also Prazosin was administered orally and both routes are the same. And administered as per body weight formula. The time was prolonged in 3 mice up to 25 to 85 mins. & the 3 mice were survived. Comparison to both, the *Kutajadi yoga* along with Prazosin is more effective than *Kutajadi yoga* individually.

Discussion on the Probable action of Kutajadi Yoga (Statistical analysis)

In Indian Red Scorpion venom, Group I (control group) death was observed after 35 min (average) and in Group II

(experimental group) time was prolonged more than 10 to 15 min as compared to control group and out of 6 mice 3 mice were survived, which is statistically significant. P value is (P<0.01). Experimental group shows good result on the prolongation time than the control group thus the *Kutajadi yoga* shows significant result.

Group I (control group) compared with Group III (experimental group), in Group III there was also time prolonged more than 25 to 85 min as compared to control group and out of 6 mice 3 mice were survived. So it is also statistically significant. P value is (P<0.01). Also here, experimental group shows good result on the prolongation time than the control group thus the *Kutajadi yoga* along with Prazosin shows significant result. Group I (control group) compared with Group IV (standard group), there was also time prolonged after 24 hrs (average), there were all mice survived, which is also statistically significant. P value is (P<0.01). Here, standard group shows good result on the prolongation time than the control group thus the Prazosin shows significant result.

Group II compared with Group III, in both experimental group time was prolonged more in both group as compared to control group and out of 6 mice, 3 mice were survived. So it is statistically insignificant. P value is (P>0.05). Here both the group i.e. II and III show similar effect on the prolongation time. Thus the result was insignificant. Group II (experimental group) compared with Group IV (standard group), there was also time prolonged in both group. So it is statistically insignificant. P value is (P>0.05). Here both the group i.e. II and IV shows similar effect on the prolongation time. Thus the result was insignificant.

Group III (experimental group) compared with Group IV (Standard group), there was also time prolonged in both group. So it is statistically insignificant. P value is (P>0.05). Here both the group i.e. III and IV shows similar effect on the prolongation time. Thus the result was insignificant.

Impact statement: From the observation and analysis of data, it is observed that *Kutajadi Yoga* separately and also along with Prazosin against Indian Red Scorpion venom

- It delays the duration of survival time.
- It does not interact with Prazosin, the time prolongation is more of *Kutajadi Yoga* along with Prazosin.

Future Roadmap

Change in design of study: In future studies of this type of design can be changed for obtaining better results. As this study the venom was administered first and then *Kutajadi Yoga* has administered. It is only for the pre-clinical trials, not suggested for clinical trials. In future; change design will be *agad* will be administered first and then after within ½ hour venom is administered. So *agad* will get sufficient time to absorbed and reach to the blood stream then only it will tackle venom which has got in the circulation. So changed design will be able to establish the activity of experimental drug.

- *Agad* titration: Procedures like *agad* titration should be carried out before starting of trials. It indicates how much quantity of *agad* is responsible for neutralizing specific quantity of venom.
- *Sushruta* states that, there are seven *visha vegas* in human and four *vegas* in animals. Study of signs and symptoms during these *vegas* can be a topic of research.

SUMMARY

Scorpions are the insects that are included under the Phylum Arthropods, which are found in warm & dry climate. Some species belonging to the family Buthidae produce neurotoxin venom that is potent, lethal and toxic to human victims. They are dangerous to human due to their ability to give painful & sometimes life threatening stings. Depending on the toxicity of their venom, the scorpions are classified into two types. Mesobuthus tamulus (as Indian Red scorpion, called Buthus tumulus) which is dangerous to human due to their ability to produce painful & sometimes life threatening stings and Black scorpion (Palmaneus garvimanus) often seen all over Keral state are the common ones. Black scorpion is less toxicant and no systemic involvement is there. The world statistics shows the hazardous feature of this creature. Mesobuthus tamulus species are most commonly found in the state of Maharashtra, Anantpur and Karnool districts of Andhra Pradesh, Chennai, Pondicherry and Madurai in Tamil Nadu, part of Gujarat, Patna area from Bihar and Bellary district of Karnataka. Almost all the systems of Medicine have developed their own treatment modality to manage scorpion sting. In *Ayurvedic* science also much fruitful & regional knowledge is there in the management of *Vrishchika damsha* (scorpion sting), to which the efficacy is not proved and no statistical evaluation has been carried out, and thus the knowledge is not being incorporated in *ayurveda Kutajadi Yoga* is one of such medicine.

The present study – "Preclinical study of the *Kutajadi Yoga* in relation with Prozosin against Indian Red scorpion venom poisoning in albino mice," was proposed to evaluate experimentally or pre-clinically the efficacy of *Kutajadi Yoga* separately and along with Prazosin. The work is divided into Introduction, Literary review, Materials and Methods, Observations and the last chapter includes Results, statistical analysis, Discussion, Summary & Conclusion. In literary review, all the available references to the subject *Vrishchika* and Scorpion were compiled as much as possible from *Ayurvedic samhita* & modern views.

In Material & methods, detailed description about the ingredients of *Kutajadi yoga*, was given. The preclinical study was designed on albino mice. 24 mice were divided into four groups. In Each group 3 males and 3 females were included for the study after weighing in range 20-40 gm.

- Group I: received only Indian Red Scorpion venom
- Group II: received Indian Red Scorpion venom and *Kutajadi yoga*
- Group III: received Indian Red Scorpion venom + *Kutajadi yoga* + Prazosin and finally
- Group IV: received Indian Red Scorpion venom + Prazosin.

During this preclinical study, in the Ist Group (Control group) all death of mice were occurred. In Group IInd out of 6 mice, deaths of 3 mice (2 male and 1 female) were occurred. In Group IIIrd out of 6 mice, deaths of 3 mice (1 male and 2 female) were occurred. Time prolongation of Group IIIrd was

more as compared with Group II[nd]. Group IV i.e. Standard group, no death was occurred. All mice were survived. As per planned protocol Group I (control) compared with Group II (experimental) got significant result (P<0.01); Experimental group shows good result on the prolongation time than the control group thus the *Kutajadi yoga* shows significant result. Group I (control) compared with Group III (experimental) got significant result (P<0.01); Experimental group shows good result on the prolongation time than the control group thus the *Kutajadi yoga along* with Prazosin shows significant result. Group I (control) compared with Group IV (standard) got significant result (P<0.01). Standard group shows good result on the prolongation time than the control group thus the Prazosin shows significant result. Group II compared with Group III got insignificant result (P>0.05). Here both the experimental group i.e. II and III shows similar effect on the prolongation time. Thus the result was insignificant.

Group II (experimental) compared with Group IV (standard) got insignificant result (P>0.05). Here both the group i.e. II and IV shows similar effect on the prolongation time. Thus the result was insignificant. Group III comparison with Group IV got insignificant result (P>0.05) Here both the group i.e. III and IV shows similar effect on the prolongation time. Thus the result was insignificant. But time prolongation was more as compared to Group II.

Indian Red Scorpion Sting is common problem in villages of India. The life threatening complication is developed. This condition requires urgent attention & ICU care. Hence we require the primary substitution or first aid measure along with serotherapy, which will increases the survival period & decreases the mortality & morbidity. The *Kutajadi yoga* was selected which is mentioned in *Charaka samhita chikitsasthana* (*Cha.Chi*.23/206-207) which is,

- Easily available and easy to carry. It is easy to prepare.
- It has very much palatability.
- It can be proved useful in small dose.
- Route is orally which is easy to take.
- It was reduced the fatality.
- It can be used as a primary first aid measure along with serotherapy.
- It alleviates snake poisoning also. Treatment of *Madhya visha* & *Maha visha* of *Vrishchika* is similar to snake bite poisoning. So it is useful for the scorpion poisoning.

The *Kutajadi yoga* individually was prolonged the time duration of survival in mice. Also the *Kutajadi yoga* along with Prazosin was prolonged the time duration of survival. In that the time prolongation was more as compare with *Kutajadi yoga* individually. In this study it is not interact with standard drug. From detailed Experimental study it is proved statistically that, the *Kutajadi yoga* individually and also in combination with Prazosin shows significant result like Prazosin. So the experimental drugs are useful against Indian Red Scorpion venom poisoning.

CONCLUSION

The present study entitled "Preclinical study of efficacy of *Kutajadi yoga* in relation with Prazosin against Indian Red Scorpion venom poisoning in albino mice" was on attempt to study the *Kutajadi yoga* as per (Ref.-*Cha.Chi*.23/206-207). Conclusion is the vital part of the entire study of research work. Without which the study or of the research may not be completed. Sometimes this conclusion may be positive or negative. It is not necessary to get result always positive. But the work which will be carried out should be standard parameters. After a detailed conceptual study, critical review, observation and discussion the following conclusions are evolved.

- Poisoning due to Indian red scorpion presents with a variety of symptoms ranging from simple itching to life threatening situations.
- In classics we find references about insect bite and management explained under "*Keetavisha prakaran*".
- The manifestation of Indian Red Scorpion venom poisoning varies widely, from mild pain to multi organ system failure and even death. It depends on several factors; the type, age and species of scorpion, the amount of venom injected etc.
- The study drug *Kutajadi yoga* individually or in combination with Prazosin is useful as a first aid measure in scorpion venom poisoning.
- In acute toxicity study was done. The *Kutajadi yoga* is not toxic to mice.
- The data was converted into tables and diagrams for analysis. 'Wilcoxon Rank Sum test' was applied for statistical analysis.
- The research design was made on a preclinical study in authorized laboratory.
- In this study the *Kutajadi yoga was* prolonged the time duration of survival in mice. Also *Kutajadi yoga* along with Prazosin prolonged the time duration of survival, in which time prolongation is more in *Kutajadi yoga* along with Prazosin as compare with *Kutajadi yoga* individually. But both treatments were found effective for prolonged time duration of survival in Indian Red Scorpion venom poisoning.
- By studying the *Kutajadi yoga* individually and along with Prazosin in scorpion venom poisoning,

➢ It is not toxic to mice.
➢ It delays the survival period.
➢ It does not interact with Prazosin.

From the detailed preclinlcal study I conclude that, the *Kutajadi yoga* individually and also in combination with Prazosin show significant result like Prazosin by statistically. So the experimental drug is useful against Indian Red Scorpion venom poisoning.

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