Original Article

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Evaluation of Effect of Vatsanabha (Aconitum Ferox Wall.) As a Prativisha against Cobra Venom (Naja Naja) Toxicity: An Experimental Study

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Abstract

Background: Snake bite, till date remains a public health hazard in tropical countries, especially In India. A detailed review of Ayurvedic literature a unique "vishae and prativisha" concept explains Sthavar visha (vegetable poison) and jangama visha (animate poison) both kinds of poison destroy or neutralizes each other's effects when used against each other. Thus in the present study Vatsanabha (Aconitum Ferox) was taken as Prativisha (antidote) against cobra venom (Naja naja) toxicity. *Methods:* The lyophilized snake venom of cobra (Naja naja) LD50 0.49 mg/kg of rat dose was injected i.p and Snake venom antiserum was used as reference standard drug. Vatsanabha (Aconitum Ferox) given orally in two different classical dose as Madhyama (medium) & Uttama (high) dose as 16.83 mg/kg & 22.5 mg / kg body weight respectively in III & IV test group and evaluated its effect against venom on survival time, neurological observational signs, hematological parameters & Histopathology for Liver & heart. *Results:* The survival time in test (III) group (87.2±23.8 min) was observed to be increased as compared to venom (II) group (71.8±14.8 min). Reverse changes are found in all biochemical parameters. Statistically reverse changes ($p<0.01$) was found in Neutrophils, monocytes & SGOT. Histopathology reports of liver & heart of test III group show mild (+) or absent (0) when compared to the Venom Group (+++=severe). *Conclusion:* By considering the oral limitation, Vatsanabha has not shown direct Antidotal activity but there was an increase in survival time. The test III group has shown statistically reverse changes on neutrophils, monocytes & SGOT levels. The test III group (madhyama dose) had shown good protection on liver & heart cells against cobra venom (Naja naja) induced toxicity which suggests Vatsanabha may contain an endogenous inhibitor of venom-induced cell damage.

Keywords: Antidote; Cobra Venom; Naja Naja Toxicity; Vatsanabha; Visha Prativisha.

Introduction

The concept of environmental toxicology has been raised as an important issue in the present century. The varieties of bites from poisonous animals as well as insects still remain as a serious matter of consideration. Snakebite, till date, remains a public health hazard in tropical countries, especially in India. The common poisonous snakes found in India are Cobra (*Najanaja*), Krait (*Bangarus Caeruleus*), Russell's viper (*Daboiarusselli*) and saw scaled viper (*Echis Carinatus*) [1].

In 2007 World Health Organization reported 50,000 people died from snakebites every year in India, accounting for a large portion of the 125,000 deaths worldwide. People in rural areas are most at risk, particularly who work outdoors [2]. Early in 2009, snakebite was finally included in the WHO's list of neglected tropical diseases, confirming the experience in many parts of this region that snake bite is a common occupational hazard of farmers, plantation workers [3,4].

Anti-snake venom (ASV) is the only specific treatment against snake venom envenomation. It neutralizes the circulating venom only and no amount of ASV will neutralize or combine with venom once the venom is attached absorbed to target organs i.e. platelets, RBC's Vascular endothelium, Renal tubules, muscles and neuromuscular receptors. ASV composed of antibodies from immunized animals; So there are chances of adverse

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reactions due to activation of theimmune system in about 20% of patients [5,6,7,8,] such as anaphylactic shock, pyrogen reaction and serum sickness [9].

At present Traditional toxicology survives in its original form only in Kerala, India. We find that Folklore practitioners manage these emergency situations which are affordable to the majority of victims who belongs to very low socio-economic communities with a wide range of herb, herbomineral formulations and *Ayurvedic* procedures including *panchakrama* and *mantra chanting*. A detailed review of an Ayurvedic literature a unique "*vishae - prativisha*" concept led to theselection of research [10], *Sthavarvisha (vegetable poison)* and *jangamavisha* (animate poison) both kind of poison possesses opposite qualities and when used as a *prativisha*, neutralizes each others effects [11]. There are very little researches conducted on Agada tantra topics. So it is of utmost importance to conduct more research on principles of *vishachikitsa* and the *Agada's* (antidotes) mentioned in ancient *Ayurvedic* texts. Since no report of snake venom neutralization action by the *Vatsanabha* (*Aconitum ferox*) against, thus hypothesis is made to screen the *Prativisha* effect against cobra venom toxicity in an animal experimentation on albino rats.

Material and Method

1. Source of Venom & Antisnake Venom

Lyophilized Venom of *Cobra Venom (Najanaja)* was obtained from KV Institute, Rakesh Park, Sagarpali, Ballia-277506, Uttar Pradesh with Batch no. 03/15. Anti-snake venom was obtained from SurakshaBio Pharma, Ramdev Galli, Belgaum, Karnataka of Bharat Serums And Vaccines Ltd. with Batch. No: A05315123, Date of mfd: 12/2015, Date of expiry: 11/2019.

2. Plant Material

Vatsanabha kanda (root of *Aconitum Ferox)* was collected naturally. Authentication and the taxonomic identification of plant materials was confirmed by Department of Dravyaguna (Ayurvedic botony) & AYUSH approved, Central research facility Department of KLEU's Shri BMK Ayurveda Mahavidyalaya and Research Center, Belgaum, Karnataka.

3. Ethics & Animals

The Experimental procedures were approved by the institutional ethical committee in the college (IAEC) Reg. No – 1017/06/CPCSEA. 30 animals of Either sex Male and female Wistar rats weighing of 150-200 gms were purchased from theauthorized animal center and experimental study was conducted in theanimal house of KLEU's Shri BMK Ayurveda Mahavidyalaya, Shahapur, Belgaum. After 7 days of thequarantine period, animals were properly weighed and marked clearly and grouped as per the study design. Food quantity was regularly supplied and water *ad libitum* was provided in a sterile container. The environment was maintained with 20-22^{0}C; 50% Humidity and with a light and dark cycle of 12 hrs as per CPCSEA guidelines. 30 animals were divided into 5 groups & each Group contains 6 animals. (Table 1).

4. Preparation of Dosing

LD50 of cobra venom (*Najanaja*) was selected as 0.49 mg/kg of rat, from the Indian article of venom procured from theeastern part of india [12]. The classical dose of *Vatsanabha (Aconitum Ferox) as Prativisha (Antidote)* are 6 *Yava* & 8 *Yava* (1 *Yava*= 31.25 mg = as per Ancienttext) respectively as *Madhyama Matra* (medium dose) for *Madhyama Visha* (medium poisoning) & *Uttama Matra* (high dose) for *Uttama Visha* (high poisoning) human use. These were converted to animal dose According to Paget's & Barnes rule which comes out to as 16.83 mg/kg &22.5 mg/kg body weight respectively & and were used in the present study in III & IV test group.

Table 1: Shows the Grouping & study design

S. No.	Group name	Experimental Group	No. of animals	Intervention
1.	Group I	Control Group	n= 6	Normal saline as I.P
2.	Group II	Venom group (LD50 as 0.49 mg / kg per rat)	n= 6	I.P
3.	Group III	Venom + *Vatsanabha* powder (*madhyama* dose as 16.83 mg / kg body weight)	n= 6	I.P & oral
4.	Group IV	Venom + *Vatsanabha* powder (*Uttam* dose as 22.5 mg / kg body weight)	n= 6	I.P & oral
5.	Group V	Venom + Anti Snake Venom	n= 6	I.P & I.P

5. Evaluation of Antidotal activity

The cobra venom was dissolved in 0.9% w/v normal saline and centrifuged at 2000 rpm for 10 minutes and according to the weight of animal dose was injected *i.p.* After 5 min of envenomation, *Vatsanabha choorna* (in fine powder form) was added in a hot distilled water glass bowel (mg/ml), and according to body weight of each animal, it was filled in syringe with mouth gaze attached. then it was administered orally in test group III and group IV. After dosing in all the groups, individual Animals were observed and duration of time was noted for their changes on the following Parameters:

1. Severity & duration of paralytic signs & convulsion signs of cobra venom injected rats.
2. No. of death per group.
3. Duration of survival in venom group, standard group & in the experimental group.
4. Bleeding timeas per the modified procedure of Mohamed et. Al. (1969). Clotting time was determined as per Mohammad et al. & the time taken for this was recorded before the death of animals [13].
5. Blood was collected & sent for biochemical investigation to the Pathological lab of KLEU's Shri BMK Ayurveda Mahavidyalaya & Research Center, Belgaum.
6. Liver and heart were collected after death of each animal & were kept in 10% formalin. Then were sent for histopathological examination to Jeevana Laboratory, Belgaum, Karnataka.

6. Statistical analysis

Neurological signs, survival times, biochemical data were expressed as mean±SD of 6 animals per group. Parametric One Way analysis of variance (ANOVA) test was performed using graph pad prism window 5.0 software. The minimum level of significance was identified at <0.05 with Tukey multiple comparison tests as post hoc test.

Results

3. Observational Parameters Findings: Neurological toxic signs like Decreased motor activity, Paralysis of lower limbs, a Behavioral pattern like lying flat on the belly, Gasping & Convulsion: Tonic-Clonic were assessed, observed & noted in mean±SD. The effect of *Vatsanabha* in Group III revealed that there was an increase in mean time on toxicity signs in the tested group which showed the interaction of *Vatsanabha* on the nervous system, but all found to be statistically nonsignificant (Table 2).

3. Survival rate: The effect of *Vatsanabha* in Group III revealed that there was an increase in survival time (87.2±23.8 min) in comparison with venom group, but still all the animals died in the present study. (Table 3).

Table 2: Shows the result of ANOVA test on the sign & symptoms of toxicity (recorded per min) in the following groups

Signs & symptoms of toxicity (recorded per min)	Group II Mean±SD	Group III Mean±SD	Group IV Mean±SD	Group V Mean±SD	P value	Significance
1 Decreased motor activity	24.2±5.6	38.3±22.1	37.2±13.2	42.8±12.6	0.1767	NS
2. Paralysis of lower limbs	35.3±3.27	45.8±21.3	43.8±15.2	58.5±23.6	0.1887	NS
3. Behavioral pattern: lying flat on belly,	47.3±7.53	54.2±23.1	51.7±15.7	74.3±40	0.269	NS
4. Respiration: Gasping	59.2±10.2	70.3±26.8	58.8±17.3	88.8±41.6	0.2062	NS
5. Convulsion: Tonic – Clonic	68±12.2	79.5±29	63.3±19.8	100±50.1	0.2105	NS

*NS = non significant

Table 3: Showsthe Mean Survival time (in min) & Mortality in the following Group

Groups	Mean survival time (in min) Mean±SD	Mortality (Total animal survival / Total no. of animals in Group)
Group II	71.8±14.8	0/6
Group III	87.2±23.8	0/6
Group IV	68.3±19.9	0/6
Group V	111±48.8	0/6

3. Blood Parameters & Biochemical Findings: On the administration of *Vatsanabha* (*madhyam* dose) along with cobra venom in Group III, there was statistically significant (*p<0.05)* reverse decrease change was found in Neutrophils (32.5±5.12)in comparison with venom II Group (57.1±15.59). We also observed, reverse increase level in WBC's (6367±1376), Lymphocytes (63.3±6.12), monocytes (1.83±1.6), decrease in Neutrohpils / Lymphocytes Ratio (0.51±0.11) in Tested Group (III) in comparison with venom group (II) which shows the reduced level of inflammation indicating amelioration of venom-induced stress and inflammatory process in the group may be due to interaction of *Vatsanabha (Aconitum Ferox)*. All the data is listed in Table 4.

Table 4: Shows the Effect of Vatsanabha (Aconitum ferox) on Blood Parameters

Parameter	Group I (control)	Group II (venom)	Group III (test group A)	Group IV (test group B)	Group V	ANOVA P value	Significance
Haemoglobin (g/dl)	14±0.54	13.4±2.71	14.7±1.19	16±1.32	14.9±2.5	0.147	NS
WBC Count (cells/cumm) Mean ± SD	7520±756.3	4917±2067	6367±1376	5333±1041	5583±2700	0.5871	NS
Neutrophils % Mean ± SD	33.8±1.64	57.1±15.59	32.5±5.12#	39.33±11.29	36.67±13.02 @	0.0045 **	S
Lymphocytes % Mean ± SD	52.2±5.40	41.1±21.41	63.3±6.12	58.6±11.57	60.6±13.19	0.05	NS
Eosinophils % Mean ± SD	2.2±0.4472	4.16±2.04	4±2.96	2.83±0.75	3±0	0.28	NS
Monocytes % Mean ± SD	2.8±0.8	1± 0	1.83±1.6	1.16±0.4	1.33±0.5	0.0024 **	S
Platelet count Mean ± SD	308000± 86813	374833± 151152	537167± 117247	673667± 73375 ##	580000± 221994	0.0014 **	S
Neutrohpils / Lymphocytes Ratio (NLR): Mean ± SD	0.65±0.06	2.44±2.9	0.51±0.11	0.71±0.4	0.7±0.55	0.11	NS
Bleeding time (in sec) Mean ± SD	325±44.16	328.3±33.71	327.5±41.68	328.3±40.21	325.8±40.3	0.99	NS
clotting time (in Sec) Mean ± SD	70±10	50.83±8.01	45.83± 9.17	45±8.944	59.17±11.14	0.001	S
Serum Urea (mg/dl) Mean ± SD	17.4±0.54	15.1±1.9	15±1.4	14.8±3.8	15.3±1.3	0.33	NS
Serum Creatinine(mg/dl) Mean ± SD	0.88±0.08	0.91±0.25	0.81±0.16	0.65±0.22	0.71±0.09	0.09	NS
S.G.P.T (IU/L) Mean ± SD	30.2±5.02	39.4±6.78	31±4.29	31.83±2.92	32.67±3.67	0.07	NS
S.G.O.T (IU/L) Mean ± SD	29.4 ± 4.3	42 ± 7.3	29.17± 6.4 #	33.5 ± 3.9	35.17 ± 3.7	0.0028**	S

NS= non significant, S= significant, *,# p<0.05=mild significance, **,##p<0.01= moderate significant.

Group III compared with venom Group II, ## Group IV compared with venom Group II, @ Group II compared with Group V.

S.G.P.T =Serum glutamic pyruvic transaminase, S.G.O.T=Serum glutamic oxaloacetic transaminase

Table 5: Shows the Effect of Vatsanabha (Aconitum ferox) on Histopathology of liver on rats

S. No.	Microscopy	Group I (control)	Group II (venom)	Group III (test group A)	Group IV (test group B)	Group V
1	Central vein cong	+	+++	+	++	+
2	Sinusoidal cong	+	+++	+	++	+
3	Focal haemorrhage	0	+	0	0	0
4	Inflammation	0	++	0	0	+
5	Ballooning hepatocytes	0	++	0	0	++
6	Degeneration	0	++	0	0	+
7	Spotty necrosis	0	++	0	0	0
8	Kupffer cell hyperplasia	0	+	+	+	+

0=absent, + = Mild, ++ =Moderate, +++ = Severe, ++++ =Highly

The Analysis of data revealed that *Vatsanabha* (*Aconitum Ferox)* along with venom tested group (III) has shown reverse decrease change in on Serum creatinine (0.81±0.16), SGPT (Serum glutamic pyruvic transaminase) (31±4.29) in comparison with venom group (II) but all found to be statistically nonsignificant. There was statistically significant reverse change ($p<0.05$) found in the tested group (III) on SGOT (Serum glutamic oxaloacetic transaminase) (29.17±6.4) in comparison with venom (II) group.

4. Histopathological findings: The histopathological changes in liver & heart was scored as follows: 0: absent, +; mild, ++; moderate, +++; severe. (Table 5 & 6).

The liver tissue section of control group I shows normal cytoarchitecture, venom Group (II) showed Severe Central vein congestion, severe Sinusoidal congestion, severe Ballooning hepatocytes etc. Whereas the histopathology of tested Group (III) & Group (IV) showed less toxic changes with only moderate Central vein congestion mild Sinusoidal cong, mild inflammation, no Ballooning hepatocytes in comparison with venom group (II) & standard Group (V). (Fig. 1).

On examination, the heart section of control group I showed normal cytoarchitecture. Heart tissue section of venom Group II revealed moderate (++) congestion, mild inflammation, mild edema, mild focal hemorrhage, and mild intracytoplasmic, whereas the histopathology of test group III and IV showed less toxic changes with mild (+) to moderate (++) changes Congestion with no Inflammation, no Oedema, no Intracytoplasmic vacuoles (Figure 2.) in comparison with venom Group (II) & standard ASV group (V) (Figure 2).

Table 6: Shows the Effect of Vatsanabha (Aconitum ferox) on Histopathology of heart on rats

S. No.	Microscopy	Group I (control)	Group II (venom)	Group III (test group A)	Group IV (test group B)	Group V
1.	Congestion	+	++	+	+	++
2.	Focal haemorrhage	0	0	0	0	0
3.	Inflammation	0	+	0	0	+
4.	Oedema	0	+	0	0	0
5.	Intra cytoplasmic vacuoles	0	+	0	0	0

0=absent, + = Mild, ++ =Moderate, +++ = Severe, ++++ =Highly

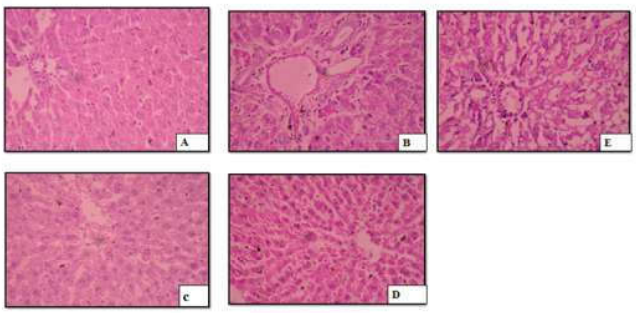

Fig . 1: Histopathology of liver at 40 X: A - control group I shows normal cytoarchitexture, B - Group II (cobra venom only), C – Group III (Venom + Vatsanabha (madhyama matra-medium dose), D - Group IV (venom + Vatsanabha (uttam matra-high dose), E- Group V (Venom + Anti snake venom).

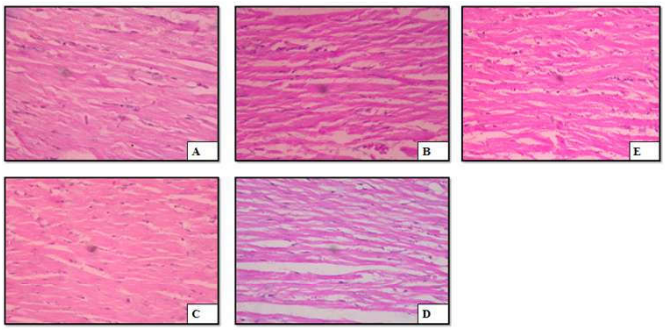

Fig. 2: Histopathology of Heart at 40 X: A - control group I shows normal cytoarchitexture, B- Group II (cobra venom only), C – Group III (Venom + Vatsanabha (madhyama matra-medium dose), D- Group IV (venom + Vatsanabha (uttam matra-high dose), E- Group V (Venom + Anti snake venom)

Discussions

In the *Ayurvedic* text, we find references about the *Jangamavisha* (animal poisoning) and their remedies explained in in a very special branch called as *Agadatantra*. In *Ayurveda*, the 24 modalities of treatment explained by *Charakacharya* are the guidelines and backbone in the management of the poisoning [14]. *Prativisha Chikitsa* (poison against poison)is one of these 24 treatment modalities and being used for *Visha* (poisonous) conditions, but it is explained in detail in *Ashtanga Hridaya Uttarsthana.* where it is well elaborated with its dose, indications, contraindications, etc. For patients of asnake bite – the vegetable poisons should use in the doses [15]. *Madhyama Matra* (medium dose) is 6 *Yava* (187.5 mg) & *Uttama Matra* (maximum dose) *is* 8 *Yava* (250 mg) to be used in humans. Therefore, it is felt that there is a necessity to evaluate the efficacy of *Vatsanabha (Aconitum Ferox)* in the management of snake bite using experimental studies.

Plant metabolites have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism. Several plant-derived small molecules monoterpens, triterpenes, sterols, a phenolic compound, benzoic acid derivatives, flavonoids, tannins, the so-called metabolites, are proved to be effective on snake venom neutralization [16].

PLA2 from snake venom has been implicated in multiple pathologies including neurotoxicity [17], nephrotoxicity [18,19], lung toxicity [20], hepatotoxicity [21], andcardiotoxicity [22,23]. Neuromuscular weakness, especially due to the non-depolarisingpost-synaptic blockade [24]. The tuberous roots of genus *Aconitum* contain alkaloids benzolymecasonine, mesaconitine, aconitine, etc. Currently, the processes aconite tubers are widely and safely used for the treatment of pain neuronal disorders and inflammation with no problematic or annoying adverse effects [25]. It has been suggested that the Neuroprotective actions of α7 nicotinic agonists arise from the activation of receptors and not from the extensive desensitization [26]. Previous studies revealed that the incremental dose of A. f*erox* produces positive inotropic and negative chronotropic effect on isolated frog heart, which are dose dependent. *Vatsanabha* (*Aconitum Ferox)* has been found to possess an excellent cardiotonic activity. It may prove an effective and safe alternative in the treatment of bradycardia [27]. These may be the reason that we observed anincrease in survival time in thetested group (III).

Snake venom PLA2s are also able to stimulate neutrophil chemotaxis, degranulate mast cells *in vitro*, and activate macrophages [28] which were well noticed in the present study. Cytotoxins exhibit activity on various cell types, including erythrocytes, lymphocytes, cardiac myocytes, spleen cells, and various tumor cells [29,30]. In the venom Group (II), we observed ahigher level of Neutrophils / Lymphocytes Ratio when compared to control Group (I). NLR is believed to reflect the balance between innate (neutrophils) and adaptive (lymphocytes) immune responses. Lymphocyte and Neutrophil counts are markers and may vary depending on the severity of inflammation. Lymphopenia is a common finding during stress responses due to increased levels of corticosteroids and lymphocyte apoptosis. Previous studies suggest the alkaloid suppresses antigen and mitogen-induced lymphocyte proliferation, natural-Killer cell cytotoxicity, histamine release by mast cells, interleukin-1 (IL-1) secretion by human monocytes and the action of PAF on platelets [31]. Studies suggest the anti-inflammatory effect is due to Increase in the plasma corticosterone level. The immunosuppressive effect of glucocorticoid represents a major effector endpoint of the counter-regulatory loop in the immune and central nervous systems. Thus there was statistically significant ($p<0.05$) reverse decrease change was found in thetested group (III) on Neutrophils in comparison with venom Group (II). There was reverse change found in NLR ration in thetested group (III) in comparison with venom group (II) which shows agoodanti-inflammatory change in the tested group (III). The increase in bleeding time in this group established the blood incoagulability. Pro-coagulability commonly found in cobra venom cause blood coagulation to occur due to its thrombin-like effect and also it can cause the activation of factor X to Xa.

In the present study, we observed ahigher level of SGPT & SGOT in venom Group (II) in comparison with control group (I) which shows cobra venom is toxic to hepatic cells and cardiac cells. SGOT & SGPT enzymes are markers for cellular damage & SGPT enzymes are essentially present in hepatocytes. Liver injury is among the common and most serious symptoms of cobra snake envenoming [31]. Hepatic injury due to cobra venom envenomation was reported by many authors. Cobra Venom found to induce intrahepatic hemorrhage, liver necrosis, and hepatotoxicity in

mice [32]. Thus Histopathological changes in the venom group (II) in comparison with control group (I) reveals that *Najanaja* envenomation caused a severe inflammatory response of the liver, as indicated by inflammatory cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes. The activation of these cells indicated the phagocytic action of the hepatic tissues as a response to cell injury and as a defense against envenoming [33]. Segelke et al., (1998) concluded that Cellular swelling might be due to the action of venom phospholipase, which causes disturbance of the cell membrane permeability [34]. Chethan kumar and Srinivas (2008) concluded that the exposure of cellular membranes to *Najanaja* venom phospholipase significantly decreased the Na+/K+ ATPase activities, there by altering the ionic gradients, disorganizing the membrane lipid bilayer and eventually leads to cell death [35]. Histopathological changes found in heart section examination of venom group (II) shows the cardiotoxic activity of cobra venom. Cytoplasmic vacuolation which is mainly a consequence of considerable disturbances in lipid inclusions and fat metabolism occurring under pathological cases. The mechanism of cytotoxicity induced by cardiotoxins in heart cells mainly involves the opening of voltage-dependent Ca+2 channels, leading to a block of the inwardly rectifying K+ channels. It has been suggested that these cytotoxins interact with protein targets in the membrane of cardiac myocytes [36]. There were very less toxic signs found in heart section of thetested group (III) which can also be compared with significant changes found in SGOT in thetested group (III) which showed good protection on cellular damage.

More over according to *Ayurveda,* the mode of action of *Visha (*poison*)* against another *Visha* (poison) as *Prativisha (*antidote*)* is due to *Prabhava* (inherent property) is mentioned. The present study confirms as *madhyama* dose (medium dose of group III) of *Vatsanabha* has shown quite a good result in comparison with *Uttama* dose (high dose of group IV) of *Vatsanabha*. According to the text, *madhyama* dose of *visha* should be neutralized by *madhyama* dose of *Prativisha (Vatsanabha*). Here if we consider LD50 as *Madhyamavisha* of cobra venom, so *madhyama* dose shows good result in comparison with selected *Uttama* dose. This shows that dose selected as *Uttama* dose (high dose) may be not sufficient to neutralize the LD50 of cobra venom in present study thus dose selection of *Prativisha* against *visha* plays animportant role. It needs further studies to prove this hypothesis.

In *Ayurveda, visha* are never used alone in snake bite treatment. Many *Visha Vaidy as* (*Ayurvedic* physicians) use the *Visha (*poison*)* drug as aspecial ingredient in the formulation for special effect against snake bite treatment in Kerala. Study reveals that it reduces the inflammatory mediators and protects cell damages which suggest its clinical importance in *Ayurvedic* formulations against snake bite like *Mritasanjeevani Gulika* etc which contains*Vatsanabha* as one drug. Though the study hasn't shown a statistical significance we need to remember that statistics has its own limitations. Thus further studies are required for clear understanding

Conclusions

Oral protection from *Vatsanabha* has not shown direct antidotal activity in both the tested Group as far as mortality is aconcern. The goals of pharmacotherapy are either to neutralize the toxin or to reduce morbidity or to prevent complications. The *Vatsanabha* has shown significant reverse changes inneutrophils, NLR ratio, and SGOT. Because of such interaction can lead to modification of venom toxins, thereby reducing the toxicity and consequently the extent of tissue damage. Thus *Vatsanabha* had shown good protection on liver & heartcells against cobra venom (*Najanaja*) induced toxicity which suggests its clinical importance in *Ayurvedic* formulations. Present parameters have some limitation so Further experiment could address the fractioning of the *Vatsanabha* in order to identify the bioactive compounds responsible for these observations.

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