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Experimental Study on Acute Oral Toxicity of *Vatsanabha* (Aconitum ferox wall. ex Seringe) and Antidote Effect of *Sweta Chitraka* (Plumbago zeylanica Linn.) *Patra Swarasa* W.S.R to Haematological Parameters in *Vatsanabha* Induced Poison

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ABSTRACT

BACKGROUND:
The visha is dire condition where it needs fast action and do the reversible changes. One Such drug which is explained as the prathyoushada, among the popular traditional text books of Keraliya visha chikitsa is Chitraka patra swarasa to counter Vatsanabha. Vatsanabha is one among mahavisha a potent herb which is currently known for the mankind and used to manage few diseases after processing it and when it is accidently consumed or used in the inappropriate methods it cause varied signs and symptoms which will leads to life threatening condition. Antidote is lifesaving substance which abolishes the ill effects of poison thus decreasing the mortality. Chitraka is one such drug quoted which is available easily and has wide therapeutic utility. Chitraka is mentioned as antidote for the Vatsanabha. Hence the study is undertaken to evaluate the antidote effect of the Chitraka patra swarasa.
OBJECTIVE: To determine the toxicity profile of vatsanabha. And to evaluate the effect of chitraka patra swarasa as an antidote in vatsanabha poisoning METHODS: Wistar albino rats were used for the present study. Determined the LD50 value of Vatsanabha according to the OECD 425 guidelines, the rats were grouped into three groups 1st group is normal control, 2nd group is toxic control and third group is test drug group and study was conducted for 28 days and The data expressed as Mean ± SEM. Difference among the groups would be assessed by employing one way ANOVA with Dennett's multiple t test.
RESULTS: The LD50 value is 29.57 mg / kg and chitraka is having the mild to moderate effect on the haematological parameters.
CONCLUSION: The sweta chitraka patra swarasa is having the mild antidotal effect on the vatsanabha induced poison w.s.r to haematological parameters.

Key Words: *Visha, SthavaraVisha, Antidote, Vatsanabha, Chitraka*

INTRODUCTION

Ayurveda mainly there are eight branches[1] which can be found in the classical treatises. One among them is Agadatantra, where the mode of different route of administration of poisons and their signs and symptoms along with their different modalities of treatment are mentioned. The term *Visha gara virodhika prashamana*[2] is mentioned by the *Charaka Samhita,* in *Susrutha Samhita* it is mentioned as the *Agadatantra*[3] and *Damstra chikitsa*[4] name given by the Acharya *Vagbhata*.We can find the classification of visa as *Sthavaravisha* (plant origin) and *jangama visha*[5] (animal origin). Sthavaravisha is further classified into the *mahavisha* and *upavisha*[6], amongst *mahavisha vatsanabha*[7] is an important drug that has multi utility in therapeutics.

Vatsanabha is one of the cardiac poison which is categorized under the Mahavisha. It includes toxic alkaloids like pseudo aconitine, picroaconitine, aconine, etc. The part it used in the medicine is Moolakanda, it is mentioned in the management of various conditions.

There are plenty of formulations which contain vatsanabha as main ingredient like Sanjivini vati, Mrutyunjaya rasa, Kaphaketu rasa,Anadabhirava rasa and so on. If it is used improperly there is a possibility of the adverse drug reaction (ADR) or in case of poisoning, it produces the symptoms like nausea, vomiting, diarrhoea, palpitation, pulmonary hypertension, pulmonary oedema, hippus, and mydriasis. Everything in the earth can be utilized as a medicine even visha can be used as the medicine after proper planning according to Ayurveda.

Antidote is the substance or agent which counteracts or neutralizes effect of poison. They can be classified into has Mechanical, Chemical, Physiological or pharmacological. Timely and judicious use of antidotes will help in restoration of patient's health. So it's necessary to conduct the studies to find out an effective antidote for this poison.

Globally there is rise in the incidence poisoning due to consumption of vegetable poison with its media-legal importance. Amongst them vatsanabha is important because of increase in incidence of accidental poisoning and due its therapeutic utility, it is sometimes it may take accidently by confusing with the horse radish and sometimes it is mixed with beverages to enhance the kick. So it is essential to develop the good antidote for Vatsanabha.

MATERIALS AND METHODS

Drug preparation

Vatsanabha is procured from the Naradevi region, Kathmandu, Nepal, authentication from the Department of *Dravyaguna.* The drug is made into fine powder and used in the study. *Chitraka* had collected freshly from herbal garden of SDM

College Ayurveda, UDUPI, authentication from the Department of *Dravyaguna*

Animals and Husbandry

Wistar strain albino rats of either of sex weighing between 150-250g were used for the experimental study with the following conditions.

The animals were obtained from animal house attached to Pharmacology and toxicology laboratory S.D.M centre for Research in Ayurveda and allied sciences ETHICAL COMMITTEE NO: SDMCRA/IAECI/AG-16 Date 26/03/2018

They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature, humidity. The dry rice husk used has a bedding and daily cage had changed. And normal tap was provided

Acute oral toxicity test

According to OECD 425 guidelines acute oral toxicity test had been carried out with limit of 17.5 mg to 175 mg / kg. Total 7 numbers of rats were used and marked as head, neck, body tail, no mark, head and neck and Forelimb as marked

After the animals dosing, they were observed for the constantly 4 hours. The carefully cage side observation had done without disturbing the animals attention and at the end of the every hour the animal was individually exposed to open arena for recording the behavioural changes like decreased or increased motor activity, catatonia, spasticity, opisthotonus, convulsion, straub's reaction, muscle spasm, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing, mode of respiration, changes in skin colour, etc. exitus, CNS depression- hypo activity, passivity, relaxation, ataxia, narcosis, etc.

Mortality:

All the animals were observed at 1, 2, 3, 4, 24, 48 hrs after dosing and there after daily once for mortality during the entire period of study. (i.e. 14 days)

Sub-acute toxicity test and antidote effect of *Chitraka* (Plumbago zeylanica Linn.) *Patra swarasa* w.s.r to haematological parameters.

The study was conducted for 28 days total 30 rats of either of sex were used for the study. The dose which is obtained from the AOT study of *vatsanabha*

Animals were divided into like Group 1 as the Normal control, Group 2 as the Toxic control has feed with *vatsanabha moola choorna* ($1/5^{th}$of LD50 as per requirement) and Group 3 as test drug feed with v*atsanabha mula choorna, Swetha Chitraka patra swarasa* in TED Found using Paget and Barnet table.

Drug Preparation: *Vatsanabha mula choorna* is taken 11.82 mg in 20ml of tap water as stock solution in each group. 12gms of C*hitraka patra swarasa* held up to 5 ml. of *swarasa*.

Experimental protocol:

Animals were kept for acclimatization for 7 days the test formulation was administered orally once a day for 28 consecutive days.

Haematologyparameters:

On 28^{th} day all animals were kept fasting for overnight and on next day blood was collected from retro orbital plexuses puncture using micro capillary tubes under mild ether anaesthesia at the

end of periods or collected in just prior to as part of the procedure for the killing animals for estimation of haematological. The following list of parameters were measured using the automatic cell counter where applicable – haematocrit, haemoglobin concentration, erythrocyte count, total count and platelet count.

Statistical analysis:

The data expressed as Mean ± SEM. Difference among the groups would be assessed by employing one way ANOVA with Dennett's multiple t test for the determine the level of significance of the observed effects, as Post HOC test if 'P' value of less than 0.05 was considered as statistically significance. Level of significance is noted and interpreted accordingly.

RESULTS AND DISCUSSION

Evaluation Acute oral toxicity test study of *Vatsanabha mula* is-

As per the guidelines 175mg/kg of *Vatsanabha mula* was administered to rat dose is. 2.25ml of solution. Observed that animal died in 2 hour due to respiratory distress and convulsions.

As there was mortality in previous rat, for the next rat 55mg/kg of drug *Vatsanabha mula* was given as per the protocol and verified with AOT software. 2.5ml of solution was administered. Observed that animal was died in 1 hour 30 minutes due to respiratory distress and convulsions

As previous dosed rat died, for next rat 17.5mg/kg of drug *Vatsanabha mula* was given. 2.01 ml of solution was administered to the animal. Observation- Rat did not die. There was a blanching, diarrhoea, nasal secretion and decreased motor activity.

The LD50 value was found to be 29.57 mg/kg with confidence limit of 17.5 to 55 mg/kg. The data generated using the AOT software can be seen further.

In acute toxicity test the female rats were used according to 425 test has carried out and single sex were used to utilized and to reduce the number of animals are used minimum. The LD50 value is may slight sensitivity variable if the both of the sex, among that females are little higher sensitive compared to both the sex. The value has been founded are 29.57mg/kg body weight. There was a blanching, diarrhoea, nasal secretion, decreased motor activity and death has occurred.

In Sub-acute toxicity and antidote effect on the haematological parameters

Table 1 Effect of the drug on the Haematological parameters

S.NO	PARAMETERS	CONTROL	TOXICCONTROL @	TEST DRUG#
1.	**Haemoglobin**	16.55±0.91	15.63±0.33	15.30±0.16
2.	**RBC**	7.96±0.413	7.63±0.16	7.47±0.10
3.	**TC**	13230±3186.9	9000±1361.5	12000±565.10
4.	**Packed cell volume**	41.99 ± 2.19	45.51±2.73	39.11±0.65
5.	**Mean corpuscular volume**	52.83 ±0.88	51.93 ±0.66	52.4 ±0.80
6.	**Mean corpuscular haemoglobin**	20.71 ±0.39	29.7±4.24 *	20.43±0.21*

7.	**Mean corpuscular haemoglobinconcentration**	39.31±0.22	39.36±0.21	39.08 ± 0.28
8.	**Red cell distribution width Coefficient variation**	14.5 ± 0.41	13.2 ±0.33*	14.01 ±0.30
9.	**Red cell distribution width Standard Deviation**	27.23 ±0.75	25.23 ±0.52	26.42 ± 0.54
10.	**Platelet**	5.96 ± 0.79	7.49 ± 0.52	6.89 ± 0.41

Data: MEAN± SEM, * P<0.05

@-compared with control #-compared with *Vatsanabha root* group.

Haemoglobin in toxic control observed found to be statistically non-significant decrease may due to acute inflammation[8]. In *Vatsanabha root + sweta chitraka patra swarasa* group the observed decrease was found to be statistically non-significant. *In Test drug administered group* showed mild antidotal effect on the *vatsanabha*.

In total count in *Vatsanabha root* group the observed decrease was found to be statistically non-significantmay be due to inflammation of connective tissue diseases[9]. In Vatsanabha *root+ sweta chitraka patra swarasa* group, the observed increase was found to be statistically non-significant and had showed the reversible action and the moderate antidote effect.

The data showed that there was a decrease in red blood cells in *toxic control* group was found to be statistically non-significant decrease, may be due haemolysis[10]. In Vatsanabha *root+ sweta chitraka patra swarasa* group, the observed decrease was found to be statistically non-significant and had showed the mild antidote effect on the *vatsanabha*.

The data shows there was an increase in packed cell volume in toxic control group was found to be statistically non-significant increase. This may be due to the severe dehydration[11]. In Vatsanabha *root+ sweta chitraka patra swarasa* group, the observed decrease was found to be statistically non-significant and also showed the mild antidote effect.

The data showed that there was a decrease in mean corpuscular volume in *toxic control* group was found to be statistically non-significant decrease. In *Vatsanabha root+ sweta chitraka patra swarasa* group, the observed increase was found to be statistically non-significant.

The data showed that there was an increase in mean corpuscular haemoglobin in *Vatsanabha root*, the observed increase was found to be statistically significant. In *test drug* group, the observed decrease was found to be statistically significant and had moderate antidote effect.

The data shows that there was an increase in mean corpuscular Haemoglobin concentration in *Vatsanabha root*, the observed increase was found to be statistically non-significant. In *Vatsanabha root+ sweta chitraka patra swarasa* group, the observed decrease was found to be statistically non-significanthad reversible action.

The data shows there was decrease in RDWCV in *Vatsanabha root* group, the observed decrease was found to be statistically significant. In *Vatsanabha root+ sweta chitraka patra swarasa* group, the observed increase was found to be statistically non-significant.

The data shows there was decrease in Red cell distribution width Standard Deviation in *Vatsanabha root* group the observed decrease was found to be statistically non-significant. In *Vatsanabha root+ sweta chitraka patra swarasa* group, the observed decrease was found to be statistically non-significant.

The data shows there was increase in platelet in *Vatsanabha root* group the observed increase was found to be statistically non-significantmay be due to haemolytic condition[12]. In *Vatsanabha root* + sweta *chitraka patra swarasa* group, the observed decrease was found to be statistically non-significantshowed the reversible action on the platelet and mild antidote effect.

CONCLUSION

In acute oral toxicity of vatsanabha moola choorna calculated LD_{50} was found to be 29.57mg/kg bodyweight. In haematological parameters antidote effect of test drug on vatsanabha induced poison showed reversible action on mean corpuscular haemoglobin, platelets and rdwsd. Hence test drug showed the mild to moderate antidote effect on vatsanabha moola choorna.

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