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An experimental study to evaluate the antidotal activity of *Neeli Moola (Indigofera tinctoria) Kalka* w.s.r to haematological parameters in *Vatsanabha* (*Aconitum ferox*) induced toxicity

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ABSTRACT

Background: *Vatsanabha* is a potent cardiac poison comes under the category of *Mahavisha* with its main active principles aconitine, picraconine and pseudaconine. In the literatures there are descriptions about toxicants and in some traditional Malayalam textbooks antidotes has been explained under the concept "*Prathyoushadha*". *Neeli Moola* has been mentioned as a *Prathyoushadha* for *Vatsanabha* poisoning in a renowned textbook '*Visha Vaidya Jyotsnika*'. Hence to evaluate the antidotal activity in *Vatsanabha* poisoning, *Neeli Moola Kalka* has been used and the haematological parameters were analyzed to screen the results. **Methods:** Wistar strain albino rats were used in this study which were divided into 3 groups, normal control, toxic control and the test drug group. The duration of the study was 28 days .The data generated was mentioned as Mean±SEM. Difference among the groups was assessed by employing one way ANOVA followed by Dunnet's multiple 't' test. **Results:** Reversible action has been observed after the administration of *Neeli Moola Kalka* in the hematological parameters which has shown toxicity changes due to administration of *Vatsanabha*. **Conclusion:** *Neeli Moola Kalka* is having mild to moderate antidote effect in *Vatsanbha* induced toxicity.

Key words: *Vatsanabha, Neeli Moola, Prathyoushadha, Antidote, Aconite, Indigofera, Cardiac posions.*

INTRODUCTION

Ayurveda is an ancient and customary arrangement of medication which has been practiced since ages. *Ashtangas* or the eight branches lay the foundation of *Ayurveda*. One among these branches is *Agadatantra* which deals with the signs, symptoms and management of poisoning resulting from the bites of snakes, insects and worms, spiders, rodents etc and various other poisons produced by the improper combination of substances or drugs.[1] *Visha* is basically classified into two i.e. *Sthavaravisha* (plant origin) and *Jangamavisha* (animal origin). *Sthavaravisha* is further classified into *Mahavisha* and *Upavisha.*[2] *Vatsanabha* comes under *Mahavisha* and is a potent cardiac poison which is being extensively used in various *Ayurvedic* formulations like *Sanjivanivati, Anandbhairava Rasa, Hinguleshwara Rasa* etc.[3]

Since *Vatsanabha* is being used in various formulations there are chances of accidental poisoning due to improper preparation, overdose etc. All parts of the plant are poisonous, but the root chiefly acts as poison. So, it is essential to develop an effective antidote which can at the least reduce the toxic manifestations produced by *Vatsanabha.*

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Antidotes are agents, which counteract the effects of poisons. They are divided into non-specific and specific antidotes.[4] In *Visha Vaidya Jyotsnika* antidotes has been mentioned under the concept of *Prathyoushada*. For *Vatsanabha, Neeli Moola* has been mentioned as the *Prathyoushada*.[3] Hence this study has been undertaken to experimentally assess the antidotal activity of *Neeli Moola Kalka* in *Vatsanabha* induced toxicity.

OBJECTIVES

To experimentally evaluate the antidotal activity of *Neeli Moola Kalka* in *Vatsanabha* induced toxicity w.s.r. to haematological parameters.

MATERIALS AND METHODS

Vatsanabha was collected from the Naradevi region, Kathmandu, Nepal. Authentication has been done from the Department of Dravyaguna, Shri Dharmasthala Manjunatheshwara College of Ayurveda, Udupi. *Neeli Moola* has been collected from an authenticated seller from Kerala. Wister strain albino rats of either sex were selected from animal house of SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The rats were divided into 3 groups containing 10 rats in each group.

Since the dose of *Neeli Moola* was not mentioned in Visha Vaidya Jyotsnika, the *Samanya Kalka Matra* has been taken into account, which was further extrapolated into rat dose by referring to pagets and barnes dose conversion formula i.e

Rat dose = human dose× 0.018 × 5

i.e. 12×0.018 × 5 Thus 1.08g/kg body weight of *Neeli Moola Kalka* has been used as antidote.

Antidote effect of *Neeli Moola Kalka* w.s.r. to haematological parameters

The study was designed in 2 phases

Phase I - Acute toxicity test to determine the LD_{50} dose of *Vatsanabha* root powder.

The *Vatsanabha* dose was selected based on LD_{50} determined by acute oral toxicity study in wistar albino rats. Maximum tolerated dose has been calculated by employing OECD 425 guidelines with AOT software.

LD_{50} was calculated to be 29.57mg/kg.

Phase II - antidotal effect assessment.

- Group I - normal control group
- Group II - *Vatsanabha Moola* ($1/5^{th}$ of LD_{50}) i.e. 5.9 mg/kg body weight.
- Group III - *Vastanabha Moola* + *Neeli Moola Kalka* in TED.

Preparation of drug

In both the groups i.e. group II and group III, stock solution of 11.82 mg of *Vatsanabha Moola Churna* in 20 ml of tap water has been prepared and 2.16g of *Neeli Moola Kalka* in 20 ml tap water.

Drug administration

Vatsanabha control and test drug were administrated for 28days including experiment day in the morning session between 9-11am orally.

Haematological parameters

Blood samples were taken from the orbital plexuses, using capillary tubes, under anesthesia at the end of periods or collected in just prior to as part of the procedure for the killing animals. 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 generated was mentioned as Mean ± SEM. Difference among the groups was assessed by employing one way ANOVA followed by Dunnet's multiple 't' test as post hoc test, if p value is < 0.05 which was considered as statistically significant.

OBSERVATION AND RESULTS

Effect of *Vatsanabha* and antidote effect of *Neeli Moola Kalka* on hematological parameters are given in table no 1.

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Table 1: Haematological parameters

Parameters	Normal control	Vatsanabha control	*Vatsanabha* with *Neeli Moola Kalka* (compared with *Vatsanabha* control)
Hb %	16.55±0.91	15.63±0.33	16 ±0.82
TC	13230 ±3186.9	9000 ±1361.5	11966.66 ±706.48
RBC	7.96±0.413	7.63±0.16	7.84±0.36
PCV	41.99 ± 2.19	45.51 ± 2.73	40.81 ± 2.05
MCV	52.83 ±0.88	51.93 ±0.66	52.03 ±0.37
MCH	20.71 ±0.39	29.7±4.24*	20.31±0.10*
MCHC	39.31±0.22	39.36±0.21	39.15 ± 0.29
RDWCV	14.5 ± 0.41	13.2 ±0.33	15.51 ±0.65**
RDWSD	27.23 ±0.75	25.23 ±0.52	28.46 ± 1.12*
Platelet	5.96 ± 0.79	7.49 ± 0.63	6.03 ± 0.23

Data: MEAN± SEM, * P<0.05, **P<0.01

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

In Subacute toxicity, *Vatsanabha* and *Neeli Moola* administered group Hb%, total count, RBC, MCV, and has shown non - significant increases, PCV, MCHC, Platelet has shown non- significant decrease. Significant decrease in MCH, and significant increase in RDWCV, RDWSD

In *Vatsanabha* alone administered group, there was non-significant increase in PCV, MCHC, platelet and non- significant decrease in HB%, total count, RBC, MCV, RDWSD, RDWCV and Significant increase in MCH.

Decrease in haemoglobin has been observed in *Vatsanabha* alone administered group which was found to be statistically non- significant, which may be due to the suppression of its synthesis by red blood cells.[6] In *Vatsanabha* + *Neeli Moola* administered group the observed increase was found to be statistically non- significant. Here *Neeli Moola* has shown reversible action and mild antidote effect.

In *Vatsanabha* alone administered group, Red Blood Cells have shown non-significant decrease which may be due to haemolysis.[7] In *Vatsanabha* + *Neeli Moola* administered group the observed increase was found to be statistically non- significant Here *Neeli Moola* has shown reversible action and mild antidote effect.

Total count has shown non- significant decrease in *Vatsanabha* alone administered group which may be due to either destruction or inflammation of the cells. In *Vatsanabha* + *Neeli Moola* administered group the observed increase was found to be statistically non-significant and *Neeli Moola* has shown reversible action here and mild antidotal effect.

The data shows there was increase in Platelet count in *Vatsanabha* alone administered group which was found to be statistically non- significant which may be due to acute hemolysis.[8] In *Vatsanabha* + *Neeli Moola* administered group the observed decrease was found to be statistically non- significant and *Neeli Moola* has shown reversible action here and mild antidote effect.

Conclusion

Neeli Moola is mentioned as a good and effective *Prathyoushada* in the treatment of *Vatsanabha Visha* by *Visha Vaidya* Jyotsnika, *Kriyakoumudi* etc. Orally administered *Vatsanabha* has shown mild to moderate toxic effects in the hematological parameters when compared with the control group which has shown reversal action by the administration of *Neeli Moola,* hence it can be concluded that *Neeli Moola* is having mild to moderate antidote effect in the hematological parameters when administered in the TED dose.

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