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Research Protocol for Study of Efficacy of Vrutaka Phala Beeja (*Solanum malongena* Linn.) Swarasa in Dhatura Beeja (*Datura metel* Linn.) Poisoning in Wistar Rats

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Abstract---Background: In the treatment protocol of poisoning, antidotes are mainly used to counter the poisons. In *Ayurveda*, antidotes are termed as *agada* or *vishaghna dravyas* which either directly neutralize the poison or counter its deleterious effects. In *Ayurveda* various *agada* (antidote) formulations are mentioned out of which few are being used in clinical practice due to insufficiency in available research data. *Vrutakphala beeja Swarasa* (VPBS) is one of the antidote formulations described in *Anupana Manjiri* and *Rasajalanidhi*, which is mentioned as effective against ingested *Dhatura Beeja* (DB) poisoning. Currently no clinical as well as preclinical data is available about its efficacy. Aim and Objective: To study efficacy of *Vrutaka Phala Beeja* (*Solanum malongena* Linn.) *swarasa* in *Dhatura Beeja* (*Datura metel* Linn.) poisoning in Wistar rats. Methodology: 36 Wistar rats equally classified into 6 groups will be subjected to DB poisoning except normal control group. Efficacy study of VBPS will be carried out on experimental groups as per standard protocol. Results: Clinical toxicity signs, Sr. biochemical and Histopathological observations from all groups will be observed. Conclusion: Conclusion will be drawn strictly on the basis of results.

Keywords---agad formulation, antidotes, protocol, study efficacy, vivo study.

Introduction

According to *Ayurveda*, the substance which when comes in contact with body vitiates *dosha*, *dhatu*, *mala* etc. and disturbs sound state of body or causes death is known as *visha*¹ (poison). According to modern science, poison is defined as a substance which on ingestion, inhalation, absorption, application, injection or development within the body, in relatively small amount, produces injury to the body by its chemical action

² In *Ayurveda*, poison is classified as natural and unnatural. Natural poisons are again classified as *sthavara* (inanimate) and *jangama* (animate) *visha*. *Sthavara visha* is further classified as *vanaspatija visha* (plant poisons) and *khanija visha* (mineral poisons).³ According to *Rasatarangini*, poisons are classified into *visha* and *upavisha*.⁴ *Visha* are highly poisonous while *upavisha* are having less fatality. Total 9 *visha* and 11 *upavisha* are mentioned⁵.

Dhatura is one of the poisons mentioned in *upavisha*.⁶ *Dhatura* seeds are used as stupefying agent to rob people hence known as 'Rail-road poison'.⁷ Its accidental poisoning is found in children and in drug abuse people. Drug abuse people consume *Dhatura* seeds mixed with food or with liquor. They even smoke the seeds along with tobacco for getting hallucinogenic effects.⁸ Though poisonous, it is used as drug for the treatment of various diseases e.g. *Kanakasavam* etc. In *Ayurveda* various poisons are used as a drug effectively after adopting appropriate *shodhana* (purification) method to enhance its therapeutic properties and reduce its undesirable effects.⁹ But if these *shodhana* methods have been bypassed or improperly advocated then formulated drugs will show toxicity.

Toxicity is of various types *viz*: acute toxicity, sub-acute toxicity, chronic toxicity etc. Symptoms of acute toxicity are more intensified, some times fatal than that of chronic toxicity which can be presented as allergic reactions. These types of allergies are termed as *dooshivisha*, a chronic stage of poisoning.¹⁰ Treatment will differ as per the nature of toxicity. *Dhatura* seeds poisoning will mostly present with acute toxicity signs. In *Ayurveda*, remedies are mentioned for the treatment of *Dhatura* poisoning, but efficacy of very few of them was tested. Clinical use of these remedies is negligible due to insufficient preclinical data. In classical text, *Anupana Manjiri*¹¹ and *Rasajalanidhi*¹², VPBS is mentioned as effective formulation in treatment of ingested *Dhatura* seed poisoning. But the efficacy of this drug has not been validated yet clinically or pre-clinically. Hence, present study will be an attempt to test the efficacy of VPBS in ingested DB poisoning in wistar rats.

Aim and objectives

To study efficacy of *Vruntaka Phala Beeja* (*Solanum malongena* Linn.) *swarasa* in *Dhatura Beeja* (*Datura metel* Linn.) poisoning in Wistar rats.

Research Question: Whether *Vruntaka Phala Beeja* (*Solanum malongena* Linn.) *swarasa* is effective in *Dhatura Beeja* (*Datura metel* Linn.) poisoning in Wistar rats?

Null Hypothesis: There is no significant efficacy of *Vruntaka Phala Beeja* (*Solanum malongena* Linn.) *swarasa* in *Dhatura Beeja* (*Datura metel* Linn.) poisoning in Wistar rats.

Alternate Hypothesis: *Vruntaka Phala Beeja* (*Solanum malongena* Linn.) *swarasa* is effective in *Dhatara Beeja* (*Datura metel* Linn.) poisoning in Wistar rats.

Methodology

- Study Design: *In Vivo* Animal Study.
- Collection of toxic drug and study drug:
Toxic drug *DB* will be procured from field. Fresh, full grown fruit of *Vruntak* having abundant amount of seeds will be procured from nearby farm.
- Authentication of toxic drug and study drug:
Authentication of toxic drug (*DB*) and study drug (*Vruntak Phala Beeja*) will be done at authorized botanical laboratory.
- Analytical study of toxic drug and study drug:
Microscopic and macroscopic study, physicochemical and phytochemical study of toxic drug *DB* and study drug *VPBS* will be done by using standard parameters mentioned in *API* at authorized analytical laboratory.
- Preparation of toxic drug and study drug:
Well dried and cleaned sample of *DB* will be taken in sufficient amount and with the help of *kharala* crushed to form fine powder and will be sieved through 100 no. mesh to get fine powder. Fresh, full grown fruit of *Vruntaka Phala* will be cut and seeds will be separated and smashed with the help of *kharala* to form *kalka* (paste). *Kalka* will be kept in a fine cotton cloth and squeeze to obtained *Swarasa*. This extracted *swarasa* will again filter with cotton cloth to remove crude particles which will help in easy assimilation of drug.
- Estimation of LD₅₀ and acute toxicity dose of toxic drug and study drug:
LD₅₀ study of toxic drug *DB* and study drug *VPBS* will be conducted as per standard protocol and acute toxicity dose of toxic drug which will be the just lower to the LD₅₀ dose will be estimated.
- Dose calculation of study drug:
According to *Anupana Manjiri*, therapeutic dose of *VPBS* in human adult is 1 Pala¹³ i.e. 48 ml¹⁴. By applying rat conversion factor¹⁵, therapeutic dose of *VPBS* will be calculated as 4.32 ml/Kg body weight of rat.
- *In Vivo* study (Experimental study):
In Vivo study will be conducted at authorized animal house as per OECD guidelines.¹⁶ Before starting experimental study, permission of Institutional Animal Ethical Committee (IAEC) will be obtained. Healthy, diseased free Wistar rats of weight ranging from 160 to 240 gms of both the gender will be selected. *In Vivo* study will be conducted as per standard protocol with following inclusion and exclusion criterion.

Inclusion criteria

- Wistar rats weighting between 160 to 240 grams will be selected.
- Age of rat between 8 to 12 weeks of both sexes will be selected randomly.
- Healthy and diseased free rats will be selected.
- Females rats should be nulliparous and non-pregnant.

Exclusion criteria

- Wistar rats less than 160 grams and more than 240 grams.
- Rats of Age less than 8 weeks and more than 12 weeks.
- Unhealthy and diseased rats.
- Multipara or pregnant female rats.
- Rats under other experimental trials.
- Screening Parameters: Rise in body temperature, Redness of skin, Hyperactivity. Signs of toxicity, their time of onset, severity, reversibility, its duration along with mortality and morbidity will be noted.
- Specific Investigations: Sr. biochemical and Histopathological Examination.
- Interventions: Interventions will be done on day 1. All the groups will be observed for next 14 days.

Table 1
In Vivo study protocol

Animal species	Wistar Rat
Strain	Wistar Rat
Average weight of animal	200gm $\pm$ 20%
No. of rats	36 (18 Male, 18 Female)
Average age of rats	8-12 weeks
Sex of rat	Equal no of male and female
Period of acclimatization	7 days
Route of administration of drug	By Oral route (for DB <i>Churna</i> and VPBS) & Intravenous route (for Physostigmine).

Table 2
In Vivo study groups

Group	No of rats	Drug Administration
Group 1 (Normal Control Group)	6 (3M+3F)	Only Saline Water No DB <i>Churna</i> , No VPBS
Group 2 (Disease Control Group)	6 (3M+3F)	Only DB <i>Churna</i>
Group 3 (Experimental Group I) Minimal dose (60%)	6 (3M+3F)	DB <i>Churna</i> + VPBS (in minimal dose)
Group 4 (Experimental Group II) Sub-maximal dose (80%)	6 (3M+3F)	DB <i>Churna</i> + VPBS (in sub-maximal dose)
Group 5 (Experimental Group III) Maximal dose (100%)	6 (3M+3F)	DB <i>Churna</i> + VPBS (in maximal dose)
Group 6 (Standard Group)	6 (3M+3F)	DB <i>Churna</i> + Inj. Physostigmine

* M= Male, F= Female

Experimental procedure

Uniform suspension of DB *Churna* using suitable suspending agent, will be administer in all groups except group 1 (Normal control group) according to their body weight. Signs of toxicity, their time of onset, severity, reversibility, its duration along with mortality and morbidity will be noted. After development of acute toxicity signs, group 3, 4 and 5 will receive freshly prepared study drug VPBS and group 6 will receive standard drug (Inj. Physostigmine) according to their body weight. Observations will be noted till 24 hours for their recovery or worsening. All animals will be observed up to 7 days for any notable event and mortality. At the end of the study, on 8th day, all the survived animals will be euthanized and histopathological examination of vital organs will be done. Blood sample will be collected from the retro-orbital route from each animal before initiation of study and after 24 hours of dosing for performing serum biochemical tests like ALP, AST, Blood Urea, Sr. Creatinine and Alkaline phosphatase.

Expected results

Primary Outcomes: To study efficacy of VPBS in DB poisoning in Wistar rats.

Secondary Outcomes: To compare the effect of VPBS with proven antidote Physostigmine in DB poisoning in Wistar rats.

Discussion

Ayurveda defines poison as a substance which when comes in contact with person's body causes disturbance in its sound state or death. Incidences of poisoning are increasing day by day due to indiscriminate use of various poisons which inadvertently enters into our life style. In the treatment protocol of poisoning, use of antidotes plays a key role. Antidotes either directly neutralize the poison or counter its deleterious effects. *Agada* or *vishaghna dravyas* are the terms used in *Ayurveda* for antidotes. In classical text, various *Agada* formulations are stated in context with treatment of poisons or poisonous conditions.

Upavisha are the poisons with lower potency. *Dhatura* is one of the plant poisons mentioned in *Upavisha*. Though whole plant of *Dhatura* is poisonous but mostly seeds are used as stupefying agent to rob people. It produces toxic clinical signs like dysarthria, dysphagia, dilated pupils, diplopia, difficulty in vision, dry hot skin, drunken gait, delirium, dysuria also known as 9Ds'. Other signs are bitter taste in mouth, hyperthermia, deficit of recent memory, hyper-reflexia, convulsions etc. *Dhatura* poisoning cases are treated by using general line of treatment of poisoning and using specific treatment i.e. antidote therapy. The current established antidote therapy in *Dhatura* poisoning is inj. Physostigmine or Neostigmine. It has some drawbacks like excessive salivation, excessive sweating, nausea, vomiting, diarrhea, stomach cramps etc.¹⁷ Hence there is need of development of newer drugs which are potent, safe, cost effective and easily available.

Efforts had been made in past by many researchers to test antidote efficacy of many *Agada* formulations mentioned in classical text. One of the studies has

been tested efficacy of *Kamal Patra* as an antidote in *Dhatura* poisoning in Albino mice. Results of this study shows significant reduction in hyperthermia and increase in duration of time required for dilatation of pupils. While changes in duration of appearance of convulsions and duration of survival period both found statistically insignificant.¹⁸ Similar studies had been performed to test the efficacy of some more ayurvedic formulations like *Nimbujeeraka Yoga*,¹⁹ *Karpasasthi Yuktam Pushpa*,²⁰ *Chincha Rasa*²¹ etc.

Vruntaka Phala (*Solanum malongena* Linn.) contains arginine, aspartic acid, histidine, 5-HT, delphinidine -3 bioside (nasunin), oxalic acid, solasodine, ascorbic acid, tryptophan etc. as phytochemical constituents. It possesses proven pharmacological activities like antipyretic, miotic action on eyes, CNS depressant, anticonvulsant, analgesic, antioxidant, anti-inflammatory, anti-asthmatic, spasmogenic etc.²² These activities of *Vruntaka Phala* can be helpful in treating *Dhatura* poisoning as antipyretic property can be useful to counter hyperthermia, miotic action on eyes can reduce the dilatation of pupils, CNS depressant action can manage excitatory phase of poisoning caused by *Datura* etc. Hence there is a need to explore the potential of the drug *Vruntaka Phala*. Other related studies were reviewed ²³⁻²⁶.

To prove its efficacy, initially preclinical studies should be planned. *In vivo* study is important phase in drug development which is also known as preclinical trial. For this type of study various rodent and non-rodent animal models can be used. In this phase, along with *In vivo* efficacy, acute toxicity, sub-acute toxicity, chronic toxicity, carcinogenicity, teratogenicity of drug can be studied. This phase ensures the efficacy as well as safety of drug before human trial.

Hence present study has been planned to evaluate efficacy of VBPS (*Solanum malongena* Linn.) in DB (*Datura metel* Linn.) poisoning in Wistar rat model. Study will be executed as per methodology. The observations will be made and data will be collected and presented in tables, charts and graphs. The observations from animal study, serum biochemical study and histopathological study will be analyzed by applying appropriate statistical tests (ANOVA) to get results. The results will be thoroughly discussed to derive probable rationale. Study results will be compared with previously available study data and thoroughly discussed to find correlation if any.²⁸⁻³¹

Data Management: The data entry coding will be done by the principle investigator.

Statistical methods: ANOVA test will be applied to analyze data.

Scope: If the efficacy of VPBS will be proved then further clinical trials in human subjects can be conducted to prove its efficacy in humans.

Implications: Preclinical data of efficacy of VPBS in DB poisoning in Wistar rats will be useful for further advancement in drug development phases.

Translatory component: If the efficacy of this remedy is found significant then this will help to treat the patients where the present established treatment i.e. Physostigmine is not available. Also it will encourage the Ayurved physicians to treat *Dhatura* poisoning cases in Ayurveda hospitals and increase faith in Ayurveda. Treatment cost will also be reduced.

Strength: It will provide preclinical data about efficacy of VPBS in DB poisoning which is currently not available.

Limitation: As it is a preclinical study, the actual efficacy of VPBS in ingested DB poisoning in human subjects will not be assessed.

Conclusion

From the study observations, *In vivo* efficacy of VPBS in oral DB poisoning will be evaluated in Wistar rats.

Ethical Approval: Research ethics approval from the research ethics committee has taken Ref. No- DMIMS (DU) PhD. Reg. No. M-8643/2017-18

Competing Interests: Authors have declared that no competing interests exist.

Article Type: Study Protocol

Conflict of Interest: - None

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