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Study Protocol

Comparative in vivo evaluation of Toxicity and Aphrodisiac activity in drug-induced erectile dysfunction of *Sidha Shalmali Kalpa* (*Shalmali malabarica* formulation) with its modified form – a Research Protocol

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ABSTRACT:

Introduction: Infertility has a prevalence of 15% in couples globally. Of this, 50 % infertility cases are due to problems in males. *Sidha Shalmali Kalpa* has been mentioned in *Bhaishajya Ratnavali* for its Aphrodisiac activity. This study aims to reduce the incidence of male infertility and improve reproductive health. Current pharmacological treatment has adverse side effects and is unsuitable for long-term use. Therefore, there is a need for safer alternatives. *SSK (Shalmali malabarica* formulation*)* is described for improving male reproductive functions; its safety and efficacy are not supported by scientific evidence. Therefore, the present study demonstrates the *Vaijikarana* activity of the drugs using modern scientific parameters. It helps to integrate *Ayurvedic* formulations into evidence-based reproductive healthcare. **Methods:** This experimental study will be conducted at a certified animal house. All raw ingredients will collect from authentic sources. Then, they will verify for authenticity. *SSK* and its modified dosage form will prepare, standardized, and analyzed as per the *Ayurvedic* Pharmacopoeia of India (API). An Acute toxicity study will conduct in male rats (6 rats per group - 2 groups) in accordance with OECD guidelines. In the Efficacy study, the aphrodisiac activity will examine in both normal and drug (Paroxetine) - induced erectile dysfunction animal models (6 rats per group: 6 groups of male rats and 1group of female rats). General clinical observations, such as behavioural parameters (mount latency/frequency, intromission latency/frequency), along with biochemical marker assessments (Serum testosterone, ALP levels), will conduct. Histopathological studies of vital organs will also carry out to assess safety. **Conclusion:** This study will likely to generate scientific evidence regarding the safety and aphrodisiac activity of classical SSK and its modified form. The modified form of SSK is likely to exhibit increased therapeutic efficacy, possibly due to improved bioavailability and its constituent photochemical.

KEYWORDS: Aphrodisiac therapy, Erectile dysfunction, Herbal Aphrodisiac, Infertility, *Shalmali malabarica* formulation, *Siddha Shalamali Kalpa, Vaijikarana*, Virility therapy

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1. INTRODUCTION

Infertility is a most troublesome disorder in today's era. It affects 15 % couples of reproductive age. Globally, an estimated 60–80 million couples suffer from infertility annually, [1] with around 27.5 million affected couples in India alone. [2, 3] It accounts for 50% of cases, according to the World Health Organization. Sexual disorders result in the formation of significant personal distress, interpersonal challenges, and mental health problems such as anxiety and depression. Despite this high prevalence, sexual disorders remain undiagnosed and untreated. Most cases are effectively managed by psychiatric practice. There is a gap in safe, effective, and affordable pharmacological treatments. [4, 5] This highlights the urgent need for holistic and integrative therapeutic options.

Vaijikarana (Aphrodisiac therapy or Virility therapy) is a branch of *Ayurveda* that deals with offering a unique and time-tested approach to managing sexual and reproductive disorders. [6, 7] It enhances sexual vitality, fertility, and overall reproductive health. Aphrodisiac therapy or Virility therapy aims not only to treat conditions such as erectile dysfunction, premature ejaculation, and low sperm count, but also to promote general well-being and longevity, by using a range of herbal, herbo-mineral, and natural formulations known for their rejuvenating, strengthening, and aphrodisiac properties [8, 9]. Thus, this therapy considers an alternative approach in the management of male sexual disorders and infertility**.** [10-12]

Various formulations are mentioned in *Ayurvedic* texts. [13, 14] The *Bhaishajya Ratnavali* describes *SSK (Shalmali malabarica* formulation) as a *Vajikarana* formulation. [15, 16] It significantly enhances sexual vigor and performance, even in advanced age. [17] The Modified *SSK*is created by following the process of *Churnikriya* and *Bhavana* to adjust a classical formulation to contemporary pharmaceutical standards while preserving its traditional therapeutic benefits. The adjustment is aim to enhance the formulation's overall stability by reducing degradation of the active components during processing and storage. [18]

Sildenafil is selected as the standard comparator due to established efficacy in the treatment of erectile dysfunction, [19] providing a validated benchmark for assessing the aphrodisiac activity of SSK *(Shalmali malabarica* formulation) and its modified form. Normal and disease control groups are included to establish baseline and disease-induced changes, enabling accurate evaluation of treatment effects.

Rationale of the Present Study:

Conventional aphrodisiac medicines have side effects, which include palpitations, headaches, dyspepsia, and unintended or incomplete sexual arousal. [20] In contrast, Ayurvedic aphrodisiac formulations, such as those used in Aphrodisiac therapy [21] or Virility therapy, [22] are traditionally considered to be free from such adverse effects. However, there is a significant lack of comprehensive scientific data to substantiate these claims. There is a need to bridge the gap by performing pharmaceutico-analytical standardization, toxicological assessment, and efficacy evaluation of these formulations in accordance with modern scientific standards.

Benefit–risk analysis of interventions:

The intervention SSK and its modified form will evaluate for their effect on sexual behaviour, erectile function, and reproductive health. The study will also evaluate the pharmacological properties of these Ayurvedic formulations.

Potential risks of these interventions will include dose-related toxicity, adverse behavioural effects, changes in body weight, and possible hepatic and renal impairment. These risks will be monitored through toxicity assessment, clinical observations, biochemical investigations (including liver function tests (LFT) and kidney function tests (KFT)), and histopathological evaluation to ensure animal welfare throughout the study.

The study is designed to generate data on the efficacy and safety profile of SSK and its modified form in the experimental model.

Aim: To Evaluate Pharmaceutico-analytical Standardization

of *SSK* (*Shalmali malabarica* formulation) and its Modified Dosage form and in vivo Comparative evaluation of its Toxicity and Aphrodisiac activity in Paroxetine-induced erectile dysfunction

Objectives:

- To study the preparation of *SSK* (*Shalmali Malabarica* formulation) and its Modified Form.
- To study the Physico-chemical analysis of *SSK* (*Shalmali malabarica* formulation) and its Modified Form.
- To study the toxicological (Acute) effect of *SSK* (*Shalmali malabarica* formulation) and its Modified Form.
- To study the aphrodisiac effect in normal and Paroxetine-induced E.D. in male rats of *SSK* (*Shalmali malabarica* formulation) and its Modified form.

Research question: Whether *SSK* (*Shalmali malabarica* formulation) and its Modified Form is safe and significantly possesses Aphrodisiac activity in Paroxetine induced E.D. in male rats?

Null hypothesis (H0): *SSK* (*Shalmali malabarica* formulation) and its Modified Form are not safe and do not possess significant Aphrodisiac activity do not show significant improvement in Paroxetine induced E.D. in male rats.

Alternative hypothesis (H1): *SSK* (*Shalmali malabarica* formulation) and its Modified Form are safe and do possess significant Aphrodisiac activity do show significant improvement in Paroxetine induced E.D. in male rats.

2. METHODS

Protocol version and date: 7 and 06/08/2026

Study design: In vivo experimental study with parallel groups, randomized and controlled experimental trial in male rats.

Study Setting and Location:

Biocyte, Research and Development PVT LTD., Flat No. -11, Shriram Residency, Near a Bypass Road, Kala Nagar, Sangli, Maharashtra -416416.

This research includes the following steps-

Step 1 - Pharmaceutical (Preparation) Study –includes Purification procedure of *Parada* (Mercury)**,** Purification procedure of *Gandhaka* (Sulphur), Preparation of *SSK (Shalmali malabarica* formulation*)* and its Modified Form

Step 2 - Analytical Study -Physico-chemical studies of Prepared formulations

Step 3 - Acute Toxicity study –As per OECD Guidelines420, fixed-dose method. [23]

Step 4 - Experimental Efficacy Study –Study of aphrodisiac effect of *SSK (Shalmali malabarica* formulation*)* and its Modified Form in Paroxetine-induced E.D. in male.

Allocation ratio: 1:1 – Arandomized selected animals will equally distributed into Seven groups as - Normal group, Disease controlled group, Standard drug Sildenafil treated group, SSK (*Shalmali malabarica* formulation) treated group (Experimental group –I), Modified form of SSK (*Shalmali malabarica* formulation) treated group (Experimental group –II), Treatment group treated with SSK (*Shalmali malabarica* formulation) (Experimental group –III), Treatment group treated with Modified form of SSK (*Shalmali malabarica* formulation) (Experimental group –IV), to ensure fair comparison of toxicity and aphrodisiac activity.

Trial framework: Superiority framework - This study will follow a superiority framework. SSK (*Shalmali malabarica* formulation) and its modified form will be tested for acute toxicity and aphrodisiac efficacy, compared with standard control groups. The study is designed to demonstrate that the test formulations are superior to the disease control group in reversing Paroxetine-induced erectile dysfunction, as assessed by predefined primary sexual behaviour parameters and secondary biochemical and histopathological parameters. SSK and its modified form will also be compared to identify any pharmacological advantage of the modified dosage form over the classical preparation.

Eligibility Criteria:

Inclusive Criteria -

- Healthy male *Albino wistar* rats
- Rat of 90 - 120 days old
- Weight ranges between 150- 200 g.
- Animals showing normal base line activity and no visible signs of illness.

Exclusive Criteria -

- Unhealthy male *Albino wistar* rats
- Below 90 days and above 120 days
- Weight bellow 150 and above 200 g
- Animals showing abnormal behaviour, injury or physiological distress

Eligibility criteria for intervention handlers: All experimental procedure to be performed by trained person.

The CPCSEA and Institutional ethical guidelines will be followed during handling and dosing.

Step 1 - Pharmaceutical (Preparation) Study:

SSK (Shalmali malabarica formulation) and its modified form will be prepared using standard *Ayurved* Procedures by following the steps -

Source of raw material: All raw ingredients used in the preparation will be collected from authenticated, reliable, and GMP-certified suppliers.

Batch number – Each raw material will be assigned a specific batch number to ensure traceability and proper documentation throughout the study

Authentication: All drugs will be authenticated and verified before use by a qualified pharmacognosist, including confirmation of botanical identity and organoleptic evaluation. Standard pharmacopeial and classical Ayurvedic texts will used for Identification. It helps to confirm the botanical identity and purity.

Table no: 1- Ingredients of *SSK* (*Shalmali malabarica* formulation) and its modified form

Sr. No.	Ingredient–Sanskrit name (Common Name)	Scientific Name	Quantity	Part Used
1	*Shuddha Parada*	Mercury	1 part	--
2	*Shuddha Gandhaka*	Sulphur	2 parts	--
3	*Bhukushmanda (Vidarikanda)*	*Pueraria tuberosa*	1 part	Root - Fine powder
4	*Talmuli (Krishna Musali)*	*Curculigo orchioides*	1 part	Root - Fine powder
5	*Dhatri (Amalaki)*	*Emblica officinalis*	1 part	Fruit - Fine powder
6	*Punarnava*	*Boerhavia diffusa*	1 part	Root - Fine powder
7	*Shweta Shalmali Kwatha*	*Shalmali malbarica*	—	Root *Kwatha; Bhavana Dravya*
8	*Mahisha Dugdha*	Buffalo Milk	—	*Bhavana Dravya*

Standardization of raw ingredients:

Standardization of all the above-mentioned ingredients of *Sidha Shalmali Kalpa* and its modified form will be carried out using API-based analytical standardization parameters including Total Ash, Acid Insoluble Ash, Loss on drying, water-soluble extractive, alcohol soluble extractive pH and specific gravity.

Preparation of *SSK* (*Shalmali malabarica* formulation):

Fine powder of *Bhukushmanda (Vidarikanda), Talmuli (Krushna Musali), Dhatri (Amalaki), and Punarnava* will be taken in equal quantity, each one part (Total 4 parts); *Shudha Gandhak* will be taken half of *Shudha Parada*, that means one part of *Shudha Parada* is taken, and Half part of *Shudha Gandhaka* will be taken.

The *Kajjali* will be prepared by using *Shuddha Parada* (1 part) and *Shuddha Gandhaka* (1/2 part). A mixture of the above-mentioned ingredients is added to this and triturated well.

Bhavana of *Shweta Shalmali Kawath* and *Mahishi Dugdha* will be given 7 times each (Total 14 *Bhavana*). Then this mixture will dry in the shade.

Preparation of Modified *SSK* (*Shalmali malabarica* formulation):

Firstly, *SSK (Shalmali malabarica* formulation*)* will be prepared by following the above-mentioned steps, as explained in the Classical texts, up to *Bhavana* of *Shweta Shalmali Kawath* and *Mahishi Dugdha*.

Then Bhavana of *Bhukushmanda (Vidarikanda) kwatha, Talmuli (Krishna Musali) kwatha, Dhatri (Amalaki) kwatha,*

Punarnava mula kwatha, Shwta Shalmali Kwatha, and Mahisha Dugdha will be given 7 times each (Total 42 *Bhavana*). Then this mixture will dry in the shade.

Step 2 - Analytical Study:

Standardizations of final formulations:

Standardization of *SSK (Shalmali malabarica* formulation*)* and its modified form will be performed using API-based analytical standardization parameters, including Total Ash, Acid Insoluble Ash, Loss on drying, water-soluble Extractive, Alcohol Soluble Extractive, pH, and XRF analysis. The quality profile and standardization characteristics of both formulations will establish using these tests.

Step 3 - Acute Toxicity Study:

The toxicological investigation of *SSK* (*Shalmali malabarica Formulation*) and its modified dosage form will be conducted using groups of six animals each, in accordance with OECD Guideline 420 the fixed-dose method criteria for acute toxicity studies. The dosage of *SSK* (*Shalmali malabarica* formulation) and its modified dosage form will be 1 *Masha* (gram), or 1000 mg, twice daily, according to the classical text *Bhaishajya Ratnavali.* With this knowledge, the experimental animals will receive a 400 mg dose (2000 mg/kg) in rats all at once, and they were monitored for 14 days.

Step 4 - Experimental Efficacy Study:

In this part an animal experiment will conducted.

Type of Study - In Vivo Experimental (Preclinical) study

Preparation of Test compounds *SSK* (*Shalmali malabarica* formulation) and its modified form Test drug for intervention will be prepared by mixing *Shidha Shalmali Kalpa* (*Shalmali malabarica* formulation) and its Modified Dosage form in Normal Saline.

Table no: 2 –Details of Intervention for the Acute Toxicity of *SSK* (*Shalmali malabarica* formulation) and its modified form

Group	Group type	No. of Animal	Details	Dose	Duration	Administration	Observation Duration
Group 1	Study Group I	06 Male rat	Acute toxicity Study of *Sidha Shalmali Kalpa* (*Shalmali malabarica* formulation)	2000 mg/kg - Single Dose	1 Day – first day of Experiment	Oral	14 days
Group 2	Study Group II	06 Male rat	Acute toxicity Study of Modified dose of *Sidha Shalmali Kalpa* (*Shalmali Malabarica* formulation)	2000 mg/kg - Single Dose	1 Day – first day of Experiment	Oral	14 days

Table no: 3– Detail description of Animal used for the Efficacy study–Sample size and Recruitment Animals used

Animal Species used	Rats (*Albino wistar*)
Source of Animals	Certified Animal Research Centre
Sex of Animals	Male rats will be taken in each group (and for the performance, female rats will also be taken)
Age of Animals	Adult
Avg. wt of Animals	150-200 g.
Period of Fasting	Overnight
Feeding	Wheat bran and water ad libitum
No. of Animals	6 Rats for each group
Sex of Animals	Male rats
No. of Groups	8 (2 For Toxicity study and 7 for Experiment)
Dose form	Semi solid form
Route of administration	Oral
Duration of Exposure	Single exposure

Table No. –4: Details of experimental groups and Intervention for the Efficacy study –

Group	Group type	Dose	Duration	Administration	Observation Duration
	Evaluation of the Male Sexual Performance of Aphrodisiacs Action in Normal Rats				
Group 1	Normal Control	Nil	--	Nil	30 days
Group 2	Experimental group I Intervention – SSK (*Shalmali malabarica* formulation)	200 mg /kg	30 days	Oral	30 days
Group 3	Experimental group II Intervention – Modified form of SSK (*Shalmali malabarica* formulation)	200 mg /kg	30 days	Oral	30 days
	Evaluation of Male Sexual Performance in Drug-induced E.D. Rat Model For the creation of the E.D. Rat model, Paroxetine will administer for 21 days, followed by a 30-day experimental study.				
Group 4	Drug Control Intervention –Sildenafil	5 mg/kg	30 days	Oral	21 days + 30 days
Group 5	Experimental group III Intervention – SSK (*Shalmali malabarica* formulation)	200 mg /kg	30 days	Oral	21 days + 30 days
Group 6	Experimental group IV Intervention – Modified form of SSK (*Shalmali malabarica* formulation)	200 mg /kg	30 days	Oral	21 days + 30 days
Group 7	Disease Control (Paroxetine)	10 mg /kg	21 days	Oral	21 days + 30 days

Note – the group of 6 female rats will use as support to evaluate Aphrodisiac activity *Rats received Paroxetine (10 mg/kg) via oral route for E.D.

Six sexually receptive female *Albino wistar* rats (age: 90–120 days; weight: 150–200 g) will used exclusively to assess aphrodisiac activity through the mating performance test. Female rats will not to be assigned to any treatment group and are not to be administered any test formulation. They will serve solely as stimulus animals during the mating behavior observation sessions.

Receptivity will be induced before each observation session by subcutaneous administration of estradiol benzoate (10 µg/rat, 48 hours before testing) followed by progesterone (500 µg/rat, 4 hours before testing), as per the validated protocol. [24]

The same cohort of 6 female rats will used across all experimental groups. Each female will be exposed to a maximum of one male rat per session to avoid fatigue or stress carryover. Female rats will not be sacrificed at the end of the study and will maintain under standard housing conditions throughout the study.

The use of 6 female rats for this purpose will explicitly cover under the IAEC clearance letter no. BIRD/12/2024/06 (Date: 12/12/2024) and CPCSEA guidelines. The ethical approval will explicitly account for their use, reuse protocol, and end-of-study care.

Table No. - 5: Total Animals Used

Category	Details
Acute Toxicity Study	12 male rats (2 groups × 6 animals/group) — as per OECD 420
Efficacy Study — Male rats	48 male rats (6 groups × 8 animals/group, with 2 animals' buffer per group for attrition)
Efficacy Study — Female rats	6 female rats (used as stimulus animals across all groups; not sacrificed)
Total	60 male rats + 6 female rats = 66 animals total (covered under ethical approval)

Criteria for Intervention modification/discontinuation:

The intervention will be discontinued or modified if the animal exhibits severe toxicity signs, significant weight loss (> 20%), marked behavioural abnormality, unexpected adverse effect, or mortality related to the experimental drugs. Any such event will be documented and managed in accordance with IAEC and CPCSEA guidelines.

Adherence monitoring strategy:

Adherence to the intervention will be ensured by administrating of experimental drugs directly to all animals. Each one will be recorded in the treatment log, and any missed or incomplete dosing is documented with justification. Regular checks will be conducted to ensure compliance with the dosing schedule and study protocol.

Specify permitted/prohibited concomitant treatments:

Only the study drug interventions SSK (*Shalmali malabarica* formulation), Modified form of SSK (*Shalmali malabarica* formulation), and Sildenafil will be permitted during the experimental period. Administration of any additional drug, herbal product, supplement, or treatment will prohibit.

Outcomes:

A comprehension review will be conducted on the traditional Ayurvedic formulation *SSK* (*Shalmali malabarica* formulation), which is mentioned in Ayurvedic classical texts and also, papered Modified form of SSK (*Shalmali malabarica* formulation) for improving sexual health.

Primary outcome:

To assess its Aphrodisiac potential, a Paroxetine-induced erectile dysfunction animal model is employed, representing a clinical scenario of sexual dysfunction caused by pharmaceutical agents.

Measurable Primary Outcome (measured at Day 30 and Day 60):

Mount Latency (ML) - time in seconds from introduction of female to first mount

Mount Frequency (MF) — number of mounts per 30-minute observation session

Intromission Latency (IL) — time in seconds from introduction to first intromission

Intromission Frequency (IF) — number of intromissions per 30-minute session

Ano-genital sniffing frequency — number of events per session

Genital grooming frequency — number of events per session

Secondary Outcomes (measured at study end — Day 60):

Serum testosterone level (ng/dL) - assessed by validated ELISA diagnostic kit

Serum alkaline phosphatase / ALP (U/L) - assessed by validated diagnostic kit

Histopathological examination of kidney, prostate, testis, intestine, and heart - graded by standard scoring

Acute toxicity parameters:

mortality rate, body weight (g), food/water intake (g/mL/day), clinical signs - recorded at Day 0, Day 8, and Day 22

Pharmaceutico-analytical parameters:

Total Ash, Acid Insoluble Ash, Loss on Drying, Water-Soluble Extractive, Alcohol-Soluble Extractive, pH, specific gravity, XRF analysis

Effects and Future Perspectives:

Clinical trials, long-term toxicity studies, and investigations of molecular mechanisms will focus of future research to support therapeutic claims and bridge the gap between encouraging evidence-based uses.

Specify measurement variables:

Variables the study are measure as

Pharmaceutical and analytical variables (Total Ash, Acid Insoluble Ash, Loss on drying, water-soluble extractive, alcohol soluble extractive, pH, specific gravity, and XRF analysis)

Acute toxicity study related variables (mortality rate, body weight, water/food intake, clinical signs, and histopathological changes)

Aphrodisiac study related variables: the sexual behaviour parameters (such as ano-genital sniffing, genital grooming, mount latency, mount frequency, insertion latency, and frequency)

Biochemical variables (alkaline phosphatase and serum testosterone), mortality, and Histo-pathological examination of the Kidney, Prostate, Testis, Intestine, and Heart

Time points:

Acute Toxicity study - The key assessment will be conducted at baseline (Day 0); Acute Toxicity of *SSK* (*Shalmali malabarica* formulation) and its modified form (Day 8 to 22), and at the end of the study (Day 22), including biochemical and toxicity evaluation

Aphrodisiac study -The key assessments will conducted at baseline (Day 0); followed by disease Incubation – Paroxetine induced E.D. (Day 8 to 29); then the treatment phase (Next 30 Days) and at the end of the study (Day 30 to 60), including sexual behaviour parameters, biochemical examination, Mortality and Histo-pathological examination of the Kidney, Prostate, Testis, Intestine, and Heart.

Analysis metrics:

Matrix analysis will include comparisons of changes from baseline (Day 0) to post-treatment (Day 60) in sexual behaviour and biochemical parameters between groups. Endpoint values will also analyzed for inter-group differences by using appropriate statistical tests. Time to event analysis will used for sexual parameters such as ano-genital sniffing, genital grooming, mount latency, mount frequency, insertion latency, and frequency to assess the treatment effect dynamically over time.

Participant timeline:

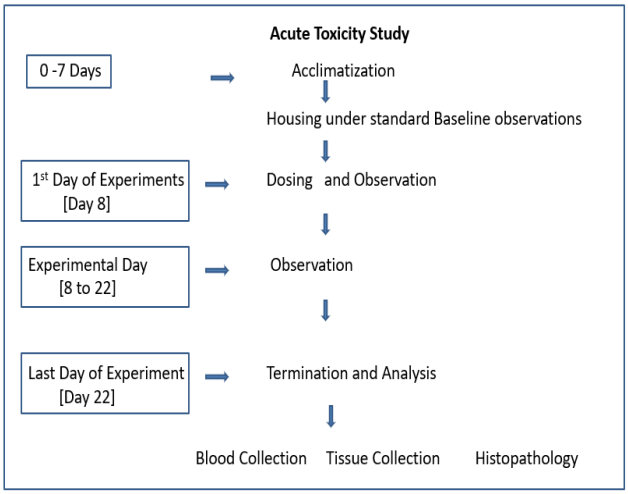

Diagram No. – 1 Timeline for Acute Toxicity Study

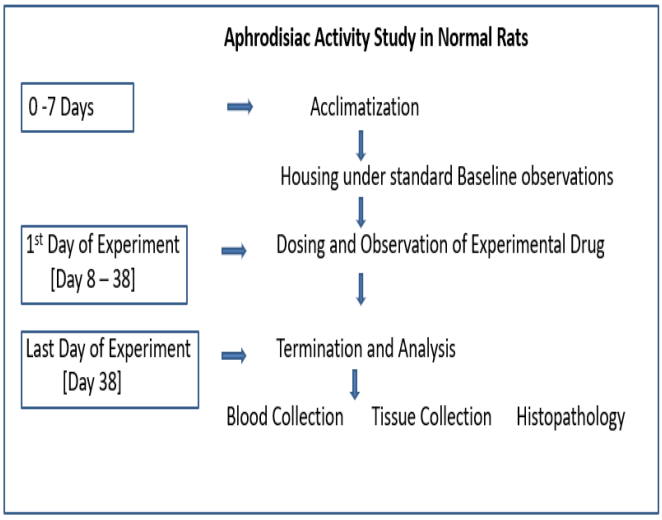

Diagram No. – 2 Timeline for Aphrodisiac action Study in Normal Rats

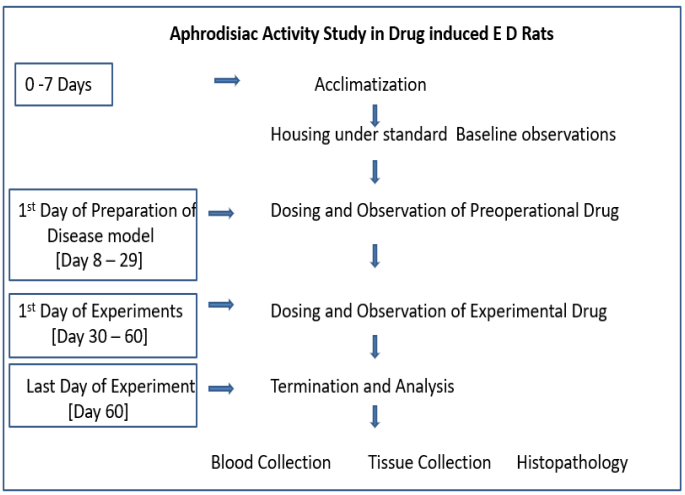

Diagram No. – 3 Timeline for Aphrodisiac action Study in Paroxetine induced E.D. Rats

Sample size and Recruitment:

Sample size will be estimated using G*Power 3.1 software [25] for a one-way ANOVA design. Based on comparable published studies evaluating aphrodisiac activity of classical Ayurvedic formulations in male Wistar rats [25], a large effect size of Cohen's f = 0.40 will assumed. With a significance level of $\alpha = 0.05$ and desired statistical power of $1-\beta = 0.80$, and with six experimental groups, G*Power estimated a minimum total sample size of 52 animals. To account for potential attrition of approximately 15%, the sample size will be increased to 60 male rats (n = 10 per group × 6 groups). An additional 12 male rats will used for the acute toxicity study (n = 6 per group × 2 groups),

following OECD 420 guidelines. Six female rats will used exclusively for the mating performance assessment. This sample size will consistent with CPCSEA guidelines for small animal pharmacological studies.

Healthy male *Albino wistar* rats meeting the inclusion criteria will be selected for an approved institutional animal facility. After acclimatization, animals will be randomly assigned to experimental groups, using a predefined randomization method to ensure unbiased distribution and comparability between groups.

Methods: Assignment of Interventions

Sequence generation:

Experimental animals will randomly allocate to study groups using simple randomization.

Allocation concealment mechanism:

Allocation concealment will maintain using a pre-generated randomization schedule. Each eligible animal will be assigned a Unique Identification number and allocated to a group according to a frequency-number-coded allocation record prepared before enrolment. The principal investigator will maintain the allocation list.

Implementation:

The principal investigator will generate the random allocation sequence, enrol eligible participants, and assign them to the respective experimental groups.

Methods: Data Collection, Management, and Analysis:

Data Collection Methods:

During this experimental study, the following areas will the focus of in-depth observations and analyses during the data collection of this Experimental phase –

Assessment of Aphrodisiac Activity:

Validated and standardized tools, such as body weight, Cage observation for behaviour, toxicity signs, and sexual parameters such as ano-genital sniffing, genital grooming, mount latency, mount frequency, insertion latency, and frequency, is recorded.

Investigating Clinical Laboratory Conditions:

Biochemical markers will examine such as alkaline phosphatase (ALP) and serum testosterone will measure using a validated Diagnostic kit.

Necropsy and Histopathological Examination:

A thorough autopsy will be conducted at the conclusion of the study. To determine any pathological alterations and analyze the safety profile of both formulations, histopathological investigation of the main organs - the liver, kidneys, heart, prostate, and testes is performed.

Each observation will systematically record at each phase of the research, by continuous daily follow-up monitoring of animals throughout the study period for body weight, cage observation for behaviour, and toxicity signs are ensured by maintaining standardized housing conditions, proper acclimatization, and minimizing stress factors, while any animal lost due to morbidity or humane endpoints will be documented and included in attrition reporting.

Data quality will be ensured through proper training in animal handling, dosing procedure, and data recording. All observations will record induplicate, in manual and electronic formats, and cross-verified by another investigator to minimize errors.

The data will accurately organize and presented by using well-structured tables and informative charts.

Data management:

Data obtained during this experimental study will be recorded in a structured record sheet, coded by animal ID and treatment group, and entered into a password-protected electronic file. Data will regularly verify for accuracy and securely stored, with access limited to the investigator.

The Data obtained from the analytical and in vivo experimental studies will graphically be represented using appropriate software tools. This will show the differences in toxicity and aphrodisiac activity between the modified and classical formulations.

Retention Strategy and handling of dropouts/replacement animals:

To minimize animal retention throughout the study, all animals will be maintained under standardized laboratory conditions with appropriate housing, nutrition, environmental enrichment, and daily health monitoring. Stress and handling-related factors will minimize through acclimatization and uniform experimental procedures.

Any animal that develops severe illness, experiences an expected mortality, or reaches humane endpoint will withdraw from the study and documented with the reason for exclusion. All dropouts and losses will record and reported in the final analysis.

No replacement animals will add after the study has started, and all withdrawals will appropriately document and considered during data analysis.

Statistical Methods:

All values will express as mean + SEM (Standard Error of Mean). The respective data will statistically analyzed by using appropriate statistical tests such as - One way ANOVA, which is used to compare multiple groups for the sexual behaviour parameters and biochemical outcome; the Kruskal-Wallis test will applied for the non-normally distributed data, and paired or unpaired 't' test will used for within-group and between-group comparison, ensuring appropriate analysis of efficacy and safety endpoints. If necessary, another statistical test will apply.

Any additional analysis will include exploratory evaluation of the secondary outcome such as biochemical, antioxidant, and histopathological parameters. Statistical methods appropriate to the data type will apply as required and all the additional analysis will clearly be specified and reported separately from the primary outcome.

A subgroup analysis will be performed within each treatment group to compare primary and secondary outcomes. Adjusted analyses will be carried out to control for baseline variation, such as body weight or behavioural differences, to ensure accurate interpretation of efficacy and safety results.

Missing data due to animal loss or technical errors will document with a reason. Affected cases will be excluded from the specific analysis, and no bias imputation is applied. All missing data will be reported transparently in the final study results.

Methods: Monitoring:

Data Monitoring:

The study data will monitor regularly throughout the experiment, which ensures completeness, accuracy, and consistency. Any deviation during the experiment at any stage will immediately be recorded and reviewed. Also, missing values and unusual observations will note.

As this is a preclinical experiment, the separate data monitoring committee is not applicable. The principal investigator will conduct continuous monitoring.

A provisional analysis will perform after completion of the in vivo toxicity assessment. If significant toxicity, unexpected mortality, or severe adverse effects are observed, the study may be stopped or modified.

The structured adverse event monitoring plan will be followed including daily observation of behaviour, body weight changes and clinical signs of toxicity.

Ethics and Dissemination:

Research ethics approval:

Institutional ethical committee and the IAEC approval will obtaine before the initiation of any experimental procedures.

IEC Clearance Letter Number: MGACHRC/IEC/Dec-2022/622 Date – 07/12/2022

IAEC clearance letter no.- Ref. No. – BIRD/12/2024/06 Date – 12/12/2024

Although informed consent is not applicable to animal studies, all experimental procedures will be performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) and the applicable CPCSEA guidelines (or relevant national regulations). Every effort will be made to ensure humane handling, minimize pain and distress, and maintain the highest standards of animal welfare.

Auditing: The study will subject to periodic internal review and audit by the institutional authorities to ensure

compliance with the approved protocol, IAEC guidelines, and data integrity.

All study activities, including data collection and analysis, will be conducted independently by the investigator without external influence, ensuring objectivity and transparency in the reporting of results.

Protocol amendments:

Any important modification to the study protocol, methodology, intervention, or analysis plan will be documented, reviewed, and approved by the Institutional Animal Ethics Committee before implementation. All amendments will properly record with version number and date.

Confidentiality: All study-related data, records, and biological specimens will be maintained in confidence and used solely for the research purposes.

Access to data:

Access to the data will restrict to the investigator, and all records will be securely stored in accordance with institutional and ethical guidelines.

The results obtained during the study will be shared with the institutional ethics committee, ensuring transparent reporting in accordance with ethical and publication guidelines.

Ancillary and post-trial care: The experimental animals will receive appropriate care, monitoring, and supportive management throughout the study period. After the study is completed, animals are handled in accordance with CPCSEA and institutional ethical guidelines.

Dissemination Policy and Publication Plan: The study findings will be disseminated through presentation in a scientific conference and through submission for publication in a peer-reviewed, indexed journal.

Biological Specimens' Handling/Storage Plan: All biological specimens (blood and tissue) will collected during and at the end of the experiment by using aseptic techniques, properly labelled with a study ID, and preserved under standard conditions to maintain sample integrity for biochemical and histopathological analysis. Blood samples will stored at 2 to 8^0 C for short-term use, and serum will preserved at - 20^0 C for biochemical analysis.

3. RESULT:

This study is planned to evaluate the safety and aphrodisiac ability of the traditional Ayurvedic formulation, *SSK* (*Shalmali malabarica* formulation), and its modified formulation in the Paroxetine-induced erectile dysfunction model. The efficacy assessment is likely to include changes in sexual behavior parameters, including ano-genital sniffing, genital grooming, mouth latency, mouth frequency, intromission latency, and intromission frequency, together with relevant biochemical markers such as serum testosterone and alkaline phosphatase levels. The study is also planned to assess the safety profile of both formulations through acute toxicity evaluation, including observation of mortality, body weight, food and water intake, and general clinical signs. Histopathological examination of kidney, prostate, testes, intestine, and heart is likely to provide additional evidence regarding the safety of the formulation. Based on the objectives of the study, the modified SSK formulation is expected to demonstrate improved therapeutic performance compared with the classical *SSK* (*Shalmali malabarica* formulation) while maintaining comparable safety. The findings are probable to contribute to the scientific validation of the potential of this classical *SSK* (*Shalmali malabarica* formulation) and provide extensive experimental evidence to support further preclinical and clinical investigations.

4. DISCUSSION:

The study is planned to provide scientific evidence regarding the assessment of safety and aphrodisiac activity of the classical *SSK* (*Shalmali malabarica* formulation) and its modified form. These effects may be approved due to the presence of several bioactive constituents including flavonoids, phenolic compounds, and plant steroids which have been reported as antioxidant, androgen-supportive and reproductive health-promoting properties in previous studies. If the findings demonstrated improvement in the sexual behavior and the reproductive biochemical

parameters, they may be explained by enhancing antioxidant defence, improved testosterone status and better penile vascular function, which are mechanisms suggested in the exsisting literature. The modified formulation of SSK is expected to produce greater therapeutic benefits than classical *SSK* (*Shalmali malabarica* formulation). After this experimental study, if these findings confirm, it would support the traditional *Ayurvedic* use of classical *SSK* (*Shalmali malabarica* formulation). The toxicity assessment is expected to demonstrate that the both formulations are at the tested dose, thereby supporting their potential therapeutic application. However, the safety evaluation will be limited to acute toxicity and additional sub-acute and chronic and clinical studies will be required for the establishment of their long-term safety profile.

Certain limitations should be considered as the study will be conducted in an experimental animal model. The result may not be directly applicable to humans. In addition, factors such as biological variability among animals and difference in photochemical constituents of the herbal raw ingredients may be influenced the observed outcome. These potential sources of bias will be minimized by adopting standardized experimental procedure, appropriate and valuable analytical methods. Future preclinical and clinical investigation will be necessary to confirm the efficacy, safety and mechanism of action of both formulations in human population.

These findings will suggest that *SSK* (*Shalmali malabarica* formulation) possesses aphrodisiac activity and is safe at the tested dose. The modified form of SSK may offer greater efficacy than the classical formulation while maintaining comparable safety.

Strengths of the present study:

The study is designed to evaluate the traditional formulation, *SSK* (*Shalmali malabarica* formulation), using predefined experimental parameters.

A comparative assessment of the classical and modified forms of *SSK* (*Shalmali malabarica* formulation) will conduct.

Pharmaceutico-analytical standardization will perform to assess the quality and uniformity of both formulations.

Acute toxicity studies will conduct to assess the safety profile of both formulations.

The study will evaluate the effect of the formulations on sexual behaviour and related biochemical parameters in normal animals and in a paroxetine-induced E.D. model.

Limitations of the present study:

This study will conduct in an animal model; therefore, the findings will limit to experimental conditions and species studies.

Chronic and long-term toxicity assessments will outside the scope of the study.

The evaluation of the formulations will be based on behavioral and biochemical parameters; molecular mechanisms will not investigate.

5. CONCLUSION:

This study will likely to generate scientific evidence regarding the safety and aphrodisiac activity of classical SSK and its modified form. The modified form of SSK is likely to exhibit increased therapeutic efficacy, possibly due to improved bioavailability and its constituent photochemical. The findings obtained after this study may contribute to the scientific validation of this traditional *Ayurvedic* formulation SSK (*Shalmali malabarica* formulation) and support its potential application in the management of male sexual dysfunction. Also, the study is likely to provide a basic foundation for future pharmacological investigations and well-designed clinical trials to further evaluate its efficacy, safety, and underlying mechanism of action.

Abbreviations:

ALP - Alkaline phosphatases

API - *Ayurvedic* Pharmacopoeia of India

E.D. - Erectile dysfunction

SSK - Sidha Shalmali Kalpa

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Declaration of Generative AI

The authors declare this manuscript was written without the use of generative artificial intelligence tools. All the content, including text generation, data analysis and references was developed and reviewed by the author without assistance from AI technologies.

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REFERENCES:

1. Direkvand-Moghadam A, Sayehmiri K, Delpisheh A. The global trend of infertility: an original review and meta-analysis. International Journal of Epidemiologic Research. 2014;1(1):35-43. Available from: https://api.semanticscholar.org/CorpusID:4660092
2. Devranoğlu B, Gürbüz T, Yurci A. Male infertility: an overview of etiology, diagnosis and management. Journal of Controversies in Obstetrics & Gynecology and Pediatrics. 2024;2(2):34-37. Available from: 10.51271/JCOGP-0031
3. Velu A, Prasad G. Epidemiologic aspects of male infertility. International Journal of Reproductive Contraception Obstetrics and Gynaecology. 2017 Aug.;6(8):3362-3365. Available from: https://www.ijrcog.org/index.php/ijrcog/article/view/3306/2631
4. Gül M, Fode M, Urkmez A, *et al.* A clinical guide to rare male sexual disorders. Nature Reviews Urology. 2024;21:35–49. Available from: https://doi.org/10.1038/s41585-023-00803-5
5. Sogi MS, Prashantha AS. Review on klaibya (erectile dysfunction). Journal of Ayurveda and Holistic Medicine (JAHM), 2022 Feb;10(1):26-40. Available from: https://jahm.co.in/index.php/jahm/article/view/503
6. Khan MB, Rathi BJ, Rajput D, Wanjari A. A Review on classical *Vajikarana* formulations of *Shweta Musali*. Journal of Indian System of Medicine. 2019;7(4):205-211. Available from: https://api.semanticscholar.org/CorpusID:216082196}
7. Rao RG (translator). Kaviraj Shri Govinda Das Sen. Bhaijyaratnavali, Volume II, Chapter 74, verse no. 02. First Edition, Varanasi; Chaukhambha Orientalia; 2014;681.
8. Singh R, Ali A, Gupta G, Semwal A, Jeyabalan G. Some medicinal plants with aphrodisiac potential: A current status, Journal of Acute Disease. 2013;2(2):179-188. Available from: https://doi.org/10.1016/S2221-6189(13)60124-9.
9. Sandroni P. Aphrodisiacs past and present: a historical review. Clinical Autonomic Research. 2001 Oct;11(5):303-7. Available from: doi: 10.1007/BF02332975. PMID: 11758796.
10. Staines MJ, Sengottuvelu S, Sherief SH, Lalitha V. (2023). Aphrodisiacs: A short review on naturally available sexual boosters. Annals of Phytomedicine: An International Journal. 2023;12(1):39-50. Available from: http://dx.doi.org/10.54085/ap.2023.12.1.93.
11. Rao RG (translator). Kaviraj Shri Govinda Das Sen. Bhaijyaratnavali, Volume II, Chapter 74, verse no. 06. First Edition, Varanasi; Chaukhambha Orientalia; 2014;682.
12. Kulkarni SV, Patil SS. Rejuvenating sexual health holistically: an approach in ayurveda. Journal of Ayurveda and Integrated Medical Science. 2024;9(11):225-239. Available From: https://jaims.in/jaims/article/view/3792
13. Sharma M, Dharmendra A, Bhagour K, Gupta RS. Natural aphrodisiac and fertility enhancement measures in males: A review. Current Medicine Research and Practice.2017;7(2):51-58. Available From: https://doi.org/10.1016/j.cmrp.2017.02.007
14. Owaba AD, Etim EI, Johnson EC, Umoh UF. Aphrodisiac agents used in traditional medicine and their mechanism of action-A Review. Journal of Pharmacognosy and Phytochemistry. 2021;10(3):126-53. Available from: DOI: https://doi.org/10.22271/phyto.2021.v10.i3b.14085
15. Rao RG (translator). Kaviraj Shri Govinda Das Sen. Bhaijyaratnavali, Volume II, Chapter 74, verse no. 59-62. First Edition, Varanasi; Chaukhambha Orientalia; 2014;689.
16. Dalal PK, Tripathi A, Gupta SK. Vajikarana: Treatment of sexual dysfunctions based on Indian concepts. Indian Journal of Psychiatry. 2013 Jan; 55(Suppl 2):S273-6. doi: 10.4103/0019-5545.105550. PMID: 23858267; Available from: PMCID: PMC3705695.
17. Koparde PK, Wahurwagh A, Koparde SP, Pharmacological study of Sidha Shalmali Kalpa with special reference to Bhaishajyaratnavali - A review article.Ayurved Darpan Journal of Indian Medicine (ADJIM). 2022 Dec; 7(4):17-21. Available from: http://ayurveddarpan.com/AyurVed/journal/252_1.pdf
18. Shingadiya RK, Bedarkar PB, Patgiri BJ, Prajapati PK. Effect of churnakriya in the management of several life style disorders: a review through research works carried out at RSBK department, Jamnagar, India. International Journal of Research in Ayurveda and

Pharmacy. 2017 June;8(2):68-72, Available from: DOI:10.7897/2277-4343.08285

19. Hatzimouratidis K. Sildenafil in the treatment of erectile dysfunction: an overview of the clinical evidence. Clinical Intervention in Aging. 2006;1(4):403-14. doi: 10.2147/ciia.2006.1.4.403. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC2699643/ :
20. Dundar M, Kocak I, Dundar SO, Erol H. Evaluation of side effects of sildenafil in group of young healthy volunteers. International Urology and Nephrology. 2001;*32*(4):705–708. Available from: https://doi.org/10.1023/a:1015056416501
21. Rahman AU, et al. Evaluating the aphrodisiac potential of *mirabilis jalapa* l. Root extract: phytochemical profiling and in silico, in vitro, and in vivo assessments in normal male rats. Molecules. 2023;*28*(17):6314. Available from: https://doi.org/10.3390/molecules28176314
22. Nisargandha MA, Parwe SD. Evaluation of Spermatogenic Action in the Management of Oligospermia. International Journal of Life Sciences and Pharma Research. 2021;11(2):218-223. Available from: http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.2.P218-223
23. OECD, Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris; 2002. Available from: https://doi.org/10.1787/9789264070943-en.
24. Gurav N, Gurav S, Wanjari M, Prasad S, Wayal S, Rarokar N. Development and evaluation of aphrodisiac potential of a classical ayurvedic formulation, 'Kaamdevghrita' in rat model. Journal of Ayurveda and Integrative Medicine. 2021;12(2):294-301. Available from: https://doi.org/10.1016/j.jaim.2020.09.007
25. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods. 2007;39(2):175–191. Available from: https://pubmed.ncbi.nlm.nih.gov/17695343/