



Research Article

SPERMATOGENIC EFFICACY OF AYURVEDIC FORMULATION *GOKSHURADI CHURNA* AND ITS EXTRACT

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ABSTRACT

Infertility affects approximately 15% of couples globally, with male factors contributing to 20-70% of cases. The male reproductive system is highly sensitive to environmental, lifestyle, and various physical and chemical factors. Semen analysis identifies the cause in 40-50% of cases. While modern drugs are available, they may cause undesirable side effects. *Vajikarana*, one of the eight branches of Ayurveda, offers herbal and herbomineral formulations with aphrodisiac properties, such as *Gokshuradi Churna*. This polyherbal formulation includes *Gokshura* (*Tribulus terrestris* Linn.), *Ikshura* (*Asteracantha longifolia* Nees.), *Mash* (*Phaseolus mungo* Linn.), *Atmagupta* (*Mucuna prurita* Hook.), and *Shatavari* (*Asparagus racemosus* Wild.), as described in the classical text *Ashtanga Hridaya*. **Aim:** To evaluate the spermatogenic activity of *Gokshuradi Churna* and its extract. **Objectives:** Literary review, Pharmaceutical and analytical study, pharmacognostic study, In-vivo spermatogenic activity evaluation **Materials and Methods:** *Gokshuradi Churna* was prepared per classical methods, and various extract samples were formulated. Organoleptic parameters (taste, odor, appearance) and physicochemical parameters (loss on drying, total ash, water-soluble and alcohol-soluble extractives, pH, particle size) were analyzed. Tests included HPTLC, microbial limit, and heavy metal analysis. In-vivo spermatogenic activity was evaluated using Albino Wistar rats, analyzing sperm count, motility, non-motile sperm, body weight, and reproductive organ weight after 30 days. **Results and Observations:** Analytical results for all samples were within permissible limits. A statistically significant increase in sperm count ($F=371.03$, $P=0.0001$) and motility ($F=11.13$, $P=0.0001$) was observed across all groups. Sperm count improvement: Extract (low dose) < *Gokshuradi Churna* < Extract (high dose). Sperm motility improvement: Extract (low dose) < Extract (high dose) < *Gokshuradi Churna*. Non-motile sperm: Standard > Extract (low dose) > Extract (high dose) > *Gokshuradi Churna*. **Conclusion:** Both *Gokshuradi Churna* and its extract demonstrated significant spermatogenic activity, with varying efficacy depending on the dose and form.

INTRODUCTION

Health is the cornerstone of wealth, happiness, and spiritual fulfilment. Poor health and diseases disrupt life's harmony and hinder societal progress.^[1]

Ayurveda, the ancient holistic medical system introduced by Sage Bharadwaja, emphasizes maintaining balance through *Hetu Gyan* (knowledge of causes), *Linga Gyan* (knowledge of symptoms), and *Aushadi Gyan* (knowledge of medicines).^[2] Among its eight branches, *Vrishya Chikitsa* specializes in male reproductive health, particularly infertility treatment, by enhancing and nourishing *Shukra Dhatu* (reproductive tissues).^[3]

Infertility is defined as the inability to conceive after one year of unprotected intercourse and affects up to 15% of couples globally. Male factors contribute

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to 30-40% of infertility cases, with oligozoospermia (low sperm count) and poor sperm quality being the primary causes.^[4] In Ayurveda, these conditions are often associated with *Shukra Dushti* (vitiation of reproductive tissues) caused by imbalances in *Vata*, *Pitta*, and *Kapha Doshas*. Advanced allopathic treatments, while effective, have limitations, including significant side effects and high costs, making them inaccessible to many.^[5]

Gokshuradi Churna, a classical Ayurvedic polyherbal formulation, is widely recognized for its *Vrishya* (aphrodisiac) properties and efficacy in managing male infertility. It comprises five key herbs: *Gokshura* (*Tribulus terrestris* Linn.), *Ikshura* (*Asteracantha longifolia* Nees.), *Mash* (*Phaseolus mungo* Linn.), *Atmagupta* (*Mucuna prurita* Hook.), and *Shatavari* (*Asparagus racemosus* Wild.).^[6] These ingredients work synergistically to improve reproductive health by balancing *Doshas* and enhancing *Shukra Dhatu*. However, issues like poor palatability and inconsistent dosing of the traditional *Churna* form limit its acceptability.^[7]

The present study aims to address these challenges by converting *Gokshuradi Churna* into an extract form. The extraction process enhances its therapeutic potency, ensures standardization, and improves patient compliance. This study evaluates the spermatogenic activity of both *Gokshuradi Churna* and its extract, offering a safe, cost-effective, and accessible solution for male infertility.^[8]

AIM AND OBJECTIVES

Analytical Study

The following tests were conducted for the physio-chemical evaluation

S.No	Analytical Parameters	For Churna	For Extracts
1	Organoleptic Test	Appearance, colour, odour, taste, consistency	Appearance, colour, odour, taste
2	Physio-chemical parameters	- Loss on drying - Total ash - Acid-insoluble ash - Water-soluble extractive - Alcohol-soluble extractive - Particle size	-Loss on drying -Total ash -Acid-insoluble ash -pH -Water-soluble extractive - Alcohol-soluble extractive
3	Identification	TLC/HPTLC	TLC/HPTLC
4	Microbial Contamination	- Total bacterial count - Total fungal count	- Total bacterial count - Total fungal count
5	Test for heavy metals	Lead, cadmium, mercury, arsenic	Lead, cadmium, mercury, arsenic
6	Test for specific pathogens	<i>E. coli</i> , <i>Salmonella spp.</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i>	<i>E. coli</i> , <i>Salmonella spp.</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i>

Aim

To evaluate the spermatogenic activity of *Gokshuradi Churna* and its extract.

Objectives

1. Preparation of *Gokshuradi Churna* by classical method.
2. Hydro-alcoholic extraction of *Gokshuradi Churna* ingredients.
3. Evaluation of analytical parameters of both formulations.
4. Assessment spermatogenic activities of both (*Churna* and extract) by *in-vivo* study.

MATERIAL AND METHODS

1. PHARMACEUTICAL STUDY

a) Procurement

Drugs were collected from a local herbal drug stockist.

b) Authentication

Procured drugs were authenticated by P.G. Department of Dravyaguna Rishikul Campus, UAU, Haridwar.

c) Preparation of *Gokshuradi Churna*

Were be prepared by Ashtanga Hridaya Uttaratantrar 40/34 as reference. *Gokshura*,

Ikshura, *Mash*, *Atmagupta* and *Shatavari* all were finely grinded

d) Hydro-alcoholic extract *Gokshuradi Churna*

Were carried out by using a suitable solvent with the help of Soxhlet apparatus.

Drug Review

The literature on *Gokshura*, *Ikshura*, *Mash*, *Atmagupta*, and *Shatavari* has been reviewed from various Ayurvedic texts and research articles.

Table 1: Ingredients of Gokshuradi Churna

Contents	Botanical Name	Family	Parts Used
<i>Gokshura</i>	<i>Tribulus terrestris</i> Linn.	Zygophyllaceae	Seeds
<i>Ikshura</i>	<i>Asteracantha longifolia</i> Nees	Acanthaceae	Seeds
<i>Mash</i>	<i>Phaseolus mungo</i> Linn.	Leguminosae	Seeds
<i>Atmagupta</i>	<i>Mucuna prurita</i> Hook	Leguminosae	Seeds
<i>Shatavari</i>	<i>Asparagus racemosus</i> Wild.	Liliaceae	Roots

Table 2: Raspanchak of Gokshuradi Churna

Drug	Rasa	Guna	Veerya	Vipaka	Karma
<i>Gokshura</i>	Madhura	Guru, Snigdha	Sheeta	Madhura	Vaatapitta-shamak, Vrishya, Garbhasansthapak
<i>Ikshura</i>	Madhura	Snigdha, Pichhila	Sheeta	Madhura	Vaatapitta-shamak, Mutral, Vrishya, Rasayana
<i>Mash</i>	Madhura	Guru, Snigdha	Ushna	Madhura	Vaatahamak, Vrishya, Stanyajanan, Mutral
<i>Atmagupta</i>	Madhura, Tikta	Guru, Snigdha	Ushna	Madhura	Vaatahamak, Vrishya, Mutral
<i>Shatavari</i>	Madhura, Tikta	Guru, Snigdha	Sheeta	Madhura	Vaatapitta-shamak, Shukral, Stanyajanan

Table 3: Pharmacological Properties of Drugs- (Sharma PC, Database on Medicinal Plants Used in Ayurveda, Vol 1, 3, 4, 2005, pp. 200, 229, 320, 418)

Drug	Phyto-constituents	Pharmacological Actions
<i>Gokshura</i>	Saponins, polyphenolic compounds, alkaloids, furostanol, spirostanol, kaempferol	Aphrodisiac, anti-inflammatory, analgesic, antispasmodic, anti-bacterial
<i>Ikshura</i>	Alkaloids, saponins, steroids, phenolic compounds, tannins, flavonoids, terpenoids, protein, amino acids, anthraquinones	Aphrodisiac, <i>Balya</i> (stamina booster), <i>Shukrashodhaka</i> (purifies semen anomalies), <i>Vatraktahara</i> (useful in gout), <i>Ashmarihara</i> (lithotriptic), <i>Shothahara</i> (anti-inflammatory)
<i>Mash</i>	Genistein, glycinol, kievitone, eugenol, beta-sitosterol, phloretin	<i>Vrishya</i> (aphrodisiac), diuretic, strengthening (<i>Balya</i>), <i>Brumhana</i> (bulk promoting), <i>Stanyajanana</i> (galactagogue)
<i>Atmagupta</i>	Steroids, flavonoids, tannins-dopa, mucunine, prurienine, palmitic, oleic, linoleic, stearic	<i>Balya</i> (stamina booster), <i>Shukrala</i> (aphrodisiac), <i>Vatavyadhihar</i> (useful in disorders of <i>Vata</i> humor)
<i>Shatavari</i>	Steroidal saponins, quercetin, rutin, polysaccharides	<i>Balya</i> (stamina booster), <i>Rasayana</i> (rejuvenation), <i>Vrishya</i> (aphrodisiac), <i>Stanyajanana</i> (galactagogue), anti-stress, anti-inflammatory

Spermatogenic Study (In-Vivo)

Preparation of Animals: Healthy male 30 albino Wistar rats weighing 140-200gm were randomly selected, marked and kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions.^[9]

Housing & Feeding conditions: The temperature in the experimental animal room were 22°C ±3°C. Although the relative humidity was at least 30% and preferably not exceeding 70% other than during room cleaning the aim should be 50%-60%. Lightening should be artificial. The sequence is 12hrs light, 12hrs

dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.^[10]

Spermatogenic activity on male rats: Male Wistar strain albino rats were divided into 5 groups of six animals each (n=6). Lead Nitrate were given to induce oligospermia in Albino Rats for duration of 30 days Group A animals served as control and were receive only vehicle normal saline 1ml/kg. Group B animals were served as standard control and were receive testosterone propionate suspension (0.5mg/kg). Group C were received *Gokshuradi Churna*. The group D were received hydro-alcoholic extract of *Gokshuradi Churna* in lower dose and group E were receive a hydro-alcoholic extract of *Gokshuradi Churna* in a higher dose. The mode of administration were an oral-dependent manner by feeding the needle for 30 days.^[11]

Experimental dose calculation

Calculated by Barnes and Paget's rule

Conversion factor for rats: Human dose \times 0.018=Xg/200g rats

$X \times 5 = Yg/kg$ of rat

X = dose for 200gm of rats (Applicable for *Churna* & Extract both) **Y**= dose per kg body weight (Applicable for *Churna* & Extract both) **Humans dose**=5-10gm/kg/days. 30 Albino Wistar rats weighing between 140-200gm were be selected for the study purpose

Experimental Study Design

The study involves five groups of animals (rats) to evaluate the effects of *Gokshuradi Churna* and its extracts under different conditions.

Group	Type	No. of Animals	Drugs Used	Dose
Group-A	Control	6 rats	Normal saline	1 mL/kg (P.O)
Group-B	Standard	6 rats	Lead nitrate (0-30 days) Testosterone propionate suspension (1-30 days)	Lead nitrate: 80mg/kg (P.O) Testosterone propionate suspension: 0.5mg/kg (S.C)
Group-C	Experimental Group	6 rats	Lead nitrate (0-30 days) <i>Gokshuradi Churna</i> (1-30 days after 30 days of lead nitrate)	Dose calculated using Barnes and Paget rule
Group-D	Experimental Group	6 rats	Lead nitrate (0-30 days) Low-dose <i>Gokshuradi Churna</i> extract (1-30 days after 30 days)	Dose calculated using Barnes and Paget rule
Group-E	Experimental Group	6 rats	Lead nitrate (0-30 days) High-dose <i>Gokshuradi Churna</i> extract (1-30 days after 30 days)	Dose calculated using Barnes and Paget rule

Note: The addition or elimination of 8

The parameters can be done as per the need of the study and availability of resources. After 30 days following parameters were evaluated.

- Effect on the weight of sexual organs and histological studies.
- Sperm count, motility and pH etc.

Collection of Semen- After 30 days, rat's semen was collected either by epididymal puncture or rats were sacrificed after the last administration of the drug. Testis were immediately removed and blood samples were collected.^[12]

Effect on the weight of sexual organs and histological studies- After 30 days of treatment as described earlier the body weight of animals were recorded. The testis, seminal vesicles, epididymis and prostate gland were carefully removed and weighed. Testis and epididymis of animals were cut into small pieces and fixed in Bovine's fixative. After dehydration with varying percentages of ethanol, sections were cut,

stained with hematoxylin and eosin and then analyzed microscopically.^[13]

Sperm count & Motility- One drop of sperm were placed on a glass slide and ten random feeds would manually score for the number of motile and non-motile sperm. The pH of semen would be determined by a digital pH meter.^[14] After 30 days, the rats would be subjected to tissue biopsy. Rat's testis were removed and fixed overnight and processed into paraffin and sections were be stained with hematoxylin and eosin.^[15]

Type of Study

Pharmaceutico - Analytical and Experimental study

Period of Study

- Pharmaceutical study: 6 months
- Analytical study: 6 months
- Spermatogenic Study: 2 months
- Data interpretation: 4 months

MATERIALS AND METHODS

This section is divided into the following subheadings:

Pharmacognosy Study

Pharmacognosy, derived from the Greek words *Pharmakon* (drug) and *Gignosco* (to acquire knowledge), encompasses the study of identification, evaluation, and biological properties of drugs of natural origin. A pharmacognostical evaluation ensures the quality and therapeutic efficacy of plant drugs by applying macroscopic and microscopic criteria, as adulteration of raw materials can compromise the formulation's standards.^[16]

Authentication of Raw Materials

All herbs used in *Gokshuradi Churna* were authenticated (Brochure No. DG/RC/UAU-239, Date: 05-10-2024) at the P.G. Department of Dravyaguna, Uttarakhand Ayurved University, Rishikul Campus, Haridwar.

Macroscopic Study

Samples of the raw ingredients (*Gokshura*, *Ikshura*, *Mash*, *Atmagupta*, and *Shatavari*) were studied organoleptically using a magnifying lens to evaluate parameters such as shape, size, surface, texture, taste, odor, and color.

Ingredient	Botanical Name	Family	Part Used
<i>Gokshura</i>	<i>Tribulus terrestris</i> Linn.	Zygophyllaceae	Fruits
<i>Ikshura</i>	<i>Asteracantha longifolia</i> Nees	Acanthaceae	Seeds
<i>Mash</i>	<i>Phaseolus mungo</i> Linn.	Leguminosae	Seeds
<i>Atmagupta</i>	<i>Mucuna prurita</i> Hook	Leguminosae	Seeds
<i>Shatavari</i>	<i>Asparagus racemosus</i> Wild.	Liliaceae	Roots

Individual Ingredient Preparation

Each ingredient was dried, powdered, sieved (85#), and stored in airtight containers.

Preparation of *Gokshuradi Churna*

The prepared powders of all five ingredients were mixed in equal proportions, sieved, and stored in airtight stainless-steel containers.

Yield Data for *Gokshuradi Churna*

Ingredient	Weight of Raw Material (g)	Weight of Powder (g)	Yield (%)	Loss (%)
<i>Gokshura</i>	200	196	98	2
<i>Ikshura</i>	200	180	90	10
<i>Mash</i>	200	170	85	15
<i>Atmagupta</i>	200	180	90	10
<i>Shatavari</i>	200	190	95	5

Analytical Study

Analytical parameters, including organoleptic and physico-chemical evaluations, were conducted on both *Churna* and its hydroalcoholic extract.

Powder Microscopy

Microscopic studies were performed as per the *Anonymous API* (2008) method:

- **Preparation:** Powdered samples (5 g each) were sieved through an 85-mesh sieve and washed with water.
- **Procedure:** Slides were prepared by soaking the fine powder in distilled water for 1 hour, staining with safranin, and adding a drop of concentrated HCl. Observations were made under a microscope, and illustrations were drawn using a camera lucida.

Pharmaceutical Study

Procurement of Raw Materials

- Ingredients were sourced from authentic suppliers.
- Solvents and excipients were procured from Vijay Scientific, Haridwar.

Preparation of *Gokshuradi Churna*

Each ingredient (*Gokshura*, *Ikshura*, *Mash*, *Atmagupta*, *Shatavari*) was processed individually, and the final formulation was prepared by mixing equal proportions.

In-Vivo Study

Semen Collection and Evaluation

Semen samples were collected from test animals via epididymal aspiration and analyzed for sperm count,

motility, pH, and proportion of motile to non-motile sperm.

Measurement of Body Weight and Sexual Organs

The body weights of animals were recorded pre- and post-experiment, along with the weights of reproductive organs to evaluate the formulation's efficacy.

Hydroalcoholic Extraction Using Soxhlet Apparatus Procedure

- Sample Preparation:** 150g of powdered *Gokshuradi Churna* ingredients was packed in a filter paper thimble.

Batch No.	Sample Weight (g)	Solvent Used (mL)	Extract Weight (g)	Yield (%)
1	150	1000	45	30
2	150	1000	43	28.6
3	150	1000	48	32

Precautions

- Boiling chips were added to prevent solvent bumping.
- Rotatory evaporation pressure was adjusted to avoid frothing.

Pharmacognosy

Macroscopic and Microscopic Study of *Mucuna prurita* (Hook)

This section provides a comprehensive analysis of the morphological and microscopic features of *Mucuna prurita* (Hook) in its powdered form, which appears cream-colored with a characteristic odor and taste.^[17]

Macroscopic Study

- Appearance:** Cream-colored powder with a consistent texture.
- Odor and Taste:** Characteristic odor and taste, indicating the presence of intact volatile compounds and bioactive components essential for its medicinal properties.
- Relevance:** These sensory properties are crucial in traditional medicine for preliminary quality assessment, ensuring the sample is not adulterated or degraded.

Microscopy Study

a. Starch Grains with Hilum

- Structure:** Oval or circular with a distinct hilum indicating storage energy reserves.
- Relevance:** Confirms the seed-based origin of *Mucuna prurita*, contributing to its nutritional and therapeutic profile.

b. Oil Globules

- Structure:** Small, rounded structures indicating lipid presence.

- Soxhlet Setup:** The thimble was inserted into a Soxhlet extractor (500ml), connected to a 1000ml round-bottom flask containing 50% ethanol and 50% water.

3. Extraction Process:

- The setup was heated at 70–80°C for 8 hours daily until the solvent in the chamber became colorless.
- Extracts were filtered and concentrated using a rotary evaporator.

4. Yield

- Average yield: 30%

- Relevance:** Reflects the herb's richness in fats and oils, which play roles in neuroprotection and reproductive health.

c. Simple Starch Grains

- Structure:** Smaller and simpler than grains with hilum.
- Relevance:** Contributes to the carbohydrate and nutritional content of the powder.

Macroscopic and Microscopic Study of *Gokshura (Tribulus terrestris)*

This section evaluates the macroscopic and microscopic features of *Gokshura (Tribulus terrestris)*, a yellowish-brown powder with characteristic sensory properties.

Macroscopic Study

- Appearance:** Yellowish-brown powder.
- Odor and Taste:** Characteristic properties confirm its authenticity and therapeutic quality.

Microscopy Study

a. Trichomes

- Structure:** Hair-like structures present on the plant surface.
- Relevance:** Confirms the inclusion of aerial plant parts, contributing to protective properties.

b. Non-Lignified Thin-Walled Fibers

- Structure:** Flexible fibers without lignin deposits.
- Relevance:** Ensures the anatomical integrity of the herb.

c. Oil Globules

- Structure:** Rounded lipid structures indicative of essential oils.
- Relevance:** Supports anti-inflammatory and antioxidant properties.

d. Calcium Oxalate Crystals

- **Structure:** Byproducts of metabolism present as crystals.
- **Relevance:** Acts as a marker for plant identification.

e. Spiral Vessels from Vascular Strands

- **Structure:** Coiled xylem vessels aiding water and nutrient transport.
- **Relevance:** Confirms vascular integrity essential for medicinal efficacy.

Macroscopic and Microscopic Study of Ikshura (Asteracantha longifolia)**Macroscopic Study**

- **Appearance:** Reddish-brown, fibrous powder.
- **Odor and Taste:** Characteristic properties confirming authenticity.

Microscopy Study**a. Epiblema with Hairs**

- **Structure:** Unicellular or multicellular outgrowths.
- **Relevance:** Ensures the presence of external protective structures.

b. Pitted Vessels

- **Structure:** Part of the vascular system, aiding nutrient transport.
- **Relevance:** Indicates the retention of bioactive compounds.

Macroscopic and Microscopic Study of Mash (Phaseolus mungo)**Macroscopic Study**

- **Appearance:** Creamish, fibrous powder.
- **Odor and Taste:** Characteristics aiding in identity confirmation.

Microscopy Study**a. Border Pitted Vessels**

- **Structure:** Specialized xylem cells facilitating water flow.
- **Relevance:** Confirms the sample's anatomical features.

b. Starch Grains

- **Structure:** Abundant rounded structures indicative of carbohydrate reserves.
- **Relevance:** Reflects the seed-based nutritional value.

Macroscopic and Microscopic Study of Shatavari (Asparagus racemosus)**Macroscopic Study**

- **Appearance:** Cream-colored, fibrous powder.
- **Odor and Taste:** Consistent sensory characteristics confirming herb authenticity.

Microscopy Study**a. Non-Lignified and Lignified Pitted Vessels**

- **Structure:** Scalariform thickening observed in vascular tissues.
- **Relevance:** Indicates vascular integrity for nutrient transport.

b. Raphides

- **Structure:** Needle-like calcium oxalate crystals.
- **Relevance:** Aids in confirming plant identity.

c. Starch Grains

- **Structure:** Energy storage components found in roots.
- **Relevance:** Supports the herb's rejuvenating properties.

Analytical Study

The analytical study is an essential component of research, encompassing qualitative and quantitative analysis to ensure the quality, safety, and efficacy of formulations. In ancient times, Ayurvedic scholars used traditional methods for standardization, such as *Paka Siddhi Lakshana* and *Sanrakshana Vidhi*. However, modern advancements have necessitated robust analytical techniques to counter challenges like adulteration, substitution, and malpractices in manufacturing.^[18]

The evaluation of *Gokshuradi Churna* and its extract through analytical studies aims to ensure uniformity, purity, and stability, thereby establishing their authenticity and therapeutic reliability. The study adheres to protocols outlined by the Government of India, Ministry of AYUSH, and Pharmacopeial Laboratory for Indian Medicines.^[19]

Parameters Studied

The parameters for this study were adopted from the "Protocol of Testing of Ayurvedic, Siddha, and Unani Medicines." The analysis included:

1. Organoleptic characters
2. Physico-chemical parameters
3. Heavy metal testing
4. Microbial contamination and specific pathogen testing
5. High-Performance Thin Layer Chromatography (HPTLC)

Place of Work

The analytical study was conducted at the Vasu Research Centre, Vadodara, Gujarat.

Parameters for Analysis**Organoleptic Characters**

The organoleptic evaluation involved assessing appearance, odor, and taste using sensory observations to determine the quality of *Gokshuradi Churna* and its extract.

Physico-Chemical Parameters

The following physico-chemical parameters were evaluated:

Total Ash Content

The ash content was determined by incinerating the sample at 450°C to remove carbon. The residue represented the inorganic content of the sample, calculated as a percentage.

Acid Insoluble Ash

The ash was treated with dilute HCl, and the insoluble residue was filtered, dried, and weighed to determine the percentage of acid-insoluble ash.

Alcohol-Soluble Extractive

5 g of the sample was macerated with 100 mL of alcohol for 24 hours, filtered, evaporated, and weighed to determine the percentage of alcohol-soluble extractives.

Water-Soluble Extractive

A similar procedure was followed using distilled water instead of alcohol to determine water-soluble extractives.

Loss on Drying

The sample was dried at 105°C until a constant weight was achieved to calculate the percentage of moisture content.

pH Measurement

A 5% aqueous solution of the sample was analyzed using a calibrated pH meter.

Particle Size Distribution

Samples were sieved, and the residue on each sieve was weighed to determine particle size distribution.

Heavy Metal Analysis

Heavy metals (lead, cadmium, mercury, arsenic) were analyzed using Atomic Absorption

Spectrophotometry (AAS) to ensure safety limits as per guidelines.^[20]

Microbial Contamination

Microbial analysis determined the total bacterial and fungal counts and identified specific pathogens (*E. coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) using pour plate methods and selective media.

- Total Bacterial Count: SCDA plates were incubated at 30–35°C for 3 days, with results expressed in CFU/g.
- Total Fungal Count: SDA plates were incubated at 20–25°C for 5 days.
- Pathogen Testing: Selective media and incubation methods were used to identify pathogens, confirming the absence or presence of specified organisms.

High-Performance Thin Layer Chromatography (HPTLC)

Chromatographic Conditions

- Stationary Phase: Silica gel 60 F254 on aluminum sheets.
- Mobile Phase: Toluene: Ethyl acetate: Acetic acid (7:3:0.1 v/v).
- Sample Volume: 8 µL.
- Visualization: Absorbance at 433nm, 540nm, and 560 m after derivatization with anisaldehyde-sulphuric acid.

Procedure

- Sample Preparation: 1gm of the sample was refluxed in 20 l of methanol for 15 minutes and filtered for use in HPTLC fingerprinting.
- Development and Visualization: The developed plate was visualized under UV and after derivatization to detect active compounds.

Table 1: Parameters for Analysis

S.No	Parameter	Churna	Extract
1	Organoleptic Characters	Appearance, odor, taste	Appearance, odor, taste
2	Physico-chemical Parameters	Total Ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, loss on drying, particle size, pH	Total Ash, acid-insoluble ash, water-soluble extractive, alcohol-soluble extractive, loss on drying, pH
3	Heavy Metals	Lead, cadmium, mercury, arsenic	Lead, cadmium, mercury, arsenic
4	Microbial Contamination	Total bacterial count, total fungal count	Total bacterial count, total fungal count
5	Specific Pathogens	<i>E. coli</i> , <i>Salmonella spp.</i> , <i>S. aureus</i> , <i>P. aeruginosa</i>	<i>E. coli</i> , <i>Salmonella spp.</i> , <i>S. aureus</i> , <i>P. aeruginosa</i>
6	Chromatographic Technique	HPTLC	HPTLC

In-Vivo Study

Ethical Clearance

This preclinical experimental study was approved by the Institutional Animal Ethics Committee under protocol number DMIHER/IAEC/24-25/31. The study was carried out at the Central Preclinical Research Facility of Jawaharlal Nehru Medical College, DMIHER-DU, Wardha, Maharashtra.

MATERIALS AND METHODS**Animals**

- **Strain:** Albino Wistar rats
- **Number:** 30 (Male)

Housing and Feeding Conditions

1. Temperature: Maintained at 22°C ± 3°C.
2. Humidity: 30–70%.
3. Lighting: 12-hour light/dark cycle.
4. Diet: Rats were fed a conventional laboratory pellet diet with ad libitum access to drinking water.

Preparation of Animals

- Rats were randomly selected and individually marked using Picric acid for identification:
- **H** (Head), **B** (Back), **T** (Tail), **HT**, **BT**, and **HBT**.

Group Design

The study included 30 Albino Wistar rats divided into 5 groups (6 rats per group). The study period was 30 days.

Group	Name	No. of Rats	Induced Drug	Drug for Study	Dose
Group 1	Control Group	6	-	Normal saline	1 mL/kg (P.O.)
Group 2	Standard Group	6	Lead nitrate	Testosterone propionate	0.5 mg/kg (S.C.)
Group 3	Experimental Group 1	6	Lead nitrate	<i>Gokshuradi Churna</i>	54 mg/kg (P.O.)
Group 4	Experimental Group 2	6	Lead nitrate	<i>Gokshuradi Churna</i> Extract (Low dose)	4.5 mg/kg (P.O.)
Group 5	Experimental Group 3	6	Lead nitrate	<i>Gokshuradi Churna</i> Extract (High dose)	9 mg/kg (P.O.)

Experimental Dose Calculation

The dose was calculated using the Paget and Barnes rule based on body surface area (BSA).

Human Doses

- *Churna*: 3 g
- Extract: 500 mg

Formula for Rat Dose

Rat Dose (mg/kg) = 0.018 × Human Dose (mg) × 5
 $\text{Rat Dose (mg/kg)} = 0.018 \times \text{Human Dose (mg)} \times 5$

Calculated Rat Doses:

- *Gokshuradi Churna*: 54 mg/kg
- *Gokshuradi Churna Extract*:

- Animals were acclimatized to laboratory conditions for 7 days before dosing.

Administration of Doses

1. Route: Oral gavage with a stainless steel curved, ball-tip feeding needle (16-20 G).
2. Preparation: Animals were fasted overnight, with food withheld for 3–4 hours post-dosing. Water was provided throughout.
3. Dosing Schedule: Rats were weighed before dosing, and the appropriate test substance or drug was administered based on calculated doses.

OBSERVATIONS

1. Animals were observed individually after dosing, with special attention during the first 4 hours and daily for 14 days.
2. Observations included:
 - Changes in skin and fur, eyes, and mucous membranes.
 - Behavioral changes: Salivation, eating habits, lethargy, sleep, coma, convulsions, or tremors.
 - Signs of toxicity: Diarrhea, morbidity, and mortality.
 - Individual records were maintained for each animal.

- Low dose: 4.5 mg/kg
- High dose: 9 mg/kg

Parameters Studied

The following parameters were assessed during the study:

Semen Collection

Epididymal aspiration was performed to collect semen samples.

Sperm Analysis

- Sperm Count: Measured under a microscope using a hemocytometer.
- Motility: Percentage of motile vs. non-motile sperm was determined.
- pH: Assessed using a calibrated pH meter.

Body Weight

Recorded pre- and post-study to assess weight changes.

Sexual Organ Weight

Reproductive organs (testes, epididymis, seminal vesicles, and prostate gland) were dissected and weighed to evaluate treatment effects.

OBSERVATIONS AND RESULTS**Result of the Pharmaceutical Study**

1. During the preparation of individual *Churnas*, the yield from the raw drug was found to be 98%, 90%, 85%, 90% and 95% in *Gokshura*, *Ikshura*, *Mash*, *Atmagupta* and *Shatavari* respectively.
2. The percentage yield of *Gokshuradi Churna* was found to be 91.6%.
3. The percent yield of *Gokshuradi Churna* extract was found to be 30.33%.

Result of the Analytical Study

1. The analytical results remarked that *Gokshuradi Churna* had a creamish Brown colour while the extract was specifically brown in colour with both having a characteristic odour and taste.
2. The total ash value was found to be 7.55% in *Churna* and 3.16% in extract.
3. The obtained value of acid-insoluble ash in *Churna* was 0.88% and in extract was 0.00%.
4. The water-soluble extractive value was found to be 25.87% and 90.75% for *Churna* and extract respectively.
5. The alcohol-soluble extractive for *Churna* and Extract was 18.51% and 26.78% respectively.
6. The moisture content for *Churna* and Extract was 9.54% and 1.68% successively.
7. The pH value was found to be 6.95 and 7.08 for *Churna* and extract respectively.
8. The particle size for *Churna* by sieve method was 74.15% (20 mesh), 42.42% (40 mesh), 18.08% (60 mesh), 13.80% (80 mesh), 9.82% (100 mesh) and for Extract, it was not applicable.
9. Heavy metals and total microbial count in *Churna* and extract were within permissible limits.

HPTLC:

- The Rf values for *Gokshuradi Churna* at 433nm were 0.18, 0.38, 0.42, 0.56, 0.61, 0.73, 0.83 (7 spots) and for extract were 0.11, 0.18, 0.21, 0.42, 0.56, 0.61, 0.73, 0.83 (8 spots).
- At a wavelength of 540nm, Rf values for *Gokshuradi Churna* were 0.11, 0.20, 0.24, 0.30, 0.46, 0.58, 0.65 (7 spots) and for extract were 0.11, 0.20, 0.24, 0.30, 0.40, 0.46, 0.52, 0.58, 0.65 (9 spots).

- At a wavelength of 560nm, Rf values for *Gokshuradi Churna* were 0.17, 0.23, 0.29, 0.39, 0.65, 0.73, 0.84 (7 spots) and for Extract were 0.29, 0.39, 0.47, 0.55, 0.65, 0.67, 0.73, 0.84 (8 spots).

Statistical Analysis of *in-vivo* study

Statistical Analysis was done using the "ANOVA test".

Statistical Significance of *Gokshuradi Churna* and its Extract in improving the Total Body weight and weight of sexual organs:

- ✓ It interprets that Control group was significantly more efficient than Standard group- Testosterone propionate, *Gokshuradi churna*, low dose and high dose of extract respectively in increasing the mean body weight of rats.
- ✓ Hence, it can be Interpreted that *Gokshuradi churna*, low dose of extract and high dose of extract, were equally efficient in improving the weight of right testis as the standard and the control group.
- ✓ It can be interpreted that *Gokshuradi churna*, low dose of extract and high dose of extract, were equally efficient in improving the weight of epididymis as the standard and the control and standard group.

Statistical Significance of *Gokshuradi Churna* and its Extract on Sperm Count and Sperm Motility and pH

- ✓ It signifies that *Gokshuradi churna*, and high dose of extract were more efficient in increasing the sperm count. However, extract in high dose was more efficient than *Gokshuradi churna* and low dose of extract of *Churna* in increasing the sperm count.
- ✓ It signifies that *Gokshuradi churna* was more efficient than low dose of extract in increasing the sperm motility. However, *Churna* and high dose of extract, were equally efficient in increasing the sperm motility.
- ✓ It can be interpreted that *Gokshuradi churna*, low dose of extract and high dose of extract, were not significantly efficient in changing the non-motile sperms score than the standard and the control group.
- ✓ it can be interpreted that *Gokshuradi churna*, low dose of extract and high dose of extract, showed Equal change in the pH value of collected fluid in epididymis before and after study as the control group.

The results from the studies outlined in the materials and methods section are summarized as follows:

Results of Pharmaceutical Study**Table: Yield of Gokshuradi Churna and its Extract**

S.No.	Sample	Raw Drug (g)	Final Product (g)	Yield (%)	Loss (%)
1	<i>Gokshuradi Churna</i>	1000	916	91.6	8.4
2	<i>Gokshuradi Extract</i>	600	182	30.33	70.0

Results of Analytical Study**Organoleptic Characters**

S.No.	Parameter	<i>Gokshuradi Churna</i>	<i>Gokshuradi Extract</i>
1	Appearance	Brown	Dark Brown
2	Odor	Characteristic	Characteristic
3	Taste	Astringent	Bitter
4	Texture	Granular Powder	Paste

Physicochemical Parameters

S.No.	Parameter	<i>Gokshuradi Churna</i>	<i>Gokshuradi Extract</i>
1	Total ash content	7.55%	2.72%
2	Acid-insoluble ash content	0.88%	0.19%
3	Water-soluble extractive	25.87%	70.84%
4	Alcohol-soluble extractive	18.15%	31.77%
5	Loss on drying	9.54%	11.68%
6	pH (1% solution)	6.57	7.0
7	Particle size (20 mesh)	74.15%	NA

Phytochemical Analysis**Instrumental Analysis of Biomarkers (HPTLC Findings)**

Parameter	<i>Gokshuradi Churna</i>	<i>Gokshuradi Extract</i>
Diosgenin Assay (%)	0.662%	0.332%
Shatavarin Assay (%)	0.364%	0.182%
L-dopa Assay (%)	0.186%	0.022%

Heavy Metal Analysis

Parameter	Permissible Limit	<i>Churna</i>	<i>Extract</i>
Mercury	NMT 1 ppm	0.147 ppm	0.243 ppm
Cadmium	NMT 0.3 ppm	0.089 ppm	0.023 ppm
Arsenic	NMT 3 ppm	0.650 ppm	0.815 ppm
Lead	NMT 10 ppm	1341 ppm	470 ppm

Microbial Contamination

Pathogen	<i>Churna</i>	<i>Extract</i>	Limit
<i>E. coli</i>	Absent	Absent	Absent/g
<i>Salmonella spp.</i>	Absent	Absent	Absent/g
<i>S. aureus</i>	Absent	Absent	Absent/g
<i>Pseudomonas aureus</i>	Absent	Absent	Absent/g

Results of In-Vivo Study**Total Body Weight**

Group	Initial Weight (g)	Final Weight (g)
Control	153.33 ± 24.01	280.00 ± 63.32
Standard	165.83 ± 19.60	204.16 ± 41.28
Exp. 1	153.33 ± 17.79	211.66 ± 17.22
Exp. 2	142.66 ± 5.92	232.83 ± 13.86
Exp. 3	152.50 ± 10.83	210.00 ± 21.67

Sperm Count and Motility

Group	Sperm Count (10 ⁶)	Sperm Motility (%)
Control	34.00 ± 0.89	86.33 ± 3.26
Standard	32.33 ± 0.81	86.00 ± 1.67
Exp. 1	43.66 ± 1.36	91.50 ± 1.37
Exp. 2	39.00 ± 1.26	88.83 ± 1.16
Exp. 3	54.00 ± 1.09	91.00 ± 0.89

Weight of Sexual Organs

Group	Right Testis (mg)	Right Epididymis (mg)
Control	872.16 ± 16.03	179.00 ± 9.52
Standard	879.83 ± 11.25	189.16 ± 6.64
Exp. 1	871.16 ± 16.27	174.00 ± 5.65
Exp. 2	885.33 ± 20.79	180.66 ± 3.93
Exp. 3	898.00 ± 7.15	187.66 ± 8.31

Statistical Analysis of In-Vivo Study**Body Weight and Sexual Organ Weight**

Statistical analysis using One-Way ANOVA and post-hoc Tukey tests revealed significant differences in final body weight and sexual organ weight among the groups, confirming the spermatogenic effects of *Gokshuradi Churna* and its extracts.

DISCUSSION**Discussion on Pharmaceutical Processing of *Gokshuradi Churna***

The primary objective of pharmaceutical processing is to transform natural raw ingredients into a standardized and effective dosage form that ensures optimal absorption and therapeutic efficacy. In preparing *Gokshuradi Churna*, traditional Ayurvedic techniques were followed, such as the cleaning of raw ingredients to remove impurities, followed by sun and shade drying. These methods preserved the *Virya* (potency) and *Guna* (qualities) of the ingredients while balancing *Vata* and *Kapha*. Adhering to the classical guidelines of *Sharangdhara Samhita* ensured authenticity and efficacy, highlighting the Ayurvedic emphasis on precise formulation methods.^[21] Standardized powdering and sieving were employed to achieve uniform particle size, which is critical for

enhancing absorption and bioavailability. Equal proportion mixing of ingredients maintained their synergistic effects, and airtight storage preserved potency, preventing degradation. The overall yield of 91.6% demonstrated efficient processing with minimal wastage, showcasing the formulation's therapeutic integrity.^[22]

Discussion on Extraction Process

Extraction serves to isolate active medicinal components from raw materials, concentrating their therapeutic properties for enhanced efficacy. The Soxhlet extraction method, used for *Gokshuradi Churna*, ensured efficient extraction of bioactive compounds. The process involved controlled heating for eight hours, providing sufficient time for thorough extraction while preserving heat-sensitive compounds. Continuous solvent exposure was maintained until the solvent turned colorless, indicating complete extraction. Post-extraction filtration removed impurities, and the solvent was evaporated using rotary evaporation to achieve a concentrated, solvent-free extract. The final product, a potent extract of *Gokshuradi Churna*, displayed improved bioavailability and therapeutic value, aligning with Ayurvedic

principles of enhancing potency through purification and concentration.^[23]

Comparative Analysis of Gokshuradi Churna and Extract

The comparative analysis of *Gokshuradi Churna* and its extract revealed differences in their physical properties, chemical composition, and therapeutic potential. The granular texture of the *Churna* contrasted with the paste-like consistency of the extract, reflecting their distinct processing methods. While both displayed characteristic odor and taste, the extract showed higher water and alcohol-soluble extractive values, indicating its fast-acting therapeutic nature. HPTLC analysis demonstrated variations in bioactive compounds like Diosgenin, Shatavarin IV, and L-Dopa, supporting the use of the *Churna* for anti-inflammatory and neurological benefits, while the extract proved more effective for adaptogenic and reproductive health. Heavy metal and microbial analyses confirmed the safety of both formulations, with the extract showing superior microbial control and lower heavy metal content due to additional purification steps.^[24]

In-Vivo Study Outcomes

The in-vivo study demonstrated the potential of *Gokshuradi Churna* and its extract in supporting reproductive health. Significant differences in body weight, sperm count, sperm motility, and sexual organ weight across treatment groups highlighted their efficacy. The extract, particularly at a high dose, showed superior results in enhancing sperm motility and count, indicating its therapeutic effectiveness in improving male fertility. Epididymal pH remained within the optimal range for sperm function across all groups, confirming the safety and compatibility of the treatments with reproductive health. The statistical analyses, including ANOVA and post-hoc tests, validated these findings, emphasizing the potential of *Gokshuradi Churna* and its extract as reliable therapeutic options.^[25]

Probable Mode of Action

The mode of action of *Gokshuradi Churna* can be attributed to its *Guru*, *Snigdha*, and *Madhura* properties, which promote *Shukra Dhatu* (reproductive tissue) formation as per the principle of *Samanya Vriddhikaranam*. The high antioxidant content of the formulation protects against oxidative stress, preserving mitochondrial health in sperm cells and supporting spermatogenesis. Specific ingredients like *Mucuna prurita* (rich in L-Dopa) offer aphrodisiac and neuroprotective effects, while *Shatavari* contributes adaptogenic properties. Together, these actions enhance reproductive health, making

Gokshuradi Churna a potent natural formulation for addressing male infertility and related conditions.^[26]

RESULTS AND FINDINGS

Pharmaceutical Study

- **Yield Efficiency:** The preparation of *Gokshuradi Churna* yielded 91.6%, with minimal wastage, while its extract had a yield of 30.33%. The difference in yield reflects the concentration and purification during the extraction process.
- **Processing Standards:** Cleaning, drying, powdering, and sieving (85 mesh) ensured uniformity, enhancing bioavailability and therapeutic efficacy.
- **Storage Conditions:** Airtight containers preserved the formulation's stability and potency.

Analytical Study

Organoleptic Properties

- *Gokshuradi Churna*: Brown granular powder with an astringent taste and characteristic odor.
- *Gokshuradi* Extract: Dark brown paste with a bitter taste and characteristic odor.

Physicochemical Analysis

- *Gokshuradi Churna* had higher total ash (7.55%) and acid-insoluble ash (0.88%) compared to the extract (2.72% and 0.19%, respectively), indicating lower residue in the extract.
- The extract exhibited significantly higher water-soluble extractive (70.84%) and alcohol-soluble extractive (31.77%) values than the *Churna*, highlighting its concentrated active compounds.

HPTLC Analysis:

- Bioactive markers such as Diosgenin (0.662% in *Churna*, 0.332% in extract), Shatavarin IV (0.364% in *Churna*, 0.182% in extract), and L-Dopa (0.186% in *Churna*, 0.022% in extract) were quantified. These findings suggest differing therapeutic applications based on compound concentration.

Heavy Metal Analysis

- Both formulations were within permissible limits for mercury, cadmium, arsenic, and lead. The extract demonstrated lower heavy metal content due to its purification process.

Microbial Contamination

- The extract showed superior microbial purity, with total microbial plate count significantly lower (24cfu/g) than *Churna* (2829cfu/g). Both formulations were free of specific pathogens (*E. coli*, *Salmonella*, *S. aureus*, and *Pseudomonas*).

In-Vivo Study

Body Weight Analysis

- **Initial Weight:** No significant baseline differences among groups.

- Final Weight: Significant variation was observed ($p = 0.008$). Groups treated with high-dose extract showed improved weight stability compared to standard and low-dose treatments.

Sperm Parameters

- Sperm Count: Significant improvement in groups treated with *Gokshuradi Churna* and high-dose extract ($p = 0.0001$). Group E (high-dose extract) showed the highest sperm count.
- Sperm Motility: Group C (*Churna*) and Group E (high-dose extract) demonstrated significant improvements in motility compared to the control and standard groups ($p = 0.0001$).
- Sperm Non-Motility: The *Churna* and high-dose extract significantly reduced non-motile sperm counts compared to the control and standard groups.

Weight of Sexual Organs

- Right Testis: Group E (high-dose extract) showed a significant increase in testicular weight compared to other groups, indicating enhanced testicular health.
- Right Epididymis: Significant improvement in epididymal weight was observed in Group E, supporting its role in sperm maturation and storage.

pH of Epididymal Fluid

- All groups maintained the pH within the optimal range (7.35–7.46) for sperm health. Group D (low-dose extract) exhibited the highest pH, suggesting enhanced alkalinity without exceeding physiological norms.

Statistical Analysis

- **ANOVA and Post-Hoc Tests:** Significant differences were observed for sperm count, motility, and sexual organ weights across groups, validating the therapeutic efficacy of *Gokshuradi Churna* and its extract.
- **Tukey and Dunnett Tests:** Highlighted the superior efficacy of high-dose extract (Group E) and *Churna* (Group C) in improving reproductive parameters compared to standard treatment and control groups.

Key Findings

- *Gokshuradi Churna* and its extract demonstrated significant therapeutic effects on sperm count, motility, and testicular and epididymal health.
- The extract, particularly in high doses, exhibited superior bioavailability, safety, and efficacy compared to the *Churna* alone.
- Both formulations were microbiologically safe and met heavy metal safety standards, ensuring their suitability for clinical use.

CONCLUSION

There are five formulations named *Gokshuradi Churna/Yoga* mentioned in the classical Ayurvedic texts collectively known as *Brihatrayi*. The formulation studied in this research has been specifically taken from *Ashtanga Hridayam Uttartantra* (40/34). All the ingredients of the formulation have been described by various *Acharyas* as possessing *Vrishya* (aphrodisiac) and *Shukrala* (spermatogenic) properties, underscoring their traditional role in supporting male reproductive health. The *Gokshuradi Churna* was prepared using traditional methods as per Ayurvedic guidelines, while its hydro-alcoholic extract was obtained through the Soxhlet apparatus. The yields for the *Churna* and its extract were 91.6% and 30.33%, respectively, reflecting efficient preparation methods and concentration of bioactive compounds in the extract. All required quality control parameters for the final formulations, including organoleptic, physicochemical, microbial, and heavy metal analyses, were within permissible limits. This confirms that both *Gokshuradi Churna* and its extract are safe for therapeutic use. The experimental study demonstrated that both the *Churna* and its extract, in low and high doses, were effective in increasing sperm count and improving sperm motility. The efficacy index of the formulations revealed distinct advantages based on the type and dose. For sperm count, the order of efficacy was observed as: low dose of extract < *Churna* < high dose of extract. For sperm motility, the efficacy order was low dose of extract < high dose of extract < *Churna*. This highlights that while the extract shows enhanced concentration effects for sperm count, the traditional *Churna* formulation maintains superior efficacy for sperm motility.

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