

**EVALUATION OF SPERMATOGENIC ACTIVITY OF GOKSHURADI CHURNA  
(TRIBULUS TERRESTRIS COARSE POWDER) & ITS EXTRACT: PROTOCOL**Somveer Singh<sup>\*1</sup>, Usha Sharma<sup>2</sup>, Manish Purushottam Deshmukh<sup>3</sup>, Shuchi Mitra<sup>4</sup> and Khem Chand Sharma<sup>5</sup><sup>1</sup>P.G. Scholar, P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Haridwar, India.<sup>2</sup>Professor, P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Haridwar, India.<sup>3</sup>M, Pharma. Ph.D. Director Interdisciplinary Research DMIHER-DU, Wardha, Maharashtra, India.<sup>4</sup>Associate Professor, P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Haridwar, India.<sup>5</sup>Professor and HOD, P.G. Department of Rasa Shastra and Bhaishajya Kalpana, Rishikul Campus, Uttarakhand Ayurved University, Haridwar, India.**\*Corresponding Author: Somveer Singh**

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**ABSTRACT**

**Introduction:** Infertility is an important and growing health problem affecting nearly 15% of couples worldwide. It is estimated that male factors contribute to 20-70% of cases depending on the latitude. The male reproductive system is susceptible to many factors such as environment, lifestyle as well as numerous physical and chemical factors which lead to infertility. In 40 to 50 % of cases, a semen analysis can rule out the cause of the infertility. Many drugs in the market are available but they might produce various side effects that cannot be ignored. *Vajikarana* is one of the eight branches of Ayurveda. In Ayurveda, various herbal and herbomineral formulations take place in *Vajikarana chikitsa* owing to their aphrodisiac property. *Gokshuradi Churna* is a versatile formulation with *Vajikarana* properties and is used in male infertility. This formulation consists of five herbal ingredients i.e. Gokshura (*Tribulus terrestris* Linn.), Ikshura (*Asteracantha longifolia* Nees.), Mash (*Phaseolus mungo* Linn.), Atmagupta (*Mucuna prurita* Hook.) & Shatavari (*Asparagus racemosus* Wild.). It is a polyherbal formulation illustrated in the classical text of Ashtanga Hridaya. Many formulations are available in various texts under the same name *Gokshuradi Churna* with different contents and different *Rogadhikara* (indications). This article compiles the probable mode of action and Spermatogenesis activity of each ingredient of *Gokshuradi Churna*.

**KEYWORDS:** *Gokshuradi Churna*, Infertility, spermatogenesis activity.**INTRODUCTION**

Infertility is the major reproductive health problem in today's era. About 15% of couples have infertility problems out of which 40% are related to male factors. Oligozoospermia is a condition related to infertility that is associated with low sperm concentration. The objective of this study was to observe the effect of ayurvedic medicine on infertile men and to observe changes in sperm concentration, sperm motility, and sperm morphology in the albino Wistar rats before and after treatment. (*Nisargandha M. A. and Parwe S. D. 2021*) There are many causes of male infertility like infection, trauma, cystic fibrosis, varicoceles, etc but in the current scenario in the Indian population changes in lifestyle, nature of work and daily routine are the major factors contributing to increasing infertility.

Male reproductive function is susceptible to many factors such as physical and chemical agents which leads to infertility. Infertility is defined as the inability of a sexually active, non-contraception couple to achieve pregnancy in one year. (*WHO 2000*) In 40 to 50 % of cases, a semen analysis can rule out the cause of the abnormality. It can easily determine the total sperm count, motility morphology of the sperm, etc. Many drugs in the market are available but they might produce side effects that cannot be ignored.

There are various formulations used as aphrodisiac treatment in Ayurveda. *Gokshuradi churna* (*Tribulus terrestris* Linn *Corase powder*) is one of such versatile formulation that possesses aphrodisiac properties and is used in male infertility. This formulation consists of five

herbal ingredients which are *Gokshura* (*Tribulus terrestris* Linn), *Ikshura* (*Asteracantha longifolia*), *Mash* (*Phaseolus mungo*), *Atmagupta* (*Mucuna prurita* hook) & *Shatavari* (*Asparagus racemosus*). Hence *Gokshuradi churna* is selected here, to evaluate its Spermatogenic activity pharmacologically.

## REVIEW OF LITERATURE

### 1. REVIEW OF AYURVEDIC LITERATURE

*Gokshuradi Churna* (*Tribulus terrestris* Linn *Corase powder*) is an Ayurvedic formulation mentioned in the ancient classical text *Ashtang Hridaya Uttar tantra*, Chp 40, verse no 34, 831.

This formulation consists of five ingredients i.e *Gokshura* (*Tribulus terrestris* Linn), *Ikshura* (*Asteracantha longifolia* Nees), *Mash* (*Phaseolus mungo* Linn), *Atmagupta* seeds (*Mucuna prurita* Hook) & *Shatavari* (*Asparagus racemosus* Wild).

Many formulations are available in various texts under the same name *Gokshuradi Churna (Powder)* with different ingredients and different *Rogadhikara*.

### 2. REVIEW OF MODERN LITERATURE

- The extraction technique will be reviewed from different authentic books of biochemistry and biotechnology along with research articles.
- Literature regarding the Spermatogenic study of extracts will be reviewed from different authentic books and research articles.

### 3. DRUG REVIEW

The literature regarding *Gokshura*, *Ikshura*, *Mash*, *Atmagupta* and *Shatavari* will be reviewed from different *Ayurvedic texts* and research articles. Ingredients of *Gokshuradi Churna* Ayurvedic Pharmacological properties are mentioned in Table No.1, 2 and 3 respectively.

**Table No. 1: Ingredients of *Gokshuradi Churna (Tribulus terrestris linn Corase Powder) (P.V sharma 2006)*.**

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 No. 2: Taste of *Gokshuradi churna (Tribulus terrestris Powder) (P.V sharma 2006)*.**

Drug	taste	qualities	potency	digestive state of drugs	Pharmacological actions
<i>Gokshura</i> ( <i>Tribulus terrestris</i> Linn)	Sweet	Heavy, Unctuousness	Cold	Sweet	Hypoglycemi, Adatogenic Immunomodulatory Hypolipidemic,
<i>Ikshura</i> ( <i>Asteracantha longifolia</i> Nees)	Sweet	Unctuousnes, Pichhila	Cold	Sweet	Hepatoprotective Aphrodisiac, Antioxidant Antimicrobial
<i>Mash</i> ( <i>Phaseolus mungo</i> Linn)	Sweet	Heavy, Unctuousnes	Hot	Sweet	Rejuvenating Nutritional, Nervine Asporidisiac
<i>Atmagupta</i> ( <i>Mucuna prurita</i> Hook)	Sweet Bitter	Heavy Unctuousnes	Hot	Sweet	Aprodisiac Neuroprotective Antidepressent
<i>Shatavari</i> ( <i>Asparagus racemosus</i> Wild)	Sweet Bitter	Heavy Unctuousnes,	Hot	Sweet	Adaptogenic, Hormonal balancer, Aphrodisiac Galactagogue

**Table No. 3: Pharmacological Properties of Drug (Sharma PC, Database on medicinal plants used in Ayurveda Vol 1,3,4,2005, pg.200,229,320,418).**

Drug	Phyto-constituents	Pharmacological Actions
<i>Gokshura</i> ( <i>Tribulus terrestris</i> Linn)	Saponins, Polyphenolic compounds, and alkaloids, Furostanol and Spirostanol, Kaempferol	Aphrodisiac, anti-inflammatory analgesic antispasmodic antibacterial,
<i>Ikshura</i> ( <i>Asteracantha</i>	Alkaloids, Saponins, Steroids, Phenolic compounds,	Aphrodisiac Purify the semen anomalies

<i>longifolia</i> Nees)	Tannins, Flavonoids, Terpenoids, Protein & Amino acids Anthraquinones	Useful in gout Lithotriptic Anti-inflammatory
<b>Mash</b> ( <i>Phaseolus mungo</i> Linn)	Genistein, Glycinol Kievitone, Eugenol Beta-sitosterol, Phloretin	Aphrodisiac Diuretic Strengthening Bulk prompting Galactagogue
<b>Atmagupta</b> ( <i>Mucuna prurita</i> Hook)	Steroid, Flavonoids, Tannins-dopa, mucunine, prurienine, palmitic, oleic, linoleic, stearic	Stamina booster Aphrodisiac Useful in disorder of vata humor
<b>Shatavari</b> ( <i>Asparagus racemosus</i> Wild)	Steroidal, saponins, Querecitin, Rutin Polysaccharides	Stamina booster Rejuvenation Aphrodisiac activity galactogogue Anti-stress activity Anti-inflammatory activity.

## MATERIAL AND METHODS

### 1. PHARMACEUTICAL STUDY

#### a) Procurement

Drugs will be collected from a local herbal drug stockist.

#### b) Authentication

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

#### c) Preparation of *Gokshuradi Churna* (*Tribulus terrestris* Linn *Corase Powder*)

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

*Ikshura*, *Mash*, *Atmagupta* and *Shatavari* all will be finely grinded

#### d) Hydro-alcoholic extract *Gokshuradi Churna* (*Tribulus terrestris* Linn *Corase Powder*)

Will be carried out by using a suitable solvent with the help of Soxhlet apparatus

### 2. ANALYTICAL STUDY

For physio-chemical study, various test will be carried out which are given below:

S. No	Analytical Parameters of Churna	Analytical Parameters of Extracts
<b>Organoleptic test</b>	Appearance Colour Odour Taste Consistency	Appearance Colour Odour Taste
<b>Physio-chemical Parameters</b>	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
<b>Identification</b>	TLC/HPTLC	TLC/HPTLC
<b>Microbial Contamination</b>	Total bacterial count Total fungal count	Total fungal count Total bacterial count
<b>Test for heavy metal</b>	Lead Cadmium Mercury Arsenic	Lead Cadmium Mercury Arsenic
<b>Test for specific pathogens</b>	<i>E-coli</i> <i>Salmonella</i> spp. <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	<i>E-coli</i> <i>Salmonella</i> spp. <i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>

**Note-** Other analytical tests will be carried out as per need and availability of equipment.

### 3. SPERMATOGENIC STUDY (IN-VIVO)

- **Preparation of Animals-** Healthy male 30 albino Wistar rats weighing 140-200gm will be randomly selected, marked & kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions.
- **Housing & Feeding conditions-** The temperature in the experimental animal room will be 22°C ±3<sup>0</sup>C. Although the relative humidity will be 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.
- **Spermatogenic activity on male rats:** Male Wistar strain albino rats will be divided into 5 groups of six animals each (n=6). Lead Nitrate will be given to induce oligospermia in Albino Rats for duration of 30 days Group A animals served as control and will receive only vehicle normal saline 1ml/kg. Group B animals will serve as standard

control and will receive testosterone propionate suspension (0.5mg/kg). Group C will receive **Gokshuradi Churna**. The group D will receive hydro-alcoholic extract of **Gokshuradi Churna** in lower dose and group E will receive a hydro-alcoholic extract of **Gokshuradi Churna** in a higher dose. The mode of administration will be an oral-dependent manner by feeding the needle for 30 days.

#### Experimental dose calculation

Calculated by Barnes and Paget's rule:

**Conversion factor for rats:** Human dose × 0.018 = Xg/200g rats

$$X \times 5 = Y \text{g/kg of rat}$$

**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 will be selected for the study purpose

### EXPERIMENTAL STUDY

No of group		No of animal	Drugs used for study	Dose
Group-A	Control	6 rats	Normal saline	1ml/kg (P.O)
Group-B	Standard	6 rats	Lead nitrate-on 0 to 30 days Then after 30 days Testosterone propionate suspension-on 1 to 30 days	Lead nitrate-80mg/kg Testosterone propionate suspension-0.5mg/kg (S.C)
Group-C	Experimental Group	6 rats	Then after 30 days <i>Gokshuradi Churna</i> on 1 to 30 days	calculated by Barnes and Paget rule
Group-D	Experimental Group	6 rats	Lead nitrate- on 0 to 30 days Then after 30 days <i>Gokshuradi Churna</i> extract at low dose-on 1 to 30 days	Animal dose of extract calculated by Barnes and Paget rule
Group-E	Experimental Group	6 rats	Lead nitrate – on 0 to 30 days then after 30 days <i>Gokshuradi Churna</i> extract at high dose-on 1 to 30 days	Animal dose of extract calculated by Barnes and Paget rule

**Note:** The addition or elimination of the parameters can be done as per the need of the study & availability of resources.

After 30 days following parameters will be 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 will be collected either by epididymal puncture or rats will be sacrificed after the last administration of the drug. Testis will be immediately removed and blood samples will be collected.

**Effect on the weight of sexual organs and histological studies-** After 30 days of treatment as described earlier

the body weight of animals will be recorded. The testis, seminal vesicles, epididymis and prostate gland will be carefully removed and weighed. Testis and epididymis of animals will be cut into small pieces and fixed in Bovine's fixative. After dehydration with varying percentages of ethanol, sections will be cut, stained with hematoxylin and eosin and then analyzed microscopically.

**Sperm count & Motility-** One drop of sperm will be placed on a glass slide & 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.



After 30 days, the rats would be subjected to tissue biopsy. Rat's testis will be removed & fixed overnight and processed into paraffin and sections will be stained with hematoxylin and eosin.

## DISCUSSION

As the current scenario is changing, the human population is facing a lot of challenges due to hectic, busy and sedentary lifestyle. Around 10-15% of couples in the reproductive age range experience infertility, which is a global health issue. Infertility is defined as the inability of a sexually active, non-contraception couple to become pregnant within a year. Acharyas have recommended *Vajikarana* or *Vrishya Chikitsa* as a solution to such kind of issues. *Vajikarana* or *Vrishya Chikitsa* is one of the eight branches of Ayurveda which is concerned with aphrodisiacs, virility and improving the health of progeny.

There are numerous *Vajikarana* formulations in different classical texts. *Gokshuradi Churna* is a polyherbal formulation comprising of five ingredients i.e., *Gokshura*, *Ikshura*, *Mash*, *Atmagupta* and *Shatavari*, used for spermatogenesis activity. *Tribulus terrestris* contains three groups of active phytochemicals. They are Dioscin, protodioscin, and diosgenin. Protodioscin is a potent natural precursor of the testosterone enhancer. It also increases the production of Testosterone in another natural way. *Tribulus terrestris* leads to the production of the luteinizing hormone (LH). When the LH levels are increased, the natural production of testosterone also increases. LH is a hormone that also deals with sex drive. LH has been used to increase fertility and helps to relieve impotence. *Asparagus racemosus* Wild. aids in the enhancement of spermatogenesis by providing the regeneration of seminiferous tubules. *Phaseolus Mungo* Linn. improves strength, the bulk of feces, and fertility quickly. It contains a small amount of isoflavones, an excellent source of B-complex vitamins such as B6, thiamin (B1), pantothenic acid (B5), riboflavin (B2), niacin (B3), and folates (B9). Folate along with vitamin B12, is one of the essential factors for DNA synthesis and cell division. It is also an incredible source of minerals like iron, calcium, copper, magnesium, zinc, and phosphorus. *Asterantha longifolia* Nees. results in a rise in fructose content in seminal vesicles, which is a suggestion of an increase in testosterone synthesis in the body. The seminal vesicles provide fructose substances to the seminal pathway, which is necessary to generate normal sperm motility. These phytochemicals enhance spermatogenesis activity by improving the production of Testosterone, luteinizing hormone (LH), the regeneration of seminiferous tubules, and provide essential nutrients for testosterone synthesis.

With the above properties, all the constituents of *Gokshuradi Churna* have *Madhura Rasa*, *Guru* and *Snigdha Guna*, *Sheeta Virya* (except *Mash* and *Atmagupta*), and *Madhura Vipaka* which results in *Shukra Vridhi*. Thus, it indicates the *Vajikarna* property

of *Gokshuradi Churna*.

## CONCLUSION

Men's reproductive health & infertility is one of the unexplored areas of research which has a lot of potential. Ayurveda has been used in this field for ages, but due to a lack of scientific validation, these are not well established. By reviewing all Ayurvedic literature along with various research work it is accomplished that all the constituents of *Gokshuradi Churna* (*Tribulus terrestris* linn corase powder) have spermatogenesis activity. Therefore, *Gokshuradi Churn* (*Tribulus terrestris* linn corase powder) can be used in improving men's reproductive health. This article provides a platform for researchers to further explore this formulation and other potent formulations related to men's reproductive health.

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