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# An Experimental Evaluation of Shukrala Karma of Kakamachi (Solanum nigrum Linn) with Special Reference to Spermatogenic Activity

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## Abstract

**Introduction:** Infertility affects 8-12% of couples worldwide. Approximately 40-50% of infertility is due to male factor. Nearly 7% of all men exhibit suboptimal sperm parameters including low sperm count, poor sperm motility or abnormal morphology. *Kakamachi (Solanum nigrum* Linn) commonly known as 'Black nightshade', belonging to Solanaceae family, is a prominent herb in Ayurveda which is used as food and medicine since long ago. It is used as a single drug and in compound formulations and various folklore uses. *Kakamachi* helps to increase *Shukra Dhatu*. It is *Shukrala, Rasayana,Vrishya, Tikta, Snigdha, Sheeta*, and *Laghu*. It is taken for the present study to see its *Shukrala Karma*. Thus, the study was carried out to evaluate *Shukrala Karma* of *Churna* of whole plant of *Kakamachi* .

**Materials and Method:** In vivo spermatogenic activity was evaluated in male Wistar albino rats. Rats were divided into 4 groups, each of 6 rats. Normal control rat groups, each of 6 rats. Normal control rats were given plain water, standard group was given Testoviron injection (0.05mg/kg BW) intramuscularly and test drug groups were given with drug *Churna* for TED (1.08gm/kg BW) and TEDx2 (2.16 gm /kg BW) orally for 60 days

**Result:** Body Weight *Churna* of whole plant of *Kakamachi* had shown good result in sperm count, weight of prostate, testes and seminal vesicle, sperm morphology, bio-chemistry of blood and histological evaluation of seminal vesicle, testes and prostate.

**Conclusion:** *Kakamachi(Solanum nigrum* Linn), which is effective in *Shukrala Karma* (Spermatogenic activity), can be accepted regarding sperm morphology, sperm motility, secretion of prostate and maturity of sperm with good evidence of spermatogenesis in histological evaluation of testes.

**Keyword:** *Kakamachi, Solanum nigrum Linn, Shukrala Karma,Churna*

## INTRODUCTION

*Kakamachi (Solanum nigrum* Linn), Solanaceae family, is a prominent herb in Ayurveda which is used as food and medicine since long ago. *Kakamachi (Solanum nigrum* Linn.) is widely described in the Ayurvedic classics and also have references in Vedas. This herb has its own ethno-medicinal importance since it plays a significant role in the treatment of various diseases. It is having both curative and nutritive values. It is used as single drug and in compound formulations. In the Vedas description regarding Kakamachi is mentioned in Samaveda; by author Sayana by the name of ''Nitatin''. Its description is also present by the same name in Atharva Veda (1500 BC), Kaushikasutras and Amarakosha1,2.

Description of *Kakamachi* is found in all the Ayurvedic literature. The drug is explained first time in vedic Granthas (12th century) and was also widely used in Samhita period especially in the form of *Shaka Dravya* (vegetable). *Gadanigraha* is the one who mentioned a special chapter in *Rasayanadikara* in *Kakamachi*. The description of *Kakamachi* is explained first time in Vedic Granthas of Koushikasutra. It is described along with *Bhringraj* for the prevention of Kesha dosha. In Keshava Paddhati the fruits were used for Kesha vruddhi as Phalamani Bandhan. All Samhita have described *Kakmachi* for *Shaka* Dravya as well as *Oushadha* Dravya. It is observed that the drug is very popular in those days, which was studied well and used widely in the therapeutics and in dietary supplement. They have explained the drug in the form of therapeutic application as well as contradicted with some combination and application.

*Kakamachi* has been mentioned under *Tikta Skandha in Charaka Samhita; Surasadi Gana in Sushruta Samhita, Ashtanga Hridaya; Pippalyadi Gana in Astanga Nighantu; Karaveeradi Varga in Dhanwantari Nighantu, Shodhala Nighantu; Vrikshadi Varga in Shabdachandrika; Guduchyadi Varga in Madanapala Nighantu, Bhavaprakasha Nighantu, Shaligrama Nighantu; Shatavaryadi Varga in Raja Nighantu; Aushadhi Varga in Kaiyyadeva Nighantu; Kantakaryadi Varga in Nighantu Adarsha; Shatapushpadi Varga in Priya Nighantu.*[3]

Infertility is a disease of the reproductive system defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. (*WHO-ICMART glossary, 2009*).4 Male infertility refers to a male's inability to result pregnancy in a fertile female. Male factor infertility is seen due to alteration in quantitative and qualitative semen parameters. Sperm count, sperm motility and sperm morphology are the leading factors. Infertility affects 8-12% of couples worldwide and 4-16% of couples in India. Male infertility accounts for 40-50% of total infertility.5 Recent research updates shows mean sperm count of healthy men is declined by 1% every year.6,7 A study in south India over a period of 13 years shows 30.31% decline in sperm count, 22.92% decline in sperm motility, 51.25% defect in sperm morphology.8

## MATERIALS AND METHODS

The study was conducted in the following phases:

1. Drug collection and processing

2. Pharmacognostic study

3. Experimental study

4. Statistical analysis

**Drug Collection and Processing**

**Source of Drug:** Whole plant of *Kakamachi (Solanum nigrum* Linn) was collected, in the month of June -July and authenticated at Department of *Dravyaguna*, SDMCAH, Hassana.

**Processing**: Collected whole part of drug was washed thoroughly, physical impurities and plant parts were removed and shade dried.

Drugs thus obtained were pulverized into fine powder for *Churna* dosage form and into coarse powder for Aqueous extract dosage form.

**Aims and Objectives:** To evaluate the spermatogenic activity of *Churna* of *Kakamachi (Solanum nigrum* Linn) in experimental models.

**Experimental Study on Spermatogenic Activity**

Different experimental animal models are available for analyzing the process of spermatogenesis, including transgenic animals and strains that inherently lack spermatogenesis. Among the models available male wistar albino rats are considered to be the better option for experimental study of spermatogenic activity.9-11Spermatogenic activity in male wistar albino rats was monitored by using ponderal, biochemical, sperm parameter and histological changes in testes, prostate and seminal vesicle.

**Materials and methods of experimental study**

Test Drugs

Whole plant of *Kakamachi (Solanum nigrum* Linn).

Standard Drug

The reference standard drug used for spermatogenic activity evaluation is Testosterone. It was purchased from the market with the trade name Testoviron® Depot 250mg/ml, Mfd- 12.06.17, Exp- 12.06.22, Lot. 075J. Manufactured by Schering's Pharmaceuticals AG Berlin, Germany. Imported by Schering (Bangkok) Ltd. Nonthabuti, Thailand.

**Chemicals:** All the chemicals and reagents used in the experimental study were procured from standard and reputed firms and are of analytical grade regularly used in the laboratory. The biochemical and enzymatic kits for biochemical investigations were obtained from ERBA Diagnostic Mannheim, Transasia Biochemicals Ltd., Daman.

**Animals:** The study was carried out in male Wistar strain albino rats of body weight ranging from 150 - 300 gm. They were obtained from well-established animal house attached to S.D.M Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. They were maintained on feed of "Sai Durga feed and food, Bangalore" and tap water was given *ad-libitum*. The temperature and humidity were kept at optimum and animals were exposed to natural day night cycles. Experimental procedures were undertaken according to the principle guidelines of animal care with prior permission from Institutional Animal Ethical Committee (SDMCRA/IAEC/HSN-DG- 06).

**Drug Preparation:** *Churna* of drug was prepared at Department of *Dravyaguna*, SDMCAH&H, as per the standard method. *Churna* was sieved with the sieve of 60mm size.

**Dose Fixation:** Dose of the standard drug, test drug and vehicle were calculated by extrapolating the therapeutic dose of human to rat dose on the basis fact of sure area ratio by referring to the table of Paget and Barnes (1964).12

| Dose Calculation for *Churna* | |
|---|---|
| Suggested human dose = 12 gm | |
| Rat dose | = HD x 0.018 x 5 / kg body weight |
| | = 12 x 0.018 x 5 / kg body weight |
| | = 1.08gm/kg body weight |
| | = 1.08/gm body weight of rat |
| | = 108mg/100gm body weight of rat |

**Dose Calculation for Reference Standard (Testoviron)**

Rat dose = 0.5 mg / kg = 0.05 mg /100 gm body weight of rat.

**Drug Dosing Schedule**

**Test Drug Administration**

| | |
|---|---|
| Time | 9:00-9:30 am |
| Frequency | one dose per day |
| Total no of days | 60 |
| Route of drug administration | Oral |
| Vehicle used | Normal distilled water |

Test drugs *Churna* of whole plant of *kakamachi (Solanum nigrum* Linn) was administered by oral route with the help of rat feeding tube fixed to the syringe. Normal distilled water was used as vehicle for oral administration of *Churna*.

**Standard Drug Administration**

| | |
|---|---|
| Time | 9:00-9:30 am |
| Frequency | One dose per week |
| Total no of days | 60 |
| Route of drug administration | Intra muscular |
| Vehicle used | Peanut oil . |

**Evaluation of Spermatogenic Activity**

Spermatogenic activity was assessed in animal model by comparing ponderal, seminal, biochemical and histological parameters of Test drug, Standard drug & Control group animals. Healthy male albino wistar rats weighing 150 – 250g were selected & divided into four groups of six animals in each group (n = 6) and were kept in separate cage.

**Statistical Analysis**

All the values were expressed as MEAN±SEM. The data were analyzed by one way Graph pad instant software one-way ANOVA followed by Dunnet's multiple comparison t-test as post-hoc test if $p<0.05$. A level of P 0.05 was considered as statistically significant. Level of significance was noted and interpreted accordingly.

**OBSERVATION AND RESULT**

The normal rats were treated with oral administration of test drug in four groups. Normal control rats were given only plain water and standard laboratory food and standard group was given testosterone injection intramuscularly.

The results obtained were analyzed by one way ANOVA followed by Dunnet's multiple comparison 't' test as post hoc test, by using graphic instant software. A level of $p<0.05$ was considered as statistically significant. Level of significance was noted and interpreted accordingly. The obtained data in each group are shown accordingly in given Tables.

| Parameter | Normal | | SD | | TED | | TEDx2 | |
|---|---|---|---|---|---|---|---|---|
| | Parameter | %Change | Parameter | %Change | Parameter | %Change | Parameter | %Change |
| Body Weight | 1.88±0.30 | - | 11.91±4.93 | 533↑ | 77.01±11.56** | 3996.27↑ | 16.39±3.68 | 771.8↑ |
| Wt of S.V | 0.91±0.11 | - | 0.77±0.09 | 15.38↓ | 0.95±0.09 | 4.39↑ | 0.86±0.08 | 5.49↓ |
| Wt. of Testes | 2.73±0.11 | - | 2.59±0.15 | 5.12↓ | 3.21±0.11* | 17.58↑ | 2.73±0.13 | 0 |
| Wt. Of Prostate | 0.73±0.05 | - | 0.82±0.10 | 12.32↑ | 0.94±0.07 | 28.76↑ | 0.95±0.11 | 30.13↑ |
| Sluggish Motality | 23.16±1.16 | - | 26.75±1.17 | 15.5↑ | 15±2.05** | 8.16↓ | 23.33±1.54 | 0.73↑ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Non-Motality | 76.83±1.16 | - | 73.25±1.17 | 4.6↓ | 85±2.05** | 10.63↑ | 76.66±1.54 | 0.22↓ |
| Sperm count | 3.88±0.69 | - | 4.58±0.38 | 18↑ | 4.19±0.43 | 7.98↑ | 5.30±0.52 | 36.59↑ |
| NSM | 80.66±0.61 | - | 81.37±0.59 | 0.88↑ | 76.42±0.84** | 5.25↓ | 77.16±0.47** | 4.33↓ |
| AHM | 2.5±0.22 | - | 1.37±0.26* | 45.2↓ | 1.85±0.26 | 26↓ | 2±0.26 | 20↓ |
| HLM | 15.16±0.70 | - | 15.37±0.67 | 1.38↑ | 18.28±0.35** | 20.58↑ | 17.66±0.55 | 16.49↑ |
| CTM | 1.67±0.21 | - | 1.71±0.18 | 2.39↑ | 2±0.37 | 20.48↑ | 1.83±0.16 | 10.24↑ |
| Blood Sugar | 131.16±2.84 | - | 139.87±5.82 | 6.64↑ | 120±5.26 | 8.5↓ | 126.33±5.77 | 3.68↓ |
| SGOT | 102±6.55 | - | 138.75±7.76* | 36↑ | 181±12.13** | 77.45↑ | 131±1.54 | 28.43↑ |
| SGPT | 54.16±5.73 | - | 66±4.46 | 21↑ | 77.28±5.47** | 42.68↑ | 190±1.52** | 66.17↑ |
| Blood Urea | 47.16±2.67 | - | 32.5±1.16** | 31↓ | 41.14±.73 | 12.76↓ | 50.5±3.88 | 7.08↑ |
| Blood Creatinine | 0.54±0.03 | - | 0.58±0.02 | 9.4↑ | 0.55±0.02 | 3.77↑ | 0.55±0.02 | 3.77↑ |
| Total Cholesterol | 79±3.72 | - | 60.75±3.91* | 23.1↓ | 64.14±5.65 | 18.81↓ | 49.66±3.99 | 37.13↓ |
| Latency 15$^{th}$ day | 524.5±262.94 | - | 105.67±13.18 | 79.8↓ | 339±33.06 | 35.36↓ | 262.33±19.46 | 49.98↓ |
| Latency 30$^{th}$day | 789.34±298.27 | - | 475.67±210.23 | 39.73↓ | 440±73.4 | 44.25↓ | 330.33±49.68 | 58.15↓ |
| Latency 45$^{th}$day | 422.17±218.79 | - | 138.67±28.30 | 67.15↓ | 487.33±24.4 | 15.43↑ | 501±68.67 | 18.67↑ |
| Latency 60$^{th}$day | 414.84±206.06 | - | 128±8.99 | 69.14↓ | 271.33±36.41 | 34.59↓ | 342±74.17 | 17.55↓ |
| Number of licking (15$^{th}$ day) | 2.34±0.61 | - | 7.67±0.91** | 227↑ | 4.33±0.21 | 85.83↑ | 8±0.36** | 243.34↑ |
| Number of licking (30$^{th}$ day) | 4±1.18 | - | 6±1.82 | 50↑ | 4.33±0.21 | 8.25↑ | 3.66±0.2 | 8.5↓ |
| Number of licking (45$^{th}$ day) | 3.84±1.16 | - | 7.67±0.91** | 99.73↑ | 4.33±0.21 | 13.05↑ | 3.66±0.21 | 4.43↓ |
| Number of licking (60$^{th}$ day) | 2.5±0.42 | - | 9.34±0.55** | 273.6↑ | 4.66±0.21** | 86.4↑ | 3.33±0.21 | 33.2↑ |

## DISCUSSION

Different experimental animal models are available for analyzing the process of spermatogenesis, including transgenic animals and strains that inherently lack spermatogenesis. Among the models available male wistar albino rats are considered to be

the better option for experimental study of spermatogenic activity. Spermatogenic activity in male wistar albino rats was monitored by using ponderal, biochemical, sperm parameter and histological changes in testes, prostate and seminal vesicle.

**Table Consolidated statement depicting the Spermatogenic activity profile of Solanum nigrum Linn (TED and 2TED group) in Churna form.**

| Parameters (% change) | STD | TED | TEDx2 |
|---|---|---|---|
| Ponderal Changes | | | |
| Body weight | NSI | VSI | NSI |
| Weight of Testes | NSD | SI | NSI |

| | | | |
|---|---|---|---|
| Weight of Prostate | NSI | NSI | NSI |
| Weight of Seminal vesicle | NSD | NSI | NSD |
| Sperm Parameters | | | |
| Sluggish Motility (SM) | NSI | VSD | NSI |
| Non-motility (NM) | NSD | VSI | NSD |
| Sperm Count | NSI | NSI | NSI |
| Normal Sperm Morphology (NSM) | NSI | VSD | VSD |
| Amorphous Head Morphology AHM) | NSD | NSD | NSD |
| Hook Less Morphology(HLM) | NSI | VSI | NSI |
| Curled Tail Morphology (CTM) | NSI | NSI | NSI |

NSI: Non Significant Increase NSD: Non Significant Decrease

SI: Significant Increase SD: Significant Decrease

VSI: Very Significant Increase VSD: Very Significant Decrease

Discussion on the Experimental study can be depicted under the following headings:-

1. Ponderal Changes
2. Sperm Parameters
3. Serum Biochemical Parameter
4. Latency Period
5. **Histological Study**

**1. PONDERAL CHANGES**

Results obtained from current study had shown significant effect of *Kakamachi* in ponderal parameters that may be due to presence of steroidal compounds. Improvement in body weight and other related organs is generally attributed to steroid genesis and is a biological indicator for effectiveness of herbal drugs in improving genesis of steroidal hormones.[13]

**2. SPERM PARAMETERS**

This is discussed under the following three headings:-

- Sperm motility
- Sperm count
- Sperm morphology

**Sperm Motility**

In the present study, there was a non-significant increase in sluggish motile sperms in 2TED group probably due to its hypoglycemic effect which is also evident in the serum biochemical study though the decrease in blood sugar is statistically insignificant. Also the results of the non- motile sperms in 2TED is almost similar to the standard group. Hence the results of sperm motility are inconclusive.

**Sperm Count**

The increase in sperm count could be attributed to glycosides, saponins and sterols present in whole plant of *Kakamachi*. Steroidal constituent increases the steroidogenesis and elevates androgen levels which enhances the spermatogenic activity thereby increasing in quality and quantity of sperm[14].

**Sperm Morphology**

A normal spermatozoon is having oval head shape with a regular outline &acrosomal cap covering more than one-third of the head surface. The mid piece is long, straight and regular in outline, slender, less than 1/3 of width of the head. The tail slender, uncoiled and regular in outline. When there is variation in the above said characteristics the sperm is said to have an abnormal morphology

**Normal Sperm Morphology**

According to obtained result there is significant decrease in TED and TEDx2 test drug in comparison to normal control group. But there is a non-significant increase in normal sperm morphology in standard as well, which shows that there is not much variation between standard and test groups.

**Sperm abnormalities**

Amorphous Head, hook less, curled tail Morphology of semen are abnormalities, which are indications of interference with maturation stage of spermatogenesis in the seminiferous tubules[15].

In the present study, there has been non- significant decrease in amorphous head abnormality while non- significant increase in hookless and curled tail in both the test groups. But these findings are almost similar to the standard group. Also the findings are not supported by the histopathological studies, which shows normal sperm morphology and a good spermatogenesis. Hence similar to the motility parameter, the sperm abnormality parameter remains inconclusive.

**3. Serum Biochemical Parameter**

**Blood Sugar**

According to obtained result there is non-significant decrease in blood sugar of blood in TED and TEDx2 test drug group and non-significant increase in standard group in comparison to normal control group. However, previous studies on different parts of Kakamachi have revealed antidiabetic potential16.

Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamate-Pyruvate Transaminase (SGPT)

The other biochemical parameters, like SGPT. SGOT help to assess the effect of the test drug on vital organ, liver. In the TED group, there has been significant elevation in SGOT, SGPT. But previous works have revealed hepatoprotective activity till 1g/kg body weight of aqueous extract of whole plant against CCl4-induced oxidative damage in rats17. Hence the present study with respect to effect of *Kakamachi Churna* on liver needs to revalidated.

**Blood urea, creatinine, cholesterol**

In the present study, blood urea, creatinine has shown non- significant decrease in both the test groups which suggest its safety to kidney.

**4. Latency Period**

**Latency (15th day)**

Latency is calculated as the time from the introduction of a female to the occurrence of first mount. In the present study, the latency period was significantly decreased at 15, 30, 45 and 60 days. This shows improvement in the sexual behavior of the animal, which is also a component of sexual function18.

**Number Of Licking**

One of the important aspects of sexual behaviour assessment is orientation behavioural analysis which includes self-licking which is a self-exploratory behaviour and

licking, a non-self-exploratory behaviour19. In the present study, the number of lickings have moderately increased in TED group at 15, 30, 45 and 60 days, while there had been increase only at 60th day in 2TED group. This shows that *Kakamachi churna* was capable of improving the orientation behaviour in the animal. Further studies may be undertaken to assess specific effect on sexual behaviour. The present study partly demonstrates the efficacy of *Kakamachi churna* in affecting sexual behaviour in animals.

**5. Histological Study**

Thus, the present study elucidated the efficacy of *Kakamchi churna* at a dose of 108mg/kg b.w and 216g/kg b.w administered orally for a period 60 days in increasing body weight, sperm count and improving oriental behavior in animals. The histopathological studies correlated with the observations and findings of the study. There was no significant improvement in the reduction of abnormal sperms in the study animals.

**CONCLUSION**

*Churna* of whole plant of *Kakamachi (Solanum nigrum* Linn.) TEDx2 group has shown better result in Shukrala Karma (Spermatogenic activity) than that of TED group. The study shows both therapeutic estimated drug groups are having *Shukrala Karma* (Spermatogenic activity) but has different efficacy in different dosage and specific parameter.

Hence, hypothesis *Kakamach (Solanum nigrum* Linn.) is as effective in Shukrala Karma (Spermatogenic activity) can be accepted regarding sperm morphology, sperm motility, secretion of prostate and maturity of sperm with good evidence of spermatogenesis.

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