

Original article

Effect of *Silene cucubalus* on the Reproductive System in Male Albino Rats

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Abstract

Background:To combat population explosion, major result oriented efforts have been made towards female contraception. Since the responsibility of procreation is on both the sexes, there is an intense shortfall in development of male contraception.

Aims and objectives:The study was aimed at examining the product of plant origin, *Silene cucubalus* for male contraception as a large population uses native medicine for contraception.

Material and methods:The study was conducted with *Silene cucubalus* to evaluate its effect on albino rats for which 60 male and 180 female albino rats were used. Male albino rats were divided into three groups with control receiving 1ml of distilled water, low dose group 50 mg and high dose 250mg of *Silene* extract. Females were mated with males in the ratio of 2:1 following which 50% of males from each group were killed to assess the morphology and histopathology of testis.

Results and conclusion:The study concluded that there was a decrease in size and weight of testis in low and high dose groups by 4.45% and 10.84% respectively associated with reduction in interstitial tissue and mild spermatogenic arrest in low dose group while as there was disfiguration of seminiferous tubules with complete spermatogenic arrest in high dose group and after withdrawal of drug, weight and size of testis in both groups were at par with control group which indicates that changes are reversible.

JK-Practitioner 2019;24(3-4):38-41

Introduction:

The recent years have witnessed remarkable advances, the substances and methods have become available which are effective in inhibiting or promoting human fertility. Worldwide efforts are being made for the development of an inexpensive acceptable contraceptive. An ideal contraceptive can be defined as a non toxic and completely effective substance which when self administered on a single occasion would ensure non-pregnant state temporarily or permanently¹. For centuries, the male has been sidelined by the researchers and family planners probably due to social and technological forces and for whom there are contraceptives methods like use of condoms, vasectomy or coitus interruptus that suffer from several drawbacks including inconvenience, unacceptability or unsuccessful application. Hence there is a major need and demand for newer and better methods of male fertility regulations in terms of efficiency, safety, reversibility and acceptability². It is generally agreed that the dynamics of spermatogenesis form a key factor in the potential success of fertility regulation in male³ for which have been made many attempts to identify the antigenic constituents of testis and spermatozoa and in this context, prospects of seminal inhibition of the immunocontraception has been developed for complete or partial block of fertility⁴.

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Indexed:

Scopus, INMED, EBSCO @ Google
Scholar among others

Cite This Article as:

Bali Z S, Shah MA, Mushtaq Q, Khatib ZA, Mir SA, Ashai ZA. Effect of *Silene cucubalus* on the Reproductive System in Male Albino Rats. JK-Practitioner 2019;24(3-4):38-41

Full length article available at JK-Practitioner.com one month after publication

Key words: Contraception, *Silene cucubalus*, albino rats, spermatogenic arrest, seminiferous tubules.

In spite of spectacular advances in modern medicine, a large section of population especially in rural areas of the developing countries use native system of medicine known as folklore medicine and a host of medicinal plants growing in different parts of the world have been used against conception. Although, no plant has been thoroughly studied for its effect on male reproduction, serious scientific investigation of plants derived agents having antispermatogenic effects are underway in a number of laboratory species of mammals and in that context the present study was conducted with the aim to see the effect of silence cucubalus extract (a herbaceous plant mostly found in alpine and subalpine regions of Kashmir) on reproductive system of male albino rats in two doses corresponding to human being and large dose which is five times more than first group.

Material and Methods:

The present study entitled "Effects of *Silene Cucubalus* on reproductive system in male albino rats" was carried out in the department of Pharmacology, Government Medical college Srinagar. White male albino rats of proven fertility were used due to their moderate size, relative docile nature, ease in holding, maintenance, conveniently observable sex, libido, having a relatively gestation period of 21-23 days and well characterized reproductive process⁵. After an acclimatization period of two weeks in the animal house of college, these were cohabited in wire mesh plastic cages. Sixty healthy adult males almost of same age and exhibiting normal mating behavior were selected and divided into three groups of twenty each. Likewise 180 adult healthy female rats almost of same age were selected and kept in separate cages and maintained on a balanced diet consisting of rat food pellets and water ad libitum.

Silene cucubalus, a herbaceous plant belonging to the silence family were collected from the department of Botany, University of Kashmir. These were washed and then crushed along with the roots to extract the juice which was purified using primitive methods of straining and subsequent concentration to a semi solid mass in the department of Pharmacy, University of Kashmir. This semi solid mass was used as a diet to the male rats, one with dosage appropriate to human beings and other five times more than the first. So the treatment consisted of control dose (distilled water) that is group A, low dose (dose appropriate to human

beings) that is group B and high dose (five times of the low dose) that is group C. After that Group B was given 50mg of extract and group C 250mg of extract dissolved in one ml of distilled water as Sharma et al⁶ reported that semi solid extract of silene is used in human beings as male contraceptive in dose of 2-3 gms each evening at least two hours after dinner after dissolving in cup of milk or water. The drug was given to male albino rats two hours after evening feed orally through a syringe cannula for fifteen days and at the end of fifteen days, one hundred and twenty female rats were given diethyl stibioestrol in the dose of 0.1 ml intramuscular per each rat⁷. At the expiry of 24 hrs, vaginal smears were taken from female rats to confirm the estrous after putting normal saline by a rubber dropper into the vagina. Then male rats twenty from each group were allowed to mate with their females in the ratio of 1:2 and after twelve hours vaginal smears were again taken to see the sperm to confirm mating. The male rats were separated from females and ten male rats from each group were killed and testis removed for morphological and histological examination. The mated female rats were kept in cages for eighteen days according to their group and were killed on nineteenth day to determine litter size.

After withdrawal of the drug, the remaining half of the male rats (ten from each group) were put in the separate cages for three months after which they were again allowed to mate with female rats of proven fertility in the ratio of 1:2 and vaginal smears taken from female rats. The study was repeated in similar manner after giving the drug and the male rats were killed by cutting the throat and testis were separated from other structures after identification, their weight and size determined, put in 10% formalin and sections having thickness of less than 4mm were made and subjected to histopathological examination, embeddings and block making, section cutting and staining

Results and observations:

Table-I shows the weight of rats and weight and size of testis as affected by control, low dose and high dose of drug.

| Treatment | Weight of rats (g) | Weight of testis (g) | | Size of testis cm ³ | |
|---------------------|--------------------|----------------------|------|--------------------------------|------|
| | | Right | Left | Right | Left |
| T1 (control) | 143.7 | 1.02 | 0.92 | 1.16 | 1.05 |
| T2 (Low dose drug) | 142.3 | 0.96 | 0.88 | 1.15 | 1.04 |
| T3 (High dose drug) | 140.7 | 0.73 | 0.83 | 1.14 | 1.02 |
| SE | 2.61 | 0.13 | 0.01 | 0.07 | 0.02 |
| CD | 9.36 | 0.27 | 0.04 | 0.08 | 0.03 |

Results indicate that highest weight of right testis was in control group which marked an increase of 6.25 and 39.73% over the weight of right testis in low and high dose group respectively. Similar findings were also made in case of weight of left testis as the magnitude of increase over low and high dose groups was 4.54 and 10.84% respectively. As regards size of testis the data showed that control group rats had highest size (1.05cm) which decreased significantly in low and high dose group.

Table-II Litter size of females rats (after giving the drug) as effected by control, low dose and high drug

Litter Size

| Treatment | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------------|----|---|---|---|---|---|---|---|
| T1 (Control) | 9 | 0 | 3 | 5 | 5 | 6 | 6 | 6 |
| T2 (Low dose drug) | 19 | 0 | 0 | 0 | 4 | 5 | 5 | 3 |
| T3 (High dose drug) | 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

SE1 4.48

8.44

Observation in table II reveal that the litter size of zero was recorded in 9,19 and 34 females of control, low and high dose group of rats respectively. It also indicates that no female rat from high dose group could produce litter.

Table III Weight and size of testis after withdrawal of the drug as affected by control, low dose and high dose drug.

| Treatment | Weight of Testis (g) | | Size of testis (g) | |
|-----------|----------------------|-------|--------------------|-------|
| | Right | Left | Right | Left |
| T1 | 0.88 | 0.86 | 1.10 | 1.00 |
| T2 | 0.86 | 0.83 | 1.10 | 1.00 |
| T3 | 0.83 | 0.82 | 1.09 | 0.99 |
| SE | 0.016 | 0.016 | 0.38 | 0.033 |
| CD (0.05) | 0.034 | 0.034 | 0.05 | 0.047 |

The data revealed that the highest weight and size of right testis was recorded in control group rats. Similar findings were also made in case of weight of left testis which was stastically higher over those in high dose group rats but was stastically at par with those in low dose group. No significant difference in left testis size was observed due to different treatments.

Table IV Litter size of female rats after withdrawal of drug as effected by control, low and high dose drug.

Litter Size

| Treatment | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------------|---|---|---|---|---|---|---|---|
| T1 (control) | 4 | 0 | 4 | 4 | 4 | 3 | 1 | 0 |
| T2 (Low dose drug) | 5 | 0 | 3 | 4 | 4 | 3 | 1 | 0 |
| T3 (High dose drug) | 7 | 0 | 3 | 3 | 3 | 3 | 1 | 0 |

SE 2.15

CD (0.01) 4.48

The table shows litter size of zero in 4,5, and 7 females rats in control, low dose and high dose groups respectively which was stastically at par among themselves. Also there was no significant difference in litter size due to different treatments.

Histopathology of testis in different groups after drug.

Histopathology revealed presence of seminiferous tubules separated by interstitial cells of Leydig present in angular spaces between tubules and numerous blood capillaries in the interstitial space while as in group B , there is reduction in the interstitial tissue and mild spermatogenic arrest with at some places, necrotic spermatogonial cells exhibiting pyknotic nuclei and tubule lumen containing cell debris while as in group C there is a disfiguration of seminiferous tubules with slight reduction in diameter and spermatogenesis appears completely arrested. Several germ cells exhibit vacuolization and giant cell formation in damaged tubules.

Histopathology of testis after withdrawal of drugs.

It revealed that in group A seminiferous tubules are full of mature spermatozoa with peripheral, single cell lining of mature germ cells while as in group B seminiferous tubules contain mature spermatozoa in lumen with peripheral rim of immature germ cells in most of the tubules and in group C seminiferous tubules full of mature spermatozoa and immature germ cells with their center containing mature cells surrounded by 2-3 layers of immature germ cells.

Discussion:

Approximately 48.2 % of couples of 15-49 years of age practice family planning methods in India. Contraception especially the female contraceptive has been a thoroughly investigated field which has involved a lot of research ultimately resulting in development of ideal, cheap and safe methods of contraceptive for women. Unfortunately, the same is not true for male contraception. The requirement of an ideal contraception drug that is simplicity, acceptability, reversibility and lack of toxicity have not been met with the male contraceptive. The lack of development of an effective safe contraceptive for men is principally due to the fact that it is difficult to inhibit spermatogenesis completely although men have fathered children even when sperm counts are lowered by 99%⁸

The use of plant and other herbs as antifertility

agents has been on records on the earliest times. Numerous medicinal plant growing in different parts of the world have been used against contraception. Chinese have worked on a phenolic compound extract from cotton plant which have been documented to decrease the sperm density, total sperm count with no effect on serum leuteinising, follicle stimulating hormone, prolactin, or testosterone. However it was later on withdrawn as male contraceptive because of other side effects.⁹In order to develop an antispermatogenic substance, a male contraceptive, the present study was under taken with silene cucubalus, a herbaceous plant. The study revealed that the extract of silene cucubalious exerts antifertility effect in rats and effect may be directly rendered on spermatozoa which is reversible. Similar results were reported by Pathaketal¹⁰ who observed sterility in rats due to inhibition of sperm motility by oral administration of benzene chromatography fraction of chloroform extract of seeds of carica papaya and effects were reversible following sixty days of withdrawal of the drug whereas Lohiya etal¹¹ evaluated antifertility activity of choloform extract of carica papaya seeds by oral administration in Langur monkeys. They observed that extract gradually decreased the sperm concentration since days thirty to sixty of treatment with total inhibition of sperm mortality, a decrease in sperm viability, increase in sperm abnormality and azoospermic after ninety days of treatment. However these changes were reversible after one hundred and fifty days of withdrawal of drug. In our study silene cucubulious extract significantly affected the male reproduction by interfering with protein synthesis and concentration in accessory sex organs which are androgen dependent. Reduced sialic acid and other contents of testis and accessory organs may alter the structural integrity of acrosomal membrane which ultimately affects the metabolism, motility and fertilizing capacity of spermatozoa.¹²

Summary and conclusion:

The present study concludes that the silene extract have antifertility effect in male albino rats which are dose dependant and reversible in animal model butto meet the essential criteria to develop silene cucubalus as male contraceptive needs further detailed studies.

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