ORIGINAL ARTICLE

Effect of Tulsi (*Ocimum Sanctum* Linn.) on sperm count and reproductive hormones in male albino rabbits

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ABSTRACT

Fresh leaves of *Ocimum sanctum* (OS) were used to study its effect on male reproductive function (sperm count and reproductive hormones) in male albino rabbits. Animals in the test group received supplementation of 2 g of fresh leaves of OS per rabbit for 30 days, while the control group was maintained on normal diet for the same duration. Sperm count and hormonal estimation [testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH)] were done in serum samples of both groups and compared. A significant decrease was noted in the sperm count in test group rabbits. Serum testosterone levels showed marked increase while FSH and LH levels were significantly reduced in OS-treated rabbits. The results suggest the potential use of OS as an effective male contraceptive agent.

Key words: Follicle stimulating hormone, luteinizing hormone, *Ocimum sanctum*, sperm count, testosterone

Introduction

Ocimum sanctum (OS) – popularly known as tulsi in Hindi and holy basil in English is one of the sacred herbs for Hindus in the Indian subcontinent. It has a versatile role in traditional medicine.[1] The whole plant of *Ocimum sanctum* has medicinal value, although mostly the leaves, and sometimes the seeds, are used.[2] Earlier studies with OS have indicated that the plant has hypoglycaemic,[3] hypolipidaemic,[4] adaptogenic,[5] anti-cancer,[6] radioprotective,[7] analgesic and anti-inflammatory properties.[8] Village women and Ayurvedic physicians have been reported to be using tulsi leaves for anti-fertility effect,[9] but this type of traditional practice has been limited to rural areas of India. A rational approach to this traditional practice with modern scientific methods is not available in medical literature in spite of the easy availability of OS in India. Leaves of OS have antizygotic, anti-implantation and early abortifacient effect in women and in experimental animals.[10] Since no study is available to document the effect of OS on the levels of reproductive hormones, mainly luteinizing hormone (LH) and follicle stimulating hormone (FSH), the present study was undertaken to analyse the effect of OS on the sperm count and reproductive hormones in male rabbits.

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Materials and Methods

Animals

Male albino rabbits weighing 1.5-2.5 kg were procured from the disease-free animal house of the CCS Haryana Agriculture University, Hisar (Haryana, India). The rabbits were housed under controlled conditions of light (12-h light-dark cycle) and temperature [(23 ± 2) °C] with free access to respective diets and water *ad libitum* for a period of 30 days. Fresh tulsi leaves were collected, cleaned, and weighed and used in the study. Institutional Animal Ethics Committee (IAEC) approval (IAEC/PATHO/08/2352-58 dated 18.09.08) was obtained before the experiment and care of animal was taken as per guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Table 1: Sperm count and reproductive hormones in rabbits of control and test groups (mean ± SD)

Group	Sperm Count (million/ml)	Serum Testosterone (ng/dl)	Serum FSH (mIU/ml)	Serum LH (mIU/ml)
Control	162.2 ± 7.0	303.6 ± 18.2	0.64 ± 0.05	0.53 ± 0.03
Test	110.2 ± 5.7*	>1500.00	0.13 ± 0.03*	Undetectable

Testosterone levels in all test rabbits reached the maximum recordable limit value of the apparatus, ie, 1,500 ng/dl, LH levels were undetectable, *P<0.001, as compared to control

Experimental design

Rabbits were divided into two groups of 10 each. Group I (Control) was maintained on standard chow diet, whereas Group II (Test) was administered the same diet that the control group rabbits received along with oral supplementation with 2 g fresh leaves of *Ocimum sanctum* daily for 30 days.

After 30 days, blood samples were taken from the pinna vein for assessment of hormone levels (testosterone, FSH, and LH) from both groups. The hormones were assayed by principle of chemiluminescence using chemiluminometer (Advia Centaur CP, Siemens),[11] and sperm count was assessed in all animals. The cauda epididymis from both sides were removed and washed repeatedly in 10 ml of normal physiological saline. Spermatozoa were counted by using 1-ml aliquots of sperm suspension with the help of a haemocytometer.[12] The animals were sacrificed by phenobarbitone 30 mg/kg IV dose. Data obtained were analysed statistically by applying Student's *t* test using SPSS version 14.0.

Result

In the present study, a significant ($P < 0.001$) decrease in sperm count was observed in rabbits fed on OS leaves as compared to control animals. A marked increase in serum testosterone level was observed in OS-treated rabbits as compared to control. However LH level was significantly reduced in this group. In fact, LH level was undetectable in the serum of all animals. FSH levels in the test group decreased (0.13 ± 0.03 mIU/ml) as compared to that in the control group (0.64 ± 0.05 mIU/ml) [Table 1].

Discussion

Male reproductive process is regulated by intricately balanced mechanisms involving the hypothalamus–pituitary–testis axis and accessory sex organs. It is believed that for initiation as well as maintenance of spermatogenesis in humans, both FSH and testosterone are needed. The gonadotropin-releasing hormone (GnRH) secreted by the hypothalamus regulates the synthesis and release of FSH and LH from the pituitary. FSH acts on the Sertoli cells, which are located within the seminiferous tubules in close proximity of developing germ cells, and stimulates production of various proteins including inhibin, androgen-binding protein (ABP), aromatase, anti-Mullerian hormone (AMH), etc. The LH acts on the Leydig cells located in the intertubular space and stimulates production of testosterone. Intratesticular testosterone concentration in humans is about 200-300 times higher than that in peripheral circulation. Testosterone has profound influence on germ cell development and differentiation. It exerts a negative feedback action on LH secretion, and also on FSH (at higher concentration) acting on hypothalamic–pituitary axis.[13]

Results of the present study clearly show that tulsi treatment (2 g/day) brings about a reduction in sperm count, which is in agreement with the earlier reports. Khanna *et al*, have reported significant decrease in sperm count and motility as well as decrease in the weight of testes, epididymis, seminal vesicle, and ventral prostate after long-term feeding of OS leaves.[14] The long-term feeding of fresh tulsi leaves (465 mg/kg/day) have shown to increase the body weight, while decrease the weights of testes, prostate, and adrenal gland in rats. The results suggested that infertility in male rats seems to be due to impairment of spermatogenesis as well as changes like decrease in pH, hypotonic environment, and chemical substances like mucoproteins, alkaline phosphatase and acid phosphatase in spermatogenic cells leading to formation of non-viable spermatozoa.[15] However, Seth *et al*, reported that benzene extract of OS leaves significantly altered the weight of testes but did not have any significant effect on epididymis, seminal vesicle, prostate, and vas deferens.[16] Treatment with OS leaves led to a highly significant increase in testosterone levels. FSH and LH levels also showed a significant decrease in the test group after tulsi treatment. A possible hypothesis to explain this pattern of changes in hormone levels could be that tulsi leaves probably contain some androgenic analogue, which increased the circulating testosterone levels sufficiently to inhibit LH but not sufficient to accumulate in the testis at the required concentration for normal spermatogenesis. However, the decreased LH levels will diminish intratesticular production of testosterone by Leydig cells, which results in reduced levels of spermatogenesis. For suppression of spermatogenesis, different androgens and progestins have been used either alone or in combination. Reddy and Rao administered testosterone propionate 100 mg daily intramuscularly to normal volunteers and achieved azoospermia in 100% subjects.[17] Weekly IM injections of 200 mg testosterone enanthate given to 399 normal healthy fertile males produced azoospermia or severe

oligospermia (sperm density <3 million/ml) in more than 95% volunteers. This contraceptive effect was comparable to female contraceptive pills, and was reversible.[18]

It can thus be concluded that antispermatogenic effect of OS is brought about by modulation of levels of reproductive hormones. Since this is a preliminary study, further studies are required to establish the role of OS as an effective herbal male contraceptive.

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