Research Article (Open access)

Reversible Anti-Fertility Effects of Aqueous Leaf Extract of *Ocimum Sanctum* (Linn.) in Male Mice

Aarti verma[1]*, Shail Pragya[1], V. N. Singh[1]
[1]Department of Zoology, T. M. Bhagalpur University, Bhagalpur, Bihar, India

[*]**Address for Correspondence:** Aarti Verma, Research Scholar, Department of Zoology, T. M. Bhagalpur University, Bhagalpur, Bihar, India
Received: 22 April 2016/Revised: 27 May 2016/Accepted: 14 June 2016

ABSTRACT- Present study was undertaken to evaluate the reversible anti-fertility effect of *Ocimum sanctum* Linn (Tulsi) on male mice. Aqueous leaf extract of *Ocimum sanctum* was orally administered (0.1ml) for 10 ($P < 0.05$), 20 ($P < 0.01$), 30 ($P < 0.001$), 40 ($P < 0.001$) and 50 ($P < 0.001$) days of exposure. Treatment of aqueous leaf extract of *Ocimum sanctum* showed significant decrease in sperm counts during 10 ($P<0.1$), 20 ($P<0.01$), 30 ($P<0.01$), 40 ($P<0.001$) and 50 ($P<0.001$) days treatment than the control. Motility of spermatozoa also declined significantly in treated group after 10 ($P<0.01$), 20 ($P<0.01$), 30 ($P<0.001$) 40 ($P<0.001$) and 50 ($P<0.001$) days treatment than the control. However, abnormality of spermatozoa increased significantly in treated group of mice during 10 ($P<0.01$), 20 ($P<0.01$), 30 ($P<0.001$) 40 ($P<0.001$) and 50 ($P<0.001$) days treatment than the control. Thus significant decrease in sperm counts, motility and increased abnormality of spermatozoa altered the seminal quality which caused infertility among treated group of mice. Effect of the treatment on reproductive organs and fertility was also investigated. The results revealed that *Ocimum* treatment caused decrease in the weight of reproductive organs viz., testis ($P<0.001$), epididymis ($P<0.01$), seminal vesicle ($P<0.001$), vas deferens ($P<0.01$) and ventral prostate ($P<0.001$) significantly when compared to the control group. The recovery group of animal, which also received 50 days treatment, was maintained for 90 days without any treatment to check the reversibility. All the animals after the recovery period showed normal fertility rate. Thus *Ocimum sanctum* adversely affects fertility in mice and showed anti-fertility effect among them. From this we can conclude that *Ocimum sanctum* can be used as a potent anti-fertility agent which is reversible.

Key-words- *Ocimum sanctum*, Anti-fertility, Sperm count, Motility, Sperm abnormality

----------IJLSSR----------

INTRODUCTION

One of the important concerns today's is the problem of over population. If the population increase is not controlled or checked, it will lead to several problems (Sailani and Moeini, 2008). The solution to this predicament is population control. India opted for family planning to control the increase in population (Sarkar, 1996). Various methods of contraception were used for fertility control. There are a variety of methods available and are in use for female contraception (Bajaj, 1999). In contrast, except for the barrier method and vasectomy, there are no methods available for male contraception. Thus, there is need to develop multiple male contraceptive methods. The risk obtained by the drugs has triggered the need to develop newer molecules from medicinal plants.

Access this article online	
Quick Response Code:	Website: www.ijlssr.com
ISSN 2455-1716	DOI: 10.21276/ijlssr.2016.2.4.17

Hence there is need to search suitable products from indigenous medicinal plants, that could be effectively used (Absar *et al.*, 2006). Many local plants have been identified and tested for their anti-fertility effect in male rats and mice (Sharma *et al.*, 1999).

Tulsi (*Ocimum sanctum* L.; Family-*Lamiaceae*) is a religious, aromatic and annual herb with diverse medicinal property wound healing (Shetty *et al.*, 2008), anticancer (Pandey, 2009), cardioprotective (Suanarunsawat *et al.*, 2010). Leaves of *Ocimum sanctum* has anti-zygotic, anti-implantational and aboritifacient activity in women and in the experimental animals (Chopra *et al.*, 1956, Chopra *et al.*, 1958); Batta and Santhakumari, 1970; Vohra *et al.*, 1969). *Ocimum sanctum* leaves show anti-fertility property in male mouse (Kasinathan *et al.*, 1972) as it possesses anti-spermatogenic (Prakash and Gupta, 2005). Benzene, extract of *Ocimum sanctum* in albino rats also decreases the total sperm counts and sperm motility (Ahmed *et al.*, 2002) which leads to infertility among them. From the above said medicinal property, the present study has been undertaken to observe the effect of *Ocimum sanctum* on Swiss albino male mice to see the reversible anti-fertility effect in male.

MATERIALS AND METHODS

The young fresh leaves of *Ocimum sanctum* were collected from the minigarden of University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, India. Leaves were washed 2-3 times in the tap-water. 10 grams of leaves were then grounded with 10 ml of distilled water with sterilized pestle and mortar. The homogenized mixture was filtered twice through a cotton cloth and centrifuged at 5,000 rpm for 10 minutes. Supernatants were collected and diluted with 30 ml of distilled water to obtain a concentration of 250mg/day/kg body wt. of mice.

Adult swiss albino male mice weighing between 25-30 gm. were divided into four groups each consisting of six mice. One group of six mice was considered as control group while other were considered as experimental. These four groups of mice were maintained at uniform animal husbandry condition (12 h photoperiod, 25±2°C temp.). Control group of mice was fed with 0.1 ml distilled water and experimental groups were fed with 0.1 ml aqueous leaf extract of *Ocimum sanctum* for 10, 20, 30, 40 and 50 days.

At the end of feeding period, all the mice were sacrificed by cervical dislocation. The Testis and Epididymis were excised, dried thoroughly by blotting on a filter paper, weighed accurately and both the cauda epididymis was taken into watch glass containing 2ml of normal saline. The weights of the dissected organs were calculated for 100g body weight of animal by using the following formula:

Weight of organ = Wet weight of organ/Body weight × 100 = g/1000g b wt. Body weight

Both the cauda epididymis of each mouse was teased and seminal content were sieved by metallic filter. Sperm counts were done by using methods described by Eliasson (1975), motility of spermatozoa was observed after the method of Tijee and Oentoeng (1968) and the morphology of stained sperms was observed under high magnification of the microscope. Abnormalities if present were noted. If 70 % sperms had head, tail and mid-piece the animals were considered to have normal sperms (Sood, 1987).

Tail abnormality - Broken tail, double tail
Head abnormality - Broken head, joint head
Presence of immature spermatozoa

RESULTS

Organ weight

In the treated groups, the weight of the testis and other reproductive organs decreases significantly when compared to the control group (Table 2). Complete recovery of the testicular and other reproductive organs weight is observed in this group.

Sperm count, Motility and Sperm abnormalities

The treatment of *Ocimum sanctum* leaf extract causes significant decline (P<0.001) in sperm counts of mice after 10 to 50 days treatment in the treated group than the control group of mice. In the treated group of mice, motility of spermatozoa reduces significantly (P<0.001) during 10 to 50 days treatment in the treated group than the control. However, abnormality of spermatozoa increases significantly (P<0.001) in treated group of mice than the control (Table-1).

Table 1: Effect of *Ocimum sanctum* L. on seminal **profile of mice**

Groups	Sperm counts (million sperm/ml)	Motility (%)	Sperm abnormality (%)
Control (6)	21.00±2.22	85.16±0.47	4.83±0.4773
10 days (6)	19.83±2.19*	81.66±0.71*	8.33±0.7070**
20 days (6)	16.66±1.86**	72.06±1.47**	17.33±1.475**
30 days (6)	15.37±1.76**	49.00±1.06**	31.60±1.064***
40 days (6)	13.83±1.02***	35.0±0.64***	20.47±0.74***
50 days (6)	10.48±1.09***	27±0.004***	35.20±0.79***
90 days (Recovery) (6)	26.66±2.64	86.00±0.63	5.03±0.54

Data presented as mean±SEM; *, **, *** shows significance of 0.1, 0.01 and 0.001 level with the value in control Number within parenthesis denotes number of samples.

Table-2: Effect of *Ocimum sanctum* L. on reproductive organs weight (mg) of male mice

GROUPS	Organ's weight (mg/100g b wt)				
	Weight of testis	Weight of epi-didymis	Wt of vas def-erens	seminal vesicle wt.	Ventral prostate wt.
Control (6)	0.791±0.008	0.263 ± 0.005	0.115 ± 0.001	0.586 ± 0.10	0.227 ± 0.001
10 days (6)	0.787±0.01	0.252±0.010	0.112 ± 0.004	0.582±0.01	0.225±0.001
20 days (6)	0.753±0.01	0.252±0.010	0.109±0.004	0.567±0.01	0.221±0.002*
30 days (6)	0.736±0.01**	0.246±0.010*	0.105±0.005*	0.560±0.01*	0.218±0.001**
40 days (6)	0.719±0.003***	0.238±0.003**	0.93±0.005**	0.546±0.004 **	0.215±0.004***
50 days (6)	0.659±0.003***	0.231±0.003**	0.085±0.001**	0.508±0.004***	0.196±0.002***
Recovery Group (6)	0.727±0.01	0.270 ± 0.010	0.117 ± 0.004	0.590±0.01	0.251±0.267

Data presented as mean ± SEM; *, **, *** shows significance of 0.1, 0.01 and 0.001 level with the value in control. Number within parenthesis denotes number of samples.

DISCUSSION

In this study the significant reduction of testis weight is found, which is known to be mostly related to number of spermatozoa present in the tissue. The significant reduction in the weight of reproductive organs indirectly supports the reduced availability of androgen (Zeherea *et al.,* 1998). Androgen deprivation not only suppresses spermatogenesis, leading to low sperm concentration, but also alters the epididymal milieu which affects the maturation and survival of the spermatozoa (Setty, 1979 & Rao, 1988; Rao and Mathur, 1988; Rao and Shah, 1988).

Leaf extract of *Ocimum sanctum* treatment causes significant decline in sperm counts and motility of spermatozoa in the treated group of mice than the control. This observation corroborates the study of Khanna *et al.,* 1986 and Kantak *et al.,* 1992. Such reduction in sperm counts and motility of spermatozoa may be due to decline in testosterone level as sperm counts and their motility are androgen dependent. This suggests that *Ocimum sanctum* causes decrease in sperm counts and their motility by modulating testosterone level in treated group of mice. Various plants like *Solanum xanthocarpum* (Rao. 1988), *Banbusa arundinacea* (Kumari *et al.,* 1989), and *Carica papaya* (Lohiya & Goyal 1992), have been reported to possess anti-fertility activity. Treatment with above said plant materials could reduce sperm count, fertility and viability and increase the amount of abnormal sperm (Ghosesawar *et al.,* 2003). In the same manner, treatment with *Ocimum sanctum* decreases the sperm count and increase the abnormality of sperm which leads to decrease in the concentration of sperm in the treated animals.

Clinical assessment of male anti-fertility agents should include acceptability, safety and efficacy during and after the treatment. Such agents must have reversible anti-fertility effect. Our present data shows the reversible effect of the treatment. Complete recovery of fertility was observed following the withdrawal of the treatment. In the present work after the recovery period of 90 days, all the animals were able to reproduce normally when compared to the control groups. The similar results are found when the administration of *Carica papaya* seed to the rat (Chinoy *et al.,* 1999), *Curcuma longa* to the rat (Mishra and Singh, 2009), *Allamanda cathartica* to the male mice (Singh and Singh, 2008).

CONCLUSIONS

From the above observations it can be concluded that the aqueous leaf extract of *Ocimum sanctum* decreases the sperm counts, motility, and testicular and accessory reproductive organs weight and increases the abnormality of spermatozoa which results into infertility among treated groups of mice by alternating the seminal quality. This suggests that *Ocimum sanctum* shows reversible anti-fertility effects in the treated group of male mice.

REFERENCES

[1] Sailani, M.R., and Moeini, H (2008) Effect of Ruta graveolens and Cannabis sativa alcoholic extract on spermatogenesis in the adult Wistar male rats. *Indian J. of Urology.* pp 257-260.

[2] Sarkar, H.B.D (1996) Principles of vertebrate reproductive biology. *Ganapathy Graphics,* pp 320-362.

[3] Bajaj Rajalakshmi Mand, J.S (1999) Male contraception: present and future (eds) New Delhi: New age international. pp 117-188.

[4] Absar. A., Qureshi, Dhirendra, B, Sanghai, and Padgilwar S.S (2006) Herbal options for Contraceptions: A Review Pharmacology magazine. *Antifertility effects of male swiss albino mice* 664.

[5] Sharma, S., Kumar, M., Goyal, R.B., Manivannan, B., and Lohiya, N.K (1999) Reversible antispermatogenic effect of Gossypol in longur monkeys (Presbytis entellus). *Adv. Contraception,* 15, 15.

[6] Shetty, S., Udupa, S. and Udupa, E. (2008). Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* L. in rats. Evid. *Based complement Alternal Med*. 5:95-101.

[7] Panday, G., (2009). An overview on certain anticancer natural products. *J. Pharam. Res.* 2 (12):1799-1803.

[8] Suanarunsawat, T., Boonnak, T., Ayutthaya, W.D., and Thirawarapan, S. (2010). Antihyperlipidemic and cardioprotective effects of *Ocimum sanctum* L. fixed oil in rats fed a high fat diet. *J. Basic Clin. Physiol. Pharmacol*. 21 (4): 387-400.

[9] Chopra, R.N., Nayer, S.L. and Chopra, I.C.1956. Glossary of Indian Medicinal Plants 330. *Council of Industrial and Scientific Research Publication. New Delhi* (1959).

[10] Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapur, L.D. 1958. "Indigenous drugs of India*". U.N. Dhar and sons Pvt. Ltd. Calcutta* 816.

[11] Batta, S, K. and Santhakumari, G. 1970. The antifertility effect of Ocimum sanctum L. and Hibiscus Rosa sinensis. *Indian J. Med. Res*. 59:777-781.

[12] Vohra, S.B., Garg, G.K.Choudhary, R.R. (1969). Antifertility screening of plants part III. Effect of six indigenous plants on early pregnancy in albino rats. *Indian J. Med. Res.* 57: 893-9.

[13] Kasinathan, S., Ramakrishan, S. and Basu, S.L. (1972). Antifertility effect of Ocimum sanctum L. *Indian J. Exp Biol*.10: 23-5.

[14] Prakash, P and Gupta, N. (2005). Therapeutic use of Ocimum sanctum Linn. (Tulsi) with a note on eugenol and its Pharmacological actions: A short review. *Indian J. Physiol. Pharmacol*. 49 (2):125-131.

[15] Ahmed, M., Ahamed, R.N., Aladakatti, R.H. and Ghosesawar, M.G.2002. Reversible antifertility effects of benzene extract of Ocimum sanctum leaves on sperm parameters and fructose content in Rats. *J. Basic Clin. Physiol. Pharmacol*. 13:51-9.

[16] Elission, R. 1975. Analysis of semen, Rm progress in infertility. Eds. Behrman, S.J. and Kistner, R.W. Little Brown and Company Boston, Vol-II in edition chapter 33.pp. 693.

[17] Tijee DY, Ontoeng S. (1968). The viscosity of Human Semen and the percentage of motile spermatozoa, *Fertile Steril*. 19: 562-565.

[18] Sood, A text book of Lab. Techniques Chapter 13, Semen Analysis. (1987); 258- 260.

[19] Zeherea, M.N., Reddy, S.P., Reddy, S. P., Ravindra, and saraswati, B. Patil (1998) Antispermatogenic and androgenic activities of Momordica Charantia (Karela) in albino rats. *J. Ethnopharmacol*. 61, 9-16.

[20] Setty, B.S (1979) Regulation of Epididymal function and sperm maturation endocrine approach to fertility control in male Endocrinology. 74,100-117.

[21] Roa, M.V. (1988) Effect of alcoholic extract of *Solanum xanthocarpum* seeds in aldult male rats. *Indian J. Exp. Biol.* 26, 95-98.

[22] Roa, M.V., and Mathur, N (1988) Estrogen induced effects on mouse testis Epididymal spermatozoa. *Experimental and clinical Endocrionology*. 92,231-234.

[23] Roa, M.V., and shah, K.D (1988) Endocrine approach to male fertility control by steroid hormone combination in rat Rattus Norvegicus. L. *Indian Journal of Expt Biol*; 36: 775-779.

[24] Khanna, S., Gupta, S.R. and Grover, J.K. (1986). Effect of long term feeding of Tulsi (*Ocimum sanctum* L.) on reproductive performance of adult albino rats. *Indian J. Exp. Biol*. 24:302-304.

[25] Kantak, N. M., and Geogate, M.G (1992) Effect of short term administration of tulsi (Ocimum sanctum Linn) on reproductive behavior of adult male rats. *Indian J. Physiology and Pharmacology*. 36,106-111.

[26] Kumari, V.G., Manonyaki. S., Padma, S., and Malini, T (1989) Antifertility effect of *Bambusa arundinacen* shoot extracts in male rats. *J. Ethnopharamacol*. 25, 173-180.

[27] Lohiya, N.K. and Goyal, R.B. (1992) Antifertility investigations on the crude choloroform extract of *Carica papaya* linn seeds in albino male rats. *Ind. Pharmacognosy.* 30, 308-320.

[28] Ghosesawar, M.G., Ahamed, N. R., Ahmed, M., and Aladdkatti, R.H (2003) Azadirachata indica adversely affects sperm parameters and fructose levels in vas deferens fluid of Albino Rats. *Basic and Clinical Physiology and Pharmacol.*14940, 387-395.

[29] Chinoy, N.J., and Mehta, D (1999) Effects of protein supplementation and deficiency on fluoride induced toxicity in reproductive organs of male mice. Fluoride. 32 (4): 204-214.

[30] Mishra R.K., and Singh S.K. (2009) Antispermatogenic and antifertility effect of fruits of *Piper nigrum* L., in mice. *Indian Journal of Experimental biology*. 47, pp: 706-714.

[31] Singh, A., and Singh, S.K (2008) Reversible antifertility effect of aqueous leaf extract of *Allamanda cathartica* L. in male laboratory mice. Andrologia. 4(6), pp 337-345. Seth, S.D., Johri, N. Sundaram, K.R. (1981). Anti-spermatogenic effect of *Ocimum sanctum. Indian J. Exp. Biol*. 19:975-6.

Source of Financial Support: Nil

Conflict of interest: Nil

