https://doi.org/10.48047/AFJBS.6.5.2024.9314-9322

African Journal of Biological Sciences

The Efficacy of *Ocimum sanctum* (Linn.) Leaves Extract Exhibiting Anti-Fertility Activity in Male Albino Mice

Vinod Ragade[a], Rahi Sarnobat[a], Shekhar Phadtare[a*], Preetha Achary[a], Veena Menon[a], Kiran Kharat[a], Abhay Morajkar[a], Rajani Sirsath[a], Ajit Kengar[a*],

[a]Dept. of Zoology, KET's V. G. Vaze College (Autonomous), Mulund, Mumbai, India,400081

Corresponding Author

*Shekhar Phadtare, KET's V. G. Vaze College (Autonomous), Mulund, Mumbai, India, 400081 Email- biologicalscience98@gmail.com, Contact No.- 9096183082

*Ajit Kengar, KET's V. G. Vaze College (Autonomous), Mulund, Mumbai, India, 400081 Email- ajitkengar@vazecollege.net, Contact No.- 9920978380

Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi:*10.48047/AFJBS.6.5.2024.9314-9322*

ABSTRACT

Ocimum sanctum Linn. (Family: Lamiaceae), commonly known as 'Tulsi' is used as a traditional medicine in India due to its versatile therapeutic properties, which contain certain secondary metabolites affecting physiological and biochemical process in humans. In the present study, we have investigated the effect of *Ocimum sanctum* on the fertility of albino mice. The oral dosage administration of the leaves extract of *Ocimum sanctum* in male mice was monitored for 15 days to 30 days. The sperm motility was evaluated with sperm counting using a hemocytometer and the presence of different biomolecules that is secondary metabolites and other herbal compounds in the leaf extract of OS was analyzed using Quadrupole Time of Flight Mass Spectroscopy (QTOF). The phytochemical screening depicted presence of alkaloids, flavonoids and saponins in the leaf extract of the plant. The antifertility effect of the plant might be due to the chemical compounds such as apigenin, luteolin, hopanes, galangin, pyrrolidine and tryptohypol present in the extract. The results reported the progressive decline in the sperm count, sperm motility, whereas a significant increase in sperm abnormalities was observed in case of the test male albino mice. This indicated that the secondary metabolites present in the plant shows the negative effect on the process of spermatogenesis in mammals. Thus, the leaves of *Ocimum sanctum* confirmed as an effective anti-fertility agent in the albino mice.

Keywords: *Ocimum sanctum*, sperm motility, spermatogenesis, anti-fertility.

1. INTRODUCTION

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). The different parts of *Ocimum sanctum*. Linn. (Leaves, stem, flower, roots, seeds and even whole plant) have been proved beneficial for the treatment of ailments and diseases in humans. The juice of the Tulsi leaves possesses diaphoretic, antiperiodic, stimulating and expectorant properties. Eugenol (1-hydroxy-2-methoxy-4- allylbenzene), the active constituent present in *Ocimum sanctum* Linn has been found to be largely responsible for the therapeutic potentials of Tulsi. Various herbs have been used from a long time to induce infertility, and modern research has tested and confirmed anti-fertility effects in most of the herbs (Sharma, et.al., 2003). In India, the medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day-to-day practice. About 1200 to 1500 are used in Ayurveda and 300 plants are known to cause interference in spermatogenesis which can be used as the oral contraceptives. The study of Poli and Challa (2019) revealed that the administration of EUG, and OS Linn. leaf extract has significant antifertility activity, because of the presence of EUG in the OS Linn. leaf extract. The mechanism of action of EUG, the active constituent of this plant is not yet fully established. The plant is a rich source of various components including eugenol (EUG), Vicenin- 2, linoleic acid, oleic acid, rosmarinic acid, Ocimarin, isorientin, orientin, isovitexin, aesculectin, aesculin, chlorgrnic acid, galuteolin, circineol, gallic acid, Citronellal, Camphene, Sabinene, Dimethyl benzene, Myrecene, Ethyl benzene, Limocene, Vitamin C, Calcium, Phosphorous and many more (Joseph and Nair, 2013). The efforts have been made on plant-based drugs and a large number of plants have been tested experimentally to find out their antifertility activity as the prolonged use of oral contraceptives have a negative impact on human health. The present study was undertaken to investigate the effect of the leaf extract of *Ocimum sanctum* and its compounds on the fertility of male mice.

2. MATERIALS AND METHODS

2.1. Collection of the plants and the crude extract of leaves:

The leaves of Ocimum sanctum were collected locally from garden area. The extract was given orally to the male albino mice weighing 42 g each, purchased from Haffkine Institute, Parel, Mumbai. The mice were maintained in a well-ventilated animal house with standard mouse pellet and water.

Figure No. 1 Leaves of *Ocimum sanctum* Linn.

2.2. Grouping of Mice for Experimental Work:

The mice were divided into two groups comprising two individuals in each group.

Group I (Control Mice): The mice were ingested approximately 1.0 ml of distilled water and 3.54 g of standard mouse pellet food per mouse per day for a period of 19 days.

Group II (Test Mice): The mice were administered approximately 1.0 ml of *Ocimum sanctum* leaf Extract in water per mouse per day along with standard mouse pellet diet. (Approximately, 3-4 g per mouse per day) for a period of 19 days.

The weight of the mice was monitored for the Control group and Test group. After anaesthetizing the rats, the Cauda epididymis from the mouse were dissected and all fat and blood were cleaned off. This was achieved by placing the organs on a paper tissue. The cleaned organs were transferred to a watch glass containing PBS buffer (pH 7.4) and using forceps the epididymis was minced and the sperms were gently squeezed out of the epididymis. The Morphology (abnormality) was evaluated on sperm from the caudal epididymis. The Sperm counts were detected by using hemocytometer.

2.3. Evaluation of Sperm Motility

A sample of semen was diluted in PBS buffer (pH 7.4). About 10 to 20 microliter of this sample was pipetted onto a clean, prewarmed microscope slide with a cover slip onto the sample, avoiding formation of air bubbles. The slide was examined using a microscope with a 40X objective. At least 4 widely spaced fields were examined to provide an estimate of the percentage of motile sperms.

2.4. Determination of Sperm Count using Hemocytometer

A 1:100 dilution of the sperm sample was obtained accurately by pipetting 0.1 mi of semen into 9.9ml of PBS Buffer (pH 7.4). Both sides of the hemocytometer need to be filled and counted. The microscopic analysis was done using 10X objective focusing on one chamber grid, further to 40X objective and the sperms dispersed in the middle square and the four corner squares of the 25 squares in the grid were counted.

Calculations:

The numbers obtained from counting the first and second chamber should be averaged.

To obtain the number of sperm cells per ml of the ejaculate the following formula was used.

$$\textbf{Spz/ml = N *5* Dilution factor* 10,000}$$

Where,

N is the average number of sperm cells counted per chamber

5 is a correction factor needed because on the chamber only 5 of the 25 chambers are counted i.e., 25/5=5.

10,000 is a correction factor needed because the volume beneath the cover slip is 0.0001 ml per chamber.

When the Dilution Factor is 1:100, then Spz/m1=5*N where the number of sperms cells is in millions.

3. RESULTS AND DISCUSSION

For Control Mouse:

Number of cells counted in chamber 1:14

Number of cells counted in chamber 2: 8

Average number cells counted per chamber (N): 14 + 8/2 = 22/2 = 11

Spz/ml = 5 * 11 = 55 million sperms/ml

For Test Mouse:

Number of cells counted in chamber 1:4

Number of cells counted in chamber 2: 4

Average number cells counted per chamber (N): 4 + 4/2 = 4

Spz/ml = 5 * 4 = 20 million sperms/ml

PARAMETERS	CONTROL MOUSE	TEST MOUSE
SPERM MOTILITY	68.57 %	31.0 %
SPERM COUNT	55 million sperms/ml	20 million sperms/ml
CHANGE IN BODYWEIGHT	Increase By 1-1.5 g	Decrease By 6-7 g

Table No. 1. Parameters of control mouse vs test mouse

The remedy to control human fertility has become the need of time with measures such as vasectomy, tubectomy, tubal ligation, oral contraceptives are quite effective and the risks associated with them have triggered newer plant products to be used as anti-fertility agents. The present study focused on the anti-fertility effect of aqueous crude leaf extract of *Ocimum sanctum* on male albino mice. A significant decrease in the sperm motility, sperm count and an increase in sperm abnormalities was observed in case of the test mice as compared to the control mice, thus have proven to be an effective anti-fertility agent. The leaves of *Ocimum sanctum* have been reported to contain active compounds viz Ursolic acid which possess antiandrogenic properties. The phytochemical analysis of *Ocimum sanctum* Linn. has revealed the presence of alkaloids, carbohydrates, flavonoids and saponins, which includes apigenin, lureolin 7- neohesperidoside, hopane-29-acetate, N-(14-Methylhexadecanoyl) pyrrolidine, galangin and tryptohypol. The antifertility effect of the plant might be due to the presence of these chemical constituents in the leaf extract. There are many chemical constituents present in *Ocimum sanctum* such as, oleanolic acid, rosmarinic acid, ursolic acid eugenol, linalool, carvacrol, β elemene, β caryophyllene, germacrene and thus, considered to have diuretic, stimulant property (Falagas ME and Bliziotis IA, 2007).

Ocimum sanctum has various properties such as antistress, antiseptic, analgesic, anti-inflammatory, antimicrobial, immunomodulatory, hypoglycemic, hypotensive, cardioprotective and antioxidant (Tanwar et al., 2015). The prolonged estrous cycle in eugenol treated rats potentially lowered frequency of ovulation and result in the impairment of fertility, which is in accordance with the findings of Poli and Challa (2019). The aqueous crude extract of *Ocimum sanctum* leaves is expected to produce a similar anti-androgenic effect in case of human beings too. Moreover, studies have revealed Tulsi to be a Reversible Anti-Fertility Agent. Tulsi-derived fertility inhibitors, besides being reversible in nature would have such es nausea, hemorrhage; infections are expected by consumption of Tulsi leaf extract Classically, a, typical dosage of 10- 20 ml of fresh Tulsi –Leaf extract per day is expected to bring about the desirable effect.

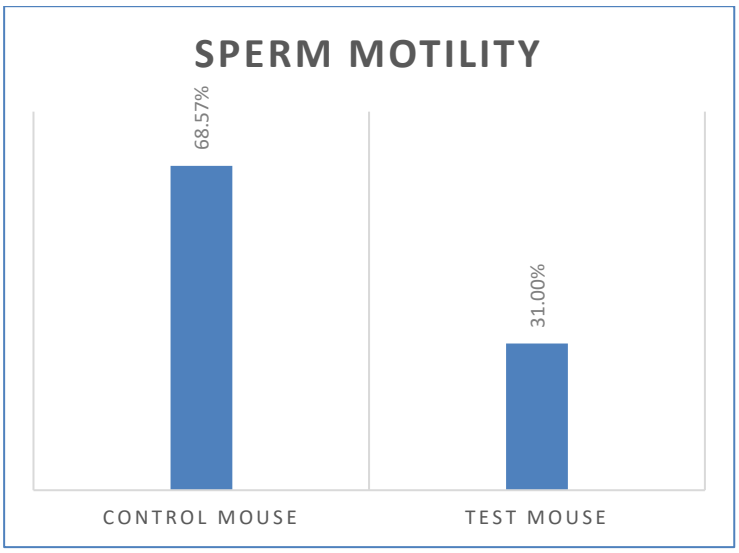

Figure No. 2. Percentage of Sperm Motility between Control Mouse vs Test Mouse

The 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 validates 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.

3.1. Decrease in Sperm Motility:

Sperm motility comes from sperm maturation which takes place in the epididymis under the influence of epididymal proteins Epididymal provides are androgen dependent. The percentage of sperm motility depicted decreased value of 31.08% in test mice, whereas the control mice reported 68.57% of sperm motility. Thus, androgen deprivation caused by the active compounds present in *O. sanctum* leaves would adversely affect sperm maturation.

3.2. Decrease in Sperm Count:

Sperm count is directly dependent on the hormonal input from the hypothalamic pituitary-testis axis. Lowering of FSH and testosterone by the active compounds of *O. sanctum* leaves would slow down the process of spermatogenesis and thus reduce the sperm count considerably.

3.3. Determination of Sperm Abnormalities

In the study of sperm morphology, the abnormalities such as double headed sperms, sperms with bifurcated tail, sperms with elongated head, sperms with detached head, sperms with bifurcated heads, two tailed sperms and comma shaped sperms were reported in the treated mice. The sperms of the control mouse were found to be normal except for a very few (1 in 100) were found to have coiled tails. No other abnormalities were recorded.

On the 20th day the weight of the control mice and test mice was observed as follows:

Mouse	**Weight of day 1 (in gms)**	**Weight of day 20 (in gms)**
Control mice	38, 40	39.5, 41
Test Mice	42, 42	40, 35

Table No. 2. Body weight of the control mice and test mice

3.4. Increase in Abnormalities in Sperm Morphology:

The production of normal sperms takes place under the influence of proper proportion of sex hormones in the body of an individual. A low proportion of androgens is expected to cause several. abnormalities in the sperm morphology, thus, making the sperms non-functional or abnormal. In accordance with the effects caused by androgen deprivation due to *Ocimum sanctum* leaf extract, it would be of great value to develop plant-derived fertility inhibitors that would be selective to the human reproductive system**.**

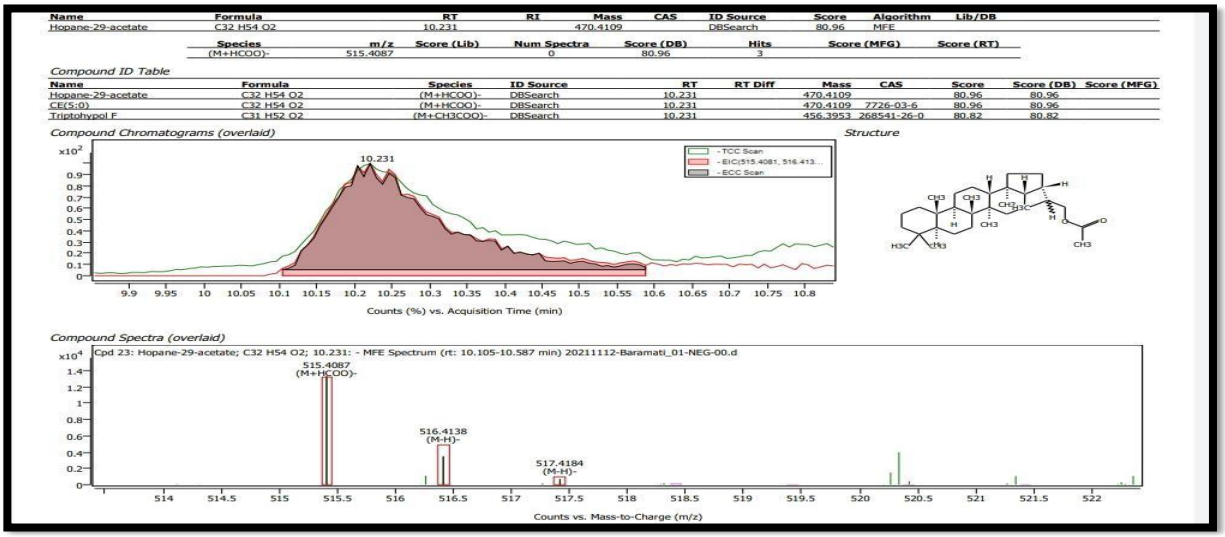

Figure No. 3. Compound name: N-(14-Methylhexadecanoyl) Pyrrolidine

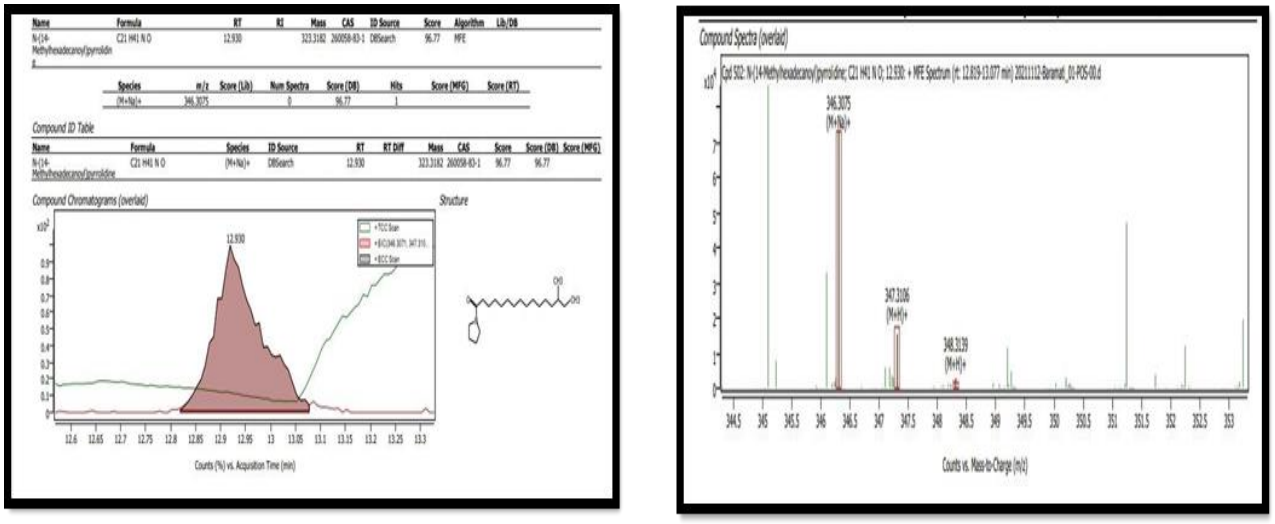

Figure No. 4. Compound Name: Hopane-29-acetate

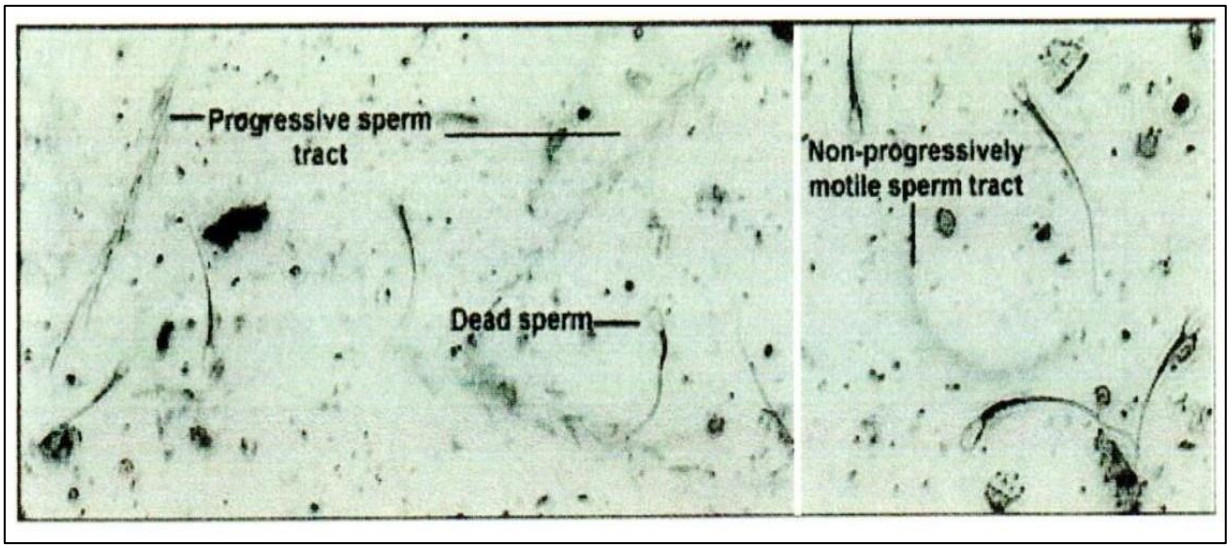

Figure No. 5. Micrographs of Progressive sperm tract and non-progressive motile sperm tract

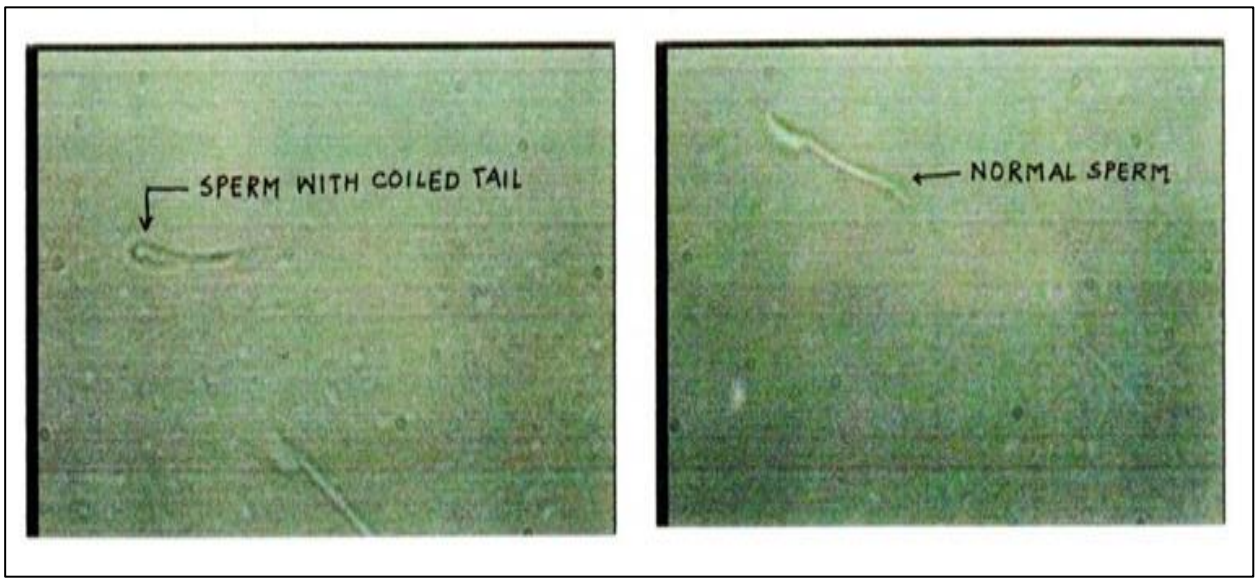

Figure No. 6. Microscopic images of sperm with coiled tailed and normal sperm

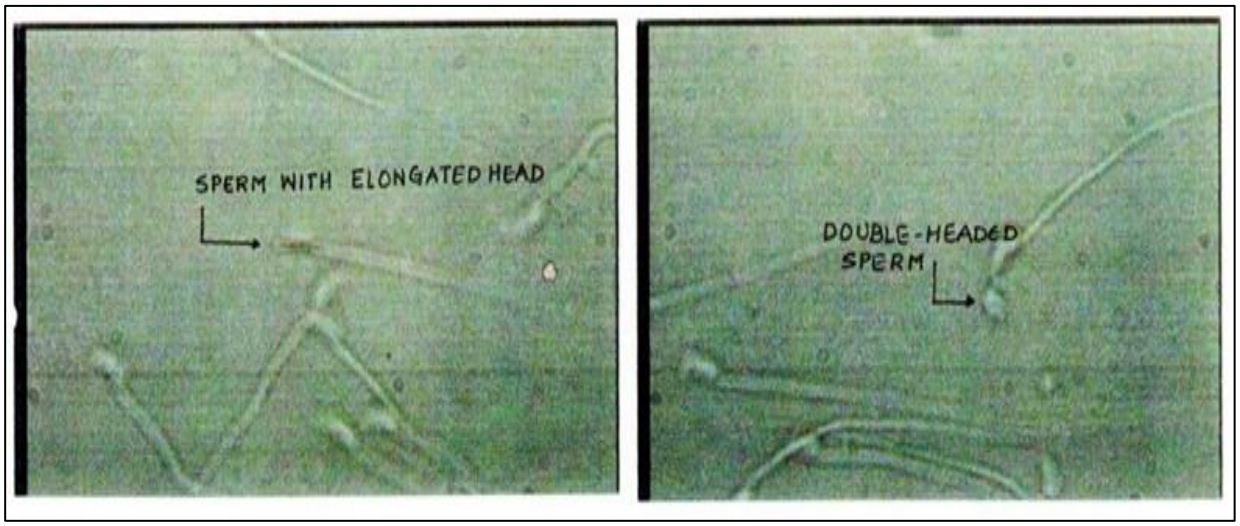

Figure No. 7. Microscopic images of sperm with elongated head and double-headed sperm

4. CONCLUSION

The present study concluded that the effects are due to androgen deprivation caused by anti-androgenic property of *Ocimum sanctum* leaf extract. The reduction in the number of sperms and motility might be due to low concentration of testosterone, which is due to the presence of chemical compounds such as apigenin, luteolin, hopanes, galangin, pyrrolidine and tryptohypol present in the treated leaf extract of *Ocimum sanctum*. Thus, the leaf extract of *Ocimum sanctum* can prove to be a promising and effective anti-fertility agent.

5. REFERENCE

1] Falagas, M. E. and Bliziotis, I. A., (2007). Pan drug-resistant Gram-negative bacteria the dawn of the post antibiotic era. *International Journal of Antimicrobe Agent*. 29, 630-636.

2] Joseph, B., and Nair, V. M. (2013). Ethanopharmacological and Phytochemical Aspects of *Ocimum sanctum* Linn- The Elixir of Life. *Journal of Pharmaceutical Research International*. 3(2), 273- 292.

3] Li, H., Zhang, M., Yan, F., Zhang, Z. and Li, Z. (2010). Effect of apigenin on the reproductive system in male mice. *Health*, 2, 435-440.

4] Panday, G., (2009). An overview on certain anticancer natural products. *Journal of Pharmaceutical Research.* 2 (12), 1799-1803.

5] Poli Venkataramanaiah and Challa Changamma (2019). A comparative study of eugenol and Ocimum sanctum Linn. leaf extract on the antifertility effect in female albino rats, *Journal of the Chinese Medical Association*,82(3), 231-234.

6] Sharma, R.K., Chopra, G., Bhushan, G. and Munjal, K. (2003). Use of Indian plants in fertility regulation in mammals. *Journal of Experimental Zoology- India*, 6, 57-74.

7] 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. *Evidence Based Complementary and Alternative Medicine*. 5, 95-101.

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. *Journal of Basic and Clinical Physiology and Pharmacology*. 21 (4), 387-400.

9] Tanwar, R., Pahare, A. and Naqvi, S. M. A. (2015) Ocimum sanctum (tulsi), excellent source of phytochemicals. *Indo-American Journal of Pharmaceutical Research*, 12(5), 2231-6876.

***