



# International Journal of Research in Pharmacy and Life Sciences

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## Research Article

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### Female Antifertility Activity of *Leonotis Nepetifolia* in Wistar Albino Rats

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#### ABSTRACT

*Leonotis nepetifolia* is a folk medicinal plant with antifertility potentials. Present investigation is to study the effect of ethanolic extract of whole plant of *Leonotis nepetifolia* on hormone and reproductive parameters of female rat. Ethanolic extract of plant was administered orally to female rat for 20 consecutive days. Estrous cycle, reproductive hormones (LH, FSH and progesteron) and weight of reproductive organ were studied in both control and extract-administered groups by using standard methods. The female albino rats after oral administration of different doses of ethanolic extract of *Leonotis nepetifolia* showed a significant antifertility activity. FSH and LH level was significantly decreased in both drugs while amount of progesteron in ethanolic extract of high dose treated animals was found to be increased. The extract decreases the weight of ovary and increases the body weights. Based on the evidenced results it was concluded that ethanolic extract of whole plant of *Leonotis nepetifolia* cause endocrinological and physiological changes in the reproductive system of female albino rats after oral administration of different doses of ethanolic extract which justifies for its anti fertility activity.

**Keywords:** Serratiopeptidase, Serratia marcescens, Matrix tablets, Ethyl cellulose, Hydroxy propyl methyl cellulose.

#### ARTICLE INFO

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**Article History:** Received 31 May 2015, Accepted 25 July 2015, Available Online 24 November 2015

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PAPER-QR CODE

**Citation:** I. Sarath Chandiran, et al. Female Antifertility Activity of *Leonotis Nepetifolia* in Wistar Albino Rats. *Int. J. Res. Pharm, L. Sci.*, 2015, 3(2): 325-329.

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## 1. Introduction

Ancient Indian literature abounds information on a large number of plants reported to have abortifacient, contraceptive and sterilizing properties and scholars of Ayurveda have also mentioned several plants preparations. Medicinal plants have been used by the women of rural communities and especially by tribals to prevent conception. [1] *Leonotis nepetifolia* is an erect, branched herb belongs to family lamiaceae. It is commonly referred to as Lion's ear and Christmas candlestick [2]. *Leonotis nepetifolia* is found in the all over the world especially tropical Africa and southern India. The whole plant is used for the menstrual pain and unspecified female complaints. The plant is being used by the local peoples and tribal of Maharashtra as ethno medicine on various ailments. The infusion of leaves is traditionally being used to cure the stomach pain of the children and also to cure cough and cold by tribals of Melghat (MS) India [3]. The present study was undertaken to find out the unexplored antifertility and hormonal activities of the ethanolic extract of whole plant of *Leonotis nepetifolia*.

## 2. Materials and Methods

### Experimental Animals

Female Wistar rats (*Rattus norvegicus*), three months old, weighing 110 to 150g were acclimatized for 7 days. The animals were housed in standard cages, three per cage, in a controlled temperature room (22°C), with a 12 h light: 12 h dark cycle. Standard laboratory food pellet and tap water were available ad libitum.

### Plant material

The whole plant of *Leonotis nepetifolia* was collected from Kalakadu, Thirunelveli district. Plant material was dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. 50g of powdered plant was extracted with 500mL of ethanol at 60°C. Then extract was poured in petri plates and allowed to air dried [4].

### Screening of Antifertility Activity

For the evaluation of anti fertility activity of plant extract following studies was carried out. Effect of the extract on estrus cycle, study of biochemical parameters FSH, LH and progesterone level and study on body weight and reproductive organ weights.

### Experimental Design

The animals were divided into 4 groups 3 animals per group. Group 1 – Control Group 2 -100mg/kg (plant extract), Group 3 -200mg/kg, Group 4 - 300mg/kg. Estrus cycles of all animals were determined by taking vaginal smear and it has been monitored for 7 days. Prior to the treatment the animals were maintained without water for 24 hours. At the estrus stage, the animals in Group1, 2, 3 & 4 was orally fed with vehicle, 100mg/kg, 200mg/kg and 300mg/kg of plant extract as single bolus in 300µL of 0.1% ethanol in saline. The treatment for the animal was continued for 20 days. After treatment leave it for 24 hours and when the estrus cycle is observed, weight of the rat is noted. Estrus cycles of all animals were determined by taking vaginal smear and it has been monitored for 7 days.

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### Effect on estrous cycle

#### Preparing the Vaginal Smear

During one month, every morning between 8:00 and 9:00 a.m., vaginal smear cytology was performed for determination of rat estrous cycle phases and to ensure regular cycles. Vaginal secretions were collected using a cotton-tipped swabs softened with a drop of saline (NaCl 0.9%). After 1-2 inches of the swab had been inserted, the end had been rotated through 2-3 revolutions, which will allow the cotton tip to pick an adequate load of cells. The swab was then gently withdrawn. The smears were prepared immediately after withdrawal of the swab by rolling (not sliding or rubbing) (lay cotton tip along the length of a glass slide. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40 x objective lenses. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases [5, 6].

### Effect on sex hormones, body weights and reproductive organ weights

After the treatment, the control and treated rats were dissected with the help of chloroform. Female rat organs of kidney, liver, uterus were washed with saline, antibiotic solution for three times. Blood is collected by cardiac puncture with help of the 5ml syringe, poured into 15 ml centrifuge tube and kept without disturbance for serum separation. The organs such as kidney, liver, uterus were taken out and kept in 15 ml centrifuge tube filled with saline + antibiotic solution. Organs are weighed in weighing machine with the help of aluminium foil and stored in freezer -20°C until use. The blood is centrifuged at 2500 rpm for 5 minutes. Supernatant solution (serum) is collected in micro centrifuge tube.

### FSH Analysis

Serum FSH levels were measured using enzyme-linked immunosorbent assay kit. Take 96 well plate placed in well stand. Gently thaw the FSH kit and serum. Add 50µl of calibrate A, B, C, D, E, F to the respective wells and add 50µl of serum to the next wells. And incubate for 15 minutes. Add 100µl of conjugate to the respective wells and incubate for 45 minutes. Decant the solution in the discarding jar and tap the well with the help of the tissue paper. Wash the wells with 200µl of water for 2 times and decant the solution in discarding jar and tap the well with the help of the tissue paper. Add 100µl of substrate and incubate for 20 minutes at darkness. The color was changed into blue color. And then add 100µl of stop solution for stopping the reaction. The color was change into yellow color. The reading was taken at 450 nm in spectrometer

### LH Analysis

Serum LH levels were measured using enzyme-linked immunosorbent assay kit. Take 96 well plate and kept in well stand. Gently thaw the LH kit and serum. Add 50µl of calibrate A, B, C, D, E, F to the respective wells and add 50µl of serum to the next wells. Incubate for 15 minutes. After incubation add 100µl of conjugate to the wells.

Incubate for 45 minutes and after incubation decant the solution in the discarding jar and tap the well with the help of tissue paper and wash with distilled water twice and decant the solution. Add 100 $\mu$ l of substrate to each well. The color changes to blue color. Incubate for 20 minutes at darkness. Add 100 $\mu$ l of stop solution for stopping the reaction. The color changes into yellow color. The reading was taken at 450nm in spectrometer.

#### Progesterone Analysis

Serum progesterone levels were measured using enzyme-linked immunosorbent assay kit. Take 96 well plate and placed in well stand. Add 25 $\mu$ l of standard solution, control solution in each well and test solution to the respective wells. Add 100 $\mu$ l of prepared conjugate and 50 $\mu$ l of anti progesterone reagent to the respective wells. Gently mix the solution for 30 seconds. Then incubate the solution at room temperature for 90 minutes. After that discard the solution in discarding jar and tap the wells with the help of the tissue paper and add 300 $\mu$ l of distilled water and decant the solution in discarding jar and tap the wells with the help of the tissue paper. Add 100 $\mu$ l of substrate and incubate at dark room temperature for 20 minutes. The solution changes into blue color. Finally add 100 $\mu$ l of stop solution and mix thoroughly. The blue colored solution is turned into a yellow color solution and absorbance (OD) should be taken immediately within 10 minutes at 450nm.

#### Statistical analysis

All data for control and experimental groups were subjected to statistical evaluation, using analysis of variance (ANOVA) for significant differences between the controls and experimental groups at  $p < 0.05$ . All data were recorded systematically in preformed data collection sheet. Statistical analysis was performed by using SPSS for windows version 16.0. 95% confidence limit was taken.

### 3. Results and Discussion

#### Effect of Ethanolic Extract of *Leonotis nepetifolia* in Estrus Cycle

With regard to each estrus cycle stage animals treated for 20 days showed differential sequence of changes in the cytology of the vaginal smear. In rats receiving the plant extract especially at 300mg/kg showed an increase in the appearance of meta estrus phase while estrus phase of all animals in group 3&4 are decreased with 4 hour and 9 hours comparatively with control animals [Table 1, Figs. 1&2 ].

#### Effect of Ethanolic Extract of *L. nepetifolia* on LH, FSH and Progesterone Hormone in Female Rats

Ethanolic extract at different dose of levels of *L. nepetifolia* administered for 20 days exhibited its effect on ovarian endocrinology that is LH quantities. The unit of hormones such as FSH, LH and progesterone are expressed in mIU/ml. The plant extract does not seem to have any effect on FSH and progesterone level with increasing dose of plant extract. The concentration of LH hormone varies among control and treated animals [Table. 2].

#### Effect of Ethanolic Extract on Body and Female Reproductive Organ Weights

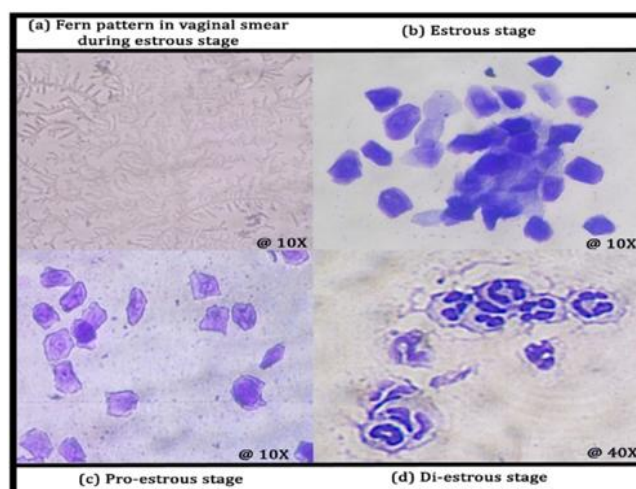
After oral administration of ethanolic extract of *L. nepetifolia* to mature female rats, animals showed no

change in their body weight. Ovaries along with oviduct are weighed in compare with the control showed slight decrease in their weight [Table. 3].

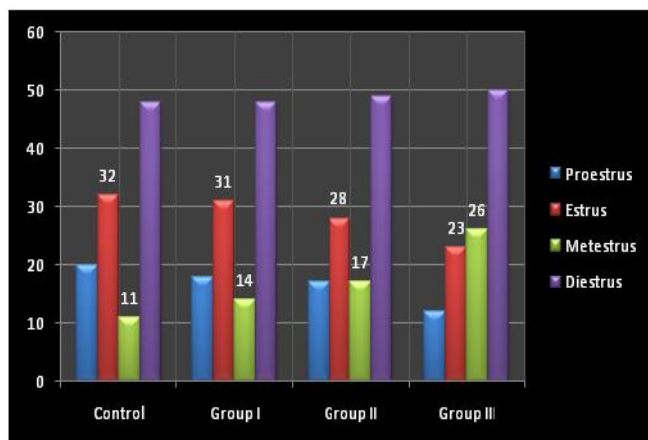
#### Discussion

*L. nepetifolia* did bring about adverse changes in the parameters of female reproduction, namely body weight, reproductive organ weight and estrous cycle, hormonal analysis. The female estrous cycle is generally considered as the time between periods of estrus (period of sexual receptivity). It comprises the recurring physiologic changes that are induced by reproductive hormones. The estrous cycle consists of four stages, viz. proestrus, estrus, metestrus (or diestrus 1), and diestrus (or diestrus 2). The study showed that administration of high dose of the ethanolic extract of *L. nepetifolia* to female rat's results in decreased estrus phase with a prolonged metestrous phase. Hence, anoestrus was not observed. It is generally believed that anoestrus, a period of reproductive quiescence between cycles, is permanent in healthy cycling rats and that estrus cyclicity only ceases during pseudo-pregnancy, pregnancy and lactation. But, the total number of estrous cycle days was not altered throughout the study.

The sex hormones are known to regulate the reproductive functions and characteristics in female organisms. Measurement of serum sex hormones profile is therefore very useful in assessing the reproductive integrity in both animals and humans. Serum level of LH, FSH and progesterone were lower than those of controls. On the contrary, Progesterone levels of serum were increased in high dose of extract treated groups. The compound (s) either may be having direct estrogenic effect or after getting metabolized the products formed might be acting in many ways. Although the specific mechanism(s) by which the plant extract cause a decrease in the levels of serum LH is not clearly understood [7]. The treatment of *L. nepetifolia* extracts (100, 150, 200mg/kg/bw for 55 days) to rats found that there is no effect on body weight. The reproductive organ weight decreased all the treated groups when compared to the control groups.



**Figure 1:** Photomicrograph of crystal violet stained smear of Control and *L. nepetifolia* ethanolic extract treated rats showing different stages of estrous cycles.



**Figure 2:** Comparison of different phases of rat estrous cycle between control and *L. nepetifolia* ethanolic extract treated rats.

**Table 1:** Mean no. of hours in each phase of estrus cycle in the Control and treated with *L. nepetifolia* extract on female rats

Groups	Treatment	No of animals	Proestrus (hr)	Estrus (hr)	Metestrus (hr)	Diestrus (hr)	Total Days
Group 1	Control	5	20	32	11	48	4.6
Group 2	<i>L. nepetifolia</i> (100mg/kg)	5	18	31	14	48	4.62
Group 3	<i>L. nepetifolia</i> (200mg/kg)	5	17	28	17	49	4.6
Group 4	<i>L. nepetifolia</i> (300mg/kg)	5	12	23	26	50	4.6

Values represent the Mean  $\pm$  SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with hoc testing least significant difference.

**Table 2:** Effect of control and treated female rats with *L. nepetifolia* extract on FSH, LH and Progesterone

Groups	Hormones (ng/ml)		
	FSH	LH	Progesterone
Group 1	5.05 $\pm$ 0.19	16.17 $\pm$ 0.23	0.221 $\pm$ 0.01
Group 2	5.025 $\pm$ 0.12	14.13 $\pm$ 0.14	0.196 $\pm$ 0.003
Group 3	4.57 $\pm$ 0.09	12.53 $\pm$ 0.21	0.167 $\pm$ 0.001
Group 4	3.55 $\pm$ 0.12	9.67 $\pm$ 0.27	0.181 $\pm$ 0.003

Values represent the Mean $\pm$ SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with hoc testing least significant difference.

**Table 3:** Effect of Control and treated with *L. nepetifolia* extract on Body and Reproductive organ weights in female rats.

Groups	Treatments	Body weight(g)		Reproductive organ weights(g)	
		Initial	Final	Ovary wt. (actual)/(mg)	Relative wt. of ovary (mg/100gm b.wt)
Group 1	Saline	125 $\pm$ 7.07	132 $\pm$ 3.05	2.2 $\pm$ 0.03	0.67 $\pm$ 0.02
Group 2	<i>L. nepetifolia</i> (100mg/kg)	120 $\pm$ 0.03	126 $\pm$ 0.43	1.45 $\pm$ 0.02	0.45 $\pm$ 0.01
Group 3	<i>L. nepetifolia</i> (200mg/kg)	116.6 $\pm$ 5.77	121 $\pm$ 1.52	1.37 $\pm$ 0.04	0.33 $\pm$ 0.02
Group 4	<i>L. nepetifolia</i> (300mg/kg)	123.3 $\pm$ 3.84	126 $\pm$ 2.80	1.15 $\pm$ 0.02	0.24 $\pm$ 0.03

Values represent the Mean $\pm$ SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with hoc testing least significant difference

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Values represent the Mean $\pm$ SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with hoc testing least significant difference.

## 4. Conclusion

The ethanolic extract of whole plant of *L. nepetifolia* as concluded from the results elevated estrogenic activity, which inhibits FSH secretion from pituitary therefore prevent the development of new follicle in ovary resulting inhibition of ovulation and impairment of fertility and thus contraception. Therefore, there remains definite scope for further research with this plant as they provide safe contraception, without disturbing general physical conditions, as they did not show any toxic effect in present study.

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