



## EUGENOL INDUCED CHANGES IN THE OVARIAN-ADRENAL-THYROID AXIS OF FEMALE ALBINO RATS

**Kulkarni D.S**

Associate Professor

Department of Zoology, Bharatiya Mahavidyalaya

Amravati, India

### Abstract

*Ocimum sanctum* Linn. (Tulsi), family Lamiaceae, is a sacred plant of India has been used from time immemorial in Ayurveda for treating and preventing various ailments. Eugenol, one of the bioactive compounds of *Ocimum sanctum*, was investigated for anti-fertility property in rats and its potential impact on the ovarian-adrenal-thyroid axis of female albino rats. The animals were divided in two groups as G1= test group was administered with eugenol mixed with olive oil (0.2ml/day/rat) for 30 days and G2= control group administered with corresponding dose of olive oil for the same duration. Vaginal cytology was studied daily by vaginal smear technique. At the end of the treatment the hormonal estimations (TSH, FSH & LH) were carried out in serum samples of both the groups and compared. Vaginal cytology revealed a notable disturbance in estrous cycle with a significant increase in duration of diestrous phase in experimental female rats. Histological observation of ovary showed significant decrease in healthy follicles as compared to control. The sections of adrenal showed the hypertrophy of the cells in the zona fasciculata and zona glomerulosa and zona reticularis was almost got compressed. The sagittal section of thyroid revealed follicle hypertrophy and hyperplasia as well as reduction in colloid in lumen of follicles. Pituitary after administration of eugenol resulted into hypertrophy of corticotropes. These histological alterations were associated with significant decrease in the serum levels of the hormones (TSH, FSH & LH). The suggested antifertility effect of eugenol is probably due to imbalance in the ovarian-adrenal-thyroid (OAT) axis.

**Keywords:** Eugenol, Antifertility, Thyroid, Adrenal, Pituitary, Albino Rats.

### Introduction

During the last few years, the use of herbal medicine has been rapidly growing all over the world. 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).

*Ocimum sanctum* (OS), commonly known as Tulsi or Holy basil, is a sacred plant of India has been used from time immemorial in Ayurveda, for treating and preventing various ailments. OS possesses properties such as Anti-diabetic, Anti-cancerous, Analgesic, Anti-inflammatory, Radioprotective, Hepatoprotective, Anti-microbial, Immunomodulatory effect, cardioprotective, Anti-coagulant, Anti-fertility, Antioxidant, Neuroprotective. The plant is a rich source of various components including eugenol (EUG), Vicenin-2, linoleic acid, oleic acid, rosmarinic acid, Ocimarin, isorientin, orientin, isovitexin, aesculetin, aesculin, chlorogenic acid, galuteolin, circineol, gallic acid, Citronellal, Camphene, Sabinene, Dimethyl benzene, Myrcene, Ethyl benzene, Limonene, Vitamin C, Calcium, Phosphorous and many more (Joseph and Nair, 2013). Effects of various extracts of OS and its components on reproductive performance such as gonadal function, estrous cycle, mating behavior, conception, parturition, lactation, and weaning in male and female rats have been studied by various researchers (Raina et al., 2018; Vohora et al., 1969; Kasinathan et al., 1972; Seth et al., 1981; Katak and Gogate, 1992; Akbarsha et al., 1998; Sardesai et al., 1999; Kulkarni 2011; Mankapure et al., 2013; and Srinivasulu and Changamma, 2017).

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. However, the antifertility capacity of EUG was indirect evidence. The mechanism of action of EUG, the active constituent of this plant is not yet fully established.

Female fertility is a biological process regulated by female hormones. Ovarian-uterine interrelationship forms an essential prerequisite for normal operation of sexual cycles in mammals (Hafez, 1970; Devi, 1992 and Guyton, 2009). Ovarian hormones are regulated through the Hypothalamus-Pituitary-Gonadal (HPG) axis. This axis exerts effect on the end organs like adrenal, thyroid and ovaries. These end organs are tied into another organ system hormonally via an axis called Ovarian-Adrenal-Thyroid (OAT). Reproductive organs, ovaries, and uterus are intimately dependent hormonally on this axis for optimal function. Hence, the current study was designed to find out the effect of intramuscular administration of EUG on the OAT axis.

### Materials and Methods

**Animals:** Healthy adult Wistar female albino rats weighing  $165 \pm 14$ g were procured from animal house of the Dr. Panjabrao Deshmukh Memorial Medical College, Amravati (MS, India). The animals were housed under controlled condition ( $28 \pm 2^\circ\text{C}$ ; 12:12hrL:D). The animals were kept in the experimental room for one week prior to the commencement of study, for acclimatization



of experimental conditions. The care and management of experimental animals was followed strictly as per ethical committee guideline. The animals were fed with standard pellet diet and water ad libitum for a period of 30 days. Vaginal smear was taken daily, and only female rats displaying at least two consecutive 4- day estrous cycle were used.

**Drug:** Eugenol (98% pure) Loba Chem.

**Experimental Setup**

- G1= Experimental female rats=6 rats, administered intramuscularly (im) with eugenol + olive oil, 0.2ml/day/rat up to 30 days. (0.2ml contains 200 mg eugenol).
- G2= Control female rats = 6 rats, injected intramuscularly (im) with 0.2ml olive oil /day/rat up to 30 days as vehicle.

**Estrous cycle study**

Daily the vaginal cytology of the control and experimental rats was studied by vaginal- smear technique as described by Hafez, 1970. Rats exhibiting 4-5 days estrous cycle of proestrus, estrous, metestrus and diestrus were considered as normal while any deviation from this pattern in terms of duration and sequence was categorized as abnormal (Gbotolorum, 2008).

**Hormonal Analysis**

After 30 days blood sample was taken from the orbital sinus of the rats for assessment of serum hormone levels (FSH, LH, Estrogen and Progesterone) from both the groups. The hormones were assayed by RIA using the method given by Shille, et al., 1983.

**Histological Study**

After period of treatment ovaries, pituitary gland and thyroid gland, were isolated from the rats and excess blood was removed using filter paper. They were quickly weighed on a digital balance and fixed for 24 hrs. in Bouins fluid and silver nitrate, respectively. The tissues, embedded in paraffin, were cut in 5µm thickness, and stained with regular hematoxylin-eosin solution for histological study. Histological study was done under light microscope at 100X.

**Statistical Analysis**

Students “t” test was used, P< 0.05 was regarded as moderately significant and P< 0.01 as significant.

**Result**

**Estrous Cycle Study:** Administration of eugenol to female albino rats exhibited an irregular pattern of estrous cycle. These rats showed significant decrease in number of estrous cycles and duration of proestrous, estrous and metestros phases. Experimental rats showed a prolonged metestrous and diestrous pattern in each cycle as compared to control (Table -1)

**Table 1: Estrous Cycle study in olive oil and eugenol administered (im) female rats**

Sr. No.	Stage of Estrous	Control	Eugenol Treated	% Change
1	Proestrus	5.01±0.12	1.12±0.11*	77.64
2	Estrus	7.31±0.29	3.28±0.18*	55.12
3	Metestrus	7.31±0.29	2.24±0.20*	69.35
4	Diestrus	12.06±0.38	23.26±0.34*	-92.86
5	Number of Cycles	5.81±0.16	2.00±0.18	65.57

Values are mean ± SE of six animals per group. \*p<0.05

**Histological Study:** The histological study of Pituitary after administration of eugenol resulted into hypertrophy of gonadotrophic cells with increased secretory granules (Fig.1.1). Degranulation in luteinizing hormone secreting cells (LH cells) was observed, the follicle stimulating hormone secreting cells (FSH cells) became regressed and the thyrotrops (TSH cells) showed swollen nuclei and less cytoplasm as compared to control (Fig.1.2). The Gonadotrophic cells showed hypertrophy with increased secretory granules.

The slide study of the eugenol treated adrenal gland showed hypertrophied cells of zona in the adrenal cortex and the hyperactive chromaffin medullary cells with distinct granulation (Fig.1.3 a and b) over the control (Fig.1.4 a and b).

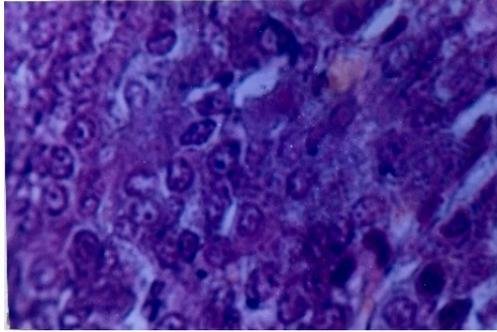


Fig.1.1: T.S. Pituitary of Eugenol 30 days

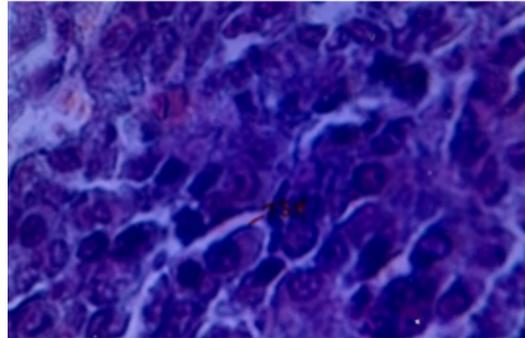


Fig.1.2: T.S. Pituitary of control olive oil administered treatment

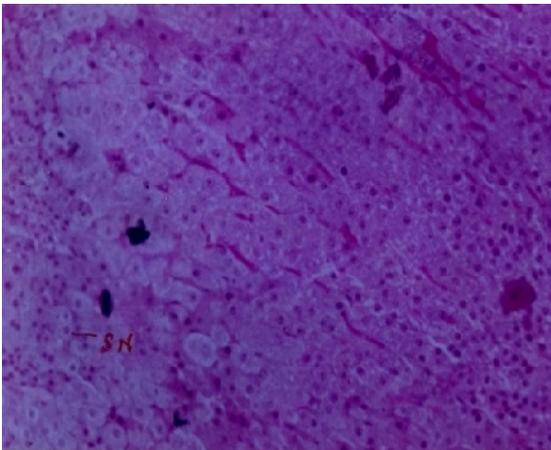


Fig.1.3a: T.S. adrenal cortex of Eugenol 30 days treatment

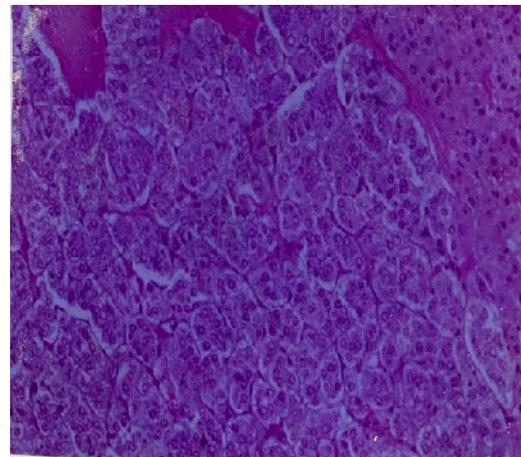


Fig.1.3b: T.S. adrenal medulla of Eugenol 30 days treatment

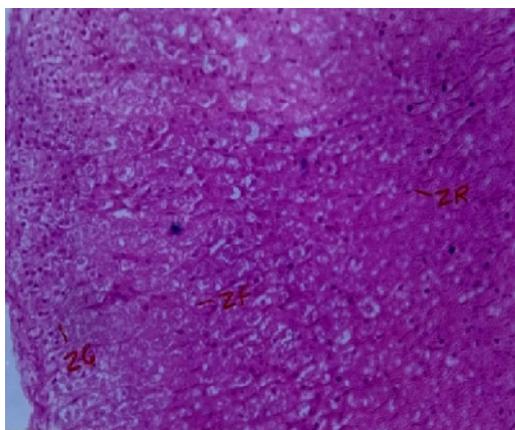


Fig.1.4a: T.S. adrenal cortex of control

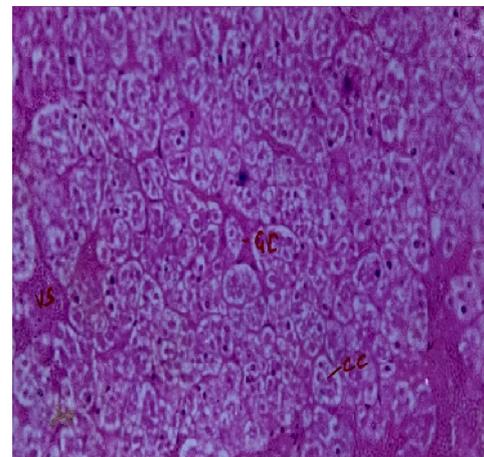
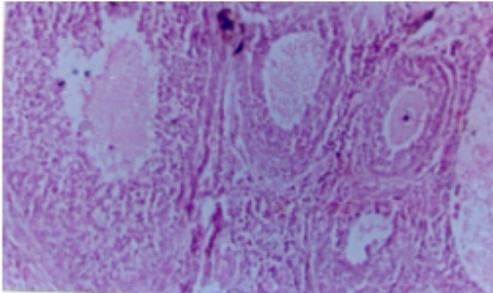
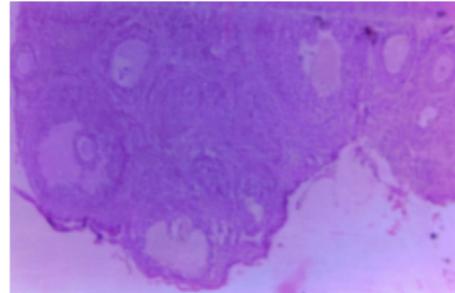


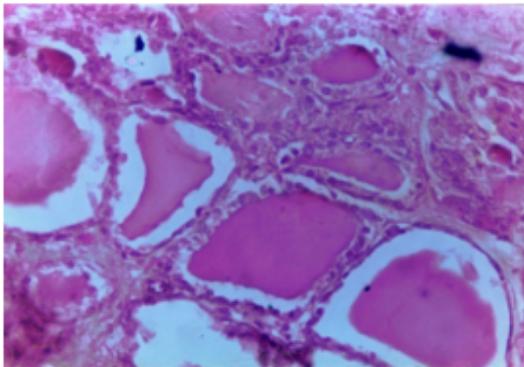
Fig.1.4b: T.S. adrenal medulla of control olive oil administered olive oil administered



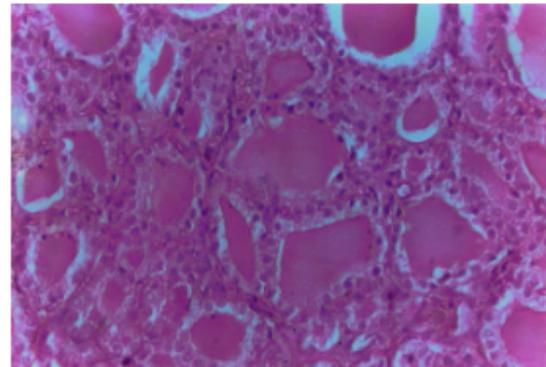
**Fig.1.5: T.S. ovary of control olive oil days administered**



**Fig.1.6: T.S. ovary of Eugenol 30 treatment**



**Fig.1.7: T.S. Thyroid of control olive oil days administered**



**Fig.1.8: T.S. Thyroid of Eugenol 30 treatment**

Considering the histological investigation on the influence of eugenol on rat's ovaries, mainly graafian follicles were seen on the photomicrographs of experimental animals. The study of follicular dynamics has revealed a significant decrease in the number of healthy follicles and increase in the number of atretic follicle in eugenol treated rats (Fig.1.5) as compared to control (Fig.1.6) indicating hypogonadotropinism. Disproportionate development of theca externa and ovarian stroma was visible on their ovaries.

The thyroid gland follicles were full of colloidal secretion (Fig.1.7). The collids showed lacunae arranged towards the periphery of colloidal secretion. The follicles were separated from the interfollicular connective tissue. The interlobular connective tissue showed vacuolation as compared with those of the olive oil injected controls (Fig.1.8).

### Hormonal study

**Table 2: Hormonal study in olive oil and eugenol administered (im) female rats**

Hormones Tested	Control	Eugenol Treated	% Change
FSH (MIU/ml)	4.25 ±0.07	1.15*±0.15	-72.94
LH (MIU/ml)	6.20± 0.05	2.15* ±0	-72.94
TSH (MIU/ml)	1.68±0.02	0.21**±0.03	-87.50
Estrogen(ng/ml)	30.20±1.15	12.70** ± 0.65	61.71
Progesterone	12.50±0.40	10.45 <sup>NS</sup> ±0.70	-16.40
Testosterone	0.98±0.04	0.65*± 0.01	-33.67
Cortisol (ng /ml)	126.79±41.83	183.74±40.24	0.05*

Values are mean ± SE of six animals per group. \*p<0.05; \*\* p<0.01, NS- not significant



The results in table 2 show that the FSH and LH levels of the experimental group were significantly decreased as compared to control. Estrogen and TSH estimated after 30 days treatment of eugenol showed highly significant decreased values in the experimental female rats. The outcome of the progesterone showed not significant decrease.

**Discussion:** The normal functioning of the reproductive tract is dependent on the normal functioning of the endocrine glands. One such intricate hormonal axis ties in the ovaries, adrenal and thyroid, called the Ovarian- Adrenal- Thyroid (OAT) axis. The important endocrine glands ovaries, the adrenals and the thyroid are dependent on each other for optimal function. These glands are in turn regulated by pituitary gland. If the adrenals are strained, this will often lead to poor thyroid function and irregular estrous cycles. Along the same lines, poor thyroid function will often damage adrenal function. Finally, ovarian hormonal imbalance will negatively affect thyroid function. In the present study the reduced size of ovarian follicles promoted by hypothyroidism could be associated with hypertrophy of the pituitary and adrenal cells. The significantly lowered levels of estrogen and progesterone were estimated to lowered FSH and LH levels in eugenol administered group of animals. In the present estimation the significant decreased levels of all the hormones indicate the disturbed AOT axis. The changes in the serum hormonal levels of OAT axis, might lead to disturbed reproductive cycle. 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).

### Conclusion:

The observations in the present investigation confirms the antifertility activity of eugenol described by various researchers. The histological and hormonal study signifies the imbalance in any of the stages of OAT axis indicate the mechanism of antifertility action of eugenol in female albino rats.

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