

# The Effect of Infertility From Some Biochemical Parameters in Eugenol and *Ocimum sanctum* linn. Leaf Extract on Female Albino Rats

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## ABSTRACT

**Background/Objectives:** The aim of the present study is to investigate infertility activities in albino female rats. Eugenol and *Ocimum sanctum* linn. effects. Leaf extracts on some biochemical parameters in ovary and uterus.

**Methods:** Healthy female albino rats are provided with EUG (99% pure) with a dose of 0.4 ml/ day/ rat and *Os linn.* Leaf extract with a dose of 500 mg/kg body weight/day/rat orally for 15 days.

**Results:** Data revealed that oral administrations of Eugenol and *Ocimum sanctum* also gets an antifertility effect in ovary and uterus. On day 15<sup>th</sup> all the animals were sacrificed and biochemical parameters were estimated.

**Conclusion:** These results suggest that it is known that the provision of estrogen has an uterotrophic effect on female rat. The uterine physiology and pathological conditions of infertility, endometrial cancer, and polycystic ovarian syndrome.

**Keywords:** Eugenol, *Ocimum sanctum*, Infertility, Ovary, Uterus

## INTRODUCTION

Anti-fertility agent is what will prevent ovulation or fertilization and ultimately intercept pregnancy [1]. Various experimental parameters, for investigating antifertility activities in women, previously reported [2,3]. At present, the most effective method to prevent conception is the use of steroids to inhibit or modify cyclic changes in endogenous hormone production. Control population in India has become a big problem with serious charges for the future. It has now been accepted in general that the method of fertility regulations is currently inadequate to meet varying personal needs and change partners at different times in their reproductive life, and in different geographical, cultural and religious settings that exist throughout the world [4]. There is a need to look for safe products that are suitable for genuine medicinal plants, which can effectively be used [5]. Many local plants have been identified and tested for the effects of their antibertility in rat and rat of women [6]. Reports that have been given to the neem antifertility effect. NADPH and oxygen are very important in 3 steps that are catalyzed enzymes required in the synthesis of estrogen in the ovaries of precursors such as androgens: Testosterone and Rostendione [7].

Eugenol is also an important chemical constituent of essential oils of many aromatic plants, such as *Eugenia Caryophyllus* [SPR].

Eugenol if Erum, and *Ocimum grasissimuml.* [8-11]. *Croton zehntneri* and *O. gyatissimum* Free *Simum* has rich essential oil content and is used in the treatment of east people in the Brazilian People as a fantastic, carminative, and intestinal antispasmodic [12].

Female fertility is a biological process organized by female hormones. The most common causes of female infertility are hormones that are generally associated with ovulation, polycystic ovarian syndrome, and pre-mature ovarian failure, damage to fallopian or uterine tubes or problems with the cervix [13]. The female reproductive cycle functions mainly by the interaction between lutenizing hormones, follicle stimulation hormones, progesterone, estradiol and testosterone [14]. Women's reproductive organs can be tested by this serum hormone-hormone level. *Ocimum sanctum* leaves is found to have an abortification effect on women. *Ocimum sanctum* also gets an antifertility effect. Benzene extract and petroleum Ether Tulsi leaves have been reported to produce 80% and 60% of antibertility activities, each in female rat [15]. *Ocimum sanctum* leaf has been shown to have anti-implantation activity in an experimental albino rat [16]. Phytochemical analysis reveals the existence of alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, glycosides, carbohydrates, protein and coumarins [17]. *Ocimum sanctum* leaf disrupts the estrus cycle and prolonged estrus stage. It also causes a decrease in the number of endometrial glands [16].

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## MATERIALS AND METHODS

### Animals study design

This research was conducted during November to January 2018. In this research healthy adult [4 months, weighing  $170 \pm 20$  g] Wistar strain female rat used. Rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. Animals are placed in a clean polypropylene cage in hygienic conditions in well ventilated air conditioned rooms, with 12 hour photoperiod light and 12 hour dark cycles, at  $25 \pm 2^\circ\text{C}$  with relative humidity of  $50 \pm 5\%$ . Rat fed with standard laboratory feed [Hindustan Lever Ltd, Mumbai] and Libitum Ad Air. The experiment was carried out in accordance with the Committee guidelines for the purpose of control and supervision of experiments in [CPCSEA] animals, the Indian Government [CPCEA, 2003]. This research is also conducted in accordance with the care and use of laboratory animals [NRC, 1996]. The use of animals was approved by the Institutional Animal Ethics Committee [IAEC] [Regd. No. 10[i]a/CPCSEA/IAEC/SVU/ZOOL/CC/ Dt.08-07-2012] at Sri Venkateswara University, Tirupati, India.

### Preparation of *Ocimum sanctum* leaf extract

Leaf extract prepared according to WHO 1983 [18] CG-04 protocol. Leaves are sliced, shed-dried, based on fine powder and extracted with 95% D/W [v/v] at  $55-60^\circ\text{C}$  for 3 hours. Solvents are filtered under reduced pressure; the resulting mass is dried under vacuum and kept at  $24^\circ\text{C}$  until it is used.

### Chemical test

Eugenol pure compound [99%] was purchased from Sigma Aldrich [St Louis].

### Dosage of animals

Female albino rat were divided into three groups, each group containing 6 rats. Early body weight of each animal is recorded.

### Experimental design

**Group I:** The first group is controlled by rat administered with 1 ml of saline [vehicle].

**Group II:** The second experimental group, administered with eugenol pure compound [99%] at the dose 0.4 ml/day for 15 days with intramuscular injection.

**Group III:** The third experimental group, administered with *Ocimum sanctum* leaf extract at the dose 500 mg/kg body weight/day for 15 days administered orally by using gastric destruction techniques [19,20].

### Tissues collection

Both controls and animal experiments are placed in a clean polypropylene cage in hygienic conditions in clean-aired air-conditioned ventilated well. Twenty-four hours after the last dose, animals are authorized and reproductive networks such as ovaries and uterus are excised at  $4^\circ\text{C}$  and are used for biochemical analysis.

### Determination of body and reproductive organs

The weight of the last object of the animal is recorded a day after

the last dose. Reproductive organs [ovary and uterine] are cut, cleaned from supporting tissues and weighed.

### Screening of antifertility activity

To evaluate the antifertility activity of plant extracts, studies followed. Extract effects on studying biochemical parameters and study on body weight and weight of reproductive organs.

### Data analysis

**Biochemical study:** The new biochemical studies removed ovaries and uterine tissue weighed for milligrams needed for biochemical analysis, such as protease activities, free amino acids, DNA, RNA, glucose, glycogen, G-6-PDH, triglycerides, phospholipids activity and lipase. Net tissue weight is estimated to be gravimetrically.

Biochemical estimates such as protease activities [21] free amino acids [21] DNA & RNA [22] glucose and glycogen [23] G-6-PDH [24], triglycerides [25] phospholipids [26] and lipase Activities [27] are estimated to use standard methods.

### Statistical analysis

Data is expressed as the average value with their elementary school. Read six different groups compared to using ANOVA Analysis One-way with a test of compression without Dunnetts. Statistical analysis was carried out using SPSS (version 11.5; SPSS Inc., Chicago, IL, USA). Use M.S. Office - 2007, Excel software, data has been analyzed for the significance of the main effects (factors) and maintenance together with their interactions [28]. The difference is considered statistically significant A-P<0.001, B-P <0.01, C-P <0.05 and the level is not significant [29].

## RESULTS

### Changes in body weight

The current investigation, body weight decreased significantly in the administration of Eugenol (% P<0.05), but no significant changes were observed in the Os administration in Table 1.

### Change of organs

The current investigation, the weight of the paired Ovary has increased significantly in both administrations. In accessory sex organs, the weight of the uterine organ decreased significantly in the Eugenol administration, while in Os administration significantly increased (% p<0.001) in Table 2.

### TSI change (Tissue somatic index)

The current investigation of a somatic ovary and uterine index which is significantly paired is increased either by the administration of more control (% p<0.001) in Table 1.

At present investigation, Ovarian and uterine protease activities increased significantly in both administrations (P<0.001) in Table 2.

### Free amino acids

Data represented in ovaries and uterus, free amino acids increased significantly in both administration (%p<0.001) in Table 2.

**Table 1:** Effect of Eugenol and *Ocimum sanctum* linn. leaf extract on Ovary, Uterus and Vagina.

Name of the parameters	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
Body weight (grams)	Initial	177.30 ± 12.80	175.95 ± 11.43 - 0.76 <sup>d</sup>	175.73 ± 11.10 - 0.88 <sup>d</sup>
	Final	197.73 ± 15.03	174.02 ± 10.21 - 11.99 <sup>c</sup>	183.92 ± 14.08 - 6.98 <sup>d</sup>
Organ weights	Paired ovary (mg)	162.60 ± 12.85	189.22 ± 15.66 + 16.37 <sup>a</sup>	180.09 ± 14.38 + 10.75 <sup>a</sup>
	Paired uterus (mg)	618.04 ± 49.67	533.67 ± 43.16 - 13.65 <sup>a</sup>	846.43 ± 73.38 + 36.95 <sup>a</sup>
	Vagina (mg)	100.33 ± 7.85	87.51 ± 6.17 - 12.77 <sup>a</sup>	110.17 ± 8.24 + 9.80 <sup>a</sup>
Tissue Somatic Index	Paired ovary	0.082 ± 0.005	0.108 ± 0.006 + 31.70 <sup>a</sup>	0.097 ± 0.004 + 18.29 <sup>a</sup>
	Paired uterus	0.312 ± 0.023	0.306 ± 0.021 - 1.92 <sup>d</sup>	0.450 ± 0.032 + 44.23 <sup>a</sup>
	Vagina	0.050 ± 0.003	0.049 ± 0.003 - 2.00 <sup>d</sup>	0.059 ± 0.004 + 18.00 <sup>a</sup>

Mean ± SD of six individual observations

+ and - percent increase and decrease respectively over control.

a- p<0.001, c- p<0.05 indicates the level of significance.

d- non significant changes

**Table 2:** Effect of Eugenol and *Ocimum sanctum* linn. leaf extract on Ovary and Uterus.

Name of the parameters	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
Protease activity (μmoles of tyrosine equivalents /mg of protein / hr)	Ovary	0.39 ± 0.02	0.71 ± 0.05 + 82.05 <sup>a</sup>	0.55 ± 0.03 + 41.02 <sup>a</sup>
	Uterus	0.55 ± 0.04	0.98 ± 0.07 + 78.18 <sup>a</sup>	0.82 ± 0.06 + 49.09 <sup>a</sup>
Free Amino acids (mg/g wet wt)	Ovary	27.43 ± 1.36	48.51 ± 3.63 + 76.85 <sup>a</sup>	39.84 ± 2.56 + 45.24 <sup>a</sup>
	Uterus	13.73 ± 1.01	23.64 ± 1.57 + 72.17 <sup>a</sup>	18.41 ± 1.17 + 34.08 <sup>a</sup>
DNA (mg/g)	Ovary	1.13 ± 0.08	2.04 ± 0.18 + 80.53 <sup>a</sup>	1.59 ± 0.11 + 40.70 <sup>a</sup>
	Uterus	2.45 ± 0.19	4.76 ± 0.38 + 94.28 <sup>a</sup>	4.72 ± 0.32 + 92.65 <sup>a</sup>
RNA (mg/g)	Ovary	1.85 ± 0.12	2.64 ± 0.20 + 42.70 <sup>a</sup>	3.40 ± 0.29 + 83.78 <sup>a</sup>
	Uterus	3.46 ± 0.27	6.37 ± 0.56 + 84.10 <sup>a</sup>	6.85 ± 0.58 + 97.97 <sup>a</sup>

Mean ± SD of six individual observations

+ and - percent increase and decrease respectively, over control.

a- p<0.001 indicates the level of significance.

## DNA & RNA

Data represented in ovaries and uterus, DNA & RNA levels increased significantly in both administration (%p<0.001) in Table 2.

Data represented in the ovary and uterus, glucose levels increased significantly in both administrations (%p<0.001) in Table 3.

## Glycogen

Data represented in Ovarian and uterine sex accessories, glycogen levels are significantly reduced in both administration (%p<0.001) in Table 3.

## G-6-PDH

Data represented in the ovary, the G-6-PDH significantly reduced both administration (%P<0.001). In uterine sex accessory organs, the G-6-PDH increased significantly in both administrations (% P<0.001) in Table 3.

## Triglycerides

Data represents Ovarian and uterine triglycerides increase significantly in both administrations (%p<0.001) in Table 3.

Table 3: Effect of Eugenol and *Ocimum sanctum* linn. leaf extract on Ovary and Uterus.

Name of the parameters	Name of the tissue	Control (Vehicle treated)	Eugenol administration % change & significance	OS administration % change & significance
Glucose (mg/g)	Ovary	3.78 ± 0.23	6.35 ± 0.43 +67.98 <sup>a</sup>	5.29 ± 0.37 +39.94 <sup>a</sup>
	Uterus	4.84 ± 0.29	7.78 ± 0.56 +60.74 <sup>a</sup>	6.65 ± 0.47 +37.39 <sup>a</sup>
Glycogen (mg/g)	Ovary	6.52 ± 0.43	3.67 ± 0.22 -43.71 <sup>a</sup>	5.35 ± 0.41 -17.94 <sup>b</sup>
	Uterus	13.44 ± 0.98	8.94 ± 0.62 -33.48 <sup>a</sup>	7.33 ± 0.58 -45.46 <sup>a</sup>
G-6-PDH (μmoles of formazan formed/mg protein/hr)	Ovary	3.46 ± 0.25	2.25 ± 0.17 -34.97 <sup>a</sup>	1.55 ± 0.12 -55.20 <sup>a</sup>
	Uterus	0.088 ± 0.005	0.124 ± 0.010 +40.90 <sup>a</sup>	0.116 ± 0.008 +31.81 <sup>a</sup>
Triglycerides (mg/g)	Ovary	36.63 ± 2.97	55.69 ± 4.10 +52.03 <sup>a</sup>	48.52 ± 3.25 +32.45 <sup>a</sup>
	Uterus	21.38 ± 1.86	30.79 ± 2.32 +44.01 <sup>a</sup>	37.85 ± 2.91 +77.03 <sup>a</sup>
Phospholipids (mg/g)	Ovary	24.59 ± 1.44	32.74 ± 2.16 +33.14 <sup>a</sup>	27.64 ± 1.35 +12.37 <sup>c</sup>
	Uterus	18.49 ± 1.08	31.13 ± 2.46 +68.36 <sup>a</sup>	26.77 ± 1.79 +44.78 <sup>a</sup>
Lipase activity (μmoles of PNPA cleaved/mg protein/hr)	Ovary	5.37 ± 0.48	2.92 ± 0.21 -45.62 <sup>a</sup>	2.22 ± 0.19 -58.65 <sup>a</sup>
	Uterus	3.82 ± 0.29	7.24 ± 0.67 +89.52 <sup>a</sup>	5.46 ± 0.45 +42.93 <sup>a</sup>

Mean ± SD of six individual observations

+ and - percent increase and decrease respectively over control.

a-p<0.001, b- p<0.01, c-p<0.05 indicates the level of significance.

## Phospholipids

The data represents the ovary and phospholipids uterus significantly increases in both administrations (%p<0.001; %p<0.05) in Table 3.

## Lipase activity

The data represents the activity of the Ovarian lipase significantly reduced either in both administration (%p<0.001). In the uterus, lipase activity increased significantly in both administrations (%p<0.001) in Table 3.

## DISCUSSION

Weight function, size and secretory is strictly regulated by estrogen [30]. The weight loss in rat may be the result of protein waste because it is not the availability of carbohydrates as an energy source [31]. Increased Ovarian weight (organ weight ratio) by administration is caused by more liquid ambitions [32]. Change sex accessories like the uterus; this is an estrogen dependency organ. The significant decrease in uterus weight is also seen in managing rat because of the no availability of hormones needed for the development of the uterus. Therefore, animals show significant prevention in loss of uterine weight, which may be caused by the effects of uterotrophic [33].

This research shows that the activities of ovarian protease significantly increase both in both administrations. Increased Ovarian protease activity can be associated with follicular atresia [34]. The increase in the activity of the uterine protease can inhibit

the release of the protease from the epithelium, and the suppression of immunity can inhibit the activity of protease leukocyte [35].

Free amino acid is a structural unit that forms protein. The process of making proteins is called the translation and involves the addition of step-by-step amino acids to a protein chain that grows with ribozymes called ribosomes [36]. The increase in free amino acids from ovaries may be caused by their degradation and the possibility of utilizing degraded products for metabolic purposes [37]. The increase in ovaries in free amino acid content may be caused by damage to proteins for energy needs and disruption of amino acid merging in protein synthesis [38]. The increase in amino acids is free of uterine weight and stimulates uterine growth, indicating estrogenic activity. It is known that the provision of estrogen has a uterotrophic effect on female rat [39].

Reproduction of the right cellular composition is controlled by genes through the action of RNA and DNA. Therefore this research focused on nucleic acid estimation. The increasing number of DNA and RNA on the ovarian tissue during the ovarian maturation shows that the synthesis of DNA and RNA correlates with an increase in the ecdysteroid titers. Increased number of DNA and RNA on the ovarian network efficiency to increase ovarian activity, including the amount and quality of oocytes without influence on the total content of DNA or RNA on the ovarian tissues [40]. Increasing the level of uterine DNA and RNA is less responsive to pregnancy stimulus in animals fed grain gluten or gelatin than in rat [41]. Increased levels of uterine DNA and RNA content showed estrogenic properties of administration Figures 1 & 2.



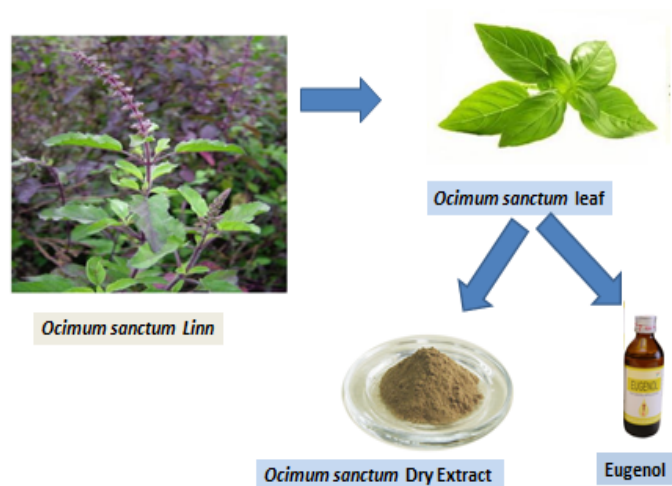


Figure 1: *Ocimum sanctum linn.* plant and extraction process.

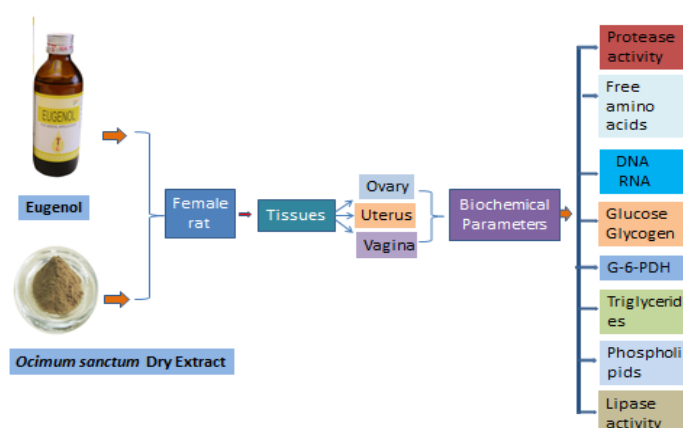


Figure 2: Extracted chemical components from *Ocimum sanctum linn.*

The level of glucose transportation into cells is governed by hormone peptides and steroids, including estrogen [42]. Estrogen has been proven to quickly activate several signal transduction path components, including those associated with glucose transporter translocation, such as cAMP, calcium and MAP kinase [43,44]. Eugenol and *Ocimum sanctum linn.* leaf extract shows significantly increased glucose in reproductive organs such as ovaries and uterus. Ovarian glucose levels significantly increase both in both administrations. Increasing ovarian glucose levels may be caused by reducing the availability of ovarian estrogen [45]. Glucose levels significantly increase in the uterus, revealing that adequate glucose absorption and metabolism are very important for the exact differentiation of the uterine endometrium towards a receptive state that can support embryonic implantation. Glucose transporter can play in normal uterine physiology and pathological conditions of infertility, endometrial cancer, and polycystic ovarian syndrome [46].

The presence of glycogen in reproductive organs is very important for many reproductive phenomena such as the formation of proliferation of *Des agumata* and uterus [47], implantation [48], Nolution [49], Uterus receipts (psychoyos, 1980) transportation in the female genital tract [50]. Eugenol and *Ocimum sanctum linn.* leaf extract shows significantly reduced glycogen in ovaries and uterus. Therefore, the decrease in ovarian glycogen levels may be caused by reducing steroidogenesis, which is associated with gonadotropin [51]. The uterus significantly decreases at glycogen levels responsible for changing the Milieu uterus that is not profitable for implantation and maintenance of pregnancy [52].

The G-6-PDH decreases significantly in the ovary, where the uterus increases in both administrations. Steroidogenesis in the ovary is under the physiological control of two dehydrogenases namely glucose-6-phosphate dehydrogenase [G-6-PDH] and  $\Delta 5-3\beta$ hydroxyteroid dehydrogenase  $\Delta 5-3\beta$ -SHD. Both extracts inhibit the activity of two enzymes significantly [53]. The G-6-PDH increased significantly on the uterus to stimulate Endo and the growth of myometrium to prepare the uterus for the possibility of pregnancy [54].

The level of triglycerides has a deep and essential effect on the uterus to stimulate endo and the growth of myometrium to prepare the uterus for the possibility of pregnancy [54]. Eugenol and *Ocimum sanctum linn.* leaf extract shows a significant increase in triglycerides in reproductive organs such as ovaries and uterus. Ovarian significantly increased in triglyceride concentration due to oxidation disorders [55]. Forbes 1986 suggested that estrogen increase lipid mobilization of the adipose tissue to offset negative energy balance by diverting non-esterful fatty acid from the adipose depot to function as a precursor of energy from various tissues. So because estrogen which was revealed in rat managed, Lipid accumulated in the uterus [56].

Phospholipids increased significantly in all networks by both administrations. Ovarian is an organ that has never rested and follicles began to grow at any time and when they developed a large number of cells. Phospholipids content has been involved in ova maturation. In this study, the accumulation of phospholipids ovaries represented, the administration disrupted follicular development, causing postponing ovum [57]. Phospholipids content has been involved in ova maturation. Hyper phospholipemia usually occurs before Hyper Cholesteremia. So because of imbalances in gonad steroids, which are important for the normal function of gonad, phospholipids increase. Phospholipids increased significantly in both administrations. In the uterus, uterine lipid metabolism seems to be oriented to oxidation of lipids that produce increased phospholipids. This can be correlated with a decrease in oxidative metabolism because of the low levels of estrogen circulating. A study on uterine phospholipids such as phosphatidyl choline, sphingomyelin, phosphatidyl inositol, phosphatidyl ethanolamine, cardiolipin, and phosphatidic acid inhibits the binding of estradiol and estrogen receptors. Therefore the decrease in estrogen levels is also responsible for accumulation of phospholipids [55].

Lipase is an enzyme that catalyzes the details of 'fat hydrolysis lipids [58]. Lipase is a subclass of Esterases. Lipase performs an important role in digestion, transportation and processing lipid diet [eg. Triglycerides, fats, oil] in most, if not all, living organisms. There was a significant decrease in ovarian lipase activity in both administrations. The decrease in lipase activity with administration clearly shows that the level of lipid accumulation is higher in the form of yolk globules during ovarian maturation [59]. In the uterus, the height of lipase activity in both administrations. The increase in lipase activity in this study supports the existence of lipoprotein lipase activities reported in the uterus of obesity rats and the relationship between enzyme activity and reproductive problems in these animals. In both species, lipase tissue activities are changed on this genetic mutant reproductive tissues compared to control. Estrogen has been shown to modulate cell lipase activity [60].

## CONCLUSION

In conclusion, weight loss in rat may be the result of protein waste because it is not the availability of carbohydrates as an energy

source. The ovaries represented, the administration disrupted follicular development, causing postponing ovum. It is known that estrogen provisions have a uterotrophic effect on female rat. Eugenol administration significantly decreases in body weight. Eugenol and *Ocimum sanctum* linn. leaf extract effect on the antifertility of female rats.

## CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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