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A comparative study of eugenol and *Ocimum sanctum* Linn. leaf extract on the antifertility effect in female albino rats

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Abstract

Background: This study was designed to evaluate the antifertility efficacy of eugenol (EUG) and *Ocimum sanctum* (OS) leaf extract in female albino rats.

Methods: The healthy female albino rats were administered with EUG (99% pure) at a dose of 0.4 ml/day/rat and OS Linn. (Tulsi) leaf extract at a dose of 500 mg/kg body weight/day/rat orally for 15 days. One-way ANOVA analysis with Dunnetts multiple comparison test is used for analyzing data.

Results: The total duration of estrous cycle was prolonged with EUG and no significant changes with OS leaf extract administration were observed. EUG elevated serum estradiol and progesterone levels but OS leaf extract elevates only progesterone levels. Elevated ovarian proteins were observed in both administrations.

Conclusion: This study concludes that the administration of EUG and OS leaf extract significantly enhanced the serum estradiol and progesterone levels leading to reduced frequency of ovulation and results in the impairment of fertility.

Keywords: Estrous cycle; Eugenol; *Ocimum sanctum* leaf extract; Serum hormone profiles

1. INTRODUCTION

Female fertility is a biological process regulated by female hormones. The most common causes of female infertility are hormones commonly associated with ovulation, polycystic ovarian syndrome, premature ovarian failure, damage to the fallopian tube or uterus, or problem with the cervix.[1] The female reproductive cycle functions primarily by the interplay between the luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone, estradiol, and testosterone.[2] The female reproductive organs can be assayed by the serum level of these hormones. The leaves of *Ocimum sanctum* (OS) were found to possess abortifacient effect in women. OS has also got antifertility effect. The benzene and petroleum ether extracts of leaves of Tulsi have been reported to produce 80% and 60% antifertility activity, respectively, in female rats.[3] Long term use of OS leaves disrupts the estrous cycle and the estrous stage is prolonged. OS leave feeding also inhibits ovarian hormones in the rats.[4] Eugenol (EUG) is one of the potent bioactive components found in tulsi. The pharmacological properties documented for tulsi are associated with EUG, which has structural resemblance to polyphenol, and has showed estrogenic properties in albino rats. Hence, the current study was designed to find out the antifertility potentials of OS and EUG in female albino rats.

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2. METHODS

In the current study, healthy adult (4-months old, weight 170 ± 20 g) female Wistar strain albino rats were used. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. The female albino rats were divided into three groups, and each group contains six rats. The initial body weight of each animal was recorded. First group was control group with rats being administered with 1 ml of saline (vehicle). Second group was experimental, with rats administered with pure compound EUG (99%) at a dosage of 0.4 ml/day for 15 days by intramuscular injection. Third group was experimental, with rats orally administered with OS leaf extract at dose 500 mg/Kg body weight/day for 15 days, using the gastric gavaging technique.[5,6] The leaf extract was prepared according to WHO 1983[7] protocol CG-04. Animals were housed in a clean polypropylene cage under hygienic conditions in well-ventilated clean, air-conditioned room, with a photoperiod of 12 hours light and 12 hours dark cycle, at 25°C ± 2°C with a relative humidity of 50% ± 5%. The rats were fed with standard laboratory feed (Hindustan lever Ltd, Mumbai) and water *ad libitum*. The use of animals was approved by the Institutional Animal Ethics Committee (IAEC) (Regd. No. 438/01/a/ CPCSEA/dt 17/07/2001) at the S. V. University, Tirupati, India. Twenty four hours after the last dose, the animals were autopsied and the reproductive tissues like ovary, uterus, and vagina were excised at 4°C and used for biochemical analysis. The blood was collected by puncturing the heart.

2.1. Determination of estrous cycle

2.1.1. Preparation of vaginal smear

Vaginal smear using saline solution was taken twice daily every day morning at 6 AM and evening at 6 PM during the entire treatment period, and the cell type obtained in vaginal smear was observed. The duration of estrous cycle together with that of

various phases was determined. All the four phases of the estrus cycle are determined according to the presence of cell types in the vaginal smear. The vaginal smear was collected as described by Marcondes et al.[8]

2.1.2. Observation of estrous cycle

Proestrus phase –The presence of nucleated or nucleated plus cornified cells.
Estrus phase –The presence of cornified cells only.
Metestrus –The presence of mixture of leukocytes and epithelial cells.
Diestrus phase –Mainly leukocytes with few epithelial cells.

2.2. Hormonal assay

The blood serum was collected by centrifugation of blood at 2000*g* for 15 min and stored at –20°C until further analysis. Serum samples were assayed for FSH, LH, and testosterone by using the chemiluminescent Immunoassay kit.[9] Prolactin, estradiol, and progesterone hormones were estimated by using the electrochemiluminescence immunoassay technique.[10]

2.3. Biochemical assay

The biochemical parameters such as total proteins,[11] total carbohydrates,[12] and total lipids[13] were estimated using standard methods.

The data were expressed as a mean value with their SD. Reading of the six different groups was compared using one-way ANOVA analysis with a Dunnetts multiple comparison test. The data have been analyzed for the significance of the main effects (factors) and treatments along with their interaction.[14] Differences were considered statistically significant: (a) $p < 0.001$, (b) $p < 0.01$, (c) $p < 0.05$, and (d) nonsignificance levels.

3. RESULTS

In Table 1, the administration of EUG enhanced the duration of proestrus, metestrus, diestrus phases, and total duration of cycles; no significant effect showed by the administration of OS leaf extract on estrus cycles. In Table 2, there was no effect on hormones such as FSH, LH, and PRL by both administrations. The testosterone levels were significantly ($p < 0.001$) reduced and progesterone levels were enhanced by both administrations. EUG enhances the estradiol levels, while no effect observed on OS administration. In Table 3, the tissue somatic index (100 g body weight) of the ovary increased significantly ($p < 0.001$), while no significant effect on sex accessory tissues like the uterus and vagina by the administration of EUG, which elevated ovarian and uterine proteins and reduced vaginal proteins. The ovarian carbohydrates were lowered, and the uterine and vaginal carbohydrates were enhanced. However, the total lipids were increased significantly in ovary and uterus, whereas in vagina these were reduced. In Table 4, the tissue somatic index (100 g body weight) was increased significantly ($p < 0.001$) in all tissues. The total proteins, total carbohydrates, and total lipids were increased significantly ($p < 0.001$) in ovary and uterus, where as in vagina these were found reduced by OS administration.

4. DISCUSSION

This study was carried out to make a preliminary investigation in EUG and OS leaf extract administered in female rats. There

Table 1

Effect of eugenol and *Ocimum sanctum* Linn. leaf extract on estrous cycle (in hours)

S. no	Name of the cycle	Control	Eugenol administered (%) change and significance	OS administered (%) change and significance
1	Proestrus	14 ± 1.01	18 ± 1.26 +28.57[a]	15 ± 1.09 +7.14[d]
2	Estrus	16 ± 1.28	16 ± 1.21	16 ± 1.23
3	Metestrus	27 ± 1.98	33 ± 2.39 +22.22[a]	29 ± 2.12 +7.40[d]
4	Diestrus	50 ± 3.82	58 ± 4.69 +16.00[a]	52 ± 3.98 +4.01[d]
5	Total duration of cycle	107 ± 6.42	125 ± 8.46 +16.82[a]	112 ± 7.35 +4.67[d]

OS = Ocimum sanctum.

Table 2

Effect of eugenol and *Ocimum sanctum* Linn. leaf extracts on sex hormones

S. no	Name of the hormones	Control (vehicle treated)	Eugenol administered (%) change between and significance	OS administered % change between and significance
1	FSH (mlU/ml)	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
2	LH (mlU/ml)	0.10 ± 0.07	0.10 ± 0.07	0.10 ± 0.08
3	Prolactin (ng/ml)	1.00 ±0.09	1.00 ± 0.09	1.00 ± 0.08
4	Testosterone (ng/m\dl)	0.18 ± 0.012	0.04 ± 0.002 –77.77[a]	0.02 ± 0.001 –88.88[a]
5	Estradiol (pg/ml)	11.36 ± 0.82	21.19 ± 1.75 +86.53[a]	11.02 ± 0.78 –2.99[b]
6	Progesterone (ng/ml)	85.42 ± 5.09	126.72 ± 9.16 +48.34[a]	103.20 ± 7.14 +20.58[a]

Mean ± SD of six individual observations.
+ and – percentage increase and decrease, respectively, over control.
[a]$p < 0.001$ indicates the level of significance.
[b]Nonsignificant changes.

Table 3

Effect of eugenol on tissue somatic index, total proteins, total carbohydrates, and total lipids in ovary, uterus, and vagina

	Ovary		Uterus		Vagina	
Name of the parameter	**Control**	**Eugenol**	**Control**	**Eugenol**	**Control**	**Eugenol**
Tissue somatic index (w/w%)	0.082	0.108	0.312	0.306	0.050	0.049
	±0.005	±0.006	±0.023	±0.021	±0.003	±0.003
		+31.70[a]		−1.92[d]		−2.00[d]
Total proteins (mg/g)	213.65	359.38	168.69	279.81	230.70	190.03
	±16.81	±28.64	±11.08	±20.87	±18.68	±12.51
		+68.20[a]		+65.87[a]		−17.62[a]
Total carbohydrates (mg/g)	6.89	5.45	5.75	6.85	6.01	8.38
	±4.01	±3.15	±3.26	±3.98	±3.32	±4.96
		−20.89[a]		+19.13[a]		+39.43[a]
Total lipids (mg/g)	38.60	74.17	59.43	94.58	44.19	37.82
	±2.95	±6.31	±4.80	±8.40	±3.79	±2.71
		+92.15[a]		+59.14[a]		−14.41[a]

Table 4

Effect of *Ocimum sanctum* on tissue somatic index, total proteins, total carbohydrates, and total lipids in ovary, uterus, and vagina

	Ovary		Uterus		Vagina	
Name of the parameters	**Control**	**OS**	**Control**	**OS**	**Control**	**OS**
Tissue somatic index (w/w%)	0.082	0.097	0.312	0.450	0.050	0.059
	±0.005	±0.004	±0.023	±0.032	±0.003	±0.004
		+18.29[a]		+44.23[a]		+18.00[a]
Total proteins (mg/g)	213.65	293.51	168.69	200.30	230.70	296.32
	±16.81	±21.73	±11.08	±15.98	±18.68	±22.66
		+37.37[a]		+18.73[a]		+28.44[a]
Total carbohydrates (mg/g)	6.89	8.92	5.75	8.18	6.01	4.96
	±4.01	±5.28	±3.26	±4.81	±3.32	±2.98
		+29.46[a]		+42.26[a]		−17.47[a]
Total lipids (mg/g)	38.60	52.73	59.43	86.34	44.19	24.55
	±2.95	±4.56	±4.80	±7.85	±3.79	±1.67
		+36.60[a]		+45.28[a]		−44.44[a]

Mean ± SD of six individual observations.+ and – percentage increase and decrease, respectively, over control.

[a]$p < 0.001$ indicates the level of significance.

was a slight reduction in body weight with EUG and OS leaf extract. The administration of EUG and OS leaf extract altered different phases of estrous cycle. The administration of OS leaf extract does not showed any effect on estrus cycle; however, EUG prolongs total duration of cycle from 107 ± 6.42 hours to 125 ± 8.46 hours. There was an increase in duration of every phase of the estrous cycle except estrus phase. Prolongation of proestrus phase indicates that maturation of the graffian follicle in the preovulatory phase was delayed, leading to nonmaturation of the graffian follicle.[15] Prolonged metestrus and diestrus pattern in each cycle lowers the frequency of occurrence of the estrus phase. Consequently, the frequency of ovulation was reduced with a resultant impairment of fertility.[16] The most common causes of female infertility are hormones. These are commonly associated with ovulation, ovarian syndrome, premature ovarian failure, damage to the fallopian tube or uterus, or problem with the cervix. Endocrine disorder results from excessive production of hormones, or insufficient production of one or more hormones, or the lack of the tissue responses to normal circulating hormones. The female reproductive cycles function primarily by the interplay between the LH, FSH, progesterone, estradiol, prolactin, and testosterone; The integrity of the female reproductive organs can be assayed by the serum level of these hormones.[2]

No significant changes were observed in FSH, LH, and prolactin levels in both administrations. However, a significant reduction in testosterone is observed in both administrations. The reduced testosterone indicates the destruction of follicles, influencing follicle-stimulating hormones that can affect reproduction.[17] It may be also due to hypopituitarism. The low levels of testosterone also represent the estrogenic properties of these administrations.[18] The elevated serum estradiol represents the alteration in hypothalamus–pituitary gonadal axis.[2] The administration of EUG caused a reduction in the functional lifespan of the corpus luteum.[19] Progesterone and estradiol are among the most important sex hormones for implantation of the blastocyst and pregnancy maintenance.[2] Progesterone controls the thickness of the uterine tissue lining. A significant increase in progesterone levels leads to the LH surge and consequently ovulation in both administrations.[20]

The elevated ovarian somatic index suggests a disturbance of the reproductive endocrine functions in both administrations.[21] The elevated uterine weight, vaginal weight, and prolonged duration of proestrus phase is possibly due to the direct oestrogen effect of OS administration.[22]

The elevated ovarian and uterine proteins may be the effect of the change of the physiology. The elevation in protein content led to the cells damage, increased free radical load might have induced DNA damage, and may be apoptosis in the ovary and uterus of female rats resulted in the reproductive impairment, which may further lead to the infertility in females. It is therefore suggested that administration of EUG and OS leaf extract affect female fertility.[23] The reduced ovarian and elevated sex accessory carbohydrates by the administration of EUG may be due to a reduction in the follicular growth and ovulation, which leads to metabolic degradation and inhibited the carbohydrate synthesis. The lipid accumulation in ovary and uterus suggests the inhibition of their functions. So the administrations

induce hyperlipidaemia, increase in lipid peroxidation, and the decrease of antioxidant enzymes in the ovary and uterus.[24] The significant reduction in vaginal lipids leads to decreased secretions and dryness of the vaginal mucosa by the administration of EUG and OS.[25]

In conclusion, the results obtained in this study 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 antifertility capacity of EUG was indirect evidence. However, further research is advised to fully establish the mechanism of the active constituent of this plant.

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