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Effect of Eugenol and *Ocimum sanctum* on Infertility Activity in Female Albino Rats

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Abstract

The purpose of this study is to explore the infertility activity of eugenol and *Ocimum sanctum* Linn. Leaf extract from reproduction of female rats, 4 months, weighing 170 ± 20 g adult female rats. Group I served as control while group II and III received Eugenol (99%) at a dose of 0.4 ml/day/rat and *Ocimum sanctum* Linn. Leaf extract with a dose of 500 mg/kg body weight/day/rat, orally respectively for 15 days. All reproductive parameters, such as biochemical parameters of ovary, uterus and liver, serum hormone levels, measurement of cytokine antioxidant enzymes, infertility activity. The effects of Eug and *OS* extracts on the concentration of sialic acid, acid phosphatase and alkaline phosphatase were also significantly reduced after the administration of Eug and OS in the ovary and uterus ($P<0.05$). These effects are related to the increase in serum levels of (cytokine) PGE_2 and $PGF_{2\alpha}$ ($P<0.05$) and the decrease ovarian of TNF-α, IL-1β and total peroxidase ($P<0.05$). The total acid phosphatase in the liver increased ($P<0.05$), the alkaline phosphatase content decreased ($P<0.05$), and there was no significant change in adenosine triphosphatase. In conclusion, it is clear that medicinal plants play an important role as an infertility agent. Ovarian weight loss after treatment may be due to reduced follicular growth and ovulation, depending on the availability of gonadotropin. There is a decrease in ovulation, therefore the antifertility activity of Eugenol and *Ocimum sanctum* Linn. Leaf extract in female wistar rats.

Keywords: Eugenol; Infertility; Liver; *Ocimum sanctum;* Ovary; Uterus

Introduction

In recent years, the use of herbal medicine has grown rapidly worldwide. For a long time, various herbs have been used to induce infertility. Modern research has tested and confirmed the antifertility effects of most herbs [1].

Population control is very important for the well-being of individuals and nations. Looking for oral contraceptives similar to recorded stories to control human fertility. Although there are various synthetic contraceptives, they cannot be used consistently due to severe side effects [2], so a method was sought to identify new antifertility drugs from natural medicines. Many personalized medicines are explained in the treatment of indigenous people and used to manage various goals related to reproduction. It is estimated that many plant preparations have proprietary regulations for fertility, and only a few plant preparations have been tested for these values.

Fertility is a biological phenomenon that can be defined as the natural ability to produce offspring. Fertility is different from fekunity, which means that reproductive potential is affected by gamete production, fertilization and pregnancy over a period of time. Anti-fertility drugs, a broad term, include drugs that can or tend to reduce or destroy fertility. The control of fertility or anti-fertility is an important and substantive medical discipline with continuous development of its scientific basis and continuous expansion of clinical applications [3].

The fresh leaves and stems of *Ocimum sanctum* extract produce some phenolic compounds [antioxidants], such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid,, as well as large amounts of eugenol. *Ocimum sanctum* leaves contain 0.7% volatile oil, including approximately 71% eugenol and 20% methyl eugenol. This oil also contains carvacrol and caryophyllene from the sesquiterpine hydrocarbons [4]. Two flavonoids orientin and andvicenin were isolated from basil leaf extract [5].

Eugenol can be separated from the buds, roots and leaves of cloves. This is mainly found in clove oil [80-90%] in nature. In addition, its existence has been confirmed in studies using various plants, such as Laurel [*Pimenta racemosa*], Tarragon [*Artemisia dracunculus L.*], Cinnamon [*Cinnamomum tamala*], Coconut [*Myristica fragrans*], Basil [*Ocimum basilicum L.* and *Ocimum gratissimum*], Japanese star anise [*Illicium anisatum*] [6].

Eugenol is easily soluble in alcohol or oil, but partially soluble in water [7]. Eugenol received more than 44 names and several synonyms; "4-allyl-2-methoxyphenol, 4-allyl-2-methoxy-phenol, 2-methoxy-4-[2-propenyl] -phenol, 2-methoxy-4- [prop-2-en-1-yl] -phenol and 1-Hydroxy-2-Methoxy-4-β-propenylbenzol" [8].

Materials and Methods

Study locations

This research was conducted between November 2017 to January 2018. In this research healthy adult [4 months, weighing 170 ± 20 g] in 18 Wistar albino 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 light and dark cycles, at 25 ± 2°C with relative humidity of 50 ± 5%. Rat fed with standard laboratory feed the crude protein and metabolizable energy (96.8%) [Hindustan Lever Ltd, Mumbai] and water ad libitum. The experiment was carried out in accordance with the guidelines of the Indian Government

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Committee for the purpose of control and supervision of experiments using animals [9]. This research was also conducted in accordance with the care and use of laboratory animals [10]. The use of animals was approved by the Institutional Animal Ethics Committee (IAEC) [Reg No. 10(i)a/CPCSEA/IAEC/SVU/ZOOL/CC/ Dt.08-07-2012) of Sri Venkateswara University, Tirupati, India.

D Preparation of *Ocimum sanctum* leaf extract

Insecticide free *Ocimum sanctum* plants were grown for 60 days. The *Ocimum sanctum* leaves collected from the Bukit Seshachalam area are part of the Eastern Ghats in the state of South Andhra Pradesh, in Southeast India. Leaf extract prepared according to WHO [1983] [11] CG-04 protocol. The fresh leaves (40 kg) were cut and washed with tap water and shed dried at room temperature (28°C) overnight. The air-dried leaves were kept in 60°C oven for 5 h when constant dry weight was achieved. The dried leaves were ground into powder form using the grinding machine. Total amount of 5.721kg of the leaf powder was soaked in 4l of 70% ethanol for 24 h at room temperature. The extract was filtered through filter paper no.3. After filtration the solid part was soaked again and filtered 24 h later. The amount of 13.5 l extract from three repetition of extraction using 70% ethanol as previously mentioned were pooled and evaporated with the evaporator (EYELA model NE) at 60°C until the extract was turned into dark brown of paste. The paste was dried in desicator for 2 weeks. The yield was 978.15 g (Figure 1).

Toxicity of *Ocimum sanctum* leaf extract

Mean lethal dose or LD_{50} was calculated after ip administration of *O. sanctum* fixed oil in mice. The fixed oil was well tolerated up to 30 ml/kg while 100% mortality was recorded with a dose of 55 ml/kg. The calculated LD_{50} of oil was 42.5 ml/kg.

Chemical test

Eugenol pure compound (99%) was purchased from Sigma Aldrich (St Louis).

Experimental design

The experiment was laid out in a complete randomized design of three treatment groups replicated thrice with each replicate having two female rats. Early body weight of each animal was recorded.

Group I: The rats in the first group were administered with 1 ml of

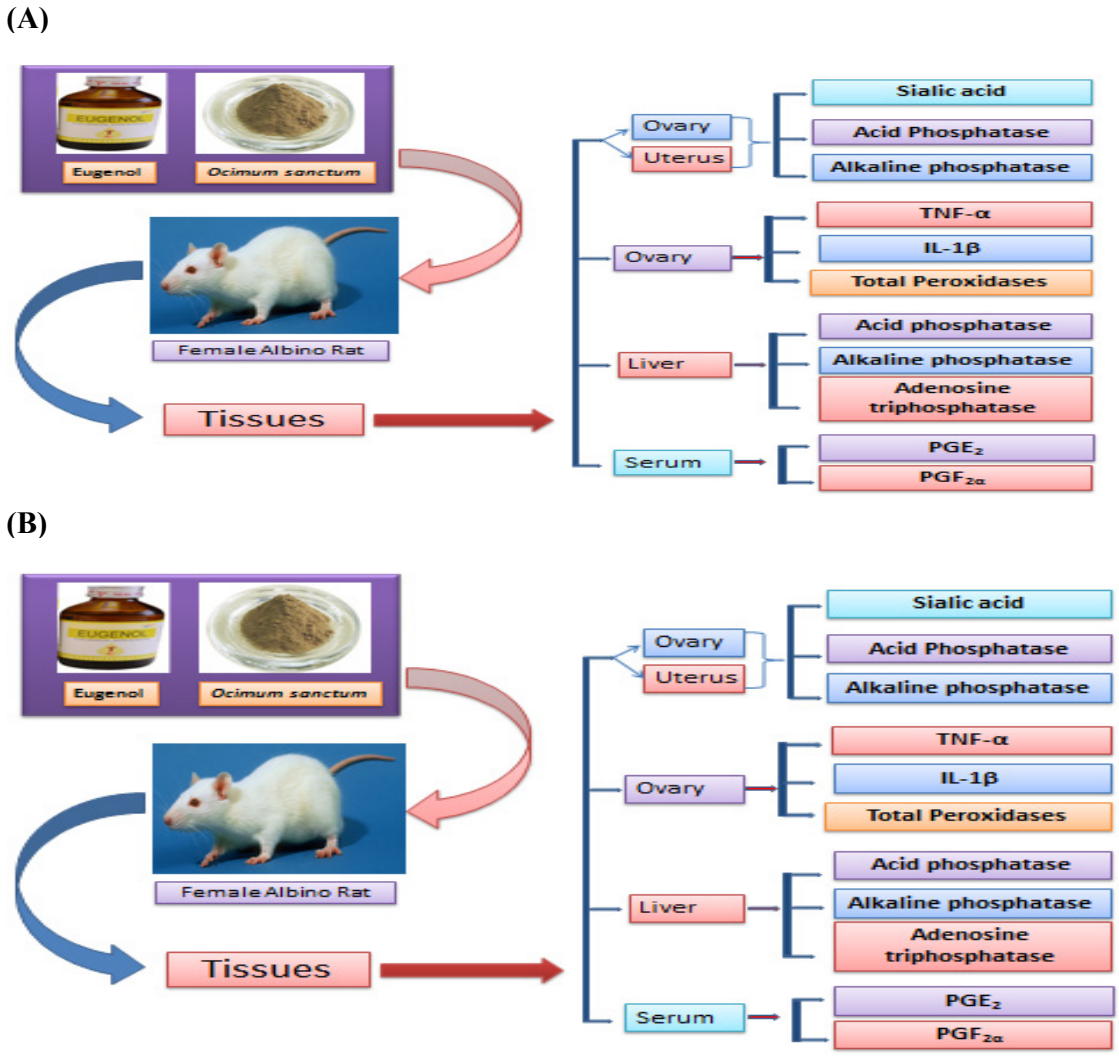

Figure 1: (A) Preparation procedure of the *Ocimum sanctum* leaf extract. (B) Reproductive Physiology Mechanism of Eugenol and *Ocimum sanctum*.

saline (vehicle) (experimental control).

Group II: The rats in the second experimental group were, administered with Eugenol at 0.4 ml/day for 15 days by intramuscular injection [standard control] [12].

Group III: The rats in the third experimental group were, administered with *Ocimum sanctum* leaf extract at the dose 500 mg / kg body weight / day for 15 days orally [13].

Tissues collection

Twenty-four hours after the last dose, animals were anesthetized and reproductive organs such as ovaries, uterus and liver were excised, blotted free of blood at 4°C and used for biochemical analysis.

Determination of body and reproductive organs

The body weight of the animals was recorded one day after the last dose. Remove reproductive organs [ovaries and uterus], non-reproductive organs [liver], remove supporting tissues and weigh.

Biochemical parameters

Cuts ovarian and uterine tissues into study pieces and homogenizes them. The homogenate is used for biochemical analysis. The following parameters were studied: Sialic acid [14], acid phosphatase and alkaline phosphatase [15], adenosine triphosphatase [16]. At least six replicates were analyzed for each organization and parameter.

Measurement of cytokine concentration

The concentration of ovarian cytokines is evaluated using ELISA kits for rat TNF-α and IL-1β (Peprotech Inc., USA).

Measurement of total peroxidases

Ovarian levels of total peroxidases were determined by the method of Habbu et al. [17] and the method description partly reproduces their wording. Briefly, ovarian homogenate [0.5 ml] was taken, and to this were added 1 ml KI solution [10 mM] and 1 ml sodium acetate [40 mM]. The absorbance of potassium iodide was read at 353 nm, which indicates the amount of peroxidase. Then 20 µl of H_2O_2 [15 mM] was added, and the change in the absorbance in 5 min was recorded. Units of peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per min. The specific activity expressed in terms of units per g of proteins was deduced by the law of Beer-Lambert [18] as follows: C = DO/ε.l.p, where C = concentration of ovarian total peroxidases [mM/g of total proteins]; DO = optical density; ε = molar extinction coefficient ($11.3m^{-1}\ cm^{-1}$); l = path length (1 cm); p = ovarian total protein level (g).

Serum analysis

The serum level of Prostaglandin-F2α ($PGF_{2\alpha}$) and Prostaglandin-E2 (PGE_2) was analysed by Enzyme Immuno Assay (EIA) using the EIA commercial kits.

Statistical analysis

Data collected were analysed using one way analysis of variance (ANOVA). Statistical analysis was carried out using SPSS (version 11.5; SPSS Inc., Chicago, IL, USA). The difference was considered statistically significant at $p<0.001$ (a), $p<0.01$ (b), $p<0.05$ (c) and non-significant (d).

Results

Sialic acid

Table 1 also showed that compared with the control group, the concentration of sialic acid was also significantly reduced after the administration of Eug and OS in the ovary and uterus ($P<0.001$).

Acid Phosphatase

Table 1 also showed that compared with the control group, the acid

S. No	Name of the Parameter	Name of the Tissue	Control (Vehicle treated)	Eugenol administration % change& significance	*OS* administration % change & significance
1	Sialic acid (mg/g)	Ovary	0.81 ± 0.06	0.53 ± 0.03 - 34.56[a]	0.64 ± 0.04 - 20.98[a]
		Uterus	0.76 ± 0.05	0.48 ± 0.02 - 36.84	0.52 ± 0.03 - 31.57
2	Acid Phosphatase (mgpi/g/h)	Ovary	5.91 ± 0.46	3.01 ± 0.19 - 49.06[a]	3.27 ± 0.21 - 44.67[a]
		Uterus	4.65 ± 0.34	2.74 ± 0.13 - 41.07[a]	2.89 ± 0.16 - 37.84[a]
3	Alkaline phosphatase (mgpi/g/h)	Ovary	5.63 ± 0.42	3.28 ± 0.23 - 41.74	4.01 ± 0.32 - 28.77
		Uterus	8.72 ± 0.75	6.37 ± 0.51 - 26.94[a]	6.13 ± 0.47 - 29.70[a]
Data presented as Mean ± SE. Groups (columns) with different superscript letters ([a]) are statistically significant (one-way analysis of variance, Tukey's post hoc, $p < 0.05$).					

Table 1: Biochemical changes of the Ovary and Uterus due to Eugenol and *OS* leaf extract.

S. No	Name of the Parameter	Name of the Tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	TNF-α (ng/ml)	ovary	0.83 ± 0.06	0.47 ± 0.02 - 43.37[a]	0.56 ± 0.03 - 32.53[a]
2	IL-1β (ng/ml)	ovary	7.21 ± 0.59	3.97 ± 0.23 - 44.93[a]	5.04 ± 0.38 - 30.09[a]
3	Total Peroxidases (mM/g of total proteins)	ovary	12.28 ± 0.96	6.38 ± 0.51 - 48.04[a]	7.26 ± 0.63 - 40.87[a]
Data presented as Mean ± SE. Groups (columns) with different superscript letters ([a]) are statistically significant (one-way analysis of variance, Tukey's post hoc, $p < 0.05$).					

Table 2: Ovarian levels of TNF-α, IL-1β and Total Peroxidases, following treatments.

phosphatase activity was significantly reduced after the administration of Eug and OS in the ovary and uterus (P<0.001).

Alkaline phosphatase

Table 1 also showed that compared with the control group, the alkaline phosphatase activity was significantly reduced after the administration of Eug and OS in the ovary and uterus (P<0.001).

TNF-α [tumor necrosis factor-α]

Table 2 also shows that the ovarian concentration of TNF-α is highest during ovulation in control rats. Compared with the control group, the ovarian TNF-α concentration was significantly reduced after Eug and *OS* administration (P<0.001).

IL-1β [interleukin-1β]

Table 2 also shows that compared with the control group, the ovarian IL-1β concentration was significantly reduced after Eug and OS administration (P<0.001).

Total peroxidases

Table 2 also showed that compared with the normal control group, the ovarian level of total peroxidase was significantly reduced by the administration of Eug and OS (P<0.001).

Acid phosphatase

Table 3 also shows that the activity of acid phosphatase also showed that compared with the control group, the administration of Eug and OS in the liver was significantly reduced (P<0.001).

Alkaline phosphatase

Table 3 also shows that compared with the control group, the alkaline phosphatase activity also showed a significant decrease in the administration of Eug and OS in the liver (P<0.001).

Adenosine triphosphatase

Table 3 also shows that compared with the control group, there was no significant change in adenosine triphosphatase activity when Eug and OS were administered to the liver.

PGE2

Table 4 also shows that compared with the normal control group, the serum level of PGE2 was significantly increased by administration of Eug and OS (P<0.001).

PGF2α

Table 4 also shows that compared with the normal control group, the serum level of PGF2α was significantly increased by administration of Eug and OS (P<0.001).

Discussion

This study aims to investigate the administration of EUG and OS extract to the female rat. Sialic acid is the main biochemical constituent of the uterus, which is directly controlled by estrogen [19].

Changes in the uterus of sialic acid levels depending on ovarian hormone [20]. In the current investigation, it was observed that the treatment of Eugenol and OS was a decrease in the content of ovarian acid and the content of alkaline phosphate and the effects of antifertility induced in Eugenol and OS of rat; this is due to the hormonal variation and the secretion of gonadotropin. Well documented that FSH is very important for the growth of the follicle and LH is needed for the formation of ovulation and corporations [21] responsible for the growth and weight of the ovary. Therefore, the reduction observed in the weight of the ovary after treatment of Eugenol & OS extract and can be caused by a reduction in the growth of follicles and ovulation depending on the availability of gonadotropins.

The decrease in the acid and alkaline phosphatase of the uterus is responsible for changing the unfavourable uterine means for the maintenance of implantation and pregnancy. Therefore, reducing the level of circulating estrogen contributes to changing the physiology of the female reproductive system. Therefore, current research shows that the fraction of steroids eugenol and OS extract of the operating system provides female rat antifertility activity.

There is a reciprocal relationship between endocrine systems and cytokines. Cytokines directly stimulate the active endocrine tissue and, on the other hand, produced by them in important concentrations [22]. In this study, we have compared the levels of cytokines [TNF-α and IL-1β] during ovulation in the ovary treated by Eugenol and OS. We

S. No	Name of the Parameter	Name of the Tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	Acid phosphatase (mg Pi/100g/hr)	Liver	197.83 ± 17.89	269.87 ± 21.15 +36.41[a]	283.26 ± 23.49 +43.18[a]
2	Alkaline phosphatase (mg Pi/100g/hr)	Liver	83.49 ± 6.93	48.74 ± 3.45 - 41.62[a]	53.62 ± 4.37 - 35.77[a]
4	Adenosine triphosphatase (nmoles of P_i/min)	Liver	1486 ± 137.32	1423 ± 129.87 - 4.23[d]	1449 ± 132.65 - 2.48[d]
Data presented as Mean ± SE. Groups (columns) with different superscript letters ([a,d]) are statistically significant (one-way analysis of variance, Tukey's post hoc, p < 0.05).					

Table 3: Changes in the Activity of Acid and Alkaline Phosphatases in Female rats Treated with a Eugenol and *OS* leaf extract.

S. No	Name of the Parameter	Name of the Tissue	Control (Vehicle treated)	Eugenol administration % change & significance	*OS* administration % change & significance
1	PGE_2 (ng/ml)	Serum	6.26 ± 0.49	10.37 ± 0.86 +65.65[a]	9.64 ± 0.71 +53.99[a]
2	$PGF_{2\alpha}$ (ng/ml)	Serum	14.73 ± 0.98	26.84 ± 1.83 +82.21[a]	24.16 ± 1.58 +64.01[a]
Data presented as Mean ± SE. Groups (columns) with different superscript letters ([a]) are statistically significant (one-way analysis of variance, Tukey's post hoc, p < 0.05).					

Table 4: Effect of Eugenol and *OS* leaf extract in the female rat serum levels.

discovered that the level of TNF-α and IL-1β decreased after eugenol and OS that could be caused by the suppression of gonadotropin [FSH and LH]. It is widely known that gonadotropin activates the production of TNF-α and IL-1β during ovulation [23]. Therefore, the decrease at the level of TNF-α and IL-1β can affect the formation of the main infertility activities.

These results indicate that they reduce the level of antioxidant enzymes [total peroxidation] in the ovary, thus stimulating the beginning of follicular development and maturation, as indicated by the growing number of Graafian follicles. Ovarian ability in Eugenol and OS extracts, animals can also be associated with reported antioxidant properties [24].

These results corrected the observations previously made by Awounfack et al. [25]. In healthy animals and support, in addition, the botanical data of Ethnno inform the efficacy of Eugenol and OS in the treatment of female infertility [26].

In addition, the increase at the PGE2 level, PGF2α played a key role of abortion by inducing separation from the fetal membranes of the uterine wall and uterine contractions [27]. In our study, the PGE2 and PGF2α level also increased in Eugenol and OS treated for groups of animals that show their abortion activity.

Conclusion

This study showed that Eugenol and OS were extracted from female rats for infertility. This effect seems to be due to a reduction in follicular growth and ovulation, depending on the availability of gonadotropins. Therefore, the reduction of IL-1β and TNF-α levels can affect the formation of major infertility activities. Therefore, based on this study, it was concluded that the administration of Eugenol and OS leaves administration infertility in female rats.

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Author Contributions

PVR: Conceptualization, Investigation, Methodology, Funding acquisition, Writing - original draft. MSR: Conceptualization, Supervision.

Conflict of Interest

The authors declare that they no regard competing financial hobbies or private relationships that might have seemed to influence the work reported in this paper.

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