Original
ISSN 2521-8492

The Potential Role of Eugenol and *Ocimum Sanctum* Extract on Female Rats: A Focus on Infertility Efficacy

Venkataramanaiah Poli, Motireddy Srinivasulu Reddy*

Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh 517502, India.
*Correspondence to: Motireddy Srinivasulu Reddy (E-mail: pvr9490641036@gmail.com)
(Submitted: 18 June 2020 – Revised version received: 10 July 2020 – Accepted: 28 July 2020 – Published online: 26 December 2020)

Abstract
Objectives: The aim of the present study was to evaluate the infertility effect of Eug and *OS* extract administrations in female albino rats.
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 20 days. The control group received saline (vehicle). On day 20 of gestation, maternal and foetal, Antifertility and Antiimplantation, ovarian follicular kinetics and Estrogenic activity parameters. ANOVA Analysis One-way with Dunnett comparison tests used to analyze data. *Ocimum Sanctum* also gets an antifertility effect.
Results: Oral administration of the extract from days 1 to 19 of gestation showed a reduction ($P < 0.05$) in the number of corpora lutea of pregnancy and number of live fetuses. The foetal and placental weights were also significantly ($P < 0.05$) decreased in Eug and *OS* extract compared with the control. The results revealed that Antiimplantation activity were 87.17% and 79.48% in Eug and *OS* extract administration. Antifertility activity was 83.33% in Eug and *OS* extract administration.
Conclusion: The results of this study concluded the evidence for the antifertility activity of the administration of Eug and *OS* extract in female rats. Administration can induce the effects of inhibition on reproductive function in female albino rats.
Keywords: Eugenol, *Ocimum sanctum,* infertility, maternal weights, foetal score, antifertility and antiimplantation, ovarian follicular kinetics, estrogenic activity

Introduction

Efforts are being made to develop antifertility products from plants. Many plants are reported to have the nature of fertility regulation in ancient Indian literature. A large number of plants have been tested for their infertility activities in laboratory animals, but so far there are no single factories that can be further developed as a strong antifertility organization. Therefore, research needs to be continued.[1]

There are increasing trends in the use of medicinal plants, botanicals or herbal preparations, especially in developing countries where these products are available. Some animal studies have revealed anti zygotic properties, blastocytotoxic, anti-implantation and abortification of organic solvent extract from many drug plants commonly used, sometimes in doses dependent. In an antifertility property investigation of triterpenoid glycosides isolated from Dalbergia Saxatilis female rats, the decrease in the body weight of the mother and inhibition of observed conception.[2] Similar observations on infertility, antiimplantation or pregnancy interceptor property suggestive effects of anovulation, antiprogesterogenic or estrogenic has been carried out on calotropis Gigantea extracts[3] and Morinda Citrifolia.[4]

However, research on other animals does not show the effects of the inhibitory plant extract on female reproductive function. For example, Carapa Guianensis seed oil is given orally during the organogenesis period, failing to damage implantation and encourage fetal death.[5] Experimental animal sensitivity, the dose of extracts used, periods and administrative routes and physiological or pharmacological mechanisms are several factors that influence the implantation process.

A number of chemical agents are known to affect utero-tubal-ovarian activity of the positive or negative. Some of these agents come from animals or plants, while others are synthetic. These agents act directly on the ovaries axis of the uterus-tubal or indirectly through the Neuro-endocrine system. The mechanism of action of these substances varies from species to species and is influenced by animal reproductive conditions.[2]

Ocimum Sanctum (*OS*) (Tulsi) has been used traditionally for thousands of years for various healing nature and has been well documented for the nature of the medicine. Chemical compounds are isolated from various parts of the plant, including eugenol, cubenol, rosmarinic acid, beta-sitosterol, apigenin, luteolin, borneol, cardinene, linolenic acid, gallic acid, palmitic acid, oleic acid, stearic acid, carnosic acid, vallinin, vitexin, vicenin, rientin, vallinin acid, circineol, vitamin C, vitamin A, iron and phosphorous. Animal studies show that chronic use of *Ocimum Sanctum* in large doses can cause infertility in females.[6]

Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), which is the main constituent of essential oils (steam is distilled from the fresh leaf of *Ocimum Sanctum* L), is responsible for the therapeutic potential of essential oils and it is a compound phenolic.[7,8]

Leaves of *Ocimum Sanctum* (Tulsi) have been observed to have antibertility and apathy activities in female rats. *Ocimum Sanctum* leaves have antibertility activities. Hibiscus Rosa Sinensis plant flower petals have been used for infertility activities by local physicians and women. Benzene extract from both plants revealed the effect of infertility as indicated by a reduction of 80% on the site of implantation on the 10th day of pregnancy. Give seeds and flowers of Butea monosperms (Leguminosae) to female mice and observe a decrease in fertility. The study clearly revealed that the hot alcohol extract of Butea Monosperm's seeds had antifertility activities because there was no implantation site on one of the eight rats administered at this extract with a weight of 300 mg/kg

B.W. Demonstration in rat that the *Ocimum Sanctum* extract inhibits implantation in more than 50% of rats.[9]

Show some large graffian follicles. Haemorrhagic Corpus Luteum was also seen in the ovary. *OS* leave feeding also inhibits the ovarian hormone in rats. The initial abortification effects of *OS* feeding leave are reported. However, Batla & coworkers cannot see the abortification effects, they confirm the effect of the *OS* antifertility by showing a reduction in 80% on the site of implantation on the 10th day of pregnancy. Singh et. al., demonstrates the absence of pregnancy in the Fed *OS* rabbit which is allowed to mate immediately after termination of eating *OS* leaves. Some workers report the formation of the vaginal plug in animals.[10]

The antifertility study of these plants in female albino rats is lacking. Based on the above information, Experimental work is done to find out the antifertility effects of Eugenol and *Ocimum Sanctum* Linn. Leaf extracts in female albino rats.

Methods

Animals

The Anti-fertility test was performed on adult female Wistar rats weighing between 170 ± 20 g. They were obtained from the animal house of Sri Venkateswara University. The rats were purchased from Sri Raghavendra Enterprises, Bangalore, India. 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 ± 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 experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (CPCSEA, 2003). This study was also carried out in accordance with the guidelines for 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.

Extraction

The *Ocimum Sanctum (OS)* leaves extract was prepared according to (WHO 1983) protocol CG-04. Leaf was sliced, shed-dried, grounded into a fine powder and extracted with 95% D/W (v/v) at 55–60°C for 3 h. The solvent was distilled off under reduced pressure; the resulting mass was dried under vacuum and kept at 24°C until use.

Test Chemical

Pure compound eugenol (99%) was purchased from Sigma Aldrich (St Louis).

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 20 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 20 days administered orally by using gastric destruction techniques.[11,12]

Anti-Fertility Study[13]

Eighteen female Wistar albino rats were used in the study. The rats were paired overnight with sexually active males in the ratio of 2:1. Successful mating was confirmed by the presence of vaginal plug and or sperm cells in the vaginal smear the following morning between 9.00 and 10.00 hours. The day sperm cells were found in the vaginal smear was considered as day 1 of pregnancy. Thereafter, the female rats were randomly divided into three groups of six rats each. 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 20 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 20 days administered orally by using gastric destruction techniques.[11,12]

On day 20 of gestation, each rat was laparatomised under high ether anaesthesia. The uterine horns were exteriorized and incised at the greater curvature of the horns. The latter was examined for sites of implantation and resorption. Number of corpora lutea of pregnancy, the number of live foetuses as well as the weights of the foetuses and placentae were also determined. The postcoitum fertility index was evaluated using the following parameters according to the methods of Uchendu et al.[2] and Tafessel et al.[14]

1. Percentage of pregnant female animals in each group (PPF)
2. Mean live foetal number per pregnant female (LFN)
3. Mean day 20 foetal crown-rump length (FCRL)
4. Mean corpus luteum number per pregnant female (CLN)

The fertility index (FI) of each group was calculated as

$$FI = \frac{LFN \times FCLR \times PPF}{CLN}$$

The anti-implantation and antifertility activities of the extract were calculated as follows:[15]

$$\text{Antiimplantation activity \%} = \frac{\text{No. of implants in control} - \text{No. of implants in test group}}{\text{No. of implants in control group}} \times 100$$

$$\text{Antifertility activity \%} = \frac{\text{No. of non-pregnant animals}}{\text{Total number of animals}} \times 100$$

Ovarian Follicular Kinetics

Ovary from another side of each animal were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 5 µm thickness of the ovary were prepared for the study of follicular kinetics. To quantitatively evaluate ovarian follicles, the methods described by Sanjay and Joshi, 1997; Hirshfield, 1991[15,16] were used in the present study. Ovarian follicles were classified as primary, small preantral, large preantral, small antral and Graafian follicle, according to the morphological classification a scheme used by Lundy et al. 1999.[17]

Estrogenic Activity

The results obtained from the anti-implantation testing indicates the potency of the Eugenol and *Ocimum Sanctum* Linn. Leaf extract in pregnant rats, which significantly increased

the weight of reproductive organs. Therefore, it was subjected to detailed investigation for its possible estrogenic-like activity.

The estrogenic potential of Eugenol and *Ocimum Sanctum* Linn. Leaf extract was evaluated in ovariectomised immature female rats. This activity was carried out according to the previously reported study.[18]

Colony bred, immature, bilaterally ovariectomized female rats (19 days) weighing between 30 to 40 g were divided into three groups consisting of six rats. 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 20 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 20 days administered orally by using gastric destruction techniques.[11,12] Doses were given for 7 days, and on the 8th day of the experiment, all of the animals were sacrificed under excess high ether anesthesia. The uteri were dissected out; surrounding tissues were removed, blotted on filter paper, and weighed quickly.

Statistical Analysis

Results were expressed as mean ± SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's '*t*' test using GraphPad version 3. P values < 0.05 were considered as significant. In each test a 95% confident interval was used.

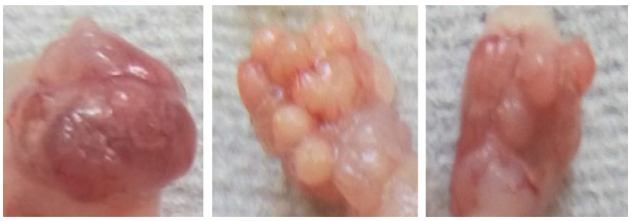

Fig. 1 **Photographs of ovaries.**

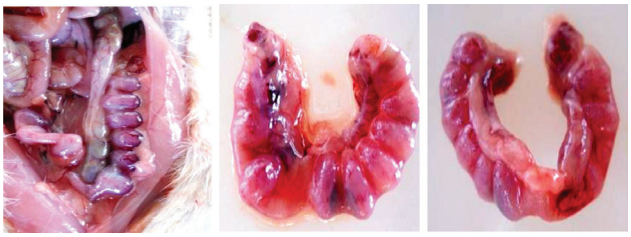

Fig. 2 **Photographs of uterus with foetuses.**

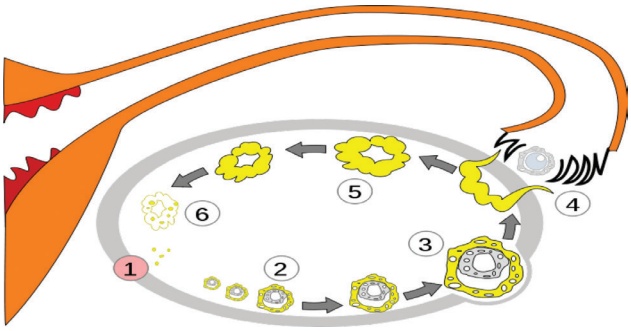

Fig. 3 **Photographs of order of changes in ovary.**
1 – Menstruation, 2 - Developing follicle, 3 - Mature follicle, 4 – Ovulation, 5 - Corpus luteum, 6 - Deterioration of corpus luteum.

Results

Effects on Mother and Fetal Parameters

The linear increase in the maternal weights and weights gained, obtained is higher in control compared to the Eug administration and *OS* extract. The weight of the foetal and placental is also significant ($P < 0.05$) decreases in the Eug administration and *OS* extract compared to control. Data is shown in Table 1.

Effects on Fetal Scores, Parameters

The Antifertility activity of Eug and *OS* extract studied in female rats. After 19 days of administrations, LFN, No. of dead foetuses, REN, FCRL, CLN and PPF (%) was determined. Based on observations above, the antifertility properties of Eug and *OS* extract are expressed with fertility index (F.I). LFN, FCRL and CLN significantly decreased ($P < 0.05$) in the administration of the Eug and *OS* extract compared to control. There is no foetal resorption. % Of pregnant female are reduced to 83.33% with Eug and *OS* extract each compared to control. The fertility index (F.I) decreased to 97.95 by Eug administration and 107.91 by the *OS* extract administration. Data is shown in Table 2.

Effects on Antifertility and Antiplantation Activities

The result revealed that anti-implantation activity was 87.17% and 79.48% in the administration of Eug and *OS* extract. Antifertility activities are 83.3% in the administration of Eug and *OS* extract. Data is shown in Table 3.

Effect on Follicular Ovarian Kinetics

Animals of administrative extract Eug and *OS* cause statistically. The results also showed a significant decrease ($P < 0.05$) in the Primary, Small preantral, Large preantral, Small antral, Graafian follicle, Total no. of follicles and Corpora lutea with simultaneously significant increase ($P < 0.05$) in atretic follicle on Eug and *OS* extract administrations ovary. Data is shown in Table 4.

Effects on Estrogenic Activities

The Eug and *OS* effect extracts administration on rat uterus, which is immature. The oral administration of the caused a significant increase in uterine weight in immature ovariectomized rats $P < 0.05$. Eug and *OS* extract the administration of the vagina opening and the smear shows the conditions of the proestrous or estrous, while all control rats have closed the vagina. The number of cornified cells in the vaginal smears is much higher (+ to ++) than the control (0 to +), but notably less than it is for Eug and *OS* extract administrations rats (+++). All rats, administration shows the open vagina. Data is shown in Table 5.

Discussion

A linear increase in the maternal weights and weights gained, obtained higher in the Eug administration and *OS* extract. In

Table 1. **Effect of Eugenol and *Ocimum Sanctum* Linn. Leaf extract on maternal and foetal weights of rats**

S. No	Parameters		Control (Vehicle treated)	Eugenol administration & significance	*OS* administration & significance
1	Maternal weights (g)	Day 1	154.62 ± 13.78	150.98 ± 11.62[a]	151.74 ± 12.13[a]
		Day 7	178.36 ± 15.14	157.63 ± 13.82[a]	159.82 ± 14.03[a]
		Day 14	201.43 ± 17.62	143.28 ± 10.56[a]	146.25 ± 11.18[a]
		Day 19	229.21 ± 18.74	175.14 ± 15.08[a]	172.39 ± 14.85[a]
	Maternal weight gain (g)	Day 0–5	13.08 ± 1.15	5.13 ± 0.47[a]	5.76 ± 0.49[a]
		Day 6–14	11.14 ± 1.02	9.23 ± 0.79[a]	9.48 ± 0.81[a]
		Day 15–19	19.03 ± 1.64	16.81 ± 1.32[a]	15.97 ± 1.26[a]
		Day 0–19	46.01 ± 3.79	38.76 ± 2.84[a]	37.85 ± 2.52[a]
2	Foetal weight (g)		4.35 ± 0.37	1.46 ± 0.12[a]	1.32 ± 0.10[a]
3	Placental weight (g)		0.05 ± 0.03	0.13 ± 0.01[a]	0.11 ± 0.01[a]

Data are represented as mean ± S.E.M. Means with different superscripts in a row are significantly different ($P = 0.05$).

Table 2. **Eugenol and *Ocimum Sanctum* Linn. Leaf extract on foetal score**

S. No	Parameters	Control (Vehicle treated)	Eugenol administration & significance	*OS* administration & significance
1	LFN	8.22 ± 0.64	3.33 ± 0.21[a]	3.47 ± 0.26[a]
2	No. of dead foetuses	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3	REN	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	FCRL (cm)	3.87 ± 0.28	2.81 ± 0.18[a]	2.87 ± 0.19[a]
5	CLN	8.12 ± 0.68	7.92 ± 0.58[a]	7.69 ± 0.51[a]
6	PPF (%)	100	83.33	83.33
7	F.I	391.76	97.95	107.91

Data are represented as mean ± S.E.M. Means with different superscripts in a row are significantly different ($P = 0.05$). LFN, Live foetal number; REN, Resorbed embryo number; FCRL, Foetal crown-rump length; CLN, Corpus luteum number; PPF, Percentage of pregnant females per group; F.I, Fertility.
The fertility index (FI) of each group was calculated as FI = LFN × FCRL × PPF/CLN.

Table 3. **Antifertility and antiimplantation activities of Eugenol and *Ocimum Sanctum* Linn. Leaf extract in female albino rats**

S. No	Parameters	Control (Vehicle treated)	Eugenol administration & significance	*OS* administration & significance
1	No. tested/No. pregnant	6/6	1/6	1/6
2	No. of dead rats	0	0	0
3	No. of rats showing implantations	6	1	1
4	Total no. of implantations	39	5[a]	8[a]
5	Antiimplantation activity (%)	Nill	87.17	79.48
6	Antifertility activity (%)	Nill	83.33	83.33

Data are represented as mean ± S.E.M. Means with different superscripts in a row are significantly different ($P = 0.05$).

Necropsy, there is no evidence of embryo resorption found in non-pregnant rats. Some reports show the antifertility activity of administrative Eug and *OS* extract[19,20] in animal models.[21] Although neither deaths nor clinically observed, administration the inhibition effects of the Eug and *OS* extract administration in pregnant rats, the change in maternal weight gives a good index of the integrity maternal homeostasis.[22] In this study, a significant decrease ($P < 0.05$) in the maternal body weights was observed when the Eug and *OS* administration was overcome given to rats.

Weights of foetuses of Eug and *OS* extract administrations female rats were also smaller ($P < 0.05$) compared to control. However, there are no gross morphological abnormalities observed.[23]

The reduction with the foetal crown-rump length (FCRL) which is a parameter for foetal growth agreed with the previous findings of the placental formation or placental insufficiency[24] and the development of the foetal.[25] Pregnant rat foetues weight reduction observed in this study has also been reported when Eug and *OS* extracts administered to

Table 4. **Effect of Eugenol and *Ocimum Sanctum* Linn. Leaf extract on ovarian follicular kinetics in female rat**

S. No	Follicles	Control (Vehicle treated)	Eugenol administration & significance	*OS* administration & significance
1	Primary	63.74 ± 5.64	58.17 ± 4.97[a]	56.32 ± 4.75[a]
2	Small preantral	51.32 ± 4.26	46.48 ± 3.94[a]	42.65 ± 3.28[a]
3	Large preantral	21.78 ± 1.63	20.12 ± 1.78[a]	19.85 ± 1.10[a]
4	Small antral	9.58 ± 0.67	8.34 ± 0.52[a]	7.96 ± 0.46[a]
5	Graafian follicle	6.03 ± 0.41	5.18 ± 0.32[a]	5.64 ± 0.38[a]
6	Total no. of follicles	152.45 ± 13.87	138.29 ± 11.05[a]	132.42 ± 10.74[a]
7	Corpora lutea	7.72 ± 0.53	6.54 ± 0.37[a]	6.38 ± 0.31[a]
8	Atretic follicle	47.16 ± 3.23	52.83 ± 2.56[a]	53.45 ± 3.08[a]

Data are represented as mean ± S.E.M. Means with different superscripts in a row are significantly different ($P = 0.05$).

Table 5. **Estrogenic activity of Eugenol and *Ocimum Sanctum* Linn. Leaf extract in ovariectomised female rats**

S. No	Parameters	Control (Vehicle treated)	Eugenol administration & significance	*OS* administration & significance
1	Uterine weight	618.04	833.67[a]	846.43[a]
2	Vaginal cornification	Not open (0 to +)	Open (+++)	Open (+++)

Data are represented as mean ± S.E.M. Means with different superscripts in a row are significantly different ($P = 0.05$). +, nucleated epithelial cells; ++, nucleated and cornified cells; +++, cornified cells.

pregnant rats during gestation.[26] Similarly, the administration of Eug and *OS* extract for pregnant rats does not affect the number and weight of the foetuses and implantation sites.[27]

Eug and *OS* extract administration from day 1 to 19 gestation showed a decrease ($P < 0.05$) in the number of corpora lutea of pregnancy and the number of live fetuses. All females on the administration of Eug and *OS* extract become pregnant, all found to have a live foetus. Based on observations above, the antifertility properties of the administration of Eug and *OS* extract are expressed by the fertility index. Administration of Eug and *OS* extract does not cause abortion or vaginal bleeding. Pregnant animals do not show signs of toxicity. All rats survived till the termination day. There is no foetal resorption. The Significant difference ($P < 0.05$) in the mean foetal number per pregnant female (LFN), the foetal crown rump length (FCRL) and the mean corpus luteum number per pregnant female (CLN) was observed in all administrative groups relative to control.[23] Rats fertility in the Eug administration and *OS* extract is significantly different ($P < 0.05$) of control as reflected by the reduced in the pregnancy and fertility index. Similar observations on the decreased fertility were carried out when rats were pregnant were the administration of Eug and OS extract.[2]

The results show that the Eug administration and *OS* extract have anti-fertility and anti-implantation activities. During the preliminary investigation with the Eug administration and *OS* extract in rat, anti-implantation/anti-fertility activity was observed in different leaves with current observations. This might be explained in terms of species variability.[28]

Substances with anti-fertility properties can administrate the effect at the ovarian level by inhibiting ovulation and or steroidogenesis. It is also supported by administrative evidence, which shows that contraceptive steroids act directly on the ovary to inhibit ovulation and/or some aspects of steroidogenesis.[29] Previously it was reported that the anti-fertility/anti-implantation effect of these agents could be caused by their estrogenic nature.[30] Although not conclusive, the absence of an implantation site in several groups of rats in this study might be caused by extract effects. Eug administration and *OS* extract to many species during the initial pregnancy have been reported resulting in a rapid passage ova through oviducts and the expulsion of ova from the uterus.[31] Decreased fertility due to the acceleration of ova not only because of the expulsion of fertilized ova from the reproductive tract, but also because of the fertilized ova degeneration while transported into the uterus is too early.[31] The reduction in the number of implants observed in several groups of rats in this study might be caused by the extracts. But this is not conclusive because the decrease in fertility or implantation can be caused by the uterine environment than fast transportation. It is possible that extract can produce anti-fertility effects through their ability to change the animal estrus cycle.[32] Therefore, the number of rats shows no implantation in this study might be caused by a prolonged phase of diastrus, which does not chance for fertilization.

There is an increase in the number of atretic follicles and a decrease in concern in the number of primary, preantral, antral, graafian follicles in Eug and administrative *OS* extract may be caused by a non-availability of required amount of extra ovarian regulators (follicle-stimulating hormone and Luteinizing Hormone). The formation of the corpus luteum is a direct continuation of the development of preovulatory follicle. The decrease in the number of corpora lutea shows that Eug and *OS* extracts administration inhibiting the conversion of the Provulation follicles ovulation of the arrest of Corpus Luteum.[33]

In a set of immature ovariectomized rat administration, we observed that extracts showed estrogenic activity as shown by increased uterine weight and vaginal cornification in immature female rats compared to control.

The egg implantation process on the uterine wall depends on the uterine hormone environment.[34] An anti-implantation agent is effective based on their hormonal attributes, namely estrogenic or progestational properties, or by hostile the effect of sex hormones (estrogen and progesterone) female. It's well established that some conventional properties of typical estrogen, such as increasing uterine weight and vaginal cornification, are useful tools for detection and confirmation of hormone properties of anti-artificial agents. In addition, some biochemical parameters of the uterus, and induction of implantation after administration of estrogenic substances in experimental animals, are also used as a tool for detection and confirmation of hormone properties of infertility agents.

Conclusion

The results of this study concluded the evidence for the antifertility activity of the administration of Eug and *OS* extract in female rats. Saponin steroids that occur naturally present in all administrations may be responsible for infertility activities. Administration can induce the effects of inhibition on reproductive function in female albino rats.

Acknowledgments

The authors are grateful to the cassava breeding team at Sri Venkateswara University (SVU) for their technical support during the course of this study.

Funding

This research received no specific grant from any funding agency in the public, commercial or non-profit sectors.

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.

References

1. Shakti Prasad Pattanayak, Papiya Mitra Mazumder. Effect of Dendrophthoe falcata L.f. Ettingsh on female reproductive system in Wistar rats: a focus on antifertility efficacy. Contraception. 2009; 80: 314–320.
2. Uchendu CN, Kamalu TN, Asuzu IU. A preliminary evaluation of antifertility activity of a triterpenoid glycoside from Dalbergia saxatilis in female Wistar rats. Pharmacol Res. 2000; 41:521–525.
3. Srivastava S, Keshri G, Bhargevan B, Singh C, Singh MM. Pregnancy interceptive activity of the roots of Calotropis gigantea Linn in rats. Contraception. 2007; 75: 318–322.
4. Muller JC, Botelho GG, Bufalo AC, Boaereto AC, Rattman YD, Martins ES, et al. Morinda citrifolia Linn Noni: In vivo and in vitro reproductive toxicology. J Ethnopharmacol. 2009; 121: 229–233.
5. Costa-Silva JH, Lima CR, Silva EJ, Araujo AV, Fraga MC, Ribeiro E Ribeiro A, et al. Acute and sub acute toxicity of the Carapa guianensis Aublet Meliaceae seed oil. J Ethnopharmacol. 2008; 116: 495–500.
6. Lalit Sharma, Aditi Sharma GL, Gupta, Gopal Singh Bisht. Protective effect of *Ocimum Sanctum* Linn. leaf extract on ethanol withdrawal syndrome in Wistar rats. Asian Pacific Journal of Tropical Medicine. 2018; 11(8):467-472.
7. Mukherji SP. Ocimum-a cheap source of Eugenol. Science Reporter. 1987; 31: 599.
8. Sen P. Therapeutic potentials of Tulsi : from experience to facts. Drugs News & Views. 1993; 1(2): 15–21.
9. Ankush Raj, Amrinder Singh, Arvind Sharma, Netrapal Singh, Pradeep Kumar, Vidur Bhatia. Antifertility Activity of Medicinal Plants on Reproductive system of Female Rat. International Journal of Bioengineering Sciences & Technology. 2011; 02(03): 44–50.
10. Singh J, Sethi J and Sood S. *Ocimum sanctum*: An Updated Review. Journal of Pharmaceutical Research. 2010; 9 3: 97–103.
11. Shankar Mondal, Bijay R, Mirdha and Suhil C, Mahapatra. The science behind sacredness of tulsi *Ocimum sanctum* Linn, Indian J Physiol Pharmacol. 2009; 53(4): 291–306.
12. Kulkarni D. Eugenol induced changes in reproductive cycle of female albino rats. Biosci Biotech Res Comm. 2011; 41: 98–101.
13. Hyacinth AA, Nwocha UC. Antifertility activity of aqueous ethanolic extract of Hymenocardia acida stems bark in female rats.Iranian journal of reproductive medicine. 2011; 9(3): 217.
14. Tafessel G, Mekonnen Y, Makonnen E. In vivo and in vitro antifertility and antiimplantation properties of Leonotis ocymifolia in rats. Afr J Trad CAM. 2005; 2: 103–112.
15. Sanjay VS, Joshi BN. Melatonin and exposure to constant light/darkness affects ovarian follicular kinetics and estrous cycle in Indian desert Gerbil Meriones hurrianae Jerdon. Gen Comp Endocrionol. 1997; 180: 352–357.
16. Hirshfield AN. Development of follicles in the mammalian ovary. Int Rev Cytol. 1991; 124: 43–101.
17. Lundy T, Smith PO, Connell A, Hudson NL, Mc Natty KP. Population of grannulosa cells in small follicles of the sheep ovary.J Reprod Fertil. 1999; 155: 251–262.
18. Hiremath SP, Hanumantha RS. Antifertility efficacy of the plant Striga lutea Scrophulariacae on rats. Contraception. 1990; 42(4): 467–477.
19. Uchendu CN, Isek T. Antifertility activity of aqueous ethanolic leaf extract of Spondias mombin Anacardiaceae in rats. Afr Health Sci. 2008; 8: 163–167.
20. Yadav R, Jain GC. Antifertility effect and hormonal profile of petroleum ether extract of seeds of Cassia fistula in female rats. International Journal of Pharmacology and Technology Research. 2009; 1: 438–444.
21. Hikim AP, Lue YH, Wang C, Reutrakul V, Sangsuwan R, Swerdloff RS. Post testicular antifertility action of triptolide in the male rat: evidence for severe impairment of cauda epididymal sperm ultra structure. J Androl. 2000; 21: 431–437.
22. Almeida FC, Lemonica IP. The toxic effects of Coleus barbatus on the different periods of pregnancy in rats. J Ethnopharmacol. 2000; 73: 53–60.
23. Nirmal G and Arun Nilkanthrao Bhasme. Anti-implantation and Antifertility activity of azadirchta indica tincture ait and azadirchta indica 30 potency ai 30 in female albino rats. International Journal of Biological & Pharmaceutical Research. 2017; 8(1): 29–33.
24. Rasheed RA, Bashir AK, Ali BH, Padmanabhan R. Effect of Rhazya stricta on the developing rat fetus. Reprod Toxicol. 1997; 11: 191–199.
25. Schwarz A, Pinto E, Haraguchi M, de Oliveira CA, Bernardi MM, Spinosa HS. Phytochemical study of Solanum lycocarpum unripe fruit and its effects on rat gestation. Phytotherapy Research. 2007; 21: 1025–1028.
26. Nana P, Asongalem ER, Foyet HS, Folefoe GN, Dimo T, Kamtchouing P. Maternal and developmental toxicity evaluation of Acanthus montanus leaves extract administered orally to Wistar pregnant rats during organogenesis. J Ethnopharmacol. 2008; 116: 228–233.
27. Iranloye B, Owokunle B. Effect of Garcinia kola seed extract on female reproductive functions in rats. Int J Pharmacol. 2008; 4: 276–281.
28. Uguru MO, Okwuasaba FK, Ekwenchi MM. and Uguru VE. Oxytocic and oestrogenic effects of Monechma ciliatum methanol extract in vivo and in vitro in rodents. Phytother. Res. 1995; 9 (1): 26–29.
29. Goldfien A. The gonadal hormones and inhibitors. Basic and clinical pharmacology, 6th ed. ed. Katzung, B. G.. Appleton and Lange, 25 Van Zant Street, east Norwalk. 1995.
30. Debella A, Haslinger E, Kunert O, Michl G and Dawit Abebe. Steroidal Saponins from Asparagus africanus. Phytochemistry. 1999; 51: 1066–1075.

31. Fransworth MR, Bingel AS, Cordell GA, Crane FA. and Fong HS. Potential Value of Plants as Sources of New Anti-fertility Agent I. J Pharml Sci. 1975; 64 (4): 535–598.
32. Mekonnen Y. Effects of ethanol extract of Moringa stenopetala leaves on guinea pig and mouse smooth muscle. Phytother Res. 1999; 13: 442–444.
33. Sarita M. Antifertility Activity of Eugenia Jambolana Seed Extract in Female Albino Rat. Biochem Physiol. 2018; 7(2): 1–5.
34. Ahirwar D, Ahirwar B, Kharya MD. Evaluation of antifertility activity of Trigonella foenum graecum seeds. J Der Pharm Sci. 2010; 1(3): 33–39.