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Scientific Validation of Ovulation Inducing Activity and Estimation of Hormonal Level of *Navamani Chendhuram* in Female Wister Albino Rats an in-vivo Study

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ABSTRACT

PCOS (Polycystic Ovary Syndrome) is a common female reproductive endocrine disorder with prevalence ranging from 22% to 26% mostly in adult women with age ranged from 18 to 45 years. *Navamani chendhuram* (NC) is a sastric preparation mentioned in "*Sigitcha Rathna Deepam*" an ancient Siddha literature. This drug was specifically indicated for the management of *Soodhaga katti* and *Soodhaga Sikkal* (Amennorhoea). The drug was prepared well as per the standard operative procedure. This study aims to scientifically validate the pharmacological activities like Ovulation inducing activity and Estimation of Hormonal level of NC through an in-vivo assay method. Experimental animals divided into 5 groups. Group I as normal control, Group II as induced control, Group III was treated with standard drug and the Group IV and group V was treated with test drug (NC) at the dose level of 5mg/kg (low dose) and 10 mg/kg (high dose) with honey along with *Seeraga kudineer*, single dose daily for 14 days. The treatment with both doses of NC reduces the size of the ovary in letrozole induced rats. The experimental group had considerably higher serum FSH and Estrogen related to the potency of the standard drug. The histopathology of the ovary showed the considerable increase in matured graafian follicles and more secondary follicles showed much similarity to standard drug that supported for the confirmation of ovulation. NC treatment had appreciable effects on the normalization of any major parameters in PCOS-induced rats.

Key Words PCOS, *Navamani Chendhuram*, Ovulation inducing activity, Letrozole, Clomiphene

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INTRODUCTION

The Siddha system is a traditional medicine practice that uses plants, metals, minerals, and animal products to treat illnesses. It includes specific remedies for women's health, focusing on both prevention and treatment.

"Women are the real architects of Society" - Hariet Beecher Stowe[1].

Due to biological, social, and behavioral differences, women's health experiences differ from men's. These biological distinctions, which range from the phenotypic to the molecular level, present specific

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risks for disease development[2]. For example, Polycystic Ovary Syndrome (PCOS), once considered a rare reproductive condition after its diagnosis in 1935, is now the most common female endocrine illness, often causing infertility[3]. It's also a genetic disorder with a 50% chance of being passed from an affected parent to their child[4].

Polycystic Ovary Syndrome (PCOS) is primarily defined by the stalled development of follicles, which normally mature into a dominant follicle[5]. This condition affects an estimated 116 million women worldwide, with symptoms present in 5% to 10% of women of reproductive age. PCOS is a leading cause of female infertility and also increases the risk of other health issues, including Type 2 diabetes, cardiovascular disease, dyslipidemia, and hyperinsulinemia. Due to these associated health risks, it is crucial to monitor and manage this syndrome to protect women's health[6,7].

Navamani chendhuram (NC) is a a traditionally used Siddha drug mentioned in "*Sigitcha Rathna Deepam Ennum Vaithiya Nool*" an ancient Siddha literature[8]. Science has not yet assessed the precise pharmacological properties of this medication. The present study shows the current study proven the therapeutic efficacy of NC by validate its ovulation inducing activity and hormonal levels by validate it using pharmacological screening of ovulation inducing response and estimation of in female Wistar albino rat models through invitro assay method in order to resolve these scientific voids.

AIM

This study aims to scientifically validate the Ovulation inducing activity, Estimation of Hormonal level of Siddha formulation *Navamani Chendhuram* through an *in-vivo* assay method.

OBJECTIVES

The following methodology was adopted to screen the pharmacological activities of the test drug *Navamani Chendhuram*.

➢ Preparing the Trial drug in accordance with the Classic Siddha literature.

Pharmacological activity

In-vivo assay method

❖ Ovulation-inducing activity in letrozole-induced PCOS animal model.

❖ Estimation of hormonal level.

MATERIALS AND METHODS

a. Drug selection

NC is a traditional Siddha mineral formulation described in *Sigitcha Rathna Deepam Ennum Vaithiya Nool*.

b. Ingredients of *Navamani Chendhuram*

1. *Veeram* (Hydrargyrum perchloride)
2. *Pooram* (Calomel)
3. *Lingam* (Cinnabar)
4. *Kandhagam* (Sulphur)
5. *Padigaram* (Alum)
6. *Vediuppu* (Potassium nitrate)
7. *Navacharam* (Ammonium chloride)
8. *Vengaram* (Borax)
9. *Ayam* (Ferrum)

c. Raw Drug collection

- All the above-mentioned raw drugs were procured from a reputed country shop in Parrys Corner, Chennai.

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- All the ingredients were purified and the medicine was prepared in the *Gunapadam* laboratory at the National Institute of Siddha.

d. Identification and Authentication of the drug:

The metal and mineral drugs were identified by Pharmacologist, Dept. of *Gunapadam*, NIS, Tambaram sanatorium, Chennai. (CN: Gun/Aut/026/21)

e. Purification of the drugs:

All the drugs mentioned here were purified as per the Siddha literature.

f. Preparation of the trial drug

The mentioned drugs were purified and powdered (except *ayam*), The powder was ground in lemon juice for two *saamam* (6 hours), and then purified mam was added to it. This mixture underwent a calcification process using 50 cow dung cakes. The calcified drug was ground using lemon juice for four *saamam* (12 hours) and made into 16 *villai*, which were then dried under sunlight. After drying, the *chenduram* were incinerated with 30 cow dung cakes. This procedure was repeated six times, and then the final product was obtained.

Dosage and vehicle:

½ - 1 *Kundri* (65- 130mg), 1times/day with honey followed by *seeraga* kudineer.

Indication:

Within 7 days, *Soothaga Katti*, *Peruvayiru* are treated. Within 3 doses, *Soothaga Sikkal* were treated.

Pharmacological study

Ovulation inducing activity in female Wister albino rat model

Method

The rats' reproductive cycles were synchronized using the following technique before beginning medication therapy. Subcutaneous injections of 100 g of estradiol in 2 ml of olive oil were administered. After 24 hours, 50 g of progesterone dissolved in olive oil was injected intramuscularly into each rat. In order to track ovulation and the estrous cycle, vaginal smears were taken by vaginal lavage after a short period of time. Vaginal smears were made by using a glass dropper to wash the vaginal opening with 0.9% w/v sodium chloride. The slide was then cleaned, and the sample was inspected under a light microscope at a magnification of 40X. All of the animals were found to be in the estrous stage when vaginal smears were examined.

Experimental design

- Group I - Normal control animals 2ml/kg of 1%CMC solution
- Group II - Letrozole 1mg/kg dissolve in 1%CMC solution
- Group III - Letrozole + Standard (clomiphene citrate) 10 mg/kg
- Group IV- Letrozole + test drug 5 mg/kg (*Navamani Chendhuram* – NC)
- Group V - Letrozole + test drug 10 mg/kg (*Navamani Chendhuram* – NC). All the drugs were given orally for 14 days.

At the end of the study, the animals were anesthetized by intraperitoneal injection of Thiopentone sodium (40mg/kg). Under anesthesia, blood was collected through Sino-orbital puncture. Blood was centrifuged, serum was separated and subjected to hormonal assay like, luteinizing hormone, follicular stimulating hormone, estradiol and progesterone. Later, all the animals were sacrificed with excess anesthesia, and the abdominal cavity was cut opened ovaries were excised, dissected and weighed. The ovaries were processed for histopathological examination[9].

Hormonal Estimations

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The method employed was Microwell Enzyme Linked Immunosorbent Assay (ELISA) using analytical grade reagents[10-13].

Estimation of serum Luteinizing hormone (LH)

Estimation of serum Follicular Simulating Hormone (FSH)

Estimation of Oestrogen levels

Estimation of Progesterone levels

Histological analysis:

After being removed from the uterus, the ovary was fixed in formalin for 20 to 24 hours. These fixed tissue samples were then immersed progressively in higher concentrations of alcohol and paraffin was covered over it. Slices of tissue were stained with hematoxylin and eosin, followed by observation and examination under a light microscope. For the purpose of evaluating the trial drug's capacity to induce ovulation, all tissue blocks were serially cut. The identification of a follicle was based on the discovery of a nucleus. The number of follicles- primordial, primary and secondary follicles etc.- was counted.

Effect of NC on Ovary and Reproductive organ weight:

There was a significant decrease in ovarian weight from 0.48 gm to 0.38 gm after the treatment of PCOS induced rats with clomiphene citrate. When the test drug NC administered to the PCOS induced rats at the dose level of 5 mg/kg low of single dose for a week, there is significant decrease in ovarian weight from 0.48 gm to 0.41 gm was recorded. At the level of high dose 10mg/kg it shows a prominent decrease in ovarian weight from 0.48 gm to 0.37 gm was recorded.

There was a significant decrease in uterus weight from 1.62 gm to 1.52 gm after the treatment of PCOS induced rats with clomiphene citrate. When the test drug NC administered to the PCOS induced rats at the dose level of 5 mg/kg low of single dose for a week, there is significant decrease in uterus weight from 1.62 gm to 1.40 gm was recorded. At the level of high dose 10mg/kg it shows a prominent decrease in ovarian weight from 1.62 gm to 1.45 gm was recorded shown in Table 1 and also represented in graph as Graph 1.

RESULTS

Table 1 Effect of *Navamani Chenduram* on weight of Uterus and Ovary

Groups	Drug Treatment	Weight of Uterus (gm)	Weight of Ovary (gm)
I	Normal Control (1% CMC, 1ml/kg)	1.40±0.04	0.35±0.02
II	Letrozole (1mg/kg)	1.62±0.03	0.48±0.03
III	Letrozole+ clomiphene (10mg/kg)	1.52±0.06	0.38±0.01
IV	Letrozole + NC (5mg/kg)	1.40±0.03	0.41±0.04
V	Letrozole + NC (10mg/kg)	1.45±0.05	0.37±0.02

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Letrozole Group.
The data are expressed in mean ± SEM. Results were analyzed using one way ANOVA followed by the Dunnet's test. Differences were considered as statistically significant if p< 0.05. when compared with control. p value ******P<0.001 is considered to be extremely significant, then *P<0.05, **P<0.01 indicates significant.

Histopathology study of ovary

Normal control:

Sections studied from the ovary of normal control showed small to medium sized ovarian follicles and matured graafian follicles. Corpus luteum lined by theca and granulosa cells with normal morphological features as shown in Figure 1.

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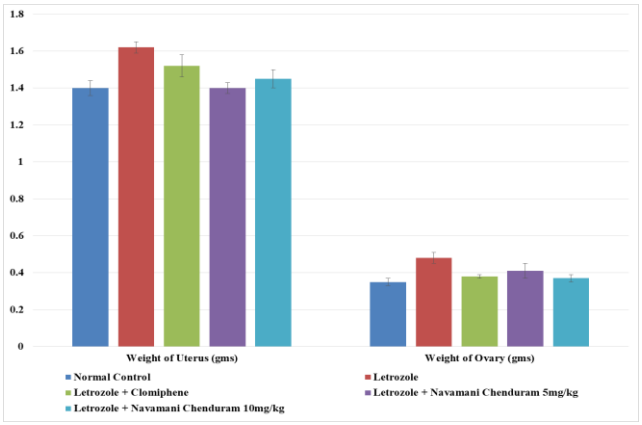
Induced control:



Graph 1 Effect of *Navamani Chenduram* on weight of Uterus and Ovary

Figure 1 Histopathology slides of ovary – Normal control

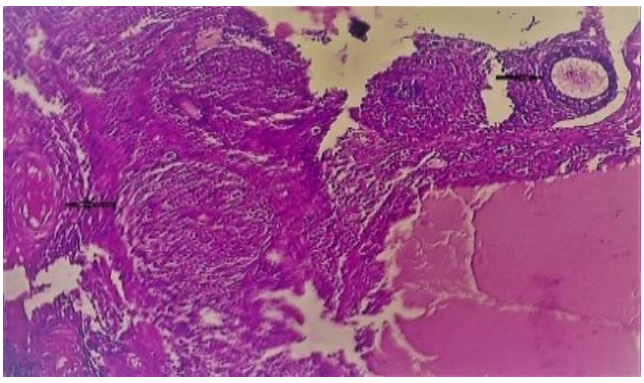


Figure 2 Histopathology slides of ovary – Induced control

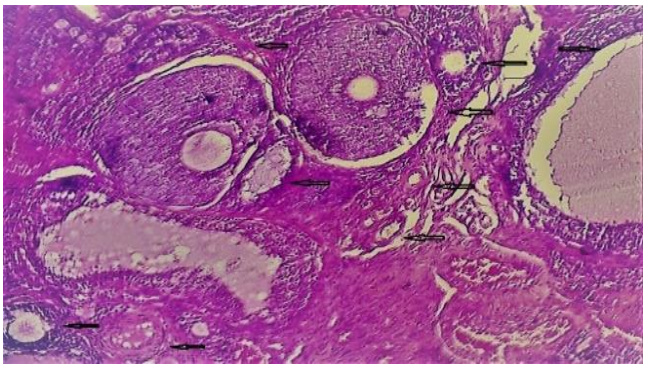


Figure 3 Histopathology slides of ovary – Standard Drug

Figure 4 Histopathology slides of ovary – Test Drug (low dose)

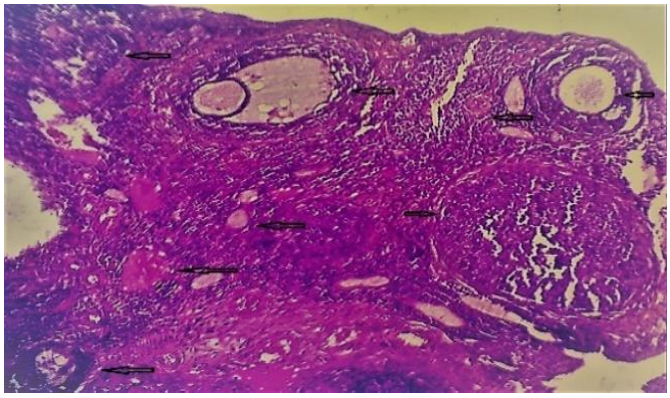


Figure 5 Histopathology slides of ovary – Test Drug (High dose)

Sections studied from the ovary of letrozole treated showed varying sizes of ovarian follicles. The section appears with few cystic follicles and lack in corpus luteum. Cortex show few cystic follicles as shown in Figure 2.

Standard drug:

Sections studied from the Ovary of Letrozole and clomiphene showed small to medium sized ovarian follicles. The section appears with increased in matured graafian follicles and corpus luteum. The section appears with healthier primary and secondary follicles as shown in Figure 3.

Test drug (low dose):

Sections studied from the ovary treated with low dose *Navamani Chenduram*, shows varying sizes of ovarian follicles with matured graafian follicles and

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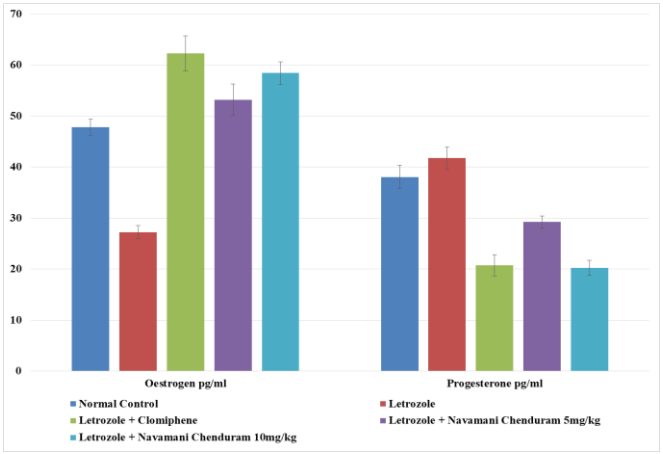
corpus luteum. Many corpus luteum with few cystic follicles appears prominent as shown in Figure 4.

Test drug (High dose):

Sections studied from the ovary treated with high dose *Navamani Chenduram,* show small, medium and large size of ovarian follicle. The section appears with increased in matured graafian follicles and corpus luteum. Cortex show primary and secondary follicles and dilated blood vessels. Few cystic follicles are also noted shown in Figure 5.

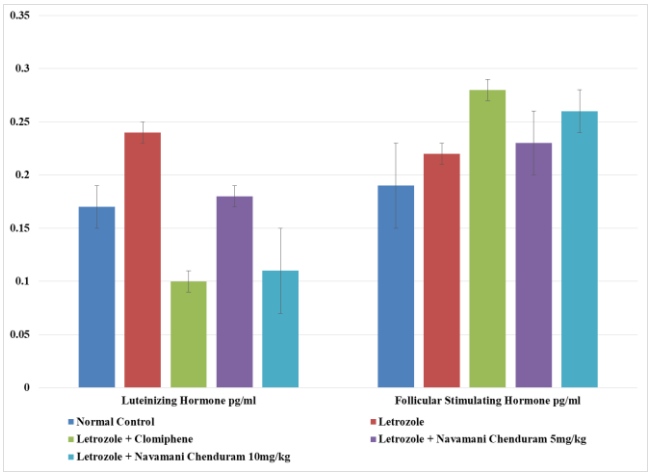
Effect of *Navamani Chenduram* on Serum Concentration of reproductive Hormones of Letrozole induced Ovulation in female rats

The level of Estrogen increased significantly from (27.24±1.24) pg/ ml to (62.27±3.48) pg/ml (P<0.001) in PCOS induced rats after the treatment with clomiphene citrate. Estrogen levels significantly increased up to (27.24±1.24) pg/ml to 53.20±3.10 (P<0.001) after administration of the test drug NC in low dose whereas in high dose it increases the estrogen level up to 58.42±2.20 (P<0.001) as represented in Graph 2.



Graph 2 Effect of *Navamani chendhuram* on Serum Oestrogen and Progesterone in rats

The level of Progesterone decreased significantly from (41.73±2.20) pg/ ml to (20.73±2.05) pg/ml (P<0.001) in PCOS induced rats after the treatment with clomiphene citrate. Progesterone levels significantly decreased up to (41.73±2.20) pg/ml to 29.22±1.21 (P<0.01) after administration of the test drug NC in low dose whereas in high dose it decreases the Progesterone level up to 20.21±1.50 (P<0.001) as represented in Graph 2.



Graph 3 Effect of *Navamani chendhuram* on Serum LH and FSH in rats

Changes occur in the level of FSH and LH in PCOS induced rats after the treatment with clomiphene citrate and the test drug (low and high doses) were recorded and shown in Graph 3.

DISCUSSION

The traditional drug '*Navamani Chendhuram'* was selected from the siddha literature "*Sigitcha Rathna Deepam*" were undergone to exerts its ovulation inducing activity and hormonal levels through in-vivo assay method.

The pharmacological activities of *Navamani chendhuram* (NC) like ovulation inducing activity, estimation of hormone level and anti-oxidant activity have shown significant outcome. The results indicated numerous histologic and biochemical findings that were compatible with human PCOS[14,15]. In the current research endeavor, similar outcomes were attained. Letrozole was administered to the rats to induce PCOS, in this the testosterone level was comparatively higher[16]. Letrozole is a non-steroidal

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aromatase inhibitor, which means that it prevents the conversion of testosterone to estrogen, according to earlier research. This results in a decrease in the production of estrogen[17]. The estrogen levels in the PCOS-induced animals were lower. NC was administered repeatedly, and this resulted in a considerable rise in estrogen levels. In PCOS induced rats, ovarian weight was higher than in induced control rats. The NC therapy stopped the weight gain in the ovaries from continuing which has been compared with the standard drug clomiphene citrate.

The treatment with both doses of NC caused an alteration in the amount FSH, which was statistically significant and reduces the size of the ovary in letrozole which indicates the ovulation. Estrogen level increased significantly from (27.24±1.24) pg/ ml to (62.27±3.48) pg/ml (P<0.001) in PCOS induced rats after the treatment with clomiphene citrate. Estrogen levels significantly increased up to (27.24±1.24) pg/ml to 53.20±3.10 (P<0.001) after administration of the test drug NC in low dose whereas in high dose it increases the estrogen level up to 58.42±2.20 (P<0.001) as in Table 2.

Table 2 Effect of *NC* on Serum Concentration of reproductive Hormones

Groups	Drug Treatment	LH (pg/ml)	FSH (pg/ml)	Estrogen (pg/ml)	Progesterone (pg/ml)
I	Normal Control (1% CMC, 1ml/kg)	0.17±0.02	0.19±0.04	47.83±1.57	38.05±2.33
II	Letrozole (1mg/kg)	0.24±0.01	0.22±0.01	27.24±1.24	41.73±2.20
III	Letrozole + Clomiphene (10mg/kg)	0.10±0.01**	0.28±0.01*	62.27±3.48***	20.73±2.05***
IV	Letrozole + NC (5mg/kg)	0.18±0.07	0.23±0.03	53.20±3.10***	29.22±1.21**
V	Letrozole + NC (10mg/kg)	0.11±0.04*	0.26±0.02*	58.42±2.20***	20.21±1.50****

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Letrozole Group

The experimental group (IV and V) that got the test drug with low and high doses had considerably higher serum levels of FSH and estrogen related to the potency of the standard drug.

In the current study, it was discovered that PCOS-induced rats had higher uterine weights. When NC was administered to PCOS-induced rats, the weights of the reproductive organs were restored to normal the outcomes showed that the administration of NC significantly increased ovarian weight as showed in Graph 1. Thus, by improving oxygen transport to granulosa cells and reducing atresia in small and medium follicles. Based on the histopathology slides, the experimental groups V has considerable increase in matured graafian follicles and more secondary follicles showed much similarity to standard drug (group III) that supported for the confirmation of ovulation.

Since serotonergic nerves and the hypothalamus are both connected, it is likely that GnRH and gonadotropic hormones are released by NC stimulation of nor adrenergic nerves in the locus coeruleus[18]. It could indicate that the test drug stimulates the hypothalamus as well as the pituitary and ovaries. The effects of NC treatment were found to be comparable to those of clomiphene citrate, and NC treatment had appreciable effects on the normalization of any major parameters in PCOS-induced rats.

CONCLUSION

Navamani chendhuram (NC), A novel siddha formulation was prepared as per the Siddha literature. The results showed that the pharmacological activity

of this study ensures that the effects of NC treatment were found to be comparable to those of standard drug clomiphene citrate. NC treatment had appreciable effects on the normalization of any major parameters in PCOS-induced rats and enhanced the ovulation by elevated the serum concentration of FSH, Estrogen and confirmed by the formation of secondary follicles. It was then concluded that, NC showed the considerable effect in reproductive diseases and enhanced the women's health. In future, wide based extensive clinical studies have to be carried out to prove the efficacy of the drug.

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REFERENCES

1. Howkin's and Bourne Shaw, Text Book of Gynaecology, J.P Brothers,14 th edition, 2004, pg:no: 589.

2. Fedorcsák P et al. The impact of obesity and insulin resistance on the outcome of IVF or ICSI in women with polycystic ovarian syndrome, Human reproduction (Oxford, England). Available at: https://pubmed.ncbi.nlm.nih.gov/11387273/

3. Stein IF & Leventhal ML (1935) Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 29: 181-186. 5. Delhi IVF Fertility Research Center. [online]. Available from: URL: http://www.delhiivf.com/PCOD.html

4. Aboul Enien, W. M., Barghash, N. A., & Mohamed Ali, F. S. (2024). Clinical, ultrasonographic and endocrine predictors of ovarian response to clomiphene citrate in normo gonadotropic anovulatory infertility. Middle East Fertility Society Journal, 242-250.

5. Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., et al. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet, 380(9859), 2163–96. https://doi.org/10.1016/S0140-6736(12)61689-4

6. Boomsma, C. M., Fauser, B. C., & Macklon, N. S. (2008). Pregnancy complications in women with polycystic ovary syndrome. Seminars in Reproductive Medicine, 26(1), 72–84. https://doi.org/10.1055/s-2007-992927

7. Kannusamipillai, C. (2014). SikitchaRatna Deepam EnnumVaidhyanool (14th ed.). B Rathinanaayagar & sons Publishers.

8. Kavitha, N., Velpandian, V., Kumar, A., Ayyasamy, S., Pitchiah Kumar, M., & Banumathi, V. (2013). Ovulation inducing and folliculogenetic activity of uppu parpam in female wistar albino rats. An International Journal of Advances in Pharmaceutical Sciences, 4(2), 213–222.

9. Rajan, R. K., et al. (2017). Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism. PharmaceuticalBiology, 55(1), 242–251. https://doi.org/10.1080/13880209.2016.1258425 PubMed: 27927075.

10. Beastall, G. H., et al. (1987). Assays for follicle stimulating hormone and luteinising hormone: Guidelines for the provision of a clinical biochemistry service. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine, 24(3), 246–262. https://doi.org/10.1177/000456328702400303

11. Dighe, A. S., & Sluss, P. M. (2004). Improved detection of serum estradiol after sample extraction procedure. Clinical Chemistry, 50(4), 764–766.

12. Hashimova, U. F., Kamilova, N. K., Babaev, K. F., Shukurova, P. A., Hasanova, S. I., & Abbasov, R. Y. (2017). Effects of saffron (Crocus sativus L. Iridaceae) on blood level of follicle-stimulating hormone and number and dynamics of body weight of offspring in female rats. Advances in Gerotology, Uspekhi gerontologii, 30(3), 436–441.

13. Barengolts, E., et al. (2009). Effects of progesterone on posto variectomy bone loss in aged rats. Journal of Bone and Mineral Research, 5(11), 1143–1147. https://doi.org/10.1002/jbmr.5650051109. Accessed 29 May 2023.

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14. Kafali, H., Iriadam, M., Ozardali, & Demir, N. (2004). Letrozole induced polycystic ovaries in the rat: a new rat model for cystic ovarian disease. Archives of Medical Research, 35, 103–108.

15. Bhattacharya, D., Mandal, S. K., Mukherjee, S., & Pradhan, M. (2005). Clinical correlation with biochemical status in polycystic ovarian syndrome. Obstetrics & Gynecology India, 55(1), 67–71.

16. Baravalle, C., Salvetti, N. R., Mira, G. A., Pezzone, N., & Ortega, H. H. (2006). Microscopic characterization of follicular structures in letrozole induced polycystic ovarian syndrome in the rat. Archives of Medical Research, 27, 830–839.

17. Manneras, L., Cajander, S., Holmang, A., Seleskovic, Z., Lystig, T., Lonn, M., et al. (2007). A new rat model exhibiting both ovarian and metabolic characteristics of polycystic ovary syndrome. Endocrinology, 148, 3781–3791.

18. Karimi, G., Hosseinzadeh, H., & Khaleghpanahi, P. (2001). Study of antidepressant effect of aqueous and ethanolic of Crocus sativus in mice. Iranian Journal of Basic Medical Sciences, 4, 11–15.