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# Effect of Siddha Formulation *"Chembaruthiver Chooranam"* on Female Wistar Rats with Estradiol Valerate induced Polycystic Ovarian Syndrome

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## Abstract

Polycystic ovary syndrome (PCOS) is a complex condition. Research suggests that 5 % to 10 % of females 18 to 44 years of age are affected by PCOS, making it the most common endocrine abnormality among women of reproductive age. In Siddha text *"Yugimunivaithiyakaaviyam"* mentioned that*"Rathasooraivaayu"*[36] which symptoms are correlated with PCOS. The aim of the present study was to evaluate the effect of Siddha formulation *"Chembaruthiver chooranam"*[37]which mentained in Siddha text *"Gunapaadam – Mooligaivaguppu"*in the treatment of Estradiol valerate induced PCOS. This was a 45 days, cohort study. Thirty animals of Wistar rat were randomly selected and divided into five groups (n=6) and housed as such (6 rats per cage). All the animals in four groups were injected with E. V by intra muscularly and the remaining one group is normal control group. The rats were allowed to establish PCOS for 30 days.**[4]** After 30 days, groups in G4 & G5 were dosed orally by gavage for 15 days, whereas rats in the standard group was dosed for 5 days. On 16$^{th}$ day, six animals from each group (Control and PCO) were randomly selected and blood samples were collected, and the serum were used for hormonal assays (FSH, LH, estradiol, progesterone and testosterone).**[4]** The ovaries were weighed, and histopathological examination was conducted on the ovaries. In conclusion ***"Chembaruthiverchooranam"***shows significant recovery of FSH, LH, estradiol & progesterone and also restored the irregular cycle and ovarian physiology to normal in the PCOS animals.

## Key words

PCOS,*Rathasooraivaayu, Chembaruthiverchooranam,* E.V-Estradiol valerate, Hormonal assay.

## Introduction

PCOS is a hormonal disorder with a potential, lead to various diseases. The disorder can be morphological or predominantly biochemical. PCOS commonly affect women of childbearing age, it also continues to be a common cause of infertility among women. Women with PCOS have higher rates of endocrine cancer, cardiovascular disease, dyslipidemia and type-2 diabetes mellitus.

To a large world population, medicinal plants are the only source to prevent and treat various diseases.Medicinal plants form main source of health care due to better acceptability and fewer side effects. Herbal plants have been used since centuries to correct disorders caused by the hormonal imbalance related to female reproductive system.[1,2,3] Current research work is to investigate the effect of *"**Chembaruthiverchooranam"*** in treatment of Estradiol valerate induced PCOS. The current research work focuses on normalization of estrous cycle in PCOS after treatment with siddha preparation*"**Chembaruthiverchooranam".***

## FORMULATION OF *"CHEMBARUTHIVER CHOORANAM"*:

**Reference:** *Gunapaadam – Mooligaivagupu*

**Incrediance:**

*Chembaruthiver* – (Root of *Hibiscus-rosasinensis)*

*Mulilavupattai*– (Bark of*Bomboxmalabaricum)*

*Thamaraikizhngu* – (Rizome of*Nelumbonucifera)*

The above drugs are taken in equal quantity, and then it is powdered separately and sieved in pure white cloth (*Vasthirakayam*). Then the powder of all the drugs are mixed and taken as a compound preparation.

## EXPERIMENTAL MODEL

For the study of poly cystic ovary syndrome an experimental model is selected in such a way that it would satisfy the following condition

- The animal should develop cyst rapidly.
- Pathological changes in the site of induction should result from PCOS formation.
- The symptom should be ameliorated or prevented by a drug treatment effective in humanbeings.
- The test drug should be administered orally.
- Drug dosage should approximate the optimum therapeutic range for human, scaled the test animal weight.

**Selection grouping and Acclimatization of Laboratory Animal**

The study is conducted after obtaining institutional animal ethical committee clearance (IAEC NO: MD(S)/TNMGRMU/KMCP/IAEC/319). As per the standard practice the rats are segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They are fed on healthy and maintained in hygienic environment in our animal house.

**Technique for inducing PCOS**

Technique for induction ofPCOS in animals is, chemically induced (using Esterodialvalerateoil, Letrazole, Androgen, Prenatal Antrogen, Dehydroepiandrosteroneetc)which have been used in experimental studies of PCOS activity.

**Induction of PCOS in the Animals**

In the present study, Estradiol valerate induced PCOS is used to evaluate the treatment of PCOS.[4]Thirty adult virgin Wistar rats of approximately 10-12 weeks of age, weighing between 180-220gm and with regular 4-5 day estruscycles as assessed by vaginal smear, were used for the study [5]. Five of the rats were kept as controls, and the others were each given intramuscular injection of 4 mg EV in an oily solution per rat.[6,7] Vaginal smears were examined daily in all animals. Cessation of cycle, which was shown by the persistent cornification of vaginal smears, was used as a criterion forselection into the PCOS group.

**Vaginal Smears**

The stage of cycle was determined by microscopic analysis of the predominant cell type in vaginal smears. Estrous cycle was monitored by vaginal smears obtained between 0800 and 1200 hours, and it was assessed by analysis at the light microscopy level of the relative proportion of leukocytes, epithelial and cornifiedcells found in daily vaginal lavages, which characteristically change during different stages of the estrous cycle. The rat estrous cycle was (estrus, diestrus1, diestrus2, and proestrus) usually lasts about 4 days, in controls or PCO rats.[8]

**Treatment Protocol**

Thirty animals of Wistar rat were randomly selected and divided into five groups (n=6) and housed as such (6 rats per cage). All the animals in four groups were injected with Estradiol valerate by intra muscularly and the remaining one group is normal control group.

The rats were allowed to establish PCOS for 30 days.[4] After 30 days, groups in G4 & G5 were dosed orally by gavage for 15 days, whereasrats in the standard group was dosed for 5 days.

**Group 1** served as the normal control.

**Group 2** served as the PCOS control. Group 1 and 2 receives normal diet and Water.

**Group 3** served as the positive control, was treated with injection Clomiphene citrate at 20 mg/kg body weight, Intra-peritoneally. [11]

**Group 4** served as the treatment control, treated with ***Chembaruthiverchooranam*** at 100 mg/kg body weight, through orally.

**Group 5** served as treatment control which was treated with ***Chembaruthiverchooranam***at 200 mg/kg of body weight, through orally**.**

On 16th day, six animals from each group (Control and PCO) were randomly selected and anaesthetized with ether. Blood samples were collected by retro orbital puncture, and the serum were used for hormonal assays (FSH, LH, estradiol, progesterone andtestosterone).[4] The ovaries were excised and weighed, and histopathological examination was conducted on the ovaries.

**Serum hormonal assays**

Serum testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol were measured usingan enzyme immunoassay kit for the quantitative determination ofthe corresponding hormones.

**Organ weight:**

On the 16th day, some body organs of rats in all treatment groups except the Positive group were excised and weighed; organs from rats in the Positive group were instead excised and weighed on the 6th day.

**Histopathological examination:**

The excised ovaries underwent histopathological examination, through which the diameter and thickness of the cystic follicles were measured. The cystic follicles were defined by thickened and fibrotic cortex with a prominent outer theca and internal layer.

**Histology**

The ovaries from toxic controls (EV-treated), standard & treated groups were removed, cleaned of adherent connective fat tissue, andtissue samples were fixed in 10%

formaldehyde buffer for histological examinations. Ovaries were imbedded in paraffin, cut in 8-µm sections, and stained with hematoxylin and eosin examined by light microscopy [hematoxylin and eosin staining). Ovaries were examined for evidence of polycystic morphology, as described previously. [12]

**Statistics**

The results are expressed as Mean ± SEM. Data was evaluated using ONE WAY ANOVA followed by Newman – Keul's multiple range test. Probability values less than ($p < 0.01$) were considered significant.

**RESULTS**

**Effect of *Chembaruthiverchooranam* on LH in EV induced PCOS rats**

Estradiol valerate(EV) treatment causes significant rise in LH levels & lowering of FSH levels in toxic control (G2) compared to normal control groups (G1) at $P<0.001$. The LH /FSH ratio was significantly different from the control groups. An elevated LH/FSH ratio observed in toxic group whereas *Chembaruthiverchooranam*treated groups i.e both doses of *Chembaruthiverchooranam*(100mg and 200mg/kg)showed a lower LH/FSH ratio & significant decrease ($p<0.01$) in LH & rise in FSH levels when compared to toxic control group**. (Table:1)**

**Effect of *Chembaruthiverchooranam*on Estradiol in EV induced PCOS rats**

There was statistically significant decrease in estradiol levels with Estradiol valerate(EV) injection after 30 days ($p<0.01$). Concurrent administration of *Chembaruthiverchooranam*for 15 days showed significant rise in estradiol levels ($p<0.01$). Animals in Standard group also showed significant rise in estradiol levels. (**Table:1)**

**Effect of *Chembaruthiverchooranam*on progesterone in EV induced PCOS rats**

Toxic control group i.e treated with estradiol valerate had shown significant lowering ofprogesterone. But treatment with *Chembaruthiverchooranam*at both doses (100mg/kg and 200mg/kg) along with EV was able to increase the progesterone levels($p<0.001$)to near normal values significantly. Similar results were also observed in standard group.(**Table:1)**

**Effect of *Chembaruthiverchooranam*ontestosterone in EV induced PCOS rats**

There was no significant rise in testosterone levels after exposure of rats to estradiol valerate ($p<0.01$) for 30 days.Treatment with *Chembaruthiverchooranam*at two doses

100mg/kg and 200mg/kg for 15days doesn't show any significant changes in testosterone levels. Similar results were observed after clomiphene treatment. (**Table:1)**

**Effect of *Chembaruthiverchooranam*on ovarian morphology:**

Ovaries of toxic control (Estradiol valerate) group exhibited more cystic follicles compared with other groups but these were not evident in extract control group. Both the 100mg/kg &200mg/kg *Chembaruthiverchooranam*showed normal follicle at different stage of development.There was evident of arteric follicles present in 100mg/kg.The group that received 200 mg/kg showed numerous healthy developing follicles.(**Table:2)**

**Effect of *Chembaruthiverchooranam* on follicular diameter & thickness:**

The follicular diameter & thickness of the cysts inPCOS treated group were increased whereas it was reduced in standard &*Chembaruthiverchooranam* treated groups.(**Table:2)**

**Effect of *Chembaruthiverchooranam*on ovarian weight:**

The ovarian weight of EV control group showed a significant decrease ($p<0.01$), when compared with other groups, whereas in treatment control group 100mg/kg &200mg/kg it was restored to near normal values.

**Table.1 Effect of *Chembaruthiverchooranam*on serum hormone in Estradiolvalerate induced PCOS**

| GROUP | LH | FSH | Estradiol | TSN | PRGSN |
|---|---|---|---|---|---|
| **G1** | 6.146±0.33 | 8.27±0.25 | 54.12±2.31 | 0.28±0.04 | 14.15±0.61 |
| **G2** | 11.36±0.75**a | 2.26±0.235**a | 14.32±0.82**a | 0.37±0.02**a | 7.055±0.712**a |
| **G3** | 5.25±0.38 | 7.02±0.46 | 46.49±1.74 | 0.32±0.02 | 12.12±0.29 |
| **G4** | 3.70±0.18**b | 6.63±0.53**b | 38.17±1.33**b | 0.34±0.01**b | 10.8±0.72*b |
| **G5** | 4.57±0.21**b | 6.13±0.56**b | 41.18±0.92**b | 0.32±0.01**b | 11.60±0.76*b |

$G_1$- Normal, $G_2$ -Toxic, $G_3$ - Standard, $G_4$ - Low dose (100mg/kg), $G_5$ - High dose (200mg/kg).

All values expressed as means ± SEM for 6 animals in each group.

**a- Values are significantly different from Normal control ($G_1$) at $P<0.001$

**b- Values are significantly different from PCOS control ($G_2$) at $P<0.001$

*b- Values are significantly different from PCOS control ($G_2$) at $P<0.01$

**Table.2 Effect of *Chembaruthiverchooranam* on ovarian morphology of PCOS rats**

| Dose mg.kg ovarian feature | Normal | Toxic control | Std control | Low dose 100mg/kg | High dose 200mg/kg |
|---|---|---|---|---|---|
| Atretic follicle | 0.00±0.00 | 4.43±0.307 | 1.12±0.05 | 3.06±0.17**b | 0.05±0.11*b |
| Cystic follicle | 0.00±0.00 | 10.55±1.21 | 3.7±10.58 | 0.00±0.00 | 0.00±0.00 |
| Cystic follicle diameter | 0.00±0.00 | 87.73±2.367 | 71.19±2.35 | 0.00±0.00 | 0.00±0.00 |
| Cystic follicle thickness | 0.00±0.00 | 42.35±1.48 | 34.63±2.18 | 0.00±0.00 | 0.00±0.00 |

$G_1$- Normal, $G_2$-Toxic, $G_3$-Standard, $G_4$-Low dose (100mg/kg), $G_5$-High dose (200mg/kg).

All values expressed as means ± SEM for 6 animals in each group.

**b- Values are significantly different from PCOS control($G_2$) at P<0.001

*b- Values are significantly different from PCOS control($G_2$) at P<0.01

## DISCUSSION

PCOS has been considered a progressive multiglandularendocrinopathy where thedelicatebalance of the hypothalamic–pituitary–adrenal– ovarian axis is disturbed, resulting in a failure of thecyclic reproductive mechanism. [101,102]

Although many models can be used to study PCOS, induction of PCOS by Estradiol valerate can also be considered as one of the best model for studying PCOS. This study investigated the effect of ***Chembaruthiverchooranam***on the serum levels of LH, FSH,estradiol, testosterone & progesterone in EV induced PCOS. After 30 days of PCO induction, animals were analyzed both harmonically &histologically, on 16$^{th}$ day after treatment with ***Chembaruthiver chooranam***, animals were also analyzed irrespective of their estrous cycle.

In PCOS condition, normal gonadotropin-ovarian axis is disturbed results in hormonal imbalancereflected by the higher levels of LH, lower FSH levels and reversal of LH: FSH ratio. An elevated LH/FSH ratio and anovulation are typical findings in women

with PCOS. [16,17] The ***Chembaruthiver chooranam*** treated groups shows better reduction in this LH/ FSH ratio indicate that ***Chembaruthiver chooranam***could reverse PCOS condition.

Estrogen similar to other steroids become altered in PCOS. [19] The level of estradiol is very minimum in PCOS rats since the metabolic conversion is very slow.Repetitive administration of ***Chembaruthiver chooranam***lead to significant rise in estradiol.Similarly, the reduction in the level of progesterone in the PCOS-induced animals could be responsible for the persistentestrusphase. [20] Elevation in the concentration of serum progesterone by ***Chembaruthiver chooranam*** may be responsible for the reversal of the luteal phase dysfunctionand restoration of the estrous cycle. Our study showed that ***Chembaruthiver chooranam*** induced an increase in serum estradiol implies that plant causes marked improvement in endocrine function and recovery of ovulary functions in the rats.Hyperandrogenism (as a result of high testosterone levels) which is evident in human PCOS. [21,22] was not present in this animal model of EV induced PCOS. [23] Therefore no effect of the ***Chembaruthiver chooranam*** on androgen levels was observed using this model of PCOS induction.

Ovarian weight in PCOS induced rats was more than the normal rats which is in accordance with earlier findings. [24,25,26,27] Treatment with ***Chembaruthiver chooranam*** prevented further increase in ovarian weight & returned to normally. The biochemical results are also supported by histopathological observation of light microscopy. After treatment with ***Chembaruthiver chooranam*** PCOS condition was reversed, number of cystic follicles reduces & foundnumerous healthy follicles at different stage of development. This indicate that treatment group shows marked recovery of ovarian tissue and the animals may probably be preparing for ovulation.

## CONCLUSION

In conclusion the Siddha formulation *"**Chembaruthiver chooranam"***shows significant recovery of FSH, LH, estradiol & progesterone and also restored the irregular cycle and ovarian physiology to normal in the PCOS animals. Thus "***Chembaruthiver chooranam"*** was effective in reversing the hormonal imbalance induced by estradiol valerate in PCOS &validate the use in treatment of infertility.Therefore, it needs further clinical study for validation.

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