Int J Trans Res Ind Med 2021, 3(1): (P) 12 - 17

Research Article

ISSN 2582-0109

International Journal of Translational Research in Indian Medicine
www.ijtrim.com
Volume 3, Issue 1 – 2021

AMELIORATIVE EFFECT OF SIDDHA FORMULATION SATHAKUPPAI CHOORANAM ON ESTRADIOL VALERATE INDUCED PCOS IN WISTAR RAT MODEL

P.M. Sathya Priyanka [1], R. Shalini Priya [2], R.Menaka[3], N.Anbu [4] , D.Sivaraman [5]

*[*1&2]P.G Scholar, Department of Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India*

[3] Lecturer, Department of Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.

[4] Professor and Head of the Department, Department of Medicine, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.

[5] Scientist, Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Road, Chennai- 600119, Tamil Nadu, India.

ABSTRACT

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of fertile age, which causes anovulation in women. The prevalence of PCOS varies between 2.5 and 7.5%. Metabolic abnormalities such as dyslipidemia, insulin resistance in PCOS responsible to make the patient more prone to diabetes, obesity, cancer, infertility as well as coronary heart diseases. Various therapeutic methods such as changing of life habits, surgery and medication like clomiphene citrate, metformin and tamoxifen have been proposed for PCOS. Today, herbal medicines are widely used as an alternative for treatment or controlling of diseases. Siddha medicines for PCOS have received attention as a form of lifestyle management in traditional medicine clinics, in which the menstrual cycle and normal serum hormones levels can be recovered. The main objective of the present investigation is to evaluate the ameliorative potential of siddha formulation Sathakuppai Chooranam (STKC) against estradiol valerate induced PCOS in wistar rat model. Results of the study indicates that treatment with STKC at both the dose level of 200 and 400 mg/kg reveals significant increase in FSH level to the maximum of 3.267 mIU/ml and provokes decrease in the level of LH to 6.083 mIU/ml. Similar activity were observed with respect to declination in the weight of the ovaries in treatment group. Histological observation justifies the reduction in number of follicular cyst in both low and high dose treated groups. In conclusion traditional medicines like Sathakuppai chooranam have significant ameliorative potential in the management of PCOS, whereas further clinical recommendation has to be made with proper clinical validations.

KEY WORDS: ***Polycystic ovary syndrome, Traditional medicine, Siddha formulation, Sathakuppai Chooranam, FSH, LH***

Corresponding Author: P.M. Sathya Priyanka, Department of Medicine, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India

1. Introduction

Polycystic ovary syndrome (PCOS), the most common female endocrine disorder, is a heterogeneous endocrine and metabolic disorder, affecting 6 - 10% of women of reproductive age [1]. Features of PCOS may manifest at any age, ranging from childhood (premature puberty), teenage years (hirsutism, menstrual abnormalities), early adulthood and middle life (infertility, glucose intolerance) to later life (diabetes mellitus and cardiovascular diseases) [2]. Several of these features increase the risk of cardiovascular diseases (CVD) in women [3] and the prevalence of hypertension in women with PCOS is about 40% in comparison with a prevalence of about 25.8 in the general population [4]. PCOS is also associated with a higher risk of myocardial infarction (relative risk) [5] and with a compromised cardiovascular profile independent from obesity in young women [6].

PCOS is a common diagnosis in women presenting with anovulatory infertility [7]. Symptoms of PCOS related to ovulation manifest as amenorrhea or oligomenorrhea [8]. Polycystic ovaries are enlarged and contain a large number of immature follicles. There is also metabolic disorder associated with PCOS such as insulin resistance and hyperinsulinemia in women [9]. During the follicle development reduced progesterone and estrogen levels are seen due to regression of the corpus luteum [10]. Consequently, release from negative feedback suppression allows a small but steady increase in FSH and LH levels, which stimulates the growth phase of ovarian follicles [11]. Hormonal contraceptives, selective estrogen receptor modulator (SERM), insulin sensitizers, gonadotropins, and ovarian surgery have been shown to be useful for improving PCOS symptoms in women [12]. These treatment approaches address a variety of PCOS symptoms, although current PCOS treatments are not successful in all phases of irregular ovarian function [13].

Clomiphene citrate, exogenous gonadotropins, Insulin sensitizers, such as metformin, are used to reduce insulin resistance which results in a reduction of ovarian androgen production and a consequent improvement in menstrual cyclicity [14]. Although many drugs have been shown to be effective in the treatment of PCOS, alternatives are continuously being searched because of actual or possible side effects ranging from arthritis, joint or muscle pain [15], psychological disturbances [16], and lactic acidosis. In traditional herbal medicine, there are many exceptional herbal drugs that have the potential competence of preventing and curing PCOS [17]; however, many more herbs are not evaluated and much research has been not done on its mechanism of action

Herbal remedies are known to be effective in reducing testosterone as well as increasing FSH and 17β-estradiol levels [18], and they have been shown to reduce polycystic ovaries and ovarian volume, improve insulin sensitivity, and normalize reproductive cycles [19]. Additionally, clinical investigations have reported no adverse effects for herbal medicines [20]. However, conclusive evidence regarding absolute therapy could not be obtained in these clinical studies due to the absence of pre-clinical data explaining the mechanism of PCOS therapy [21]. The main aim of the present investigation is to evaluate the ameliorative potential of siddha formulation Sathakuppai Chooranam (STKC) against estradiol valerate induced PCOS in wistar rat model.

2. Materials and Methods

2.1. Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ± 2o C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. IAEC ref no: SU/CLATR/IAEC/XIII/128/2019

2.2. Experimental Methodology [22-25]

Animals were checked two weeks for regular cycles by smear test. Adult wistar female rats were randomly divided into four groups of six animals each: Control (group I), PCOS (group II), and experimental groups (group III & IV). The control group received just olive

oil (i.p, 0.2 ml) as a solvent. PCOS and experimental groups were induced by 100µg estradiol dissolved in 2 ml olive oil will be injected subcutaneously. All rats after a 24 hr period, will receive intramuscular injections of 50 µg progesterone dissolved in olive oil. To induce of PCOS, estradiol valerate (4mg/kg/rat) will be injected intramuscularly. Induction of PCOS will be ascertained by consistent estrus cycle in rats and the efficacy of test drug which reverse the cycle back to the normal will be taken as an endpoint. After 28 days of PCOS induction experimental groups III and IV received 200 and 400 mg/kg doses of test drug STKC for the period of four weeks

2.3. Sample Collection [26,27]

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from retro orbital and cardiac puncture and then hormone estimation were carried out by using Cobas e411 immuno assay analyzer.

2.4. Histopathology [28]

Ovaries from the experimental rats were dissected out and fixed in 10% buffered neutral formal saline and processed. After fixation, tissues were embedded in paraffin. Fixed tissues were cut at 10 µm and stained with hematoxylin and eosin. The sections were examined under light microscope for histological changes.

3.Results

3.1. Effect of Effect of STKC on serum hormone level(s)

From the result analysis of the present investigation it was clear that the weight of ovary sample belongs to group II rats shown progressive increase when compare to group I. Treatment with STKC at both the dose level shown marginal decrease in the weight of the ovaries. From the immunoassay analysis it was predicted that FSH level of group II rats shown pronounced decrease when compare to control group I. Treatment with STKC to group III and IV at the dose of 200 and 400 mg/kg shown viable increase in the level of FSH hormone. LH level haven't shown wide variation between the control and treatment group rats. As shown in Table 1.

Table 1: Effect of Effect of STKC on serum hormone level(s)

Group	FSH (mIU/ml)	LH (mIU/ml)	Ovary Weight (mgs)
Control	6.467 ± 0.52	6.55 ± 0.12	112 ± 1.9
PCOS	1.617 ± 0.12	6.333 ± 0.17	182.3 ± 3.9
PCOS + 200 mg/kg STKC	2.467 ± 0/14	6.483 ± 0.16	166.3 ± 5.9
PCOS + 400 mg/kg STKC	3.267 ± 0.18	6.083 ± 0.24	131.5 ± 1.5

Values represent mean ± SEM of 6 experimental animals.

3.2. Effect of STKC on histopathology of rat ovary

Histology of samples belongs to group I reveals that the normal appearance of graafian and antral follicle further projection of corpora lutea (CL), atretic follicles (AF) and interstitial tissue (IT) appears normal. Histopathology of sample belongs to group II reveals that significant increase the number of follicles in the different stages and corpus luteum. Treatment with STKC at the dose of 200 and 400 mg/kg has significantly reduced the follicle number further restored the histology of corpus luteum almost similar to that of the normal control rats. As shown in Figure 1.

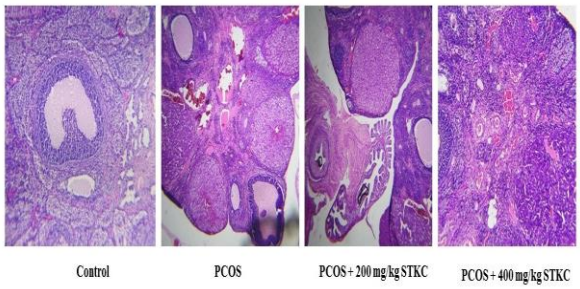

Figure 1: STKC on histopathology of rat ovary

4.Discussion

Polycystic ovary syndrome (PCOS) is a common and multifactorial disease associated with both endocrine and metabolic disorder. It affects approximately 4%–18% of all reproductive-aged women in the world [29]. PCOS is characterized by hyperandrogenism and ovarian abnormalities, resulting from a disruption in the hypothalamic–pituitary–ovarian axis. Clinically, the main cause for reproductive and metabolic abnormalities in women with PCOS are hyperandrogenism and insulin resistance [30]. The etiology of PCOS is still unknown, although environmental, genetic, and hormonal factors are all thought to be important in its development [31].

Metabolic dysfunction in women with PCOS is very common. These women often have higher levels of low-density lipoprotein (LDL), triglycerides (TG), cholesterol, and low level of high-density lipoprotein (HDL) in their blood serum that may lead to the heart disease [32]. Also, PCOS is characterized by elevated luteinizing hormone (LH) and decreased follicle stimulating hormone (FSH) levels (increased LH: FSH ratio). In fact, LH may increase in response to elevated androgens that can develop anovulation and infertility later [33-35]. Therefore, one strategy for PCOS therapy is regulating the imbalance levels of hormones and using medication that helps to improve insulin resistance. From the result analysis of the present investigation it was clear that the weight of ovary sample belongs to group II rats shown progressive increase when compare to group I. Treatment with STKC at both the dose level shown marginal decrease in the weight of the ovaries. From the immunoassay analysis it was predicted that FSH level of group II rats shown pronounced decrease when compare to control group I. Treatment with STKC to group III and IV at the dose of 200 and 400 mg/kg shown viable increase in the level of FSH hormone. LH level haven't shown wide variation between the control and treatment group rats.

Experimental polycystic ovary (PCO) in rodents resembling some aspects of human PCO syndrome, for example changes in serum levels of gonadotropin-releasing hormones (GnRH) and appearance of cysts, was induced by injecting estradiol valerate (EV). Classical neuroendocrinological studies indicate that in female rats, the neuronal component responsible for the induction of the LH surge is located in the preoptic area (POA) [36]. In fact, it has been reported that gonadotropine releasing hormone neurons in the POA express the immediate early gene, c-Fos, at the time of the LH surge suggesting that such GnRH neurons in the POA are responsible for the generation of the GnRH surge [37]. Histology of samples belongs to group I reveals that the normal appearance of graafian and antral follicle further projection of corpora lutea (CL), atretic follicles (AF) and interstitial tissue (IT) appears normal. Histopathology of sample belongs to group II reveals that significant increase the number of follicles in the different stages and corpus luteum. Treatment with STKC at the dose of 200 and 400 mg/kg has significantly reduced the follicle number further restored the histology of corpus luteum almost similar to that of the normal control rats.

5. Conclusion

Traditional herbal remedies are becoming more common in replacing established medications for the treatment of PCOS. Considering the outcome of our present investigation that the siddha drug STKC as a scope of better alternative medicine for the clinical management in the therapy for PCOS in near future.

Acknowledgement

We wish to acknowledge my thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India and The Noble research solutions, Chennai, Tamil Nadu, India for their support.

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