



RESEARCH ARTICLE

EFFECT OF PERGULARIA DAEMIA IN POLYCYSTIC OVARY SYNDROME INDUCED RAT OVARIES AND THYROID GLAND

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine disorder with multiple potential etiologies. Their symptoms are similar to hypothyroidism. The actual etiology of this syndrome is still unclear. The main aim of the study is to analyze the cellular level changes in ovary and thyroid glands in the polycystic induced rats (*Rattus norvegicus*) and the effect of *Pergularia daemia* on the treatment of polycystic ovary syndrome. Female albino Wistar rats are used to pursue this study. The polycystic condition is induced in the rats by injecting the male hormone (Testosterone propionate) intraperitoneally. The results shows remarkable changes in the ovaries with the reduced thecal layers and immature graffian follicles in the PCOS condition when compared to normal rats. The *Pergularia daemia* treated group shows the increased blood vessels size with perfect follicular cells which clearly brings out the management of polycystic ovaries. Histological sections of PCOS rat's Thyroid gland show high vacuolated areas with less colloidal substances when compared to control and *Pergularia daemia* group. Thus, the current experiment has proved that the polycystic ovarian syndrome and hypothyroidism show a minor correlated effect in the cellular level and that *Pergularia daemia* is an effective drug to cure polycystic ovarian syndrome in rats.

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INTRODUCTION

Polycystic ovarian syndrome is a disorder that can be described heterogeneously with multiple potential and unclear aetiologies. This syndrome affects more than 6 – 10% of the women who are in the reproductive stage. The term Polycystic ovarian syndrome (PCOS) was first coined by Stein and leventhal (1935). They described this disorder with the morphological structures of the ovaries and the clinical manifestations of women with the PCOS. There are three main categories of this syndrome which can be broadly classified as Clinical, Endocrinological and metabolic. In clinical category the features includes menstrual cycle abnormalities or irregular periods, acne, alopecia, hirsutism, recurrent miscarriages or frequent abortions, anovulation which finally leads to infertility or sterile condition in the female. In endocrinological category the hallmark features includes hormonal changes like elevated androgen/testosterone, Luteinizing hormone (LH), estone into estrogen and prolactin levels. Lipid abnormalities, obesity, insulin resistance and type 2 diabetes are the

features that fall under the metabolic category. (Tsilchorozidou *et al.*, 2004).

The actual definition of PCOS was stated by Metab (1998). He reported that more than 50% of the women having PCOS were quite obese. The abdominal obesity is also common due to the elevated androgen hormone that increases the adipose tissue mass in the abdomen. The hormonal imbalance brings out changes in the menstrual cycle by preventing the ovaries from releasing an egg each month and thus more immature eggs turns into multiple cysts that develops due to lack of ovulation. The ovaries appear large and studded with multiple cysts. This condition is called PCOS.

Peppler *et al.*, 2005 described that the immature follicles are small, and it does not exceed 10mm in size. Large numbers of abnormal follicles are found on the outer surface of the ovaries. They are filled with fluids in a sac. This works perfectly as a mature egg under the influence of stimulatory hormones from the brain.

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Many other disorders like hypothyroidism, glucose intolerance, diabetes and cardiovascular diseases have a link with PCOS. The signs and symptoms of hypothyroidism are irregular menstrual cycle, obesity, hair loss and hormonal changes. The common symptoms for both PCOS and hypothyroidism are menstrual cycle changes, hair loss and obesity.

The autoimmune thyroiditis is a most prevalent cause for hypothyroidism and these autoimmune thyroiditis have high prevalence effect in the patients with PCOS (Janssen *et al.*, 2004). Knochenhauer *et al.*, 1998 & Asuncion *et al.*, 2000 hypothesized that the women with PCOS have an increased estrogen to progesterone ratio and brings out the development of Auto immune thyroiditis. Several researchers and many doctors are focusing their views to correlate hypothyroidism and PCOS. Mochiziki (1997) proved that in thyroid function, hypothyroidism is one of the most common disorder that has an association with wide range of reproductive abnormalities like menstrual disorders, amenorrhea, frequent abortions and infertility. These reproductive abnormalities are treated with thyroid hormone therapy (Beamer *et al.*, 1981). Hagino (1971) and Duignan (1976) performed thyroidectomy in rats to study about female reproduction and stated that it is not an easy job to raise spontaneous hypothyroid models.

Nowadays many women do not know the exact reason for spontaneous miscarriages. Homburg (2006) stated that many studies are necessary to find out the additional infertility factors on women with PCOS. The actual mechanism of hypothyroid and PCOS is still unclear in the case of female reproduction. The ultimate aim of this study is to find out the changes in ovarian and the thyroid cells in PCOS induced rats, and a novel try to treat the defective cells caused by PCOS with the help of a single plant available in common.

MATERIALS AND METHODS

Experimental Animal

Eighteen female *Rattus norvegicus* (Albino Wistar strain) rats were purchased from Kings Institute (Chennai). They were acclimatized in Institutional Animal Ethical Committee approved (437/01/c/CPCSEA) Animal House, P.G. and Research Department of Zoology [Holy Cross College (Autonomous), Tiruchirappalli] under laboratory conditions having light:dark (12:12hrs) cycle, fed with pellet (Sai Durga Enterprisie, Chennai) and water twice daily. After 30 days, the rats (150 – 200gm) were used for further experimentation.

Plant Description

Pergularia daemia (Forsskal) Chiov, commonly called as Trellis-vine belongs to Asclepiadaceae subfamily. It is a perennial vine, collected from Golden Rock, Tiruchirappalli, Tamil Nadu. The vernacular name of this plant is called “Veliparuthi” in Tamil. It is identified and certified by the Director of The Rapinat Herbarium and center for molecular systematics Dr. S. John Britto, St. Joseph’s College, Tiruchirappalli, Tamilnadu. The Fresh leaves (Figure. 1) were washed, chopped and crushed well in the Mortar and Pestle. The fresh juice (crude extract) from 5gms leaves was collected and used for this study.



Figure.1 The aerial parts of *Pergularia daemia*

Taxonomical Classification

Kingdom	: Plantae
Order	: Gentianales
Family	: Apocynaceae
Subfamily	: Asclepiadaceae
Genus	: Pergularia
Species	: daemia

Chemical Preparation

Stock solution of Testosterone Propionate (TP) (Himedia company, Code: RM 7543, Mumbai, India) is prepared by dissolving 5gm in 100ml olive oil (vehicle). The experiment is classified into three groups as Group I (Control), Group II (TP Injected) and Group III (*Pergularia daemia* extract treated). Each group consists of six rats.

Pcos Induction

In group I (control), 1.03ml of olive oil alone (vehicle) is injected intraperitoneally (ip), to test its efficacy. In group II & III, 1.03ml of TP is injected to induce PCO condition in rats. Rats are injected with their concentration between 7-8am for six consecutive days. In group III, 1ml of *Pergularia daemia* crude extract is orally administrated from 7th to 13th day.

Histopathological Analysis

Rats from Group I, Group II are sacrificed using chloroform as an anesthetic on the 7th day morning and Group III rats are monitored by giving the crude extract and sacrificed on the 14th day. Tissues (ovary and thyroid) are immediately fixed in 10% neutral formalin fixative. Tissues are washed and processed through series of alcohol for dehydration, cleared in xylol and finally embedded in the paraffin wax. Sections of 5 μ thickness are cut using ultra microtome (LEICA, RM 2125 RT) and stained with Hematoxylin counterstained with Eosin (H & E) stain. The stained sections are mounted in DPX, observed and photographed using AXIOSTAR photomicrography unit.

RESULTS

Histological Observations of Ovary

Polycystic ovarian syndrome induced rat ovaries were compared to the control and the *Pergularia daemia* treated group. It is necessary to record the histological changes in the ovaries with the treated groups.

The control group ovaries show developing follicles in different stages. The preantral follicles and antral follicular development are clearly observed with blood vessel network. Several Secondary (S) and Graffian follicles (GF) are found. The corpora lutea (Cl) are found to be healthy in the control

ovaries in Figure 2a (100x). The graffian follicle shows three different layers of follicular cells such as Theca externa, Theca interna and Membrana granulosa which are normal and healthy. These granulosa layers are filled with follicular fluid. The theca interna have vascularized mode of cells and the theca externa have fibrous type cells. The shape of these cells varies from fusiform to epithelioid within collagen fiber surroundings. A clear follicular cavity (FC) is remarkable in the control ovarian sections in Figure 2b (400x).

In Figure 2c (100x), the TP exposed ovaries show the multiple cystic conditions in the ovaries. The follicular sizes are diminished. This state is said to be PCO condition. The immature and the atresic follicles (Af) are found. Enormous amount of non – uniform apoptotic cells are found in the inner layer of membrana granulosa (Mg). A transition appeared between the non-vascularized membrana granulosa (Mg) and the vascularized granulosa. This shows a sharp demarcation between the granulosa layer and the thecal layer particularly theca interna. Follicular wall of the cystic ovaries with follicles show irregular follicular cavity. Non-vascularized membrana granulosa (Mg), theca interna (Ti), theca externa (Te) and the stroma shows the advanced stage of atresia in Figure 2d (400x).

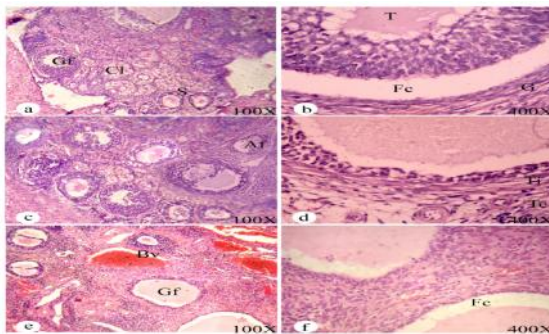


Fig.2a. - H and E stain Control Ovary (100X)
 Fig.2b. - H and E stain Control Ovary (400X)
 Fig.2c. - H and E stain Tp injected Ovary (100X)
 Fig.2d. - H and E stain Tp injected Ovary (400X)
 Fig.2e. - H and E stain Pergularia daemia treated Ovary (100X)
 Fig.2f. - H and E stain Pergularia daemia treated Ovary (400X)
 Gf - Graffian Follicles, S - Secondary Follicles,
 Cl - Corpora lutea, T - Tertiary Follicles,
 Fc - Follicular cavity, G - Granulosa,
 Af - Atresic Follicles, Ti - Theca interna,
 Te - Theca externa, Bv - Blood vessels.

Figure2

In Figure 2e (100x), the *Pergularia daemia* treated ovaries show the mature graffian follicles (Gf) with fine blood vessel network. Development of secondary follicles (S) is also found. The recovery of the sharp demarcation between the granulosa layer and the theca interna is highly remarkable. The clear follicular cavity shows the changes in the ovary which is similar to the normal Figure 2f (400x). In the membrana granulosa (Mg) layer apoptotic cell count is lowered and there is an increase in the follicular size too. This indicates that the *Pergularia daemia* has the capacity to treat PCO condition in the ovaries and the histology proves *Pergularia daemia* fresh leaf extract is highly effective in treating PCO and brings back to the normal condition.

Histological Observations of Thyroid Gland

In the control sections of thyroid gland small and large follicles are found. The follicles are filled with the colloid material in Figure 3a (100x). Clear follicular epithelial cells are observed. They are cuboidal in shape which are illustrated in Figure 3b (400x). The distribution of the Para follicular cells are also found.

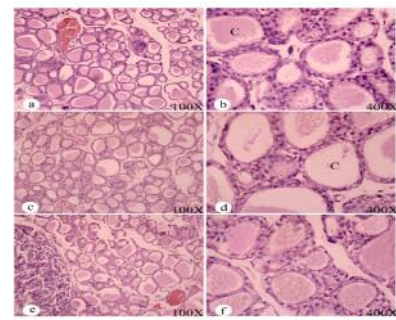


Fig.3a. - H and E stain Control thyroid gland (100X).
 Fig.3b. - H and E stain Control thyroid gland (400X).
 Fig.3c. - H and E stain Tp injected thyroid gland (100X).
 Fig.3d. - H and E stain Tp injected thyroid gland (400X).
 Fig.3e. - H and E stain Pergularia daemia treated thyroid gland (100X).
 Fig.3f. - H and E stain Pergularia daemia treated thyroid gland (400X).
 C - Colloid.

Figure3

Figure 3c (100x) are the histology of TP injected thyroid gland showing the reduced amount of colloid. In Figure 3d (400x) the Para follicular cells are lower in number, the epithelial cell lost its cuboidal shape with very lower amount of colloid. Figure 3e (100x) explains the *Pergularia daemia* treated thyroid sections showing parathyroid gland, and clear thyroid cells too with better distribution of colloid and blood vessels. Figure 3f (400x) shows well developed cuboidal epithelial cells. The distribution of the colloid shows the recovery when compared to the TP injected cells.

DISCUSSION

Ota *et al.*, (1983) observed the degeneration of the follicles and the luteinization of theca cells in the ovaries by inducing TP for 20 days in rats and proved that luteinization of the thecal layer cells are more marked in the TP treated rats when compared to that of PCOS in humans, and these symptoms were due to the excess secretion of Testosterone.

Letrozole is an another compound that induced PCOS in 21 days old rats and it was observed that multiple cysts found in the ovaries with the diminished granulosa cells and sharp demarcation was also found. Differentiations of the follicular cells are also found (Mannaras *et al.*, 2007). Similarly in the present study a sharp demarcation is found between the follicular wall and the thecal layer, resulting in the irregular follicular cavity.

Testosteronantant an oil suspension injection which helps to induce PCOS in 21 days old rats, results in showing the apoptotic cells in the inner layers of the membrana granulosa cells present in the cystic follicles and the antral follicles (Skrtic *et al.*, 2011). In our study in the injected group many atresic follicles and the huge amount of the immature apoptotic cells are found, which proves that testosterone has an ability to induce PCOS in rats (*Rattus norvegicus*).

Manni *et al.*, (2005) performed a research by injecting Estradiol Valerate (EV) in 205 to 230 gms weighed rats to induce the PCOS condition which forms the antral cysts in the ovaries. EV constrict the space in the blood vessels too. The expression of the proteins may change due to the presence of PCOS in the ovaries. The presence of antral cyst is a remarkable feature for this study. Testosterone propionate induced rats of 21 days age shows the presence of large cystic follicles and the accumulation of the immature multi – layer

preantral follicles. It was experimented and reported by Beloosesky *et al.*, (2004).

The histological structures of the thyroid gland in ducks were studied by Prachami and Fatahian Dehkordi (2012). They used both male and the female ducks and found no sex difference with the follicles of single lined epithelial cells. Khan *et al.*, (1999) described about the toxicity of the acrylamide which brings enormous changes in the thyroid gland in rats, showing the follicular shrinkage. Ghosh *et al.*, (1993) examined the role of hypothyroidism in PCOS women and suggested that hypothyroidism is invariably followed by lowering sex hormone binding globulin and increased free testosterone level and finally concluded that the route of Testosterone metabolism and estradiol acts as a rescue of ovaries from PCOS in hypothyroid condition. Maruo *et al.*, (1992) explains about the thyroid hormone role in corpus luteum function and the induction of ovulation and stated that the thyroid hormone therapy is a great value for ovulation induction and the treatment to luteal phase defective patients.

According to literature survey several herbal therapies are there to treat PCOS. Sun and Yu (2000) stated Epimedium herb (*Herba epimedii*) have a great effect in treating the PCOS. The combination of the green tea are Dodder seed (*Semen cuscutae*) flowers of Solomonseal rhizome (*Rhizoma polygonati*), Chinese fox glove root (*Raidx rehmanniae*) and lateral roots of aconite (*Radix aconiti*) brings a drastic change in obesity and anovulation in rats that are sterilized with androgen injected with testosterone propionate. Spear mint tea (*Mentha spicata*) has antiandrogenic property that helps to treat hirsutism caused by PCOS, which gradually decreases the male hormone in women with PCOS (Grant, 2010). There is no study related to PCOS and Hypothyroidism treatment in the same animal. This is a novel try to correlate PCOS and hypothyroidism in same animal.

Ota *et al.*, (1983) conclude that TP administered ovarian histology are quite similar to human PCOS. The current study also supports the same quote that the formation of PCOS by TP and their appearance is also similar to human PCOS.

The current study is a novel attempt to compare the histopathology of PCOS induced rat ovaries and the thyroid gland. The herbal therapy is also a novel one in which a common plant *Pergularia daemia* has a commendable role in treating PCOS.

CONCLUSION

The current study which is a novel try proves that *Pergularia daemia* has a hallmark effect in treating PCOS condition. Regeneration of the follicular cavity and the recovery of sharp demarcation in the ovaries in highly commendable. Similarly in the thyroid gland increase in the colloidal mass and the development of the cuboidal cells are highly remarkable. Thus we found that there is a correlation between PCOS and Hypothyroidism. We succeed that, treating methods of PCOS also has a property to treat hypothyroidism symptoms too. The future aim of the study is to perform further tests to isolate a single compound from this plant and help out the human kind to treat PCOS and its related disorders with a single medicine.

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