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Comparitive study of *Pergularia daemia* and *Citrullus colocynthis* in polycystic ovarian syndrome induced albino wistar rats

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Abstract

Polycystic ovarian is a heterogenic disorder with unknown etiology. The symptoms of PCOS are broadly classified as clinical, metabolic and endocrine that finally brings out infertility. Many herbal medicines are there to cure the PCOS but this study is a novel try for treatment of PCOS with two different commonly available plants. The ultimate aim of the current study is to compare the best natural medicine for the cure of PCOS between the two plant extracts namely *Pergularia daemia* and *Citrullus Colocynthis*. Female albino wistar strain rats were used to induce PCOS with Testosterone Propionate. The hormones that plays an essential role in the menstrual cycle are FSH, LH, Progesterone and Testosterone were immunologically analyzed and statistically analyzed which proves that *Pergularia daemia* shows significant result when compared to the *Citrullus colocynthis* whose P value is ($P < 0.5$). Thus we conclude that *P. daemia* is an effective herbal medicine that balances the hormonal levels and cures PCOS effectively.

Keywords: PCOS, LH, FSH, Testosterone, Progesterone

Introduction

Polycystic ovary syndrome (PCOS) has increased considerably in recent years with the considerably in recent years with the concept that it is a condition involving more that the reproductive system. Initially called the Stein – Leventhal syndrome after its discovery in the 1930s, the term also deals with the multisystem involvement including hyperinsulinism, hyperlipidaemia, increased androgens, endometrial hyperplasia, diabetes mellitus, obesity, anovulation, cardiac disease and infertility (Lobo, 2001 ; Norman, 2001) ^[19, 27].

The diagnosis is controversial but it is generally based on peri – pubertal onset of menstrual problems with clinical or biochemical androgenism. Several studies have suggested a prevalence of PCOS of between 5% and 10% in women of reproductive age and polycystic ovaries (PCO) alone is found in 20 – 25% of women. The women with PCO has no evidence of menstrual problem or hyperandrogenism but they have an over – exaggerated response to hormone stimulation by gonadotrophins (Follicle stimulating hormone) seen in the cysts assisted reproduction (Norman, 2001) ^[27].

PCOS is one of the most common endocrine disorders in women of reproductive age. The overall prevalence among women in this age group is between 4% and 8% (Chang, 2004) ^[2, 7], although the prevalence may be as high as 30% in women with secondary amenorrhea, 75% in women with oligomenorrhea and in women with hirsutism (Adams *et al.*, 1986) ^[1]. A complete understanding of the underlying pathophysiology of PCOS is still lacking due to the heterogeneity of this disorder. There are most likely multiple underlying pathophysiologic mechanisms. Several theories have been proposed by Tsilchorozidou *et al.* (2004) ^[37] are Alteration in gonadotropin – releasing hormone secretion results in increased Luteinizing hormone (LH) secretion, an alteration in the insulin action results in hyperinsulinemia and insulin resistance, a defect in androgen synthesis that results in increased ovarian androgen production.

LH hyper secretion is a characteristic hallmark of PCOS. LH is secreted in a pulsatile manner. Women with PCOS have an increase in both the LH pulse frequency and amplitude, resulting in increased 24 hour secretion. This increase in LH is thought to occur as a result of increased frequency of hypothalamic gonadotropin – releasing hormone (GnRH). Increased LH in turn, leads to an increase androgen production by the theca cells within the ovary (Ehrmann, 2005; Tsilchorozidou *et al.*, 2004) ^[8, 37] Increased level of LH and insulin can induce abnormality of their steroidogenesis. IN the PCOS condition elevated amount of the androgen activity may alter the gonadotrophin induced estrogen and progesterone synthesis in the follicles (Wachs *et al.*, 2008) ^[38].

The sex difference in cognitive functioning are thought to be influenced, in part by both the prenatal organization and the postnatal activational effects of sex hormones. Although a considerable number of studies have been carried out on the impact of estrogen on cognitive performance in women (Sherwin, 2004; Lablance *et al.*, 2001) [33, 17], relatively little information is available regarding the possible activational effects of testosterone on female cognitive functioning even though testosterone produced throughout the reproductive years and beyond. In women testosterone is secreted by both the ovaries and the adrenal glands. This hormone is also produced via peripheral conversion of the weaker androgens, androstenedione and dehydroepiandrosterone (Burger, 2002) [5].

Medicinal research has gained a renewed focus recently due to the interest of the researchers. The other prime reason is that other medicines will surely bring out the side effects but the herbal or the natural product brings zero side effects (Karthishwaran and Mirunalini, 2010) [14].

Pergularia daemia belongs to a milky weed family called Asclepiadaceae. This family includes more than 2000 species that can be classified under 280 genera. The plant is distributed worldwide in the tropical and the sub-tropical regions (Pankaj, 2003) [29]. The *pergularia daemia* plant have anthelmic, laxative, antipyretic and expectorant property that helps to cure infantile diarrhoea and malarial fever (Kirtikar and Basu, 1999) [16]. Latex of this plant is used for tooth ache, stem bark for cold, leaves for hepatoprotective, antidiabetic, analgesic, anti-inflammatory and antipyretic properties (Bhaskar and Balakrishnan, 2009) [4].

Citrullus colocynthis (L.) Schrad (Cucurbitaceae family) is commonly called as the bitter apple or bitter cucumber distributed in the regions like Sudan, Iran and India. The fruits of these plants are used for the diabetic treatment in Mediterranean countries. The seed extract supported for the insulin secretion and the least part of the antidiabetic activity were found in the fruits (Nmila *et al.*, 2000) [26].

The main objective of the current study is to compare the best natural medicine between *Pergularia daemia* and *Citrullus colocynthis* for the treatment of PCOS through the regulation of essential hormones.

2. Materials and Methods

2.1. Experimental Animal

Female albino wistar rats (*Rattus norvegicus*) were chosen as experimental animal whose weight ranges from 150 – 200 grams. The inbred rats from Institutional Animal Ethical Committee approved the animal house of Holy Cross College to be used for the present experiment (437/01/c/CPCSEA) (Ref. 01/2011). The rats were fed up with pellet and water *ad libitum*. The food pellets were purchased from Sai Durga Enterprise, Chennai.

2.2. Experimental Design

In the current experiment 15 pairs of animals were used and were categorized into 5 groups. They are Normal/ Placebo, Control, Testosterone Propionate (TP) injected group, *Pergularia daemia* (Plant I) treated group and *Citrullus colocynthis* (Plant II) treated group.

2.3. Chemical Preparation

5 grams of Himedia's (Mumbai, Code: RM 7543) Testosterone Propionate (TP) were dissolved in 100 ml of olive oil (Vehicle).

2.4. Plant Description

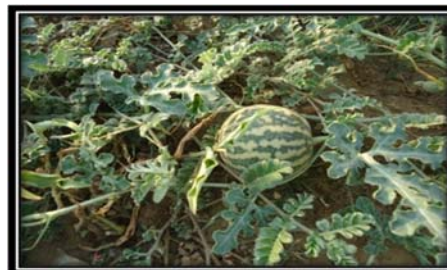
Pergularia daemia (Plant I)

Pergularia daemia (Forsskal) Chiov (SB001/20.05.2014) commonly called as Trellis – vine belongs to Asclepiadaceae subfamily. It is a perennial vine collected from Golden rock, Tiruchirappalli. The vernacular name of this plant is called “Veliparuthi” in Tamil. Fresh leaves were washed, chopped and were crushed well in the Mortar and Pestle. The fresh juice (crude extract) from the leaves were collected and used for the present study (Fig.1).



Citrullus colocynthis (Plant II)

Citrullus colocynthis (L.) Schrad (001/02.08.2014) commonly called bitter apple belongs to Cucurbitaceae family. It is a desert viny plant collected from Mamsapuram, Srivilliputhur. The vernacular name of this plant is called “Peikumutti” in Tamil. Fresh fruits were cut into small pieces and crushed with mortar and pestle. Fresh juice (extract) was collected and equal volume of castor oil were used to mix with the extract and boiled it for 30 minutes and used for the study (Fig. 2).



Both the plants were 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.

2.5. Dosage

1 ml of fresh *Pergularia daemia* leaves extract were orally given to the PCOS induced rats for duration of one week. Simultaneously 1 ml of *Citrullus colocynthis*, fruit extract were orally given to the PCOS induced rats for duration of 2 weeks.

2.6. Experimental Work

The normal / placebo group consists of 3 pairs of rats which received nothing. The control group consists of 3 pairs of rats which received vehicle alone for 4 days to test out the efficacy of the olive oil. The TP injected group consists of 3 pairs of which received 400 mg concentration of TP from the stock solution for four consecutive days to induce Polycystic Ovary Syndrome (PCOS). The Normal, Control and the injected group were sacrificed on the 7th day. *Pergularia daemia* treated group consists of 3 pairs of rats this which received 400 mg concentration of TP from the stock solution for four

consecutive days to induce (PCOS) and after seven days fresh leaves of (crude extract) were given to the rats for another seven days and the rats were sacrificed. *Citrullus colocynthis* treated group consists of 3 pairs of rats which received 400mg concentration of TP from the stock solution for four consecutive days to induce (PCOS) and after seven days fruits juice of *Citrullus colocynthis* extract boiled in castor oil extract were given to the rats for 14 days at the end of fourteenth day the rats were sacrificed. From the dissected rats the blood were collected and serum were separated out for hormonal assays like FSH, LH, Testosterone and progesterone.

2.7. Biochemical Analysis

The serum collected from the different groups was introduced for hormonal assays and were analyzed through ADVIA CENTAUR immunoassay system (auto analyzer).

2.8. Statistical Analysis

All the results were statistically analyzed and interpreted using Statistical Package for Social Sciences (SPSS V.17).

3. Results

The present study consists of five groups as Normal/Placebo, Control, TP injected, *Pergularia daemia* leaf extract treated and *Citrullus colocynthis* fruit extract treated groups. The different hormonal assays were performed and the results were statistically analyzed. Table 1 depicts the descriptive mean values of the different groups for different parameters. The mean for the normal /placebo group of the FSH is 14.3 mlU/ml, the control group is 14.1 mlU/ml, in the TP injected group the average mean is 9.03 mlU/ml, in the *Pergularia daemia* treated group the mean value is 15.85 mlU/ml and the *Citrullus colocynthis* treated group the average mean of FSH is 14.23 mlU/ml. These values indicate that the *Pergularia daemia* treated group shows higher efficacy in treating PCOS than *Citrullus colocynthis* (Fig.3).

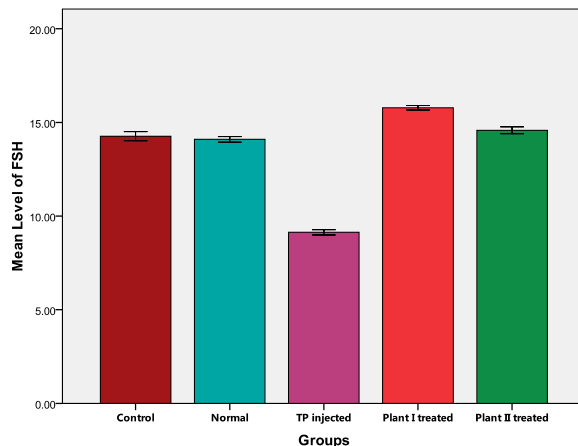
The LH descriptive mean value in the normal group is 22.33 mlU/ml, the TP injected group mean value is drastically increased 61.63 mlU/ml indicates that excess amount of serum LH level were distributed in PCOS induced animals which disturbs the ovulation, where as in *Pergularia daemia* treated group 30.4 mlU/ml which indicates that the LH level is drastically decreased and regulates the process of ovulation. Similarly in the *Citrullus colocynthis* treated group the mean value is 42.1 mlU/ml, this indicates that both the plants are treating the abnormal LH but *Pergularia daemia* shows more expressive result than the other (Fig.4).

In the TP injected group PCOS were induced by the excess amount of testosterone which were proven in by their mean value 108.33ng/dl where as in the Normal, Control, *Pergularia daemia* treated and the *Citrullus colocynthis* treated groups the mean values are 15.30, 22.16, 15.43 and 14.3ng/dl respectively. This proves that the *Pergularia daemia* brings down the testosterone value mere to the normal but the *Citrullus colocynthis* shows a sight increased efficacy when compared to the *Pergularia daemia* (Fig.5).

Progesterone hormone descriptive means of the various groups like normal, control, TP induced, *Pergularia daemia* and *Citrullus colocynthis* are 0.79, 0.86, 0.24, 0.93 and 0.87 ng/dl. This result also proves that the *Pergularia daemia* is more effective than the *Citrullus colocynthis* (Fig.6).

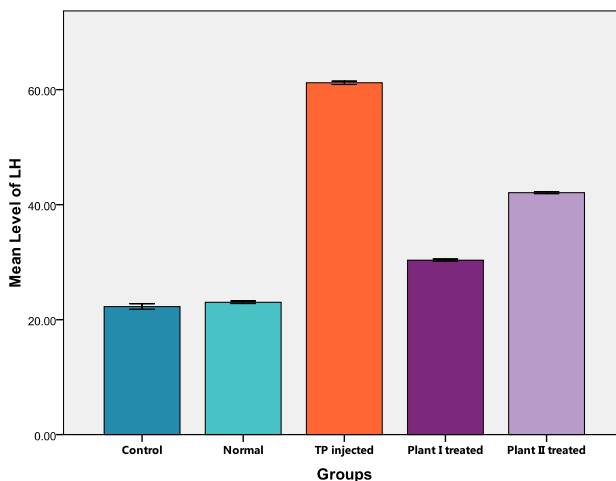
The one way analysis of variance proves that a significant difference in the hormonal levels *Pergularia daemia* (P<0.05) treated groups than the *Citrullus colocynthis* treated groups.

This indicates that in animal models the *Pergularia daemia* is an effective drug to cure PCOS than the *Citrullus colocynthis* (Table 2).



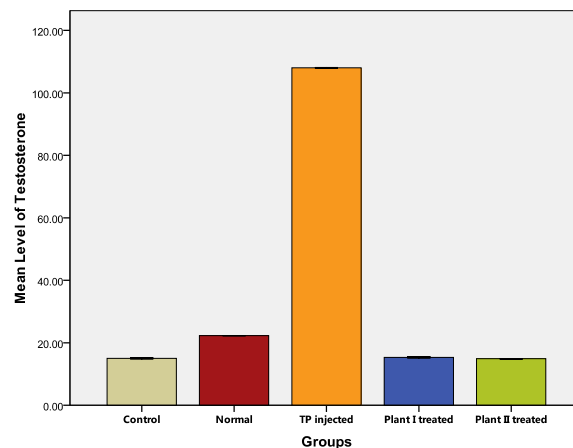
Plant I treated – *Pergularia daemia* treated
Plant II treated – *Citrullus colocynthis* treated

Fig 3: The levels of FSH in the normal, control, TP injected and extract treated groups



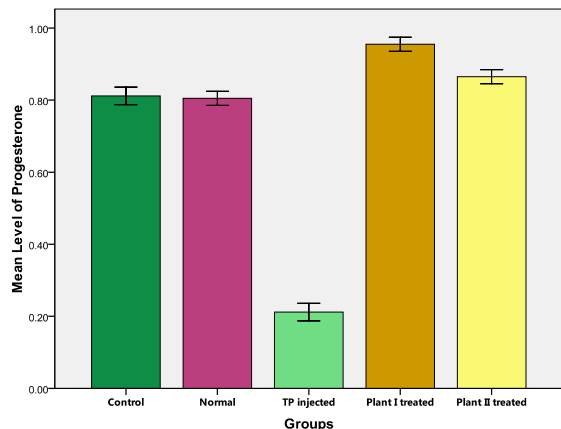
Plant I treated – *Pergularia daemia* treated
Plant II treated – *Citrullus colocynthis* treated

Fig 4: The levels of LH in the normal, control, TP injected and extract treated groups



Plant I treated – *Pergularia daemia* treated
Plant II treated – *Citrullus colocynthis* treated

Fig 5: The levels of Testosterone in the normal, control, TP injected and extract treated groups



Plant I treated – *Pergularia daemia* treated
 Plant II treated – *Citrullus colocynthis* treated

Fig 6: The levels of Progesterone in the normal, control, TP injected and extract treated groups

Table 1: The level of FSH, LH, Testosterone and Progesterone in the normal, control, TP injected and extract treated groups

Groups	FSH mlU/ml	LH mlUd/ml	Testosterone ng/dl	Progesterone ng/ml
Normal	14.3 ± 0.80	22.33 ± 1.52	15.30 ± 0.98	0.79 ± 0.015
	14.1 ± 0.16	23.4 ± 0.65	22.16 ± 1.02	0.86 ± 0.05
TP injected	9.06 ± 0.152	61.63 ± 0.66	108.33 ± 1.52	0.24 ± 0.04
	15.85 ± 0.22	30.4 ± 1.10	15.43 ± 0.65	0.93 ± 0.04
Plant I treated	14.23 ± 0.45	42.1 ± 0.20	14.3 ± 0.52	0.87 ± 0.015
	14.23 ± 0.45	42.1 ± 0.20		0.87 ± 0.015

Plant I treated – *Pergularia daemia* treated
 Plant II treated – *Citrullus colocynthis* treated

Table 2: One way analysis of variance between the level of FSH, LH, Testosterone and progesterone in the normal, control, TP injected and extract treated groups

Hormones		Sum of Squares	Df	Mean Square	F-value	Sig.
Level of Testosterone	Between Groups	40104.084	4	10026.021	787590.015	0.000
	Within Groups	0.318	25	0.013		
	Total	40104.402	29			
Level of Progesterone	Between Groups	2.099	4	0.525	1235.604	0.000
	Within Groups	0.011	25	0.000		
	Total	2.109	29			
Level of LH	Between Groups	6355.244	4	1588.811	20198.463	0.000
	Within Groups	1.967	25	0.079		
	Total	6357.211	29			
Level of FSH	Between Groups	158.170	4	39.542	1442.805	0.000
	Within Groups	0.685	25	0.027		
	Total	158.855	29			

Plant I treated – *Pergularia daemia* treated
 Plant II treated – *Citrullus colocynthis* treated

4. Discussion

The increased level of the LH and the decreased level of the FSH is the predominant sign for the cause of the PCOS (Pushpa and Kalavathy, 2013) [31]. McCartney *et al.* (2002) [23] described about the rapid increase in GnRH pulse frequency elevates the LH level in the PCOS condition. Nelson *et al.* (2001) [25] stated that the elevated pulse frequency increase the production of the androgen especially the testosterone on the PCOS state.

The PCOS can be diagnosed by the inclusion of the 21 – hydroxylase deficiency, androgen – secreting neoplasms, adrenocortical hyperactivity and the drug – induced hyperandrogenism. These disorders account for 5 – 10% of all women with the andeogen excess (Azziz *et al.*, 2004 & Carmina *et al.*, 2006) [2, 6].

The hallmark character of the PCOs symptom is LH hypersecretion – both basally and in the response to the GnRH administration. This phenomenon is due to the androgen excess which is primary abnormality in the classic symptom of PCOS (Yen *et al.*, 1970; Barnes *et al.*, 1989) [39, 3]. The pulsatile pattern of LH secretion is an indicator for the altered hypothalamic secretion of GnRH. This pulsatile secretion is required to maintain gonadotrophin synthesis and the secretion. The frequency of amplitude of GnRH pulses

determines the subunit gene expression and secretion of pituitary LH and FSH. (Marshall *et al.*, 1991; Marshall and Eagleson, 1999) [20, 21].

The low estrogen level inhibits the LH and FSH secretions at the pituitary level particularly FSH than LH (Gharib *et al.*, 1990) [11]. High amount of the oestrogen exhibits the positive stimulatory feedback with LH by inducing the LH surge at midcycle, whereas the level of estrogen raises steadily leading to a sustained elevated LH secretion (Keye and Jaffe, 1975) [15].

Low progesterone level acts at the pituitary gland level to enhance the LH response to the GnRH and are responsible for the FSH surge in the midcycle and finally suggested that the abnormalities of sex steroids concentration in the primary level have the stimulatory effect on LH secretion in PCOS, but the effect is at minimal range (Ehrmann *et al.*, 1995b) [9]. Ota *et al.* (1983) [28] injected testosterone propionate and observed the degeneration of the follicular cells in ovaries as well as elevated LH and decreased FSH level in 20 days old rats and proved the symptoms of the PCOS in rats are similar to that of the human beings.

In some cases the presence of any one symptom such as multiple cysts in the ovaries, hyperandrogenism and chronic anovulation is the prior identification for the PCOS. In series

of the adult women presenting with the amenorrhea is the only symptoms for the cause of the PCOS and 70% of the hirsutism is also considered as one of the symptom for the cause of the PCOS (Reindollar *et al.*, 1986 & Ferriman and Purdie, 1983) [32, 10].

The increased amount of the insulin also increases the serum testosterone and the androstenedione levels in the obese and non-obese women with PCOS as compared to weight – matched ovulating normal women and the testosterone levels were increased at the end of 270 – min insulin infusion with clamped glucose levels in six women with PCOS (Micic *et al.*, 1988) [24]. The current study also proves the same that the PCOS can be identified by the serum testosterone excess, elevated LH, decreased amount of the FSH and the progesterone.

PCOS can be treated in various methods. Metformin is a widely used medicine for the treatment of insulin resistance and the PCOS. Acarbose is widely used in managing type 2 diabetes that lower the serum insulin concentration (Lilliana *et al.*, 2001) [18]. Metformin shows some of the side effects when compared to acarbose, similarly acarbose is the safe and effective agent for clomiphene – resistance PCOS (Sonmez *et al.*, 2005) [34].

Mentha spicata is a spearmint tea having the antiandrogenic property that fight against the testosterone level in the female system (Grant, 2009) [12]. Short thorned epimedium herbs with the dodder seed, flowers of the solomonseal rhizome, Chinese fox glove root and roots of aconite fight against the obesity and the excess androgen (Sun and Yu, 2000) [36].

The *Citrullus colocynthis* plant contains many properties like antibacterial, anticandidal activity. But in the fruits Marzouk *et al.* (2009) [22] proved the activity against dermatology, gynaecology and pulmonary infections. The present investigations support the activity against the gynaecological problem.

Pavunraj *et al.* (2011) [30] performed a detailed analysis about the antifeedant activity of a novel 6 – (4,7-hydroxy -heptyl) quinone from the leaves of *Pergularia daemia* with the ethanolic extract. Ignacimuthu *et al.* (2009) [13] tested the antimicrobial activity against the pathogenic bacteria with the ethyl acetated crude extract of the leaves. The present study is a novel investigation to find out the effect of leaves of *Pergularia daemia* in the field of infertility.

In the present study fresh leaf extract of *Pergularia daemia* brings out a good effect in the hormonal assays of PCOS injected group when compared to the *Citrullus colocynthis* fruit extract.

5. Conclusion

The present work was carried out to know the hormonal patterns in the rat's models with the PCOS state. India is a vast country which hides many medicinal plants and herbs. Our country is renowned for its Siddha medicine. Many people nowadays are facing the infertility problem due to the hormonal balance particularly the women in the reproductive stage. Many unknown plants which brings cure for this kind of problems we had chosen two easily available plants to treat the hormonal disturbances which is a novel try. They are *Pergularia daemia* and *Citrullus colocynthis*. The leaf extract of the *Pergularia daemia* and the fruit extract of the *Citrullus colocynthis* brings a good effect in curing PCOS but the leaf extract of the *Pergularia daemia* is an effective medicine in normalizing the hormonal levels of the PCOS induced rats, this plant also shows a statistical significance between the normal, control and the extract treated group. Thus we

conclude that the *Pergularia daemia* is an effective and active natural drug to cure the hormonal problem in the reproductive stage women which is one of the sign infertility.

5. Acknowledgement

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