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EVALUATION OF LETROZOLE INDUCED POLYCYSTIC OVARY SYNDROME (PCOS) USING ETHANOL AND ETHYL ACETATE EXTRACTS OF *TECOMARIA CAPENSIS*. (FAMILY: BIGNONIACEAE)

E. Tamil Jothi[1]*, CH. Harini[2] and G. Nagarjuna Reddy[3]

[1]Department of Pharmacology, K.L.R. Pharmacy College, Palvoncha, Telangana.

[2]Doctor of Pharmacy, K.L.R. Pharmacy College, Palvoncha, Telangana.

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***Corresponding Author**
Dr. E. Tamil Jothi
Department of Pharmacology, K.L.R. Pharmacy College, Palvoncha, Telangana.

ABSTRACT

Polycystic ovary syndrome (PCOS) is a highly prevalent endocrine-metabolic disorder that implies various severe consequences to female health, including alarming rates of infertility. Ethanol extract of *Tecomeria capensis* (Bignoniaceae) was investigated for the activity against letrozole induced polycystic ovarian syndrome. Letrozole induced PCO untreated rats exhibited a significant increase in body weight compared to controls. In contrast, no further increase in body weight was observed in ethanol extract of *Tecomaria capensis* treated PCO rats. ethanol extract of *Tecomaria capensis* treatment in letrozole induced PCO rats also demonstrated showed similar kinds of rhythm in estrus cyclicity as controls and the metformin group suggesting reversion toward normal physiology Letrozole treated PCO rats showed significant increases in ovarian weights whereas ethanol extract of *Tecomaria capensis* treated PCO animals exhibited ovarian weights similar to controls. Extract showed a significant results comparing to standards. Jeans mai abh nahi loomgi. Badh.

KEYWORDS: *Tecomaria capensis*, polycystic ovarian syndrome, letrozole.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by multiple hormonal imbalances, reflecting on a clinical presentation dominated by manifestations of hyperandrogenism, which generate short and long term consequences on female health.[1] Among these, infertility is one of the most alarming associated morbidities, as it currently affects approximately 48.5 million women aged 20–44 years[2] with PCOS

accounting for 6–15% of these cases[3], although up to 70% of women with PCOS may be undiagnosed.[4] Indeed, its optimal diagnosis is often hindered due to its apparent similarities with several other pathologies remarkably, obesity as well as Cushing's syndrome, ovarian and adrenal neoplasms and congenital adrenal hyperplasia.[5]

IR, defined as a metabolic state characterized by a decrease in cellular ability to respond to insulin signalling, appears to be an essential pathophysiologic mechanism in the development of all metabolic complications of PCOS.[6] As a consequence, outstanding proportions of women with PCOS are also diagnosed with DM2 or MS, as well as isolated criteria from the latter.[7] Compensatory hyperinsulinemia appears to mediate many of these deleterious effects. This phenomenon stems as a response of pancreatic β cells in order to preserve lipid and carbohydrate homeostasis in faces of diminished insulin sensitivity.[8] This compensation leads to β cell exhaustion and the genesis of not only DM2, but also a series of collateral effects originated by hyperinsulinemia, including the aforementioned frequent comorbidities of PCOS.[9]

Notwithstanding the importance of IR in the development of PCOS, both obese and nonobese patients have specific mechanisms leading to ovarian dysfunction independent of IR, reflecting the complexity of this syndrome.[10] Therefore, considering the severe consequences PCOS exerts on the health and lifestyle of the affected women, it is of utmost importance to unravel the intricate pathophysiologic cross-talk among PCOS, IR and obesity. Insulin is a pancreatic peptide hormone produced by β-cells of islets of Langerhans.[11] The classical target organs for its action are muscles, adipose tissue and liver.[12] Insulin plays a major role in regulation of carbohydrate, fat and protein metabolism, it suppresses hepatic glucose output, inhibits glycogenolysis and gluconeogenesis and promotes glycogen synthesis. It stimulates peripheral glucose uptake in muscle and fat tissues, induces protein synthesis, cell growth and differentiation and inhibits lipolysis.[12] Insulin plays a role as a co-gonadotropin in regulation of ovarian function.[13] Co-gonadotropin is a term applied to any extra ovarian hormone, that exhibits potentiating or synergistic effect through specific receptors for gonadotropin mediating growth of follicles and steroidogenesis.[14] Insulin plays both direct and indirect roles in the pathogenesis of androgen excess in PCOS. Although women with PCOS have peripheral insulin resistance, ovarian steroidogenesis appears to be hypersensitive to insulin.[15] Insulin acts synergistically with LH to enhance theca cell androgen production in women with PCOS by activating a specific signaling pathway via its

own receptor.[16] In addition, insulin can stimulate human theca cell proliferation and can also enhance ovarian growth and follicular cyst formation in rats.[17] Hyperinsulinemia may also have adverse effects in women with PCOS through its action at non-ovarian sites including the liver, adrenal glands and pituitary.[18] Insulin also potentiates ACTH-mediated adrenal androgen production. The concept that hyperinsulinemia affects GnRH pulse frequency and inappropriate gonadotropin secretion in PCOS by acting at pituitary level is mainly based on in vitro studies in which insulin has been shown to increase LH secretion from cultured rat pituitary cells. This suggests that insulin has an important pathophysiological role in PCOS, although its role in neuroendocrine dysfunction remains unclear.[19] The course of treatment for women with PCOS largely depends on the severity of an individual's symptoms. Well-defined published data indicate a high risk for development of T2DM and CVD in women with PCOS.[20,21] Metformin is now thought to be of therapeutic value directly and/or indirectly in the management of PCOS.[22] In addition to the expected improvements in insulin sensitivity and glucose metabolism, metformin therapy also ameliorates hyperandrogenism and menstrual irregularity, the favorable effect of metformin on hyperandrogenism in PCOS. Treatment with Choline and Inositol — Inositol is a term used to refer to a group of naturally occurring carbohydrate compounds that exist in nine possible chemical orientations called stereoisomers. The most common being myo-inositol, which is often sold as a dietary supplement labeled simply as inositol. Inositol, particularly myo-inositol and another less common stereoisomer called D-chiro-inositol, plays a critical, but underappreciated, role in insulin signaling. Conditions such as hyperglycemia and diabetes are associated with disrupted inositol signaling, leading many researchers to suggest that this may be a key pathologic feature of insulin resistance.[23] Natural Treatments for PCOS Over the past few years, research into the naturopathic and nutritional approach to PCOS has revolutionized the condition's treatment. It is important to treat the factors that lead to PCOS. Following *The Natural PCOS Diet*, making lifestyle changes and taking supplements can influence a healthy outcome. One of the most important things is to address insulin resistance, if this is an apparent problem. This can be done by incorporating dietary changes and taking suitable nutritional supplements. What you eat can have a direct influence on how balanced (or unbalanced) your hormones are. This is why it's important to have a healthy diet.[24]

MATERIALS AND METHODS

The PCOS rat model was developed with adult virgin Wister female rats weighing 200 - 225g. All animals were checked daily for 4 day ovarian cycle using vaginal cytology. All animals were kept under controlled conditions of light and temperature and having free access to diet and water. The rats were divided into two experimental groups: control group of animals (n=8) received orally 1% aqueous solution of carboxymethylcellulose (CMC) and another group of animals (n=8) were treated orally with letrozole (0.5 mg/kg body weight) daily for 21 days.[25] Oral Glucose Tolerance Test (OGTT) was performed regularly every 15 day period to check glucose sensitivity. Twenty four hours after their last dose of letrozole, blood was collected to assess toxicological biomarkers. Following this, the animals were sacrificed in the late diestrum stage and their ovaries were removed. One of the ovaries was fixed in bouins fluid and histological studies were performed. The other ovary was accessed for steroidogenic enzyme activities. Letrozole treated experimental rats which demonstrated irregular estrus cyclicity, glucose intolerance and altered steroid status were considered PCOS positive animals and used for further study. These animals were further divided into three groups- PCOS untreated (control) rats and ethanol (200 mg) treated rats. The Letrozole were given orally a concentration of 1 ml daily for 45 days. The other group of animals received both letrozole (inductive agent for PCOS) and ethanol extract of *Tecomaria capensis* together for 45 days daily. All groups were continuously monitored for insulin sensitivity by OGTT. At the end of treatment, the rats were sacrificed and assessed for various biochemical parameters.

Oral glucose tolerance test

OGTT was performed after 12 hr fasting for all rats in the experiments.[26] Blood samples were collected in fluoride coated anticoagulant vials. Next, glucose (300 mg/kg body weight) was orally fed to the rats and blood samples were collected after time intervals of 30', 60', 90' and120'. The blood was subjected to 3000 rpm for 10 min and the plasma separated. Glucose was estimated using GOD - POD based kits.

Preparation of ovarian homogenate

10% ovarian homogenate was prepared in 0.1 M Tris Hcl buffer (pH--7.8) and centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was used as a source of steroidogenic enzyme assay and protein content was monitored.

Steroidogenic enzyme assays

The key steroidogenic enzymes - 3β hydroxy steroid dehydrogenase and 17β hydroxy steroid dehydrogenase were assayed to evaluate the enzyme activity of ovarian enzyme.[27] In brief, the enzyme assay was carried out in 0.1 M Tris HCl buffer (pH 7.8) containing NAD (500 µM) and the substrate DHEA (100 µM) for 3β hydroxy steroid dehydrogenase or 17β estradiol (100 µM) for 17β hydroxy steroid dehydrogenase in a total volume of 3 ml. The reaction was started by adding the enzyme (100 µl) together with the colour reagent, INT. The mixture was then incubated at 37°C for 1 hr. The reaction was terminated by the addition of 2.0 ml of phthalate buffer (pH 3.0) and read at 490 nm. The enzyme activity was calculated from the standard curve for NADH and expressed as n moles of NADH formed per hr per mg protein.

Histological Analysis

Ovaries were removed and fixed in Bouins fixative. Histological examinations of ovaries from all groups were carried out using standardized histological methods. Sections of 5 ˝m thickness were cut in paraffin-embedded block and stained with hematoxylineosin.[28]

"In vitro" experiments

To understand the direct effect of ethanol extract of *Tecomaria capensis* on steroidogenic enzymes, we performed "In vitro" assay. Ovarian protein (30-35g) was incubated both with and without ethanol extract of *Tecomaria capensis* (200 mg), for 1 hr at 4°C. In addition to these treatments, ovarian protein was also incubated with metformin [1.2 mg][29] which was used as positive control. The activities of ovarian enzymes 3β HSD and 17β HSD were estimated by the method described by Shivanandappa et al., 1997.

RESULTS ADN DISCUSSION

Rats treated with letrozole for induction of PCOS showed significant increases in body weight and ovarian weight and altered oestrus cyclicity compared to controls. PCOS positive animals exhibited an increase in glucose intolerance compared to controls. PCOS rats exhibited many small atretic cysts whereas no histological abnormalities were observed in control rats. Key ovarian steroidogenic enzymes 3β hydroxysteroid dehydrogenase and 17β hydroxysteroid dehydrogenase showed increases in activity in letrozole induced PCOS rats compared to control rats.

Letrozole induced PCO untreated rats exhibited a significant increase in body weight compared to controls. In contrast, no further increase in body weight was observed in ethanol extract of *Tecomaria capensis* treated PCO rats. ethanol extract of *Tecomaria capensis* treatment in letrozole induced PCO rats also demonstrated showed similar kinds of rhythm in estrus cyclicity as controls and the metformin group suggesting reversion toward normal physiology Letrozole treated PCO rats showed significant increases in ovarian weights whereas ethanol extract of *Tecomaria capensis* treated PCO animals exhibited ovarian weights similar to controls. The OGTT profile showed the letrozole induced PCOS group to have significant glucose intolerance compared to the control and metformin groups whereas the combined treatment (letrozole and ethanol extract of *Tecomaria capensis* treated simultaneously) as well as ethanol extract of *Tecomaria capensis* treated PCO animals exhibited an improvement in glucose sensitivity. Histological analysis exhibited a decrease in ovary atretic cysts after ethanol extract of *Tecomaria capensis* treatment of PCO rats compared to PCO controls and normal growth similar to metformin controls. As represented in Figure, ethanol extract of *Tecomaria capensis* treatment in letrozole induced PCO animals caused an improvement in ovarian 3β hydroxy steroid dehydrogenase (3β HSD) and 17β hydroxy steroid dehydrogenase (17β HSD) activities, comparable to both control and metformin treated rats suggesting improvement in steroid status. Ethanol extract of *Tecomaria capensis* treatment in PCO rats showed no effects on biomarker enzymes indicating that treatment does not affect major organ systems, namely kidney and liver function.

PCOS has many clinical manifestations, including oligomenorrhea and hyper - androgenism, leading to metabolic dysfunction.[30] In the present study, we investigated the biochemical and clinical characters of PCOS in a rat model. The inducing drug inhibited aromatase, thereby increasing ovarian androgens, leading to hyperandrogenism, a hallmark of PCOS.[31] Similarly, we found a significant weight gain in letrozole treated PCO compared to control rats which was attributable to deposition of abdominal fat.[32,33]

PCOS is positively correlated with insulin resistance. The PCOS rat model was examined for glucose intolerance, finding that these rats exhibited hyperglycemic tendencies contributing to insulin resistance, leading to full hyperglycemia and metabolic syndrome.[34] Thus, insulin resistance may be a consequence of increased truncal fat and high levels of free fatty acids.

Similarly, the present study found the ethanol extract of *Tecomaria capensis* treated PCOS rats to have returned to normo glycemic condition from their hyperglycemic condition. This may be attributable to nutritionally rich phytosterols and phyto-phenols present in the plant.[35, 36]

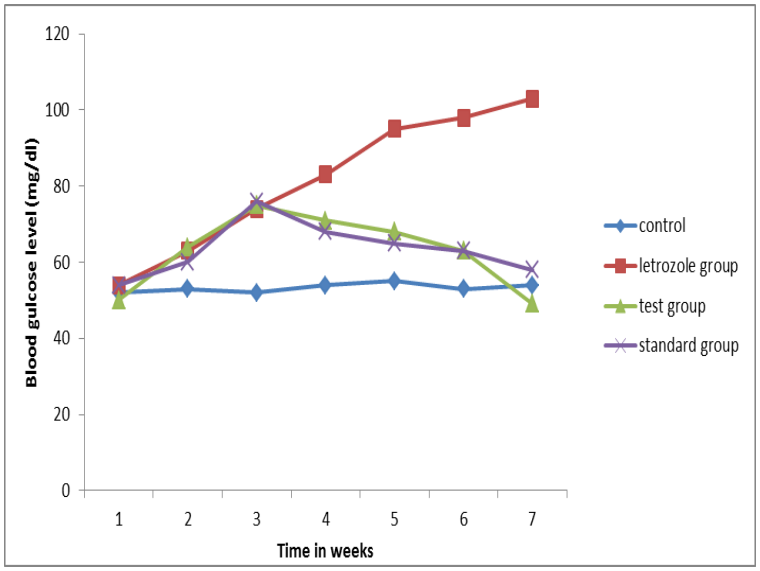
In PCOS, excess production of androgens interferes with the process of follicular maturation and selection of dominant follicles during ova formation. It also promotes early stages of follicular growth in primate ovary leading to the syndrome's insulin resistance and fat distribution. In our study, PCO rats demonstrated the formation of empty cysts filled with follicular fluid similar to reported ovarian histology. In all these ways, the rat model behaves similarly to the human system indicating that it adequately mimics the human PC ovary.
In the model, hyperinsulinemia is also positively correlated with estrogen deficiency[37] as in PCOS. As estrogen synthesis is inhibited by the use of inhibitor in our model, the 3β HSD activity is higher compared to 17β HSD activity, and androgen production will be higher than estrogen production. This will affect LH: FSH hormonal balance. Thus we can state that ethanol extract of *Tecomaria capensis* treatment brought 3β HSD activity in PCO rats back to normal levels comparable to those in the controls.

Reversion of estrus cyclicity to normal following ethanol extract of *Tecomaria capensis* treatment could be attributed to phytochemical components present in the gel that maintain steroid status, enabling fertility status to be regained.

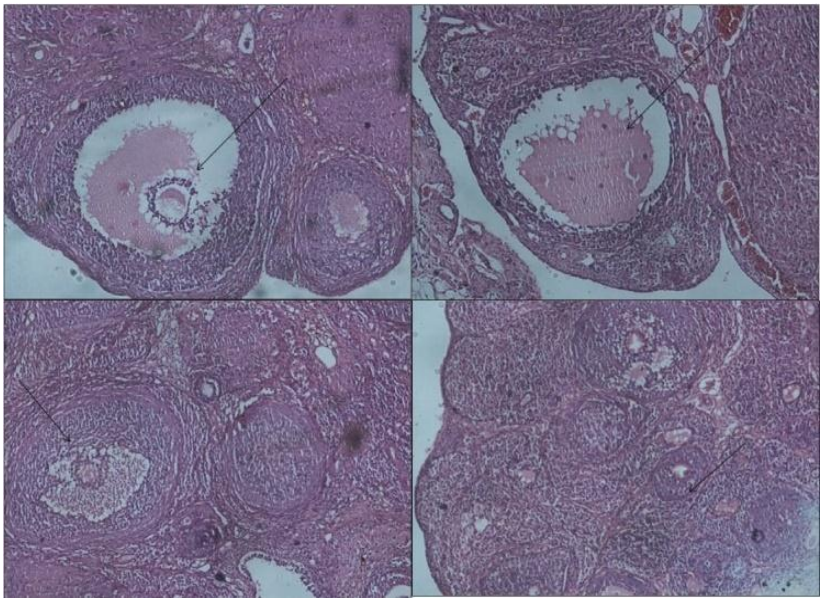
Preliminary phytochemical data found ethanol extract of *Tecomaria capensis* rich in phytosterols and polyphenols that could be active components in controlling hyperglycemic conditions and modulating steroidogenes. However, no reports of phytosterols or polyphenols affecting steroidogenic enzymes have been published. We hypothesize that these phyto-components act at various stages of the steroidogenic enzyme cascade and modulate the activity back to normal. Data from our *"in vitro"* study indicate that ethanol extract of *Tecomaria capensis* acts directly on key enzymes like 3β HSD, decreasing enzyme activity and modulating the flux toward estradiol formation. However, the specific phyto-component acting on the enzyme system needs to be identified.

Groups	Control	Letrozole (PCOS)	Letrozole+ethanol extract combined	ethanol extract group	Metformin group
(SGPT) mg/dl	59.33±2.12	59.2±1.08	46.25±1.23	59.4±2.05	61±1.28
Creatinine mg/dl	0.59±0.25	0.57±0.17	0.50±0.31	0.58±0.11	0.62±0.21

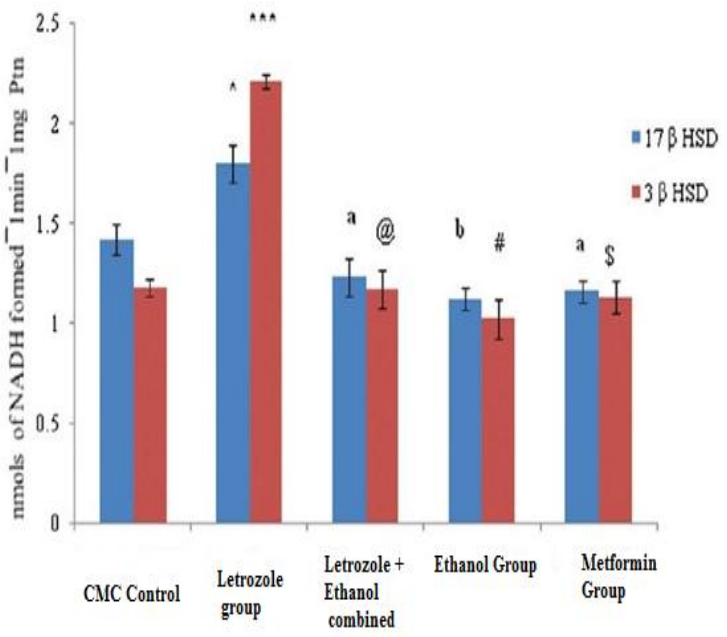
Effect of ethanol extract of *Tecomaria capensis* (1 ml orally / 45 days) on toxicity letrozole induced PCOS rats



Effect oral administration of ethanol extract of *Tecomaria capensis* (1 ml/45 days) on oral glucose tolerance test profile of letrozole induced PCOS rats. The values are represented as mean ± SEM.



Effect oral administration of ethanol extract of *Tecomaria capensis*(1 ml/45 days) on follicular growth of ovary in letrozole induced PCOS rats (a) CMC control rat showing normal follicular development (H and E ×10) b: Section of ovary from letrozole treated.



Effect oral administration of ethanol extract of *Tecomaria capensis*(1 ml /45 days) on steroid dehydrogenase enzyme activity in letrozole induced PCOS rats, n=4-6, The values are represented as mean ± SEM, **P*<0.05, ****P*<0.001, as compared to Control Group.

CONCLUSION

From the above results, it is concluded that ethanol extract of *Tecomaria capensis*, showed significant activity against Letrozole induced polycystic ovarian syndrome. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant extract against this activity. In PCOS, ethanol extract of *Tecomaria capensis* has potential efficacy in the prevention and maintenance.

The plant extracts may be further explored for its phytochemical profile to recognize the active constituent responsible for the activities.

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