Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

Therapeutic Potential of Ficus carica Extract on Protein Levels of Reproductive Tissue in PCOS Induced Mice

S. D. Gurav[1], L. P. Bhopale[2], T. C. Gaupale[3]

[1] Research Scholar, Department of Zoology, Vivekanand College (Empowered Autonomous), Kolhapur, Maharashtra, India.
[2] Assistant Professor, Department of Zoology, Rajaram College, Kolhapur, Maharashtra, India.
[3]Assistant Professor, Department of Zoology, Vivekanand College (Empowered Autonomous), Kolhapur, Maharashtra, India.

Abstract

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder characterized by hormonal imbalance and ovarian dysfunction. Experimental induction of PCOS using letrozole in animal models provides a reliable approach to study its pathophysiology and potential therapeutic interventions. The present study aimed to evaluate the effect of metformin and Ficus carica plant extract on ovarian and uterine protein levels in letrozole-induced PCOS mice. Female Swiss albino mice of 20 days old were divided into four groups: (1) Control group (2) Letrozole-induced PCOS group, (3) Metformin-treated group, and (4) Plant extract-treated group. PCOS was induced by oral administration of letrozole for 21 days. Following the treatment period for consecutive 21 days, ovarian and uterine tissues were collected, homogenized, and total protein was measured by using standard method with Folin–Ciocalteu reagent using bovine serum albumin (BSA) as the standard. Letrozole-induced PCOS mice showed a significant increase in ovarian total protein concentration compared to control, indicating altered cellular metabolism and tissue remodelling. However, the protein levels in the uterus did not exhibit significant alterations. Both metformin and Ficus carica treatments reduced elevated ovarian protein levels toward normal values. Ficus carica extract exhibited a restorative effect on ovarian protein metabolism comparable to metformin, whereas uterine tissue showed no significant biochemical alteration.

Keywords: Protein, Letrozole, PCOS, Ficus carica, Lowry method, Ovary, Uterus

1. Introduction

Polycystic ovary syndrome (PCOS) is the most frequent and complicated reproductive endocrine disease caused by endocrine and metabolism in women of reproductive age [1]. The pathophysiology of PCOS is multifactorial and involves impaired gonadotropin-releasing hormone (GnRH) pulsatility, increased pituitary luteinizing hormone (LH) secretion [2]. Hyperandrogenism is a key diagnostic feature characterized by elevated androgenic hormones, leading to clinical manifestations such as acne, hirsutism,

and alopecia [3]. Theca cells in PCOS exhibit overexpression of most steroidogenic enzymes and proteins involved in androgen synthesis, suggesting a prominent abnormality in the level and activity of steroidogenic enzymes, including P450c17, which has been highly identified. Granulosa cells prematurely luteinize primarily as a result of androgen and insulin excess [4].

PCOS has been regarded as a life course disease which besides its reproductive features has a long-term impact on the risk of type 2 diabetes mellitus (T2DM) and metabolic syndrome [5]. Metformin was the first insulin sensitising drug (ISD) to be used in PCOS to investigate the role of insulin resistance in the pathogenesis of the syndrome [6]. However, prolonged use of metformin may lead to adverse effects such as gastrointestinal discomfort and vitamin B12 deficiency, limiting its therapeutic applicability [7], [8]. This has encouraged the search for plant-based alternatives with comparable efficacy and minimal side effects.

Ficus carica (common fig) is a medicinal plant widely recognized for its antioxidant, anti-inflammatory, and antidiabetic properties. Its bioactive constituents, including flavonoids, phenolics, and triterpenoids, are known to modulate metabolic pathways and improve oxidative balance [9], [10], [11]. Since protein metabolism serves as a sensitive indicator of cellular function and tissue integrity, estimation of total protein concentration in ovarian and uterine tissues can provide valuable insight into the biochemical effects of PCOS and its therapeutic modulation.[12], [13]. Therefore, the present study aims to evaluate the effect of Ficus carica extract and metformin on total protein concentration in ovarian and uterine tissues of letrozole-induced PCOS mice. This study may help elucidate the biochemical mechanisms underlying the potential therapeutic action of Ficus carica in restoring normal reproductive function.

2. Materials and methods

2.1 Chemicals

Metformin tablet contains 500 mg of the active substance while letrozole tablet contains 2.5 mg of the active substance. Both were bought from a nearby pharmacy (Balaji Pharma Company). The remaining chemicals that were used in this study were of analytical quality.

2.2 Experimental animal

The present study was executed under CPCSEA guidelines with ethical clearance from the Institutional Animal Ethical Committee, Rajarambapu College of Pharmacy, Kasegaon, (1290/PO/Re/S/09/CPCSEA). A total of 16 Swiss albino Female mice, aging 20-day old and weighing 14–17g, were used in this study. mice were caged in standard polypropylene cages, maintained in controlled environment of 25° C temperature and a 12 h light/dark cycle with free access to food and water ad libitum throughout the study.

The mice were divided into four experimental groups (n= 04/group)

Group 1: Control group orally received 0.5ml saline daily for 3 weeks.

Group 2: PCOS was induced in mice through 1mg/kg letrozole orally once daily for 3 weeks. Studies have shown that administration of letrozole leads to PCOS-like ovarian and metabolic changes in mice [14].

Group 3: PCOS induced mice received 500mg/kg Metformin orally daily for 3 weeks.

Group 4: PCOS induced mice received 500mg/kg Ficus carica leaves extract orally daily for 3 weeks.

2.3. Preparation of Plant Extract

Fresh leaves of Ficus carica were collected from local area in Kolhapur, Maharashtra, India. The collected sample was identified and authenticated by Department of Botany, Shivaji University, Kolhapur. Further the leaves were washed, shed-dried and coarsely powdered using a mechanical grinder. The powdered sample was macerated in ethanol at room temperature for 72 hours, keeping 1: 10 proportion (sample: solvent), with occasional stirring and shaking to enhance extraction efficiency, before being filtered through a fresh cotton plug as well as Whatman (Number l) filter paper. At 40 °C, the filtrate was concentrated in vacuo using a rotary evaporator. The quantity of the crude extract was determined, as was the percentage yield. Finally, the extract of Ficus Carica leaves was dissolved in Distilled water to achieve an appropriate concentration.

2.4. Protein estimation

After completion of experimental doses, the animals were then sacrificed, ovaries and uterus excised, cleaned of fat and weighed. After excision, ovaries and uterus were freed from blood and cleaned with ice cold saline and homogenized using Distilled water to prepare homogenate of 7mg/ml and 14mg/ml respectively for protein estimation [15], [16]. Total protein was measured by the Lowry method with Folin–Ciocalteu reagent using bovine serum albumin (BSA) as the standard. All measurements were performed in triplicate and values are reported as mean ± SD.

3. Results

The total protein concentration in ug/mg was analysed in both ovarian and uterine tissues across all experimental groups, with results presented in Tables 1 and 2. In the ovary, the Letrozole-induced PCOS group exhibited a substantial increase in total protein concentration (3.33 ± 0.14) compared to the Control group (2.16 ± 0.05). Treatment with both interventions demonstrated significant modulatory effects: Metformin treatment reduced the ovarian protein concentration to 2.01 ± 0.03, while the F. Carica Extract treatment also restored the concentration to 2.19 ± 0.09, a value statistically comparable to the Control group. In the uterus, total protein concentration remained relatively stable across the groups, starting at 1.49 ± 0.12 in the control group. The PCOS induction resulted in a slight increase to 1.74 ± 0.09. Metformin treatment showed protein concentration (1.43 ± 0.06), whereas F. Carica extract treatment resulted in the protein concentration (1.47 ± 0.03). Overall, the ovary tissue demonstrated a more pronounced response to PCOS induction and subsequent treatment than the uterine tissue.

Table 1. Total protein concentration of Ovary (ug/mg)

Sr. no.	Groups	Total Protein concentration (ug/mg)
1	Control	2.16 ± 0.05
2	Letrozole-induced PCOS	3.33 ± 0.14
3	Metformin treated	2.01 ± 0.03
4	F. Carica Extract treated	2.19 ± 0.09

Table 2. Total protein concentration of Uterus (ug/mg)

Sr. no.	Groups	Total Protein concentration (ug/mg)
1	Control	1.49 ± 0.12
2	Letrozole-induced PCOS	1.74 ± 0.09
3	Metformin treated	1.43 ± 0.06
4	F. Carica Extract treated	1.47 ± 0.03

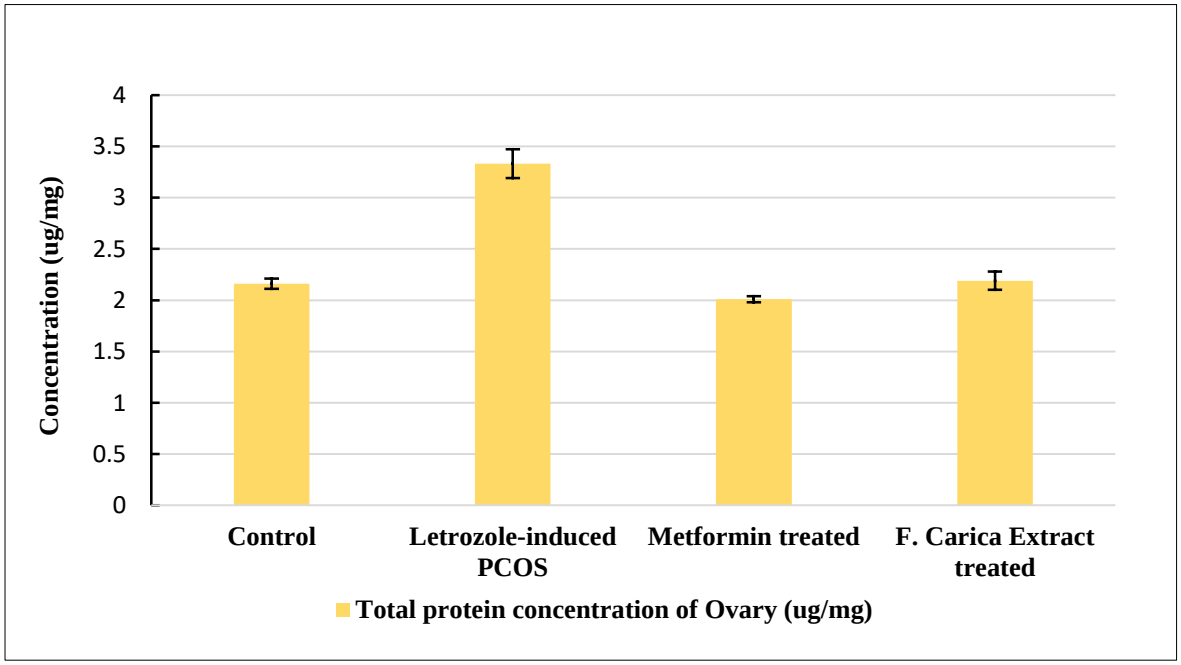

Figure 1: Graphical representation of total protein concentration (ug/mg) in ovary tissue of four experimental groups viz. 1) Control group 2) Letrozole Induced PCOS group 3) Metformin treated group 4) F. carica Extract treated group.

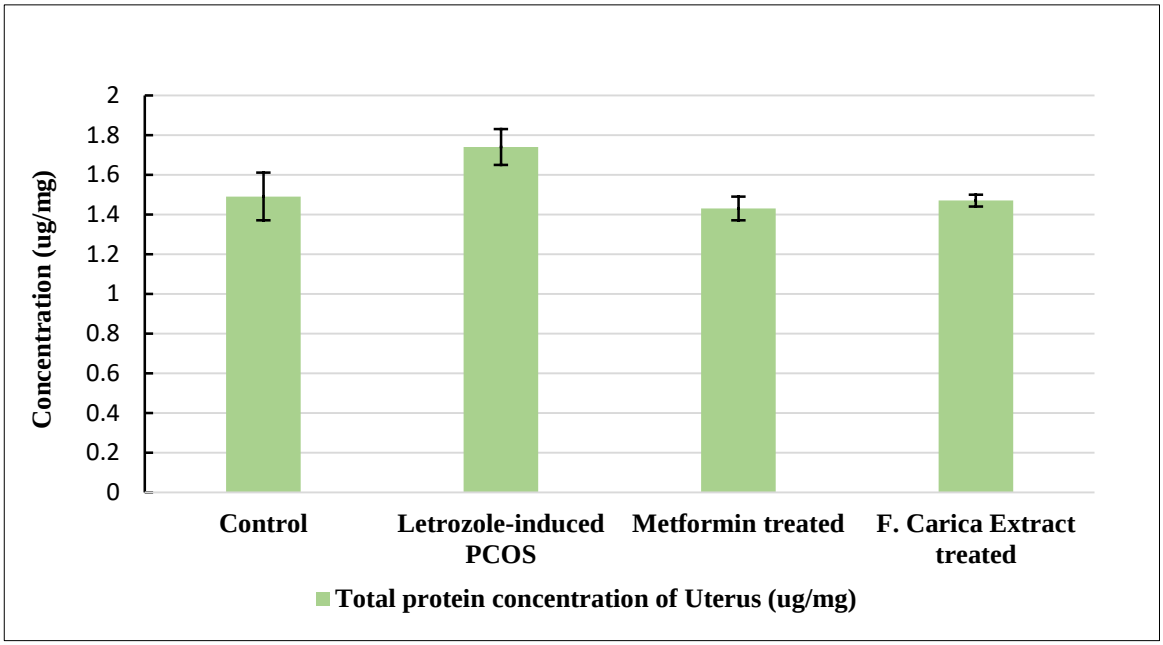

Figure 2: Graphical representation of total protein concentration (ug/mg) in Uterus tissue of four experimental groups viz. 1) Control group 2) Letrozole Induced PCOS group 3) Metformin treated group 4) F. carica Extract treated group.

4. Discussion

The Letrozole-induced Polycystic Ovary Syndrome (PCOS) model was effectively developed, evidenced by a significant increase in ovarian total protein concentration from 2.16 ± 0.05 (Control) to 3.33 ± 0.14 (PCOS). This increase aligns with findings that link Letrozole-induced hyperandrogenism and the subsequent follicular arrest to heightened ovarian inflammation, oxidative stress, and increased localized cellular stress markers [17]. Total protein concentration in ovarian tissues was significantly altered in letrozole-induced PCOS mice compared to uterine tissue. The increase in ovarian protein levels observed in the PCOS group reflects disturbances in folliculogenesis, stromal proliferation, and accumulation of cellular proteins associated with hyperandrogenism and disrupted aromatase activity which is documented features of letrozole-induced PCOS models [18]. Earlier studies also report altered expression of phosphorylated and regulatory proteins in the ovary and uterus under PCOS conditions [19], supporting the biochemical disruptions seen in our findings. The mild elevation in uterine protein concentration noted in PCOS mice aligns with reports of increased endometrial proliferation, epithelial thickening, and altered gene expression in PCOS models [20], [21]. Together, these changes suggest that letrozole-induced PCOS influences both ovarian and uterine tissue biochemistry by promoting protein accumulation linked to tissue remodelling and hormonal imbalance. Treatment with metformin reversed many of these abnormalities, evidenced by a marked reduction in ovarian protein concentration and a normalization-associated rise in uterine protein levels. These effects are consistent with studies demonstrating that metformin restores ovarian steroidogenesis, insulin sensitivity, and uterine signalling pathways in PCOS models [22]. F. carica extract treatment also reduced elevated ovarian protein levels and modulated uterine protein content, suggesting partial restoration of reproductive tissue homeostasis. The beneficial effect of F. carica may be attributed to its antioxidant, anti-inflammatory, and hormone-modulating properties, as reported in reproductive studies involving Ficus species [23], [24].

Thus, both metformin and F. carica extract showed corrective potential, with the plant extract offering a moderate yet favourable biochemical response in PCOS-affected tissues.

5. Conclusion

The Letrozole-induced Polycystic Ovary Syndrome (PCOS) model resulted in a notable, tissue-specific pathological change, characterized by a significant increase in total protein concentration within the ovarian tissue. This research validates the well-known therapeutic efficacy of Metformin, which had a significant effect by lowering ovarian protein levels close to those of the healthy control group. Importantly, the study highlights the considerable restorative capabilities of Ficus Carica leaf extract, which effectively counteracted the protein increase caused by PCOS, bringing ovarian protein levels back to a statistically similar range as the control group. The evidence indicates that F. Carica functions through corrective processes, likely addressing the oxidative stress and inflammation that contribute to the disease. On the other hand, the slight pathological changes seen in the uterus, along with its varied response to treatments, underscore the importance of considering tissue-specific mechanisms in treating PCOS. These results highlight the potential of Ficus Carica as a natural therapeutic option for addressing the primary ovarian issues linked to PCOS.

6. Acknowledgment

The authors would like to acknowledge Vivekanand College, Kolhapur (Empowered Autonomous) and Rajaram College, Kolhapur for providing laboratory facilities.

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