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RESEARCH ARTICLE

Effect of *Gymnema sylvestre* Extract on the Regulation of AMPK-GLUT4 Mediated Signaling Pathway on Insulin Resistance in a PCOS Rat Model

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Abstract

This study was intended to assess the effect of *Gymnema sylvestre* leaf extract on the AMPK-GLUT4 mediated signalling pathway on insulin resistance in polycystic ovarian syndrome-induced rats. For the induction of PCOS in rats, 1 mg/kg of letrozole was given orally for 21 days, after that metformin (300 mg/kg), *Gymnema sylvestre* leaf extract (200 mg/kg, and 400 mg/kg *p. o.*) were administered for further 28 days. Letrozole-induced PCOS rats illustrated significant estrus irregularity, and sex hormonal abnormality, and developed insulin resistance as indicated by increased fasting glucose levels, an increased rate of glucose clearance, and also decreases in GLUT4 and AMPK mRNA expression in ovarian cells. Ovarian histology in PCOS rats also showed many follicular cysts, atretic follicles, and the absence of the corpus luteum. These changes were significantly reversed by the treatment of *G. sylvestre* in a dose-dependent manner. It might be mediated through its insulin resistance modulating property by the activation of AMPK & GLUT4 expression on ovarian endometrial cells.

Keywords: *Gymnema sylvestre*, Insulin Resistance, Letrozole, Metformin, Polycystic Ovary Syndrome

1. Introduction

Polycystic Ovarian Syndrome (PCOS) is the foremost common endocrinal illness with the major source of infertility in reproductive-age women, affecting approximately a maximum of 26% globally[1]. This disease is considered to be heterogeneous and varies from oligo-or amenorrhea, chronic anovulation, cystic follicle in the ovary, and hyperandrogenism to insulin resistance and obesity[2,3]. The key pathophysiological factors of PCOS are hyperandrogenism, insulin resistance, and altered folliculogenesis. Insulin Resistance (IR) with compensatory hyperinsulinemia impact ovarian function in PCOS patients, resulting in increased androgen production and anovulation[4].

Metformin is the most often prescribed insulin sensitizer for type 2 diabetes and PCOS around the world[5]. It is used to treat IR and hyperglycemia in PCOS women, also normalizes menstrual irregularity, enhances ovulation, and lower excessive androgen[6]. Hot flushes, bloating, mood changes, joint pain, and arthritis are common side effects associated with metformin medication. Due to these constraints, innovative therapeutic approaches for PCOS are needed that have fewer side effects, are readily available, and have a broad spectrum of efficacy[7].

Gymnema sylvestre (Family: Apocynaceae) is a well-known strong antidiabetic plant utilized in Ayurveda, Siddha, Unani, and even in the modern system of medicine in India. After chewing fresh leaves of *Gymnema sylvestre*

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(*G. sylvestre*) leaves, the person cannot detect the sweet taste. Numerous human and animal model studies proved its wide range of anti-diabetic properties in both type-I and type-II diabetes melitus[8]. Antipyretic, antioxidant, stomachic, digestive, astringent, laxative, liver, cardiac, and uterine tonic are some of the other reported activities. *Gymnema sylvestre* extract is also a nutritional supplement that helps with weight loss, cholesterol, and triglyceride reduction. The secondary metabolites triterpene saponins, gymnemic acids, acidic glycosides, and anthraquinones are primarily responsible for their claimed therapeutic effects[9,10]. Besides all *G. sylvestre* is clinically preferred as a diabetes medication, because of its insulin-modulating properties like increasing insulin secretion, and improving glucose uptake through the insulin-mediated pathway by increasing insulin sensitivity[10,11]. Hence our study aimed to assess the action of *G. sylvestre* leaves extract on the AMPK-GLUT4 mediated signalling pathway on insulin resistance in PCOS rats.

2. Materials and Methods

2.1 Chemicals and Reagents

The analytical grade chemicals were used in this study. The marketed samples of letrozole were purchased from Wynclark Pharmaceuticals Pvt. Ltd., India, and metformin from USV Private Limited, India. CMC (Carboxymethyl cellulose sodium) was supplied by HiMedia in India. Biochemical analysis kits were from Thermo Fishers Scientifics, India, and hormonal immunoassay kits were from Roche Diagnostics, India.

2.2 Collection, Authentication, and Preparation of Plant Material

The *G. sylvestre* leaves were collected from Siddha medicinal plants garden, Mettur, Tamil Nadu, India in February 2021. The specimen was authenticated by Dr P. Radha, Research officer (Botany), Siddha medicinal plants garden (CCRS, Govt. of India), Mettur Dam, Tamil Nadu, India (Specimen reference no. G180221012S). It was washed with tap water to remove sand and dust. Then it was dried, powdered, and pass through sieve number 40 to get uniform powder for extraction.

2.3 Preparation of Extract

Ethanol was used as a solvent for extraction of *G. sylvestre* leaves, as previously reported studies proved that ethanolic extract of *G. sylvestre* showed considerable anti-diabetic activity in both the insulin-dependent and non-dependent diabetes meiltus[12,13]. It was mainly due to the presence of key phytoconstituents gymnemic acid, triterpene saponins, and alkaloids in ethanolic extract[14]. Hence in our study, the powdered leaves of *G. sylvestre* (150 gm) were extracted in Soxhlet's apparatus using 95% ethanol as solvent at 75°C for 16 hrs. After filtration, the extract was evaporated in a rotary evaporator under decreased pressure and at a regulated temperature (40°C) (yield=10.13%). For later use, the extract was reserved at 4°C.

2.4 Animals

Thirty inbreed virgin Wistar female rats; aged 12 weeks and weighing around 180-230 g were procured from the central animal house facility of TANUVAS, Chennai, India. Rats were acclimatized and housed a week before the experiment in the sterilized polypropylene cages at standard temperature (23 ± 2°C), and relative humidity (60 ± 10 %), with 12 hr light and dark cycle. The regular pellet diet and free access to water were provided periodically to the rats. The Institutional Animal Ethics Committee (IAEC) of Swamy Vivekanandha College of Pharmacy, Namakkal, Tamil Nadu, India, gave their approval to the study protocol (Approval no.: SVCP/IAEC/PG/1/03/2017). The rats had two consecutive estrus cycles that lasted for four to five days each and were included in this study.

2.5 Acute Oral Toxicity Study

The acute oral toxicity of *G. sylvestre* ethanolic leaf extract was assessed using the OECD-423 guideline15. Three female Albino Wistar rats were used for the acute oral toxicity study. Animals were overnight fasted before extract administration and provided water *ad libitum*. A single dose of 2000mg/kg extract was administered through gastric intubation. After administration, all the rats were individually observed for toxicological changes, mortality, and morphological, physiological, and behavioural changes, continuously for the first four

hours, sporadically in the first 24 hrs, and once a day for the next 14 days. If two out of three animals died, the dose was classified as hazardous. If one animal died, the test was repeated to ensure the toxic dose. If there are no toxic events or mortality, 1/10 of the maximum acute toxicity dose was used as a low dose, and 1/5th was selected as a higher dose for further pharmacological evaluation.

2.6 Experimental Design and Induction of PCOS

Selected thirty rats with regular estrus cycles were erratically divided into 5 groups each of six rats. Group I was designated as normal control treated with 1 ml of carboxymethyl cellulose (CMC) 0.5% orally; groups II-V were served as PCOS groups received 1 mg/kg of letrozole in 0.5% of CMC for twenty-one days to induction of PCOS[16]. After induction of PCOS, Group II act as PCOS control and received normal saline, Group III was treated with metformin 300 mg/kg orally, Group IV and V received ethanolic leaves extract of *G. sylvestre* 200 mg/kg and 400 mg/kg correspondingly through oral for 28 days. Polycystic ovarian syndrome induction in rats was confirmed by investigating estrus irregularity and evaluating the development of insulin resistance by oral glucose challenge test after 21 days of letrozole treatment.

2.7 Vaginal Smear Observation for the Evaluation of Estrus Cycle

Estrus regularity was evaluated by the vaginal smear technique from day 14 to the end of the study. The vaginal smear was prepared by collecting the vaginal fluid of all the animals with the help of a pipette containing 10 µl of normal saline, in the morning between 8.00 to 9.00 AM. The 5% methylene blue aqueous solution was used as a staining agent for the prepared smear. Under the light microscopic observation of stained smears, the different stages of the estrus cycle were determined based on the morphology of the cells[17].

2.8 Oral Glucose Tolerance Test

The Oral Glucose Tolerance Test (OGTT) was carried out on day 21 of the study after induction of PCOS and on day 50 of the study following the post-drug treatments. Glycemia was assessed by tail vein sampling using Roche diagnostics Accu Check Active glucometer after the animals had fasted for 12 hr. Blood glucose levels was assessed before (baseline) and after the single oral glucose (2 gm/kg) challenge at 30, 60, and 120 minutes[18].

2.9 Serum Lipid Profile and Sex Hormonal Analysis

Blood was obtained from the retro-orbital plexus 28 days after drug treatments under anaesthesia. The serum was separated and used to assess lipid profiles such as VLDL, LDL, HDL, and triglycerides using standard auto analyzer techniques, as directed by the manufacturer's instructions (Thermo Fisher Scientific, India). An enzyme-linked immunosorbent assay approach was applied to quantify serum testosterone, progesterone, estradiol, LH, and FSH according to the manufacturer's instruction (Roche diagnostics, India)[19,20].

2.10 Ovarian Tissue Collection and Histopathological Examination

The rats were sacrificed by an overdose of anaesthesia immediately after blood samples were collected. Both the ovary from each rat was isolated and cleaned with normal saline. The left ovary from each rat was separated, frozen quickly, and stored at –80°C for GLUT4 (glucose transporter-4) and AMPK (5'AMP-activated protein kinase) estimation. In the 10%formal saline, the right ovary was fixed and embedded in paraffin wax for histopathology analysis. The prepared blocks were sectioned at 5-micron thickness. Hematoxylin and eosin were used to stain the sections, which were then examined under a light microscope at 100 X magnification for histological alterations[20].

2.11 Quantitative Real-Time PCR (qRT-PCR) Analysis of GLUT4 and AMPK

The ovarian GLUT4 and AMPK were measured by quantitative real-time polymerase chain reaction analysis. The sequence detection system used was a Roche Light Cycler 480 (Roche Diagnostics Ltd., India). The ovarian total RNA was isolated using an

RNA extraction kit (#K0731, 0732) as per manufacturer instructions. Nanodrop spectrophotometry was used to determine the concentration and purity of the isolated RNA (Quawell 5000, USA). RNA reverse transcript cDNA synthesis kits (#K1622) was used to make the cDNA. The thermal profile of reverse transcription was set at 25°C for 10 minutes, 420C for 120 minutes, and 95°C for 5 minutes. The undiluted 1 µl of cDNA was added to a reaction mixture containing 10 µl of MAXIMA SYBR® master mix (#K0251), plus 5 µl of specific gene reverse and forward primer (Table 1). The relative expression of the β-action housekeeping gene was used to compute the fold expression of the GLUT4 and AMPK genes. The comparative expressions were determined using the 2(-Delta Delta C (T)) technique. All of the reactions were carried out in triplicate.

Table 1. Primer sequences used for gene expression analysis by quantitative PCR

Gene	Forward primer	Reverse primer
GLUT4	TCATCTTCACCTTCCTAA	CCTCAGTCATTCTCATCT
AMPK	ATCCGCAGAGAGATCCA GAA	CGTCGACTCTCCTTTTC GTC
β-actin	CAGGGTGTGATGGTGG GTATGG	AGTTGGTGACAATGCCG TGTTC

AMPK: 5'AMP-activated protein kinase, GLUT4: Glucose transporter 4

2.12 Statistical Analysis

To establish statistical significance, data were reported as mean ± standard deviation (mean ± SD). For the uniformity of variance in OGTT and estrus changes on day 21, an independent two-sample t-test was used. One-way ANOVA was used to compare rats over time or within groups, followed by post hoc Dunnett's multiple comparison test using SPSS V.17. The level of P<0.05 was judged as statistically significant.

3. Results

3.1 Effect of Ethanolic Extract of *G. sylvestre* on Acute Oral Toxicity Studies

There was no evidence of toxicity or mortality after a single 2000 mg/kg dose of ethanolic extract of *G. sylvestre*. Hence it was considered safe and the lethal dose of 50 (LD50) was found to be above 2000 mg/kg. So $1/10^{th}$ and $1/5^{th}$ (200 mg and 400 mg respectively) of the LD50 dose were selected as submaximal and maximal doses for further pharmacological screening.

3.2 Effect of Treatments on Estrus Cycle in PCOS Rats

The PCOS groups showed estrus irregularity 21 days after letrozole treatment as compared to normal rats. The diestrus duration was significantly (P<0.001) longer and the estrus stage was significantly (P<0.01) shorter in the PCOS group when compared to the normal control group. As shown in Figure 1 (A). The prolonged diestrus stage was significantly (P<0.001) reverted to normal following 28 days of drug treatment when compared to the PCOS control rats. The drug treatments did not differ in any meaningful ways (Figure 1(B)).

3.3 Effect of Treatments on Oral Glucose Tolerance Test in PCOS Rats

In the letrozole-treated PCOS rats, Fasting Blood Glucose (FBG) was significantly (P<0.001) higher, and glucose clearance after an oral glucose challenge was significantly delayed than the normal control rats (Figure 2(A)). Post-drug administration the PCOS rats demonstrated a significant (P<0.001) reduction in FBG level and improved glucose clearance when compared to the PCOS control group rats (Figure 2(B)).

3.4 Effect of Treatments on Lipid Profiles in PCOS Rats

The data in Table 2 represents a significant (P<0.001) increase in VLDL, LDL cholesterol, and triglycerides serum levels and a significant (P<0.001) reduction of HDL level in the PCOS control than the normal control. These altered lipid profiles were significantly (P<0.001) improved by the drug treatments as compared to the PCOS control. *G. sylvestre* treatments showed dose-dependent improvement in the lipid profile.

3.5 Effect of Treatments on Hormonal Profiles in PCOS Rats

The PCOS control group demonstrated a significant (P<0.001) elevation of serum testosterone, luteinizing

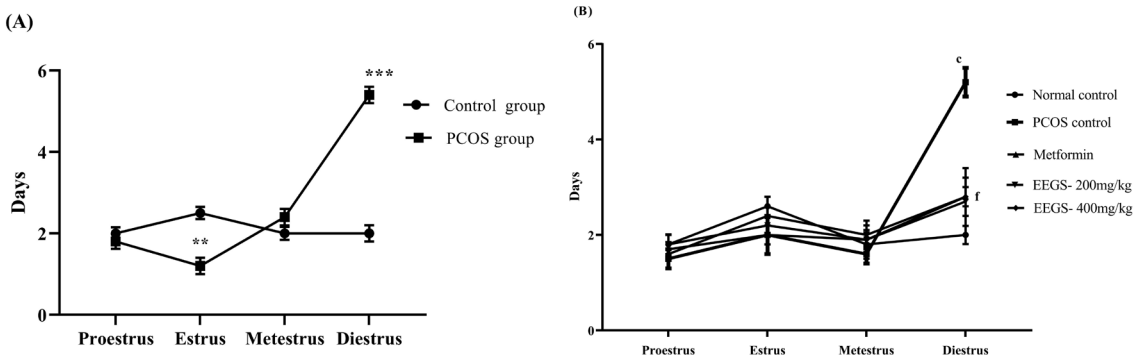

Figure 1. Effect of ethanolic extract of *G. sylvestre* on estrus cycle in letrozole induced PCOS rats. **(A)** Estrus cycle on day 22 data was analyzed by sample t-test, symbols represent statistical significance **P<0.01, ***P<0.001 vs. normal control. **(B)** Estrus cycle on day 50 data were analyzed by one-way ANOVA followed by post hoc Dunnett's test. Statistical significance is represented as [a]P<0.05; [b]P<0.01; [c]P<0.001 vs. Group I. [d]P<0.05; [e]P<0.01; [f]P<0.001 vs. Group II.

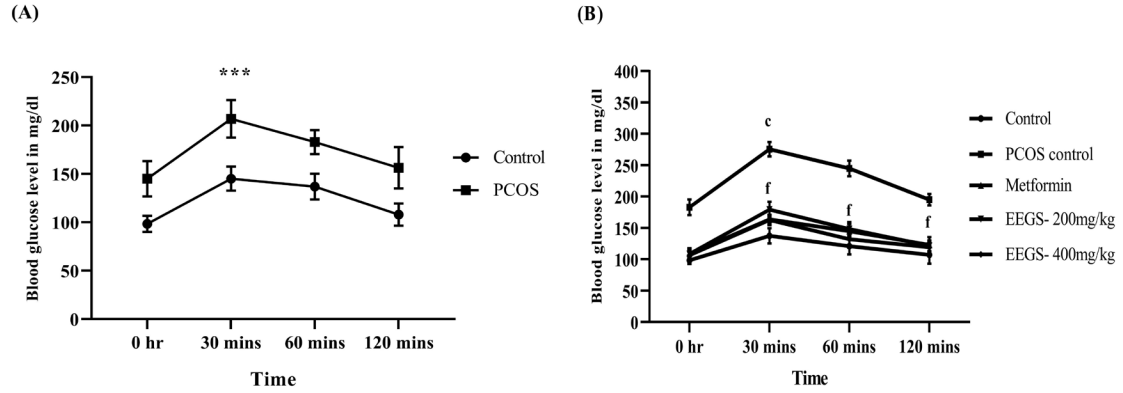

Figure 2. Effect of Ethanolic Extract of *G. sylvestre* on Oral Glucose Tolerance Test (OGTT) in Letrozole Induced PCOS Rats. **(A)** OGTT on day 22 data was analyzed by sample t-test, symbols represent statistical significance ***P<0.001 vs. normal control. **(B)** OGTT on day 50 data were analyzed by one-way ANOVA followed by post hoc Dunnett's test. Statistical significance is represented as [a]P<0.05; [b]P<0.01; [c]P<0.001 vs. Group I. [d]P<0.05; [e]P<0.01; [f]P<0.001 vs. Group II.

hormone, and decreased estradiol, progesterone, and follicle-stimulating hormone levels than the normal control rats. All the treatments produced a significant (P<0.001) reversal effect on altered serum sex hormones abnormality produced by the letrozole-induced PCOS condition. The treatment of *G. sylvestre* showed a dose-dependent effect on serum sex hormone levels (Table 3).

3.6 Effect of Treatments on Ovarian Histology

Control rats showed the normal structure of the ovary like the presence of various stages of follicles including primary, growing, and, mature follicles, and corpus luteum (Figure 3(A)). In comparison to normal control, letrozole-induced PCOS rats showed altered histoarchitecture of the ovary such as the absence of corpus luteum and increased cystic follicles and undeveloped follicles (Figure 3(B)). The metformin treatment illustrated the signs of improvement as a decreased number of follicular cysts, numerous different stages of follicles, and the existence of the corpus luteum (Figure 3(C)). The treatment of *G. sylvestre* extract also showed few follicular cysts, various stages of follicles, and the presence of the corpus luteum (Figure 3 (D and E). The efficacy of *G. sylvestre* was more evident at the dose of 400 mg/kg.

Table 2. Effect of ethanolic extract of *G. sylvestre* on lipid profile in PCOS rats

Treatment	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TG (mg/dl)
Group-I (0.5% CMC)	45.36 ± 5.32	88.53 ± 9.74	44.72 ± 8.44	75.00 ± 8.71
Group-II (Letrozole)	17.42 ± 3.71[c]	142.41 ± 14.76[c]	92.43 ± 7.73[c]	179.21 ± 17.66[c]
Group-III (Letrozole + Metformin)	28.11 ± 5.64[bd]	109.18 ± 12.19[af]	52.25 ± 9.33[f]	111.80 ± 11.45[cf]
Group-IV (Letrozole + EEGS 200mg/kg)	34.06 ± 6.24[f]	99.84 ± 15.61[f]	54.61 ± 3.15[f]	87.21 ± 5.06[f]
Group-V (Letrozole + EEGS 400mg/kg)	43.18 ± 3.49[f]	92.37 ± 8.46[f]	50.20 ± 6.00[f]	74.22 ± 5.22[f]

Data are expressed as mean ± SD, n=6 animals. Statistical significance represent as:

[a]P<0.05; [b]P<0.01; [c]P<0.001 vs. Group I.

[d]P<0.05; [e]P<0.01; [f]P<0.001 vs. Group II.

Data were analyzed by one-way ANOVA followed by post hoc Dunnett's test

Table 3. Effect of ethanolic extract of *G. sylvestre* on hormonal profile in PCOS rats

Treatment	Testosterone (ng/dl)	Estradiol (pg/ml)	Progesterone (ng/dl)	LH (ng/ml)	FSH (ng/ml)
Group-I (0.5% CMC)	69.20 ± 8.90	68.33 ± 8.17	42.40 ± 4.31	2.09 ± 0.54	84.23 ± 8.91
Group-II (Letrozole)	109.40 ± 10.43[c]	34.60 ± 8.22[c]	14.64 ± 8.54[c]	9.08 ± 0.46[c]	41.70 ± 6.32[c]
Group-III (Letrozole + Metformin)	84.80 ± 9.78[af]	47.40 ± 3.42[cf]	31.18 ± 7.21[cf]	3.46 ± 0.53[bf]	75.33 ± 5.50[f]
Group-IV (Letrozole + EEGS 200 mg/kg)	79.30 ± 6.33[f]	59.54 ± 4.75[f]	35.22 ± 3.98[f]	3.02 ± 0.64[f]	72.81 ± 5.20[f]
Group-V (Letrozole + EEGS 400 mg/kg)	72.40± 8.52[f]	62.32 ± 1.98[f]	39.36 ± 4.69[f]	2.54 ± 0.39[f]	77.31 ± 6.77[f]

Data are expressed as mean ± SD, n=6 animals. Statistical significance represent as:

[a]P<0.05; [b]P<0.01; [c]P<0.001 vs. Group I.

[d]P<0.05; [e]P<0.01; [f]P<0.001 vs. Group II.

Data were analyzed by one-way ANOVA followed by post hoc Dunnett's test

3.7 Effect of Treatments on GLUT4 and AMPK Expression in Ovarian Tissues

Letrozole-induced PCOS rats showed a significantly (P<0.001) low fold expression of ovarian GLUT4 and AMPK than the normal rats. In comparison with the PCOS control, the drugs treated rats significantly improve the ovarian GLUT4 and AMPK expressions. The treatment groups did not differ significantly, but numerically the *G. sylvestre* showed better fold expression among the treatment groups (Figure 4 (A and B)).

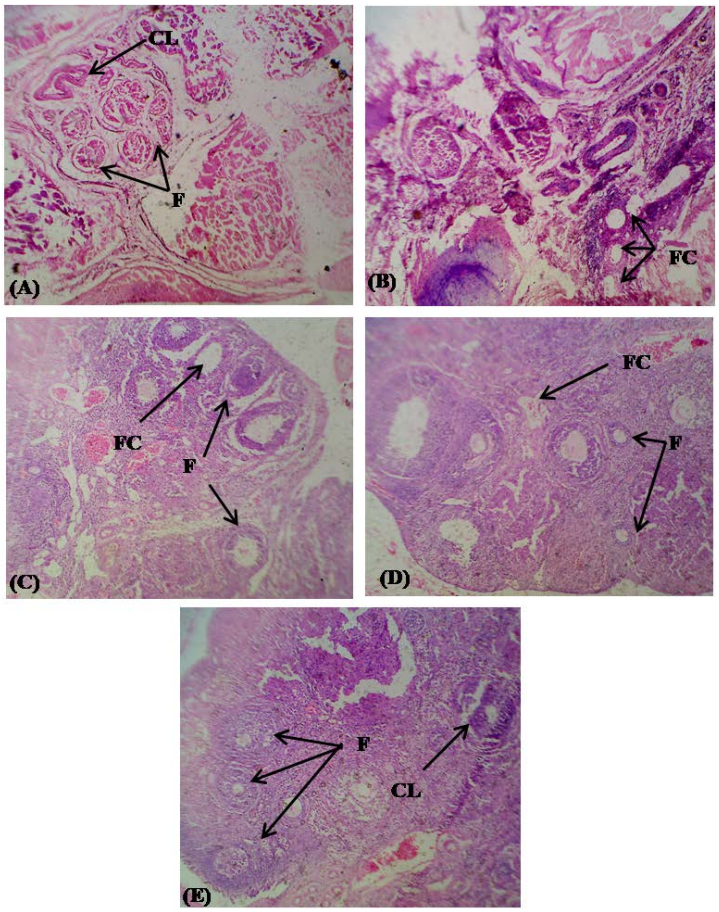

Figure 3. Histopathological photo microscopic images of ovary sections (100x). **(A)** Normal control. **(B)** PCOS control group. **(C)** Letrozole + Metformin **(D)** Letrozole + *G. Sylvestre* 200mg/kg. **(E)** Letrozole + *G. Sylvestre* 400mg/kg. FC: Follicular cyst; CL: corpus luteum; F: follicles.

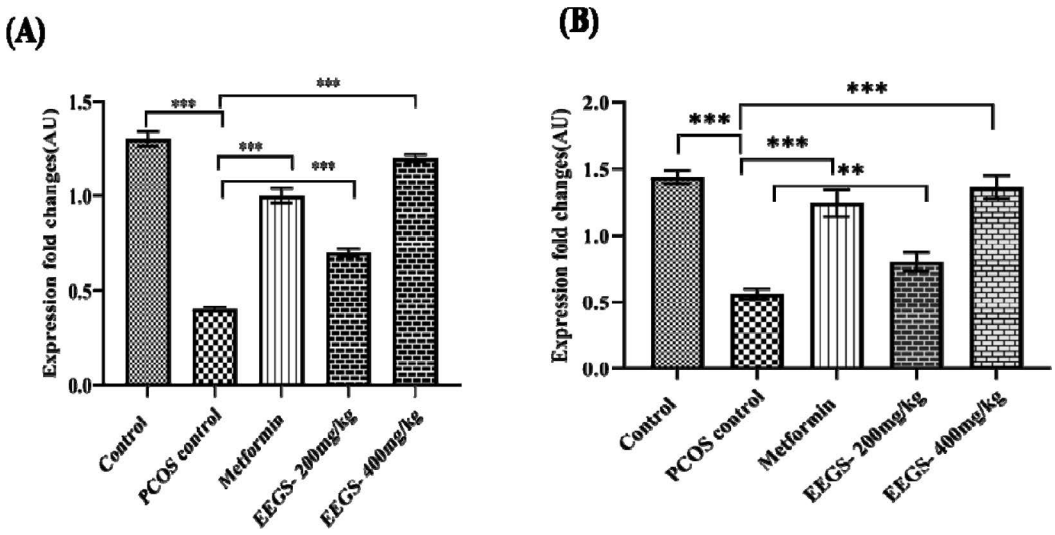

Figure 4. Effect of Ethanolic Extract of *G. sylvestre* on the fold expression level of **(A)** AMPK. **(B)** GLUT4 in Letrozole Induced PCOS Rats, symbols represent statistical significance *P<0.05, **P<0.01, ***P< 0.001 vs. PCOS control.

4. Discussion

The polycystic ovarian syndrome is a common reproductive disorder of females, characterized by the major consequence of hyperandrogenemia, hyperinsulinemia, and frequent anovulatory cycle[21]. Impaired glucose tolerance and hyperinsulinemia are present in 70–80 % of PCOS women[22]. Hyperinsulinemia, a possible factor that increases testosterone production in the ovary while lowering serum sex hormone-binding globulin, causes follicular development and ovulation to be impeded[23]. PCOS in rats was induced by 21 days of consecutive administration of 1mg/kg of letrozole. Inhibiting the aromatase enzyme letrozole prevents the conversion of androgen to estrogen resulting in excess accumulation of androgen in the ovary. The increased androgen level in the ovary arrests follicular growth and leads to anovulation and menstrual irregularity[24]. The results of our study also confirm the estrus irregularity, hyperandrogenism, hyperinsulinemia, and abnormal follicular development in the letrozole in PCOS rats. Hence it indicates the successful induction of PCOS in rats with promising PCOS clinical features.

The PCOS induction in this study was confirmed by measuring the estrus cycle irregularity and insulin resistance development 21 days after letrozole treatment. The estrus irregularity in the PCOS rats is due to the steroidal sex hormonal imbalance, intrauterine androgen-induced uterine changes, and circulatory hyperandrogenism[21]. This study's results also showed that PCOS rats had significantly higher blood testosterone, LH, and lower serum estradiol, progesterone, and FSH levels as compared to the control rats. These hormonal abnormalities induced by letrozole in PCOS condition confirm the previous results[25,26]. The treatment of *G. sylvestre* significantly normalizes the estrus cycle irregularity in PCOS rats by normalizing the hormonal abnormality induced by letrozole. This impact could be attributed to its ability to help PCOS patients normalize their sex hormone imbalance.

The development of insulin resistance is one of the key risk factors for PCOS[27]. This study's results also indicate a substantial increase in FBG levels together with impaired glucose clearance in PCOS rats. Furthermore, the considerable reduction in GLUT4 and AMPK expression in PCOS rats verifies the development of insulin resistance as compared to the normal control. By lowering FBG levels and boosting glucose clearance rate, all of the treatments decreased insulin resistance significantly and increase GLUT4 and AMPK expression in ovarian cells as well. Hence the findings of this study suggested that *G. sylvestre* therapy successfully lowers insulin resistance in PCOS via activating the AMPK and GLUT4-mediated glucose uptake pathway in the ovary.

The polycystic ovarian syndrome is commonly associated with dyslipidemia like increased serum LDL, VLDL cholesterol, and triglyceride levels, and also decreased HDL cholesterol levels[28]. The results of this study also confirm dyslipidemia associated with PCOS rats. These altered lipid profiles in the letrozole-induced PCOS condition were significantly reversed by all the treatment groups. The treatment of *G. sylvestre* showed superior dose-dependent effects on improving dyslipidemia as compared with metformin treatment. These results indicate that the treatment of *G. sylvestre* extract also has an effective impact on the metabolic complication of POCS.

Histopathological examinations of the ovary further confirm the development of PCOS in the letrozole-treated groups. Such as an increased number of follicular cysts and lack of corpus luteum indicates anovulation in PCOS rats compared to normal rats. These results are similar to the previous findings[29,30]. The metformin and *Gymnema sylvestre* treatments showed better improvement in the histology of the PCOS rat ovaries, indicated by the presence of different phases of multiple developing follicles with corpus luteum. It confirms the ovulation and estrus regularity of treatment groups. Among the treatments, *G. sylvestre* 400mg/kg treatment showed superior results of almost normal histology of the ovary with different stages of developing follicles, and an increase in the number of corpus luteum than the *G. sylvestre* 200 mg/kg and metformin-treated groups. These results proved that treatment of *G. sylvestre* extracts significantly improves ovulation by correcting the estrus irregularity in PCOS conditions.

Gymnema sylvestre is one of the familiar medications for hyperglycemia and also an insulin sensitivity-improving agent listed in the Indian pharmaceutical codex[31]. Hence the *G. sylvestre* leaves extract was selected to evaluate its impact on AMPK-GLUT4 mediated insulin Resistance in a PCOS Rat. The *G. sylvestre* extract treatment improves the menstrual irregularity of PCOS by correcting the hormonal irregularity in PCOS such as increasing the serum estradiol, progesterone, and FSH level and also

decreasing the testosterone and LH. Also correcting ovarian histoarchitecture like improved folliculogenesis and reduced follicular cysts induces ovulation and corrects menstrual irregularity. The treatment of *G. sylvestre* improves insulin sensitivity by decreasing fasting blood glucose levels and improving glucose clearance via the activation of AMPK and GLUT4-mediated ovarian pathways. When AMPK is activated in the ovary, GLUT4 is translocated from the ovarian cytoplasm to the plasma membrane, this promotes insulin sensitivity, and glucose utilization and reduces hyperinsulinemia[32]. The activation of AMPK and GLUT4 in the ovary promotes follicular growth and reduces androgen levels by altering hyperinsulinemia.

Metformin-treated groups also showed significant improvement in reproductive parameters like estrus irregularity, a hormonal abnormality, and metabolic abnormalities like insulin resistance, and hyperlipidemia in PCOS rats. The results of our study also confirm the previous study report on the beneficial effect of metformin on PCOS[32,33]. In comparison between the treatment groups, *G. sylvestre* treated groups showed a dose-dependent better effect as compared to the metformin treatment. Even though the *G. sylvestre* 400 mg/kg treatment almost completely normalize the histoarchitecture of letrozole-induced PCOS rat ovaries nearer to normal control, the metformin-treated groups showed few follicular cysts and less developed follicles.

5. Conclusion

The current study intended to investigate the effect of *Gymnema sylvestre* leaves extract on insulin resistance-mediated polycystic ovarian syndrome complications in rats through the regulation of AMPK and GLUT4-mediated insulin signalling pathways. According to the findings, *G. sylvestre* extract administration improved both reproductive and metabolic complications in letrozole-induced PCOS rats. *Gymnema sylvestre* at a 400 mg/kg dose level showed a superior beneficial effect on PCOS rats by restoring the sex hormones to the normal level, normalizing hyperlipidemia, improving insulin sensitivity, and restoring folliculogenesis to promote ovulation as confirmed by the histological study of the ovary and vaginal epithelial cells examination. These effects of *Gymnema sylvestre* on PCOS are mainly mediated by improving insulin sensitivity through activation of the AMPK-mediated GLUT4 pathway in the ovary. Hence the ethanolic leaf extract of *Gymnema sylvestre* might be considered an alternative insulin sensitivity-improving agent in both reproductive and metabolic complications associated with PCOS.

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