Keywords
Polyendocrine Metabolic Ovarian Syndrome; Luffa cylindrica; Letrozole; Network Pharmacology; Molecular Docking; In Vivo; PMOS; Phytotherapy

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Therapeutic Evaluation of Hydroalcoholic Extract of Luffa cylindrica in Letrozole Induced Polyendocrine Metabolic Ovarian Syndrome (PMOS): An Integrated In Silico, In Vitro and In Vivo Approach

Abstract

Purpose: Polyendocrine Metabolic Ovarian Syndrome (PMOS), recently renamed from Polycystic Ovary Syndrome (PCOS) through international consensus, is a multisystem endocrine and metabolic disorder affecting an estimated 170 million women globally. Current pharmacological treatments carry significant adverse effects, necessitating exploration of safer, plantbased therapeutic alternatives. This study aimed to evaluate the therapeutic efficacy of a hydroalcoholic extract of Luffa cylindrica (LCE) against PMOS through an integrated in silico, in vitro, and in vivo approach.

Methods: Active phytochemicals of LCE were identified by liquid chromatography–mass spectrometry (LCMS). Network pharmacology and molecular docking studies using Schrödinger Glide were performed to identify PMOSrelevant molecular targets and key signalling pathways. In vitro enzyme inhibition assays evaluated alphaamylase and alphaglucosidase inhibitory activity. For in vivo assessment, a letrozoleinduced PMOS rat model was established, and female Wistar rats were treated orally for 14 days with metformin (20 mg/kg) as a positive control or LCE at doses of 100, 200, and 400 mg/kg following 21 days of letrozole induction.

Results: LCMS profiling identified key phytochemicals including Rutin, Luteolin, Apigenin, Quercetin, and Neodiosmin. In vitro studies demonstrated potent inhibitory activity against alphaamylase (IC_{50}: 80.45 µg/mL) and alphaglucosidase (IC_{50}: 56.58 µg/mL), indicating significant postprandial glycaemic regulation potential. Network pharmacology identified 46 PMOSrelevant molecular targets across 16 key signalling pathways, including PI3KAkt, Estrogen, Ras, and MAPK cascades. Molecular docking revealed significantly superior binding affinities of LCE phytocompounds over metformin at EGFR, ESR2, and IGF1R. In vivo, LCE produced dosedependent restoration of estrous cyclicity, hormonal balance, lipid profiles, fasting blood glucose levels, antioxidant status, and ovarian histopathological architecture

Conclusion: LCE demonstrates promising multitarget therapeutic potential for PMOS management, modulating key metabolic, hormonal, and ovarian pathways. These findings support Luffa cylindrica as a rational phytotherapeutic candidate warranting further clinical investigation.

INTRODUCTION

Polyendocrine Metabolic Ovarian Syndrome (PMOS) officially endorsed in 2026 as the updated nomenclature for Polycystic Ovary Syndrome (PCOS) following a global consensus process [1] is one of the most prevalent endocrinemetabolic disorders among women of reproductive age. It is characterised by a constellation of features including ovarian cysts, chronic anovulation, hyperandrogenism, and significant hormonal imbalances that dysregulate the hypothalamic pituitary–ovarian (HPO) axis [3,10]. Imbalances among luteinising hormone (LH), folliclestimulating hormone (FSH), estrogen, and testosterone impair menstrual regularity and induce a spectrum of metabolic perturbations, most notably insulin resistance [2,4,47,64].

Epidemiologically, PMOS is estimated to affect between 4% and 10% of women of reproductive age worldwide, with the National Institutes of Health (NIH) diagnostic criteria being the most restrictive [1,54]. In India, a median prevalence of 15.3% has been reported, placing the country among the highestburden regions globally [2,58]. The disorder is not merely a reproductive condition; it intersects significantly with metabolic syndrome, type 2 diabetes mellitus, cardiovascular risk, and psychological comorbidities including depression and anxiety [50,57,62].

Current pharmacological therapies including combined oral contraceptives (COCs), metformin, and clomiphene citrate provide symptomatic relief but are associated with substantial adverse effects such as gastrointestinal disturbances, lactic acidosis, thromboembolic events, and renal compromise, limiting their longterm tolerability [6,15,16,49]. These limitations have renewed interest in plantderived therapeutics with multitarget mechanisms and improved safety profiles [3,9].

Luffa cylindrica (sponge gourd, Cucurbitaceae), a subtropical climbing vegetable cultivated throughout Asia, Africa, and the Americas, possesses a rich phytochemical repertoire including flavonoids, saponins, triterpenoids, cucurbitacins, and phenolic acids [7,8,9]. Several of these constituents have demonstrated antiobesity, estrogenic, antiinflammatory, and antidiabetic activities in preclinical models, making Luffa cylindrica a biologically rational candidate for PMOS management [7,23,24,34].

The present study therefore adopted a translational, multiplatform approach combining network pharmacology, molecular docking, in vitro enzyme inhibition, and a wellvalidated letrozoleinduced rodent model of PMOS to systematically characterise the therapeutic potential of hydroalcoholic LCE. This integrative methodology enables simultaneous interrogation of molecular targets, pathway modulations, and wholeorganism efficacy, providing robust evidence for the phytotherapeutic utility of Luffa cylindrica in PMOS/PMOS.

Epidemiology And Pathogenesis Of Pmos

PMOS was first clinically described by Stein and Leventhal in 1935, who documented cases of women presenting with oligo/amenorrhoea and bilateral polycystic ovaries [13]. Since this landmark description, understanding of the syndrome has evolved considerably, with modern classification acknowledging its heterogeneous phenotypic expression spanning four major subtypes as defined by the Rotterdam 2003 consensus [18,54]. Globally, approximately 116 million women were estimated to be affected as of 2012, with the worldwide prevalence rising in parallel with urbanisation and obesity epidemics [2,43,44,58].

The aetio pathogenesis of PMOS is multifactorial and involves a complex interplay of genetic predisposition, environmental exposures, neuroendocrine dysfunction, and metabolic dysregulation [10,45,53]. Central to its pathophysiology is elevated androgen production, primarily from ovarian theca cells, stimulated by abnormally elevated LH pulses originating from dysregulated GnRH secretion [46]. Hyperandrogenemia exerts hepatic and systemic insulin resistance, while compensatory hyperinsulinaemia further amplifies androgen synthesis by directly stimulating ovarian steroidogenesis and suppressing hepatic sex hormonebinding globulin (SHBG) production, establishing a selfperpetuating vicious cycle [11,47,64].

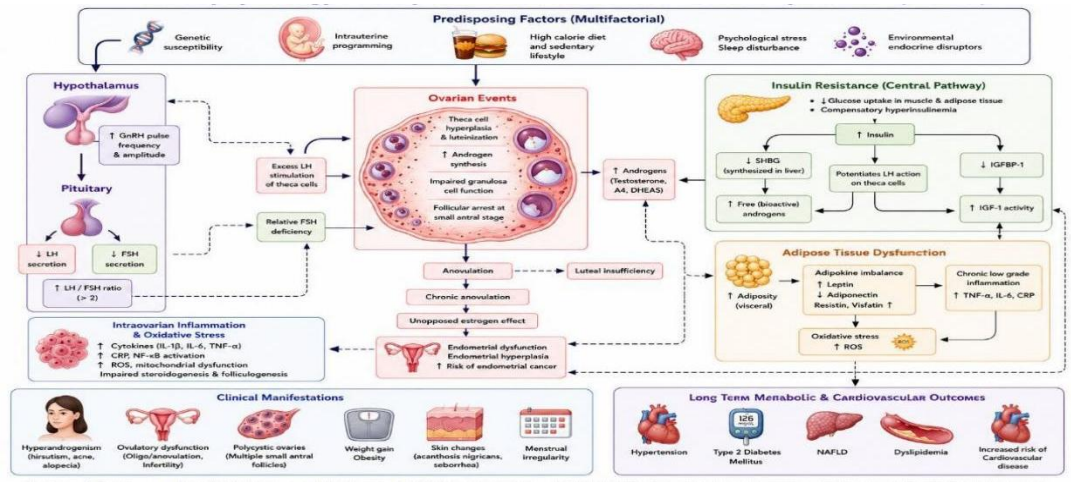


Figure 1: Pathophysiology of Polyendocrine Metabolic Ovarian Syndrome (PMOS)[11]

Anti Müllerian hormone (AMH), produced by granulosa cells of small antral follicles, is found at two to three times elevated levels in PMOS women relative to unaffected controls, serving both as a biomarker and a pathophysiological contributor by suppressing FSH mediated follicular maturation [12]. The resulting arrested follicular development manifests as polycystic ovarian morphology (PCOM) on ultrasound [18,54]. Adipose tissue dysfunction, chronic low grade inflammation, and gut microbiota dysbiosis further perpetuate insulin resistance and amplify systemic androgen excess [5,51,52]. Genetic susceptibility loci include CAPN10, CYP450 genes, the insulin gene, androgen receptor (AR), FTO, and FSHR polymorphisms [45,53]. Endocrine disrupting chemicals (EDCs) represent a growing environmental contributor [10,53].

Diagnostic Criteria

No single gold standard diagnostic criterion exists for PMOS; three major frameworks are currently used in clinical practice [14,17,18,42]

Table 1. Diagnostic criteria for PMOS [14,17,18,42].

Diagnostic Criteria	Key Features
NIH Criteria (1990)	Hyperandrogenism; Oligoovulation/anovulation; Exclusion of other NICHDrelated disorders [14]
Rotterdam Criteria (2003)	Hyperandrogenism; Oligoovulation/anovulation; Polycystic ovarian morphology 2 of 3 features required [18]
AES Criteria (2012)	Hyperandrogenism (clinical/biochemical); Oligo/anovulation OR polycystic ovarian morphology; Exclusion of other disorders [42]
NIH 2012 Extension	Ovarian dysfunction; Hyperandrogenism; Polycystic ovarian morphology (PCOM); recognition of four phenotypes [17]

Metabolic and Cardiovascular Consequences

Beyond reproductive dysfunction, PMOS confers heightened lifetime risk for type 2 diabetes mellitus (T2DM), metabolic syndrome, dyslipidaemia, and cardiovascular disease [57,60,61,62]. Insulin resistance occurs in 50–80% of women with PMOS, irrespective of obesity [55, 64]. Atherogenic dyslipidaemia characterised by elevated triglycerides, reduced high density lipoprotein cholesterol (HDLC), and increased low density lipoprotein (LDL) is prevalent and contributes to premature cardiovascular risk [31,62]. Psychological sequelae particularly depression and anxiety further diminish quality of life [32,50]. Adiposity, particularly central or abdominal obesity, is both a risk factor and amplifier of PMOS severity [56,59 ,63].

Plant Profile: Luffa Cylindrica

Taxonomical Classification And Botanical Description

Luffa cylindrica (L.) M. Roem. (synonym: Luffa aegyptiaca Mill.) belongs to the Kingdom Plantae, Family Cucurbitaceae, and Genus Luffa. It is a vigorous subtropical to tropical annual climbing plant commonly referred to as sponge gourd, smooth loofah, Egyptian cucumber, or dishcloth gourd. The plant is cultivated across China, Korea, India, Japan, Central America, and sub Saharan Africa, with China and India among the largest producers [7,23]. The fruits are consumed as a fresh vegetable when immature and are processed into fibrous skeletal networks used industrially as scrubbing pads and filtration matrices [9,23].

Ethnobotanically, various parts of the plant fruit, seeds, leaves, roots, and vascular bundles have been used across Asian and African traditional medicine systems to manage leprosy, intestinal parasitosis, sinusitis, chronic bronchitis, asthma, and spleenopathy [7,9,23]. These traditional uses have prompted systematic phytochemical and pharmacological investigations that collectively validate its therapeutic relevance.

Phytochemical Constituents and Pharmacological Activities

The pharmacological properties of Luffa cylindrica are attributed to a diverse array of bioactive compounds spanning multiple chemical classes [7,8,9,23,34]:

Table 2. Key phytochemicals of Luffa cylindrica and their pharmacological activities [7,8,9,23,34].

Phytochemical Class	Key Compounds	Pharmacological Actions
Flavonoids	Rutin, Luteolin, Apigenin, Quercetin, Kaempferol, Diosmin, Neodiosmin	Antioxidant, anti-inflammatory, antidiabetic, cardioprotective, neuroprotective [8,34]
Phenolic Acids	Gallic acid, Chlorogenic acid, Caffeic acid, Ferulic acid, pCoumaric acid	Antioxidant, antiviral, anti-inflammatory, anticancer, antidiabetic [7,34]
Triterpenoids	Oleanolic acid, Echinocystic acid, Maslinic acid	Immunomodulatory, antiviral, antimicrobial, hepatoprotective [9,23]
Saponins	Lucyoside K, Lucyoside B, Lucyoside N, Ginsenosides Re, Rg1	Anti-inflammatory, fibrinolytic, neuroprotective, antidepressant [7,9]
Cucurbitacins	Cucurbitacin B, Cucurbitacin E	Anticancer, immunomodulatory, anti-inflammatory, neuroprotective [9]
Ribosome Inactivating Peptides	Luffina, Luffinb, Luffin P1, Luffacyclin	Abortifacient, antitumour, antiHIV, antifungal [7,8]

MATERIALS AND METHODS

In Silico: Network Pharmacology and Molecular Docking

Active phytochemical components of Luffa cylindrica were retrieved from the IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database, applying drug likeness filters of oral bioavailability (OB) ≥30% and drug likeness (DL) ≥0.18, yielding 15 active components for analysis [21]. Protein targets for each compound were predicted using Swiss Target Prediction, and PMOS associated targets were retrieved from the Gene Cards database. The intersection of compound predicted targets and disease targets was identified using Venn diagram analysis, and a protein–protein interaction (PPI) network was constructed using STRING v11.5 [21]. Cytoscape 3.9 was employed to visualize the compound–target–pathway network. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment was performed to identify key signaling pathways [41].

For molecular docking, three dimensional crystallographic structures of key PMOS relevant therapeutic targets EGFR (PDB: 1M17), ESR2 (PDB: 2GIU), and IGF1R (PDB: 2OJ9) were obtained from the RCSB Protein Data Bank. Protein preparation, grid generation, and docking were executed using Schrödinger's Maestro suite (Glide SP/XP mode) [19,20]. Ligand structures of Quercetin, Neodiosmin, Luteolin, and Rutin were prepared using LigPrep. Metformin served as the reference ligand. Binding affinities were evaluated using Glide docking scores (kcal/mol) and Glide binding energies.

Plant Material, Extraction, and Phytochemical Analysis

Fresh specimens of Luffa cylindrica were authenticated by a certified botanist (Accession No. RMRC1803). The hydroalcoholic extract (LCE) was prepared by maceration of coarsely powdered plant material in a 70:30 (v/v) hydroalcoholic solvent for seven days at room temperature with intermittent stirring. The macerate was combined with a parallel Soxhlet extraction, and the pooled extract was concentrated under reduced pressure using a rotary evaporator at 40–45°C [26]. Preliminary phytochemical screening was conducted using standard Harborne methods [23]. Spectroscopic characterization was performed by Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC). Compound identification was accomplished by liquid chromatography–mass spectrometry (LCMS) using reverse phase C18 column chromatography [33].

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu colorimetric method with gallic acid as the standard (expressed as mg GAE/g dry extract). Total flavonoid content (TFC) was determined using the aluminium chloride ($AlCl_3$) colorimetric assay with quercetin as the standard (expressed as mg QE/g dry extract) [22,23]. Alpha amylase and alpha glucosidase inhibition assays were conducted at concentrations of 20–100 µg/mL, and IC_{50} values were calculated from nonlinear regression inhibition curves [25,39].

Animal Study Design and Ethical Compliance

All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC Reg. No: IAECI/RJSCOP/202526/03) and conducted in full compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines [26]. Forty two female Wistar rats weighing 150–200 g were acclimatized for seven days under standard housing conditions (12h light/dark cycle, 22 ± 2°C, free access to food and water). PMOS was induced by oral administration of letrozole (1 mg/kg/day dissolved in 0.5% carboxy methyl cellulose; CMC) for 21 consecutive days [35,36,37]. Animals were randomly allocated to six groups (n = 6 per group) as follows:

Table 3. Experimental study design [17,18,26].

Group	Treatment Protocol
Group 1 (Normal Control)	0.5% CMC vehicle (p.o.) for 35 days; no letrozole
Group 2 (Disease Control)	Letrozole 1 mg/kg in 0.5% CMC (p.o.) for 21 days followed by vehicle for 14 days
Group 3 (Standard)	Letrozole for 21 days → Metformin 20 mg/kg (p.o.) for 14 days
Group 4 (LCE 100)	Letrozole for 21 days → LCE 100 mg/kg (p.o.) for 14 days
Group 5 (LCE 200)	Letrozole for 21 days → LCE 200 mg/kg (p.o.) for 14 days
Group 6 (LCE 400)	Letrozole for 21 days → LCE 400 mg/kg (p.o.) for 14 days

Parameters assessed at designated time points (Days 7, 14, 21, 28, and 35) included body weight, food and water intake, fasting blood glucose (FBG), oral glucose tolerance test (OGTT), estrous cycle phase determination by vaginal cytology, serum hormonal levels (testosterone, estradiol), complete lipid profile (total cholesterol, TG, LDLC, HDLC), hepatic enzyme markers (ALT, AST, ALP), and antioxidant parameters (superoxide dismutase [SOD], reduced glutathione [GSH], malondialdehyde [MDA] as lipid peroxidation marker) [30,36,38]. At study termination (Day 35), ovaries and preovarian fat pads were excised, weighed, and processed for histopathological examination using haematoxylin and eosin (H&E) staining [28,29]. Statistical analyses were performed by one way ANOVA followed by Tukey's multiple comparison test using Graph Pad Prism v8 ($p<0.05$ considered statistically significant).

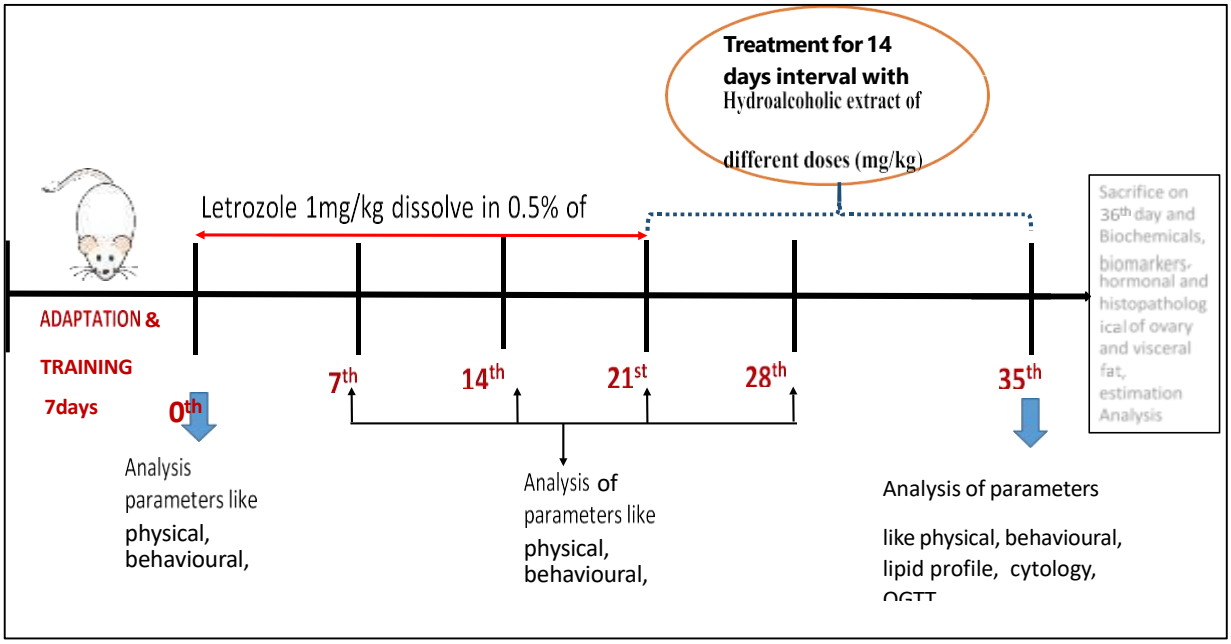


Figure 2: Designing of experimental study for PMSO using LET induced model [17,26]

RESULTS:

In Silico Results: Network Pharmacology and Molecular Docking:

Compound database screening identified 33 active phytochemicals from Luffa cylindrica meeting established drug likeness criteria [21]. Intersection analysis with Gene Cards derived PMOS targets identified 175 shared target genes, of which 46 were classified as core PMOS relevant targets after PPI network hub analysis [10,21]. KEGG enrichment analysis delineated 128 signaling pathways; 16 were strongly associated with PMOS pathogenesis and hormone regulation. The four key phyto compounds Rutin, Luteolin, Apigenin, and Quercetin collectively modulated 10–11 gene targets per compound via PI3KAkt, Estrogen, Ras, and MAPK signaling pathways [10,41].

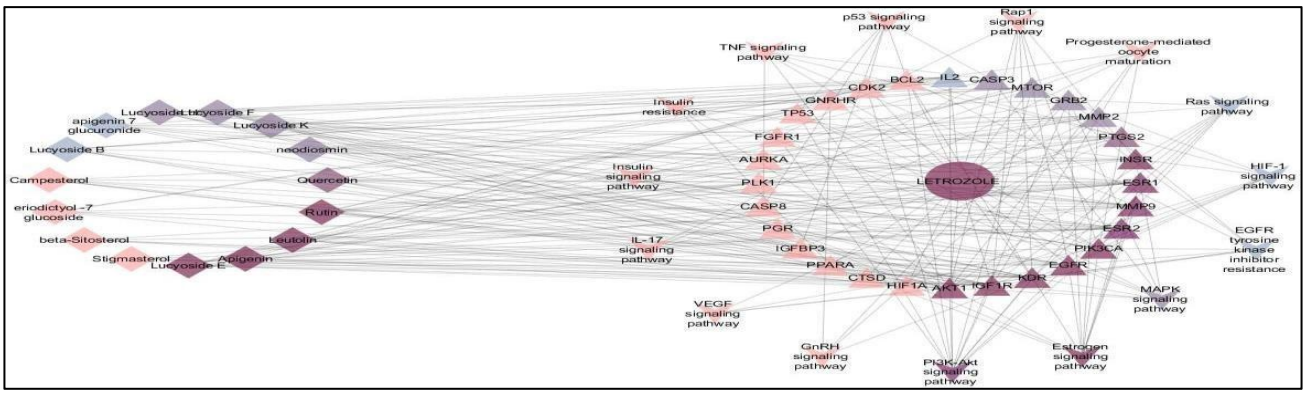


Figure 3: Network construction of Luffa cylindrica – Compound Target Pathway interaction network in PMOS [21] Molecular docking results are summarized in Table 4. All LCE phytocompounds demonstrated markedly superior Glide docking scores and binding energies relative to metformin across all three target proteins [19,20]

Table 4. Molecular docking scores of Luffa cylindrica phytocompounds vs. PMOS therapeutic targets [19,20]

Target	Phytocompound	Docking Score	Glide Energy (kcal/mol)	Key H bond Residues
EGFR (1M17)	Neodiosmin	13.029	65.471	THR830, ASN818, GLN767, THR766
EGFR (1M17)	Rutin	12.541	73.614	ASN818, THR830, THR766
EGFR (1M17)	Metformin (reference)	2.259	19.332	THR766
ESR2 (2GIU)	Quercetin	10.237	38.713	HIE475
ESR2 (2GIU)	Luteolin	10.203	21.360	HIE475, THR299
ESR2 (2GIU)	Metformin (reference)	2.733	18.293	THR299
IGF1R (2OJ9)	Rutin	12.206	58.181	GLN977, SER979, THR1127
IGF1R (2OJ9)	Quercetin	11.369	31.660	THR1053, GLN977, SER979
IGF1R (2OJ9)	Metformin (reference)	2.013	18.732	SER1059, GLN977

Phytochemical Analysis and In Vitro Enzyme Inhibition

Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycerides, and amino acids [7,23]. FTIR spectroscopy revealed a characteristic C=C aromatic stretch at 1383 cm^{-1} consistent with the flavonoid and alkaloid fractions [33].

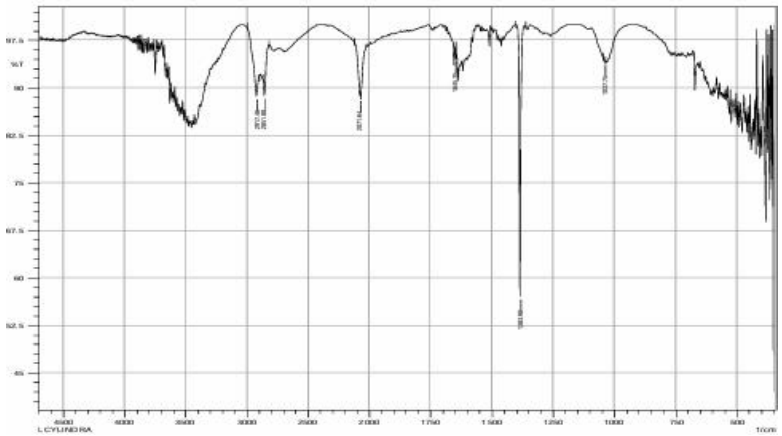


Figure 4: FTIR spectrograph

LCMS profiling identified eight major compounds: Rutin (MW 610.5 g/mol), Luteolin (286.24 g/mol), Apigenin (270.24 g/mol), Quercetin (302.23 g/mol), Neodiosmin (608.5 g/mol), Campesterol (400.7 g/mol), Eriodictyol7glucoside (450.4 g/mol), and Beta Sitosterol (414.7 g/mol) [23,33].

Total Flavonoid and Phenolic Content

Table 5. Total flavonoid and phenolic content of LCE [22,23]

Parameter	Conc. (µg/mL)	Abs.	Regression Equation	Result	R^2
Total Flavonoid Content	1000	0.256	y = 0.0045x + 0.1538	22.88 mg QE/g	0.9974
Total Phenolic Content	100	0.058	y = 0.0016x + 0.0334	14.75 mg GAE/g	0.9988

Alpha Amylase Inhibition

Table 6. Effect of LCE on αamylase inhibition (y = 0.4176x + 16.402; R^2 = 0.9988) [25].

Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
20	2.473	24.35	80.45
40	2.174	33.50	
60	1.898	41.94	
80	1.658	49.28	
100	1.366	58.21	

Alpha Glucosidase Inhibition

Table 7. Effect of LCE on αglucosidase inhibition (y = 0.8465x − 2.135; R^2 = 0.9902) [25,39].

Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
20	0.273	16.95	56.58
40	0.374	34.56	
60	0.598	50.25	
80	0.658	62.25	

Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)
100	0.787	85.25	

In Vivo: Metabolic, Hormonal, and Hepatic Parameters

Following 21 days of letrozole administration, all PMOS induced groups exhibited significant increases in body weight, fasting blood glucose, testosterone, triglycerides, LDLC, and liver enzyme levels relative to normal controls ($p<0.001$), confirming successful PMOS induction [35,36,37]. Fourteen days of LCE treatment produced dose dependent amelioration across all assessed parameters. LCE at 400 mg/kg achieved the most pronounced efficacy, significantly reducing body weight (176.83 ± 3.02 g vs. 236.5 ± 4.34 g in disease controls; $p<0.001$) and fasting blood glucose (129.5 ± 2.31 mg/dL vs. 153.83 ± 2.21 mg/dL; $p<0.001$). Oral glucose tolerance testing at Day 36 confirmed improved glucose tolerance across all LCE doses in a dose dependent pattern [36,37,38].

Table 8. Key metabolic and hormonal parameters. [a]p<0.001 vs Normal; #p<0.001 vs Disease [36,37,38]

Parameter	Normal	Disease	Standard	LCE 100	LCE 200	LCE 400

Body wt. (g) D36	159.5±2.94	236.5±4.34[a]	162±2.3#	177.33±1.73 #	196.83±2.55 #	176.83±3.02#
FBG (mg/dL) D36	76.17±1.98	153.83±2.21 [a]	95.67±3.75 #	153.83±2.21	131.5±2.36#	129.5±2.31#
Testosterone (ng/dL)	29.02±0.55	53.18±1.5[a]	32.36±0.74 #	50.88±1.51	48.29±1.09	32.36±0.74##
Estrogen (pg/mL)	47.44±1.65	23.61±0.44[a]	42.83±0.37 #	25.99±1.38	30.25±1.3#	35.22±1.16###
TG (mg/dL)	79.22±1.46	129.26±3.51 [a]	84.38±1.55 #	122.54±5.59	120.27±1.33	115.99±1.33## #
LDL (mg/dL)	39.34±0.96	74.82±1.44[a]	41.58±1.59 #	70.04±0.53	68.0±1.54	61.4±2.44#
HDL (mg/dL)	33.05±0.88	17.70±0.43[a]	32.22±1.58 #	23.42±1.3	25.04±0.28# #	26.64±0.49#

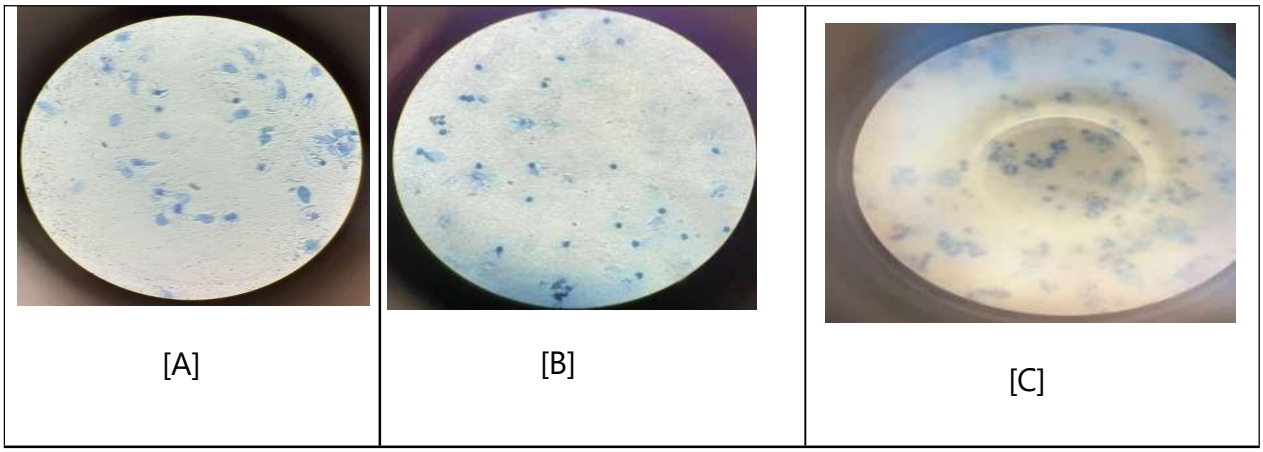
Table 9. Antioxidant markers and liver enzymes. [a]p<0.001 vs Normal; #p<0.001 vs Disease [36,37,38,40,41]

Parameter	Normal	Disease	Standard	LCE 100	LCE 200	LCE 400
SOD (U/mL)	13.29±0.19	5.22±0.14[a]	11.92±0.19#	6.58±0.17##	7.93±0.24#	8.26±0.13#
GSH (µmol/mL)	191.02±2.39	92.58±1.79[a]	166.13±1.4#	119.87±4.15#	125.24±1.42 #	139.22±2.27#
MDA (nmol/mL)	1.83±0.03	5.03±0.33[a]	2.22±0.26#	4.72±0.16#	4.0±0.14#	3.76±0.26#
ALT (IU/L)	26.68±0.91	39.3±0.46[a]	28.16±0.94#	37.14±0.75	34.94±0.69# #	32.2±0.96#

Parameter	Normal	Disease	Standard	LCE 100	LCE 200	LCE 400
AST (IU/L)	143.69±1.32	198.34±0.96 [a]	154.71±2.11 #	197.53±0.93	195.12±1.14	188.11±1.86##
ALP (IU/L)	24.76±0.56	47.9±1.85[a]	27.16±1.4#	43.23±2.13#	41.35±0.5##	37.32±1.17#

Estrous Cycle and Vaginal Cytology

Normal control rats demonstrated continuous, regular cycling through all four phases of the estrous cycle (proestrus, estrus, metestrus, and diestrus) throughout the study period [30]. Following 21 days of letrozole induction, all PMOS groups exhibited prolonged, persistent diestrus characterized by predominant leucocytes and absence of cornified epithelial cells on vaginal cytology confirming anovulatory status from Day 13 post induction [30,35,36].



[A] [B] [C]

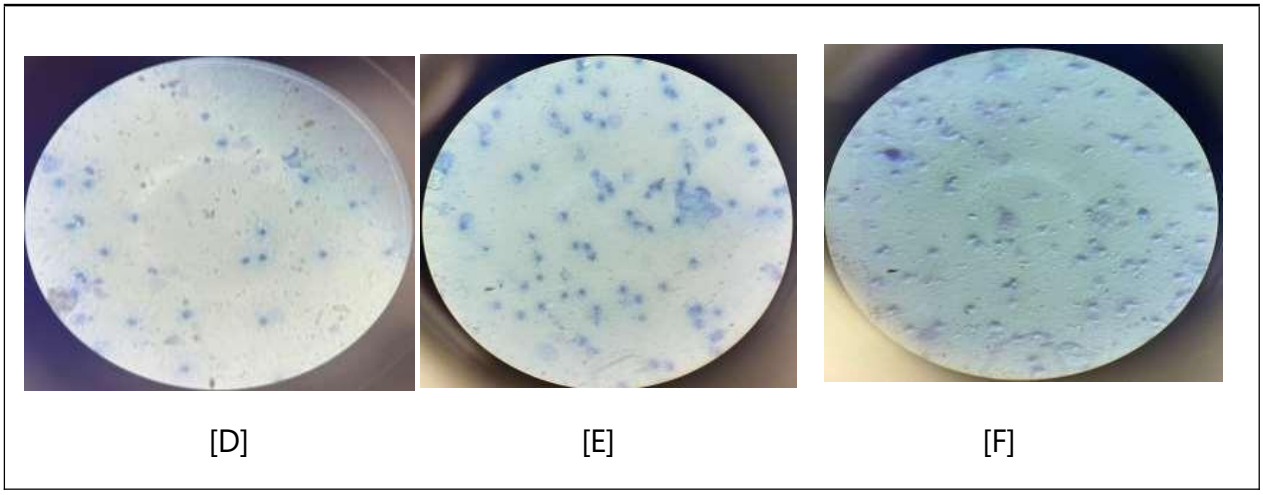


Figure 9: Estrous cycle cytology on Day 21: All letrozoletreated groups in diestrus phase [30].

[A] Metaestrus Phase (Normal), [B] Diestrus Pahse (Disease), [C] Diestrus Phase (Standard), [D] Diestrus Phase (Lec 100), [E] Diestrus Phase (Lec 200), [F] Diestrus Phase (Lec 400)

By Day 35, the metformin standard group had substantially restored proestrus/estrus cycling. LCE treated groups (100, 200, and 400 mg/kg) demonstrated progressive, dose dependent restoration of estrous cyclicity: LCE 100 achieved proestrus, LCE 200 restored estrus phase, and LCE 400 dvanced to metestrus phase, collectively indicating dose dependent ovulation reinstatement [27,30,36,37].

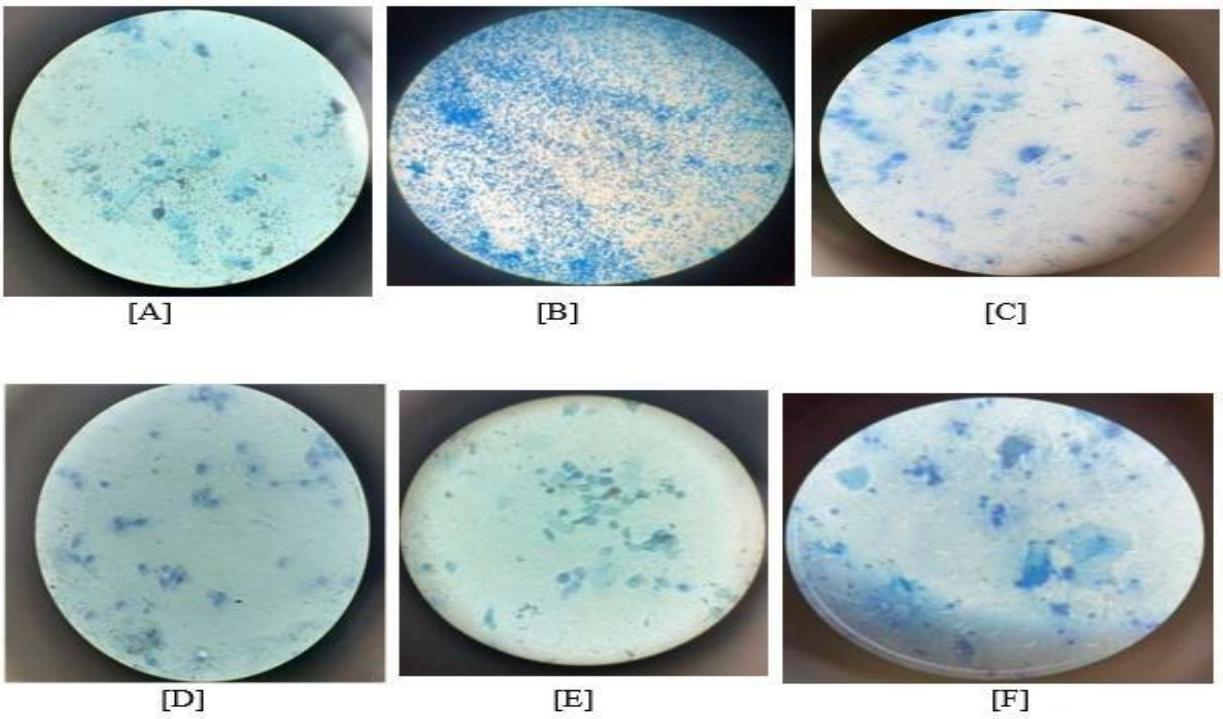


Figure 10: Estrous cycle cytology on Day 36: Treatment groups show dose dependent restoration [30].

[A] Metaestrus Phase (Normal), [B] Diestrus Pahse (Disease), [C] Proestrus Phase (Standard), [D] Proestrus Phase (Lec 100), [E] Estrus Phase (Lec 200), [F] Metaestrus Phase (Lec 400)

Ovarian Weight and Pre Ovarian Fat

Table 10. Effect of LCE on ovarian weight and preovarian fat weight (Mean ± SEM; n=6). [a]p<0.001 vs Normal; #p<0.001 vs Disease; @p<0.001 vs Standard [28]

Group	Ovarian Weight (mg)	PreOvarian Fat (mg)
Normal	42.5 ± 1.12	1637.39 ± 27.32
Disease	74.17 ± 1.66 [a]	3525.23 ± 141.7 [a]
Standard	48.75 ± 0.81 #	1728.45 ± 54.01 #
LCE 100 mg/kg	68.92 ± 0.65 @##	2774.74 ± 70.56 @#
LCE 200 mg/kg	62.67 ± 0.44 @#	2720.56 ± 56.27 @#
LCE 400 mg/kg	62.25 ± 0.40 @#	2534.02 ± 38.46 @#

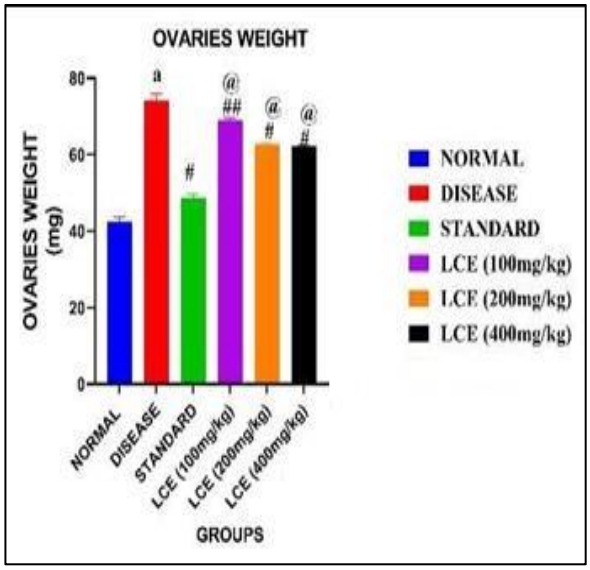


Figure 11: Effect of LCE on ovarian weight [28]

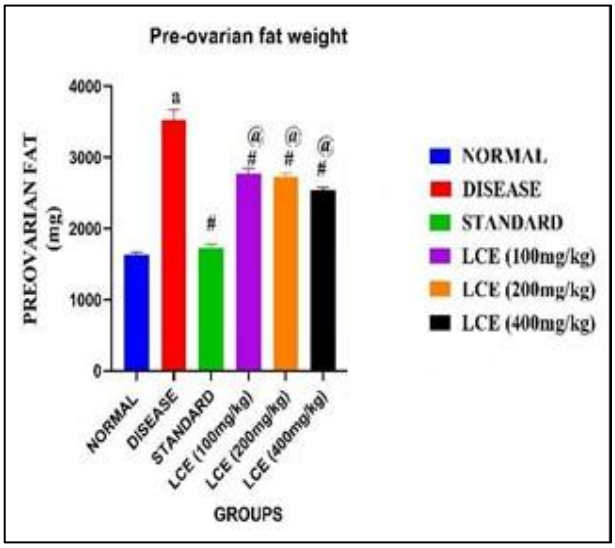
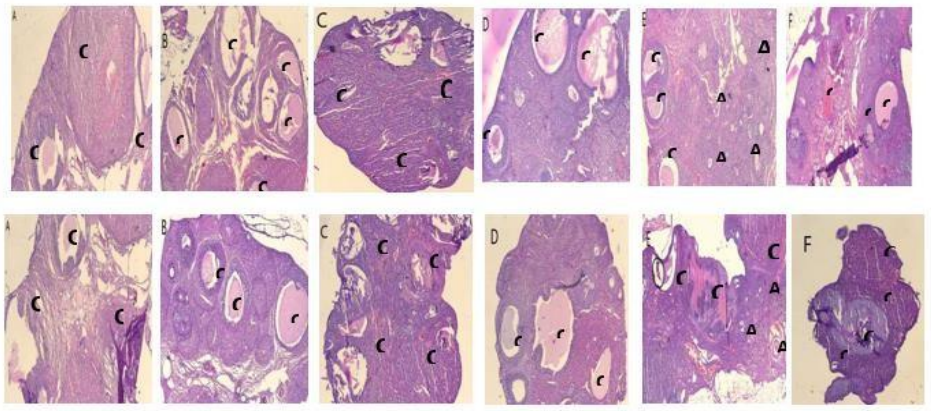


Figure12: Effect of LCE on pre ovarian fat weight

Histopathological Findings



Normal (Group 1) ovaries demonstrated characteristic histological architecture with well developed primordial, primary, secondary, and antral follicles and a corpus luteum (CL) in the absence of cystic follicles (CF) [28,29]. The disease control group (Group 2) displayed numerous enlarged cystic follicles, multiple atretic follicles (AF), a complete absence of CL, and a markedly thickened ovarian capsule features consistent with clinically diagnosed PMOS and confirming the validity of the letrozole induction model [29,35]. Metformin treated ovaries (Group 3) showed regression of cystic changes, emerging secondary follicles, and partial CL restoration. LCE treated groups demonstrated dose dependent histological recovery: LCE 400 mg/kg exhibited the most pronounced restoration, with developing antral follicles, multiple CL, and markedly reduced CF [28,29]

Figure 13: Effect of L. cylindrica extracts of histopathological study on ovarian follicular growth.

A, A (NC): Normal follicular development; B, B (DC): Letrozole treatment cause small cyst formation in the ovary; C, C (Metformin); D, D (LCE 100); E, E (LCE 200); F, F (LCE 400) Treatment with L. cylindrica cause normal follicular development. [28,29]

Pre ovarian fat histopathology in the disease group revealed marked vascular congestion, neutrophilic infiltration, irregular adipocyte morphology, and stromal oedema consistent with obesity associated adipose tissue inflammation [31]. LCE treated groups displayed progressive reduction in inflammatory cell infiltration, restoration of normal adipocyte morphology, reduced vascular congestion, and decreased stromal oedema in a dose dependent fashion, correlating with systemic anti-inflammatory actions attributed to flavonoid constituents [31,32,34].

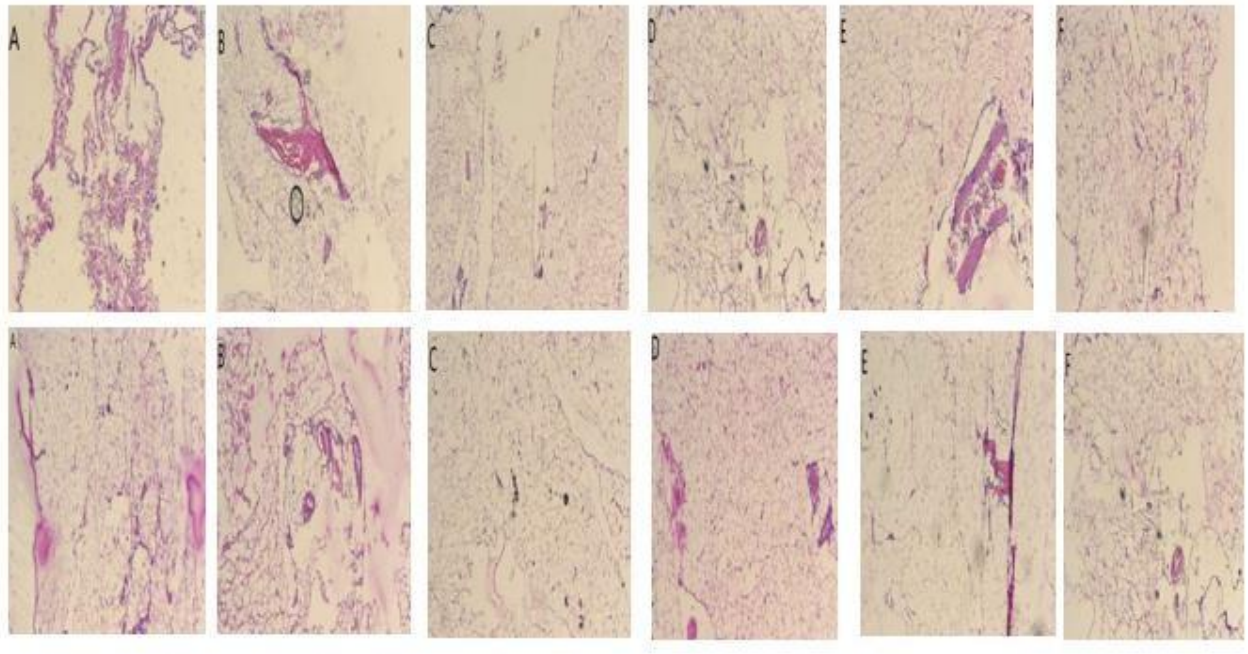


Figure 14: Effect of L. cylindrica extracts histopathological studies of preovarian fat or visceral ovarian fat [31]. A,A (NC): Normal group; B,B (DC): Letrozole treatment; C,C (Metformin); D, D(LCE 100); E, E (LCE 200); F,F (LCE 400)

DISCUSSION

This study provides integrative and multidisciplinary evidence for the therapeutic potential of Luffa cylindrica hydroalcoholic extract in the management of PMOS/PMOS. The choice of letrozole as an inducing agent is well validated; letrozole (a third generation aromatase inhibitor) suppresses oestrogen biosynthesis, elevates gonadotropin levels, promotes ovarian cyst formation, and induces insulin resistance closely recapitulating the human PMOS phenotype [35,36]. FTIR and LCMS confirmed the presence of flavonoids, alkaloids, and saponins as dominant constituents, consistent with prior phytochemical characterizations [7,8,33].

In vitro enzyme inhibition data revealed significant alpha amylase and alpha glucosidase inhibitory activity, with IC_{50} values of 80.45 µg/mL and 56.58 µg/mL respectively [25]. These values are indicative of meaningful postprandial glucose regulatory capacity, operating through mechanisms consistent with PPARγ activation and insulin sensitization pathways particularly relevant to the insulin resistant PMOS milieu [39,47,64]. Quercetin, Rutin, and Luteolin the major flavonoid constituents have individually been demonstrated to inhibit carbohydrate digesting enzymes and improve insulin signaling in multiple in vitro and in vivo paradigms [8,34,39].

Network pharmacology analysis identified a rich multi target landscape, with Luffa cylindrica phyto-compounds interacting with 175 PMOS associated genes and modulating 16 key signaling pathways [21]. Among these, the PI3KAkt pathway occupies a central role in PMOS pathogenesis: dysregulation of PI3KAkt impairs glucose uptake, promotes adipogenesis, and amplifies ovarian androgen production through insulin receptor substrate signaling defects [10,47,64]. The Estrogen, Ras, and MAPK pathways regulate follicular maturation, ovulation, and hypothalamic pituitary feedback control [46,48,53]. The simultaneous modulation of these pathways by a single botanical extract represents a significant mechanistic advantage over single target pharmacotherapy [3,6].

Molecular docking studies corroborated the network pharmacology findings: Neodiosmin and Rutin at EGFR (docking scores of −13.029 and −12.541 kcal/mol, respectively), Quercetin and Luteolin at ESR2 (−10.237 and −10.203 kcal/mol), and Rutin and Quercetin at IGF1R (−12.206 and −11.369 kcal/mol) all substantially outperformed metformin (−2.259, −2.733, and −2.013 kcal/mol, respectively) across all three targets [19,20]. These superior binding affinities, stabilized by key

hydrogen bond interactions with conserved active site residues, predict stronger pharmacological modulation of these critical therapeutic nodes. The Glide docking methodology used here has been validated in multiple pharmaco-informatics studies for its accuracy in predicting binding poses and relative affinities [40,41]. The EGFR–IGF1R–AKT axis is a known mediator of ovarian insulin sensitivity and androgen biosynthesis, and its simultaneous inhibition by multiple phyto compounds may underlie the multi domain therapeutic effects observed in vivo [19,46].

In vivo findings substantiated all in silico and in vitro predictions. LCE at 400 mg/kg produced the most comprehensive therapeutic response: significant reductions in body weight (from 236.5 to 176.83 g), fasting blood glucose (from 153.83 to 129.5 mg/dL), testosterone, TG, LDL, ALT, AST, ALP, and MDA, alongside significant elevations of estradiol, HDL, SOD, and GSH relative to disease controls [36,37,38,40]. These parameters collectively mirror the spectrum of PMOS metabolic and endocrine dysfunction reported in clinical cohorts [49,53,62]. Restoration of the estrous cycle to normal cyclicity confirmed objectively by vaginal cytology indicates reinstatement of HPO axis function, consistent with the effects of standard phyto estrogenic and anti-androgenic treatments [27,30,36].

Histopathological validation is a critical translational step. The dose dependent increase in CL formation and reduction in CF in LCE treated ovaries mirrors the response to pharmacological PMOS treatments in validated rodent models [28,29,38]. Reduced pre ovarian fat inflammation in LCE groups is particularly noteworthy given the established bidirectional relationship between perifollicular adiposity, local estrogen metabolism, and ovarian function [31,51]. The combination of systemic metabolic improvement, hormonal normalization, and localized ovarian tissue recovery positions LCE as an exceptionally holistic candidate therapeutic agent.

CONCLUSION

This investigation demonstrates the promising, multi domain therapeutic potential of hydroalcoholic extract of Luffa cylindrica (LCE) for the management of PMOS/PMOS through an integrated translational approach. LCMS characterization identified key bioactive phytocompounds Rutin, Luteolin, Apigenin, Quercetin, and Neodiosmin possessing established pharmacological activities relevant to PMOS pathogenesis. In vitro enzyme inhibition studies confirmed significant postprandial glycaemic regulatory capacity, with IC_{50} values of 80.45 µg/mL and 56.58 µg/mL for alpha amylase and alpha glucosidase inhibition, respectively.

Network pharmacology revealed modulation of 46 PMOS related protein targets across 16 key pathways, with the PI3KAkt, Estrogen, Ras, and MAPK cascades predominating. Molecular docking confirmed the superior binding affinities of LCE phytocompounds over the reference drug metformin at EGFR, ESR2, and IGF1R. In vivo evaluation in the letrozole induced PMOS rat model demonstrated dose dependent, comprehensive alleviation of PMOS symptoms: restored estrous cyclicity, normalized hormonal and lipid profiles, reduced oxidative stress, attenuated hepatotoxicity markers, and improved ovarian and adipose tissue histopathology with LCE 400 mg/kg exhibiting near standard drug efficacy.

Collectively, these findings establish Luffa cylindrica as a multi target, mechanistically plausible phytotherapeutic candidate for PMOS management. Future research should focus on bioactivity guided isolation of individual active compounds, elucidation of precise cellular and molecular mechanisms of action, pharmacokinetic and safety profiling, and ultimately, rigorously designed clinical trials to validate efficacy and safety in human PMOS populations.

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