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Research Article

Evaluate the Pharmacognostical Potential of GREWIA ASIATICA Fruit Extract in Female Rats with Letrozole-Induced Polycystic Ovarian Diseases (PCOD) by Biochemical Evaluation

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Abstract

Polycystic ovarian disease (PCOD) is characterised by hormonal imbalance, ovarian dysfunction, and metabolic disturbances, often leading to infertility and obesity. The present study investigated the therapeutic potential of *Grewia asiatica* fruit extract in letrozole-induced PCOD in female rats. Ethanolic fruit extract was prepared and subjected to preliminary phytochemical screening, revealing the presence of flavonoids, phenolics, tannins, saponins, glycosides, steroids, and alkaloids. PCOD was induced in rats by oral administration of letrozole (1 mg/kg) for 21 days, and treatment groups received either standard metformin (300 mg/kg) or *G. asiatica* extract (200 and 400 mg/kg). Parameters assessed included body weight, oestrous cyclicity, serum hormonal profile (LH, FSH, testosterone, progesterone), lipid profile, fasting blood glucose, and insulin resistance (HOMA-IR). Letrozole induced significant weight gain, hyperandrogenism, disrupted oestrous cycle, dyslipidemia, and impaired glucose homeostasis. Administration of *G. asiatica* extract demonstrated dose-dependent restoration of oestrous cyclicity, normalisation of hormonal and metabolic parameters, and reduction of body weight gain, comparable to the standard drug. The observed effects are attributed to the antioxidant, anti-inflammatory, and endocrine-modulating activities of the bioactive phytoconstituents. These findings suggest that *Grewia asiatica* possesses significant therapeutic potential as a natural adjuvant for managing PCOD, targeting both reproductive and metabolic dysfunctions.

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1. INTRODUCTION

Polycystic Ovarian Disease (PCOD), interchangeably known as Polycystic Ovary Syndrome (PCOS), is a multifactorial endocrine disorder that predominantly affects women of reproductive age. It is considered one of the most common gynaecological endocrinopathies, with a global prevalence ranging between 5% and 15%. According to the Rotterdam criteria (2013), PCOD is diagnosed if at least two of the following three features are present: (i) oligo-ovulation or anovulation, (ii) hyperandrogenism (clinical or biochemical), and (iii) polycystic ovarian morphology on ultrasonography.

PCOD is not merely a reproductive disorder but a complex metabolic and endocrine condition. Women with PCOD frequently present with menstrual irregularities, infertility, hirsutism, acne, alopecia, obesity, and metabolic disturbances, including insulin resistance, dyslipidaemia, and increased risk of type 2 diabetes mellitus. Long-term consequences include endometrial hyperplasia, cardiovascular disease, and mood disorders. The syndrome also has a profound psychosocial impact; due to cosmetic manifestations such as hirsutism and obesity, many women with PCOD suffer from depression, anxiety, and reduced quality of life.

PCOD affects approximately 6–20% of women worldwide and has become an increasing public health concern attributed to changes in lifestyle, dietary habits, and rising levels of chronic stress. The condition often begins at menarche and persists through the reproductive years, significantly affecting a woman's reproductive span, overall health, and socioeconomic productivity.

1.1 Epidemiology of PCOD

The prevalence of PCOD varies worldwide, largely due to differences in diagnostic criteria and population characteristics. In India, studies report prevalence rates as high as 20–25% among adolescent girls, with increasing numbers in urban populations. The condition is often underdiagnosed, with many women remaining untreated until infertility or metabolic complications arise.

According to a systematic screening using the National Institutes of Health (NIH) diagnostic standards, 4–10% of reproductive-age women are predicted to have PCOS worldwide. The World Health Organisation (WHO) estimated that in 2012, PCOS affected 116 million women (3.4%) globally. Although PCOS can occur at any age beginning with menarche, the majority of cases are identified between the ages of 20 and 30. PCOS affects 1.55 million women of reproductive age worldwide, resulting in 0.43 million disability-adjusted life years (DALYs). The age-standardised incidence rate of PCOS was 82.44 per 100,000 in 2017, representing a 1.45% increase compared to 2007.

Table 1.1: Prevalence of PCOD across different regions

Region / Country	Prevalence (%)
USA	6–8%
Europe	12–18%
India (Urban)	22–36%
Middle East	10–15%
Adolescents (Global)	6–20%

Source: Compiled from published literature and WHO reports

1.2 Aetiology of PCOD

PCOD is a multifactorial endocrine disorder resulting from the interaction of genetic, hormonal, metabolic, and environmental factors. A strong genetic predisposition plays a key role, as the condition often clusters in families. Insulin resistance is a central pathogenic factor; elevated insulin levels stimulate ovarian theca cells to produce excess androgens and suppress hepatic synthesis of sex hormone-binding globulin (SHBG), leading to increased free testosterone. Hyperandrogenism disrupts normal follicular development, resulting in anovulation and the formation of multiple immature ovarian follicles.

Dysregulation of the hypothalamic–pituitary–ovarian (HPO) axis, characterised by an increased LH to FSH ratio, further contributes to impaired ovulation. Obesity and lifestyle factors, including sedentary behaviour and poor dietary habits, exacerbate insulin resistance and hormonal imbalance. Additionally, chronic inflammation, oxidative stress, and exposure to endocrine-disrupting chemicals (EDCs) may influence ovarian dysfunction and metabolic disturbances associated with PCOD.

Etiological Factor	Description
Genetic Factors	Family history, gene polymorphisms (steroidogenesis, insulin signaling)
Hormonal Dysregulation	↑ GnRH pulsatility → ↑ LH / ↓ FSH ratio → anovulation
Insulin Resistance	Hyperinsulinemia → ↑ androgen by theca cells → ↓ SHBG
Metabolic Factors	Obesity, dyslipidemia, type 2 diabetes risk
Environmental Factors	Sedentary lifestyle, poor diet, endocrine-disrupting chemicals
Inflammatory Factors	↑ CRP, IL-6, TNF-α → chronic low-grade inflammation

Fig 1.1: Etiological Factors of PCOD

Figure 1.1: Summary of key etiological factors contributing to the pathogenesis of PCOD, including genetic, hormonal, metabolic, and environmental determinants.

1.3 Clinical Manifestations

The clinical presentation of PCOD is heterogeneous and encompasses a broad spectrum of reproductive, endocrine, metabolic, and psychological features. The most frequent clinical feature is menstrual irregularity, including oligomenorrhea, amenorrhea, or infrequent and delayed menstrual cycles. In some cases, menorrhagia may also be observed.

Hyperandrogenism is a hallmark manifestation clinically evident as hirsutism (present in 60–70% of cases), acne, oily skin, and androgenic alopecia. Due to impaired or absent ovulation, many women with PCOD experience subfertility or infertility. Metabolic disturbances are also prominent, with insulin resistance leading to weight gain, central obesity, difficulty in weight reduction, and an increased risk of developing type 2 diabetes mellitus and metabolic syndrome. Dermatological features such as acanthosis nigricans and skin tags further reflect underlying insulin resistance.

The key clinical domains of PCOD are summarised below:

- **Reproductive symptoms:** Oligomenorrhea, amenorrhea, anovulatory infertility
- **Hyperandrogenic features:** Hirsutism (60–70%), acne, androgenic alopecia
- **Metabolic features:** Central obesity, insulin resistance, type 2 diabetes
- **Dermatological signs:** Acanthosis nigricans, skin tags
- **Psychological aspects:** Depression, anxiety, eating disorders, reduced quality of life

1.4 Pathophysiology of PCOD

The hallmark of PCOD is hyperandrogenism coupled with insulin resistance. Increased frequency of GnRH pulses favours LH secretion, which stimulates ovarian theca cells to produce androgens. Reduced FSH secretion impairs granulosa cell aromatisation of androgens to estrogens, leading to follicular arrest and the formation of multiple small cysts. Insulin resistance is observed in 50–70% of women with PCOD. Hyperinsulinemia worsens ovarian androgen production, reduces SHBG levels, and disrupts ovulation.

PCOD is now recognised as a chronic low-grade inflammatory state, with elevated levels of C-reactive protein (CRP), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α). These inflammatory mediators contribute to oxidative stress and further impair insulin signalling and ovarian function. On ultrasonography, the ovaries are typically enlarged with more than 12 small follicles (<9 mm in diameter), arranged peripherally in a characteristic 'string of pearls' pattern.

The biochemical hallmark of PCOS is hyperandrogenemia, which manifests clinically as hirsutism, acne, and alopecia. High levels of androgens are observed in 75–90% of PCOS patients with oligomenorrhea, and their concentrations frequently increase with the severity of the phenotype. Insulin directly mimics the action of LH and indirectly raises GnRH, making hyperinsulinemia the primary driver of excessive androgen production. Sex hormone-binding globulin (SHBG), a key circulatory protein that regulates testosterone levels, is suppressed by elevated insulin, resulting in higher levels of free androgens.

Biochemical Marker	Clinical Significance	Trend
Testosterone (Total & Free)	Elevated; key androgen in PCOS pathogenesis	↑
LH : FSH Ratio	Increased (>2:1); reflects HPO axis dysregulation	↑
Insulin / HOMA-IR	Elevated due to peripheral insulin resistance	↑
SHBG	Reduced; leads to ↑ free androgens	↓
Estradiol (E2)	Variable; often normal or low	↕
CRP / IL-6 / TNF-α	Elevated; markers of chronic inflammation	↑
Prolactin	Mildly elevated in some patients	↑
DHEA-S	Elevated in adrenal hyperandrogenism	↑

Fig 1.2: Key Biochemical Markers in PCOD

Figure 1.2: Principal biochemical markers altered in PCOD. ↑ = elevated, ↓ = reduced, ↕ = variable. LH: Luteinizing Hormone; FSH: Follicle-Stimulating Hormone; SHBG: Sex Hormone-Binding Globulin; DHEA-S: Dehydroepiandrosterone Sulphate; CRP: C-Reactive Protein.

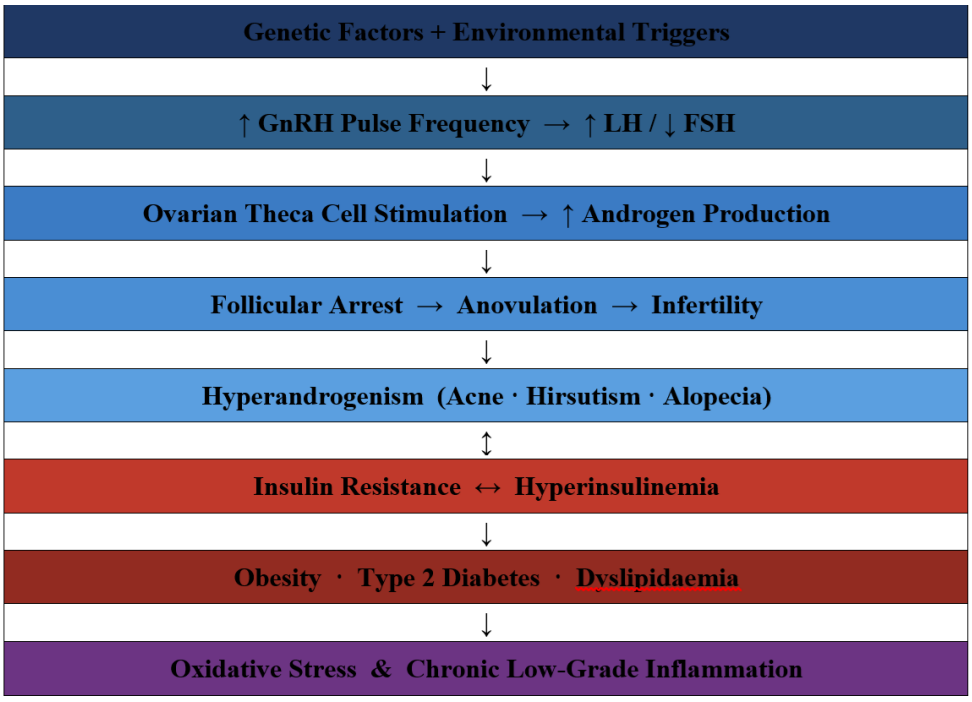

Fig 1.3: Pathophysiological Cascade in PCOD

1.5 Experimental Models of PCOD – Role of Letrozole

Animal models are essential for understanding PCOD pathogenesis and testing new therapeutic agents. The letrozole-induced PCOD model in rats is widely used because it mimics both the reproductive and metabolic aspects of human PCOD. Letrozole, a potent non-steroidal aromatase inhibitor, prevents the conversion of androgens to estrogens, thereby leading to hyperandrogenism and polycystic ovarian morphology. Rats treated with letrozole exhibit prolonged diestrus phase, increased LH/FSH ratio, cystic follicles, elevated testosterone, insulin resistance, and body weight gain.

This model is preferred over other approaches, such as the DHEA-induced model and estradiol valerate model, as it more closely represents the clinical features observed in women with PCOD. Among the various induction methods, letrozole has garnered significant attention for its efficacy in inducing PCOS-like phenotypes in rat models. Its ability to disrupt the delicate hormonal balance, particularly by inhibiting the conversion of androgens to estrogens, closely mirrors the hormonal dysregulation observed in human PCOS. The comparative attributes of common animal models are presented below:

- **Letrozole Model:** Inhibits aromatase → ↓ estrogen, ↑ androgens. Rats develop irregular cycles, cystic ovaries, and hyperinsulinemia. Most clinically relevant.
- **DHEA Model:** Mimics hyperandrogenism but lacks consistent metabolic features.
- **Estradiol valerate Model:** Reproduces ovarian cysts but with less metabolic dysfunction.

1.6 Risk Factors

1.6.1 Genetic Factors

Familial clustering and twin studies suggest a strong heritable component in PCOD. The condition is polygenic and multifaceted, with several candidate genes linked to steroidogenesis, insulin signalling, and gonadotropin secretion having been implicated. Gene–gene interactions, and interactions between genes and the environment, may also modulate an individual's susceptibility to developing PCOD.

1.6.2 Hormonal Dysregulation

Altered hypothalamic–pituitary–ovarian (HPO) axis function results in increased GnRH pulsatility, favouring luteinizing hormone (LH) secretion over follicle-stimulating hormone (FSH). This disruption in the LH: FSH ratio is central to the impaired folliculogenesis and anovulation characteristic of PCOD.

1.6.3 Insulin Resistance

Insulin resistance is a key driver of hyperandrogenism in PCOD. Elevated insulin levels increase androgen production by theca cells and suppress sex hormone-binding globulin (SHBG), resulting in elevated free testosterone. Numerous studies have demonstrated that reducing insulin resistance leads to a reduction in androgen levels and an improvement in disease outcomes.

1.6.4 Environmental and Lifestyle Factors

Sedentary lifestyle, obesity, chronic stress, and dietary patterns further worsen PCOD symptoms. Various studies have shown that environmental pollutants, including heavy metals, insecticides, and endocrine-disrupting chemicals (EDCs) such as bisphenol A (BPA), significantly affect human health and reproduction. There is mounting evidence that environmental pollutants contribute to the development and severity of PCOS. Lifestyle modifications, including regular physical exercise, maintaining a healthy body weight, and adhering to nutritious dietary habits, constitute the primary line of management for women with PCOD.

Genetic	Hormonal	Metabolic	Environmental
• Family history of PCOS • Gene polymorphisms • Polygenic inheritance • Steroidogenesis genes	• ↑ LH / ↓ FSH ratio • ↑ GnRH pulsatility • Hyperandrogenism • HPO axis dysregulation	• Insulin resistance • Obesity / overweight • Dyslipidaemia • Type 2 diabetes risk	• Sedentary lifestyle • High-carb diet • Chronic stress • EDCs (e.g., BPA)

Fig 1.4: Risk Factors Contributing to PCOD

In conclusion, PCOD is a multifaceted endocrine disorder with far-reaching consequences for reproductive, metabolic, and psychological health. Understanding its complex aetiology, pathophysiology, and risk factors is essential for the development of effective therapeutic strategies. The subsequent chapters of this dissertation explore potential pharmacological interventions, with a particular focus on the therapeutic evaluation of Grewia asiatica in a letrozole-induced PCOD rat model.

2. LITERATURE REVIEW

Jean Monod L *et al.* (2001) described the normal menstrual cycle as occurring between puberty (ages 10–16 years) and menopause (~51 years). The cycle duration ranges from 21 to

35 days (mean 28 days), with menstrual flow lasting 2–8 days and a mean blood loss of 30 ml. The ovarian cycle comprises three phases: follicular phase, ovulation, and luteal phase. The uterine cycle is divided into menstruation, proliferative (post-menstrual) phase, and secretory (pre-menstrual) phase.

Sergio P *et al*. (2002) documented the coordinated hormonal cascade of the normal menstrual cycle: the hypothalamus secretes GnRH, stimulating the anterior pituitary to release FSH and LH. FSH recruits a dominant follicle, which secretes estradiol and Inhibin A. A surge in estradiol induces the LH peak responsible for ovulation. The post-ovulatory corpus luteum secretes progesterone, which undergoes involution 14 days later in the absence of hCG (pregnancy).

Thiyagarajan M *et al*. (2003) elucidated the two-cell theory of ovarian steroidogenesis: LH activates cholesterol desmolase in theca cells, facilitating conversion of cholesterol to progesterone and androstenedione. Androstenedione diffuses to adjacent granulosa cells, where FSH-induced aromatase converts it sequentially to testosterone and then to 17β-estradiol. Elevated 17β-estradiol and progesterone exert negative feedback on the anterior pituitary, reducing FSH and LH secretion.

Al-Asmakh *et al*. (2004) described positive feedback regulation: at threshold concentrations, 17β-estradiol stimulates the anterior pituitary to increase FSH and LH output (the mid-cycle LH surge). Granulosa cells also secrete inhibin (suppressing FSH) and activin (enhancing FSH release). These feedback mechanisms are modulated by the regulation of GnRH receptors at the anterior pituitary.

Marshall *et al*. (2005) Further characterized the interplay between estrogen, progesterone, and LH in cycle regulation. Estrogen produced by granulosa cells inhibits FSH in the normal cycle. Excess LH stimulates androgen overproduction, increases sensitivity to LH-releasing factor, and promotes greater pulsatile LH release — a scenario central to PCOD pathogenesis.

Hayes *et al*. (2013) confirmed the critical regulatory role of inhibin in the menstrual cycle. Inhibin A and B, produced by granulosa and theca cells (and Sertoli cells in males), are glycoprotein dimers with α- and β-subunits. Inhibin A (βA-subunit) suppresses FSH via negative feedback; Inhibin B (βB-subunit) reduces LH secretion. This dual inhibin-based feedback precisely regulates gonadotropin output at the anterior pituitary.

Mohan H *et al*. (2015) assessed the influence of lifestyle and demographic factors on menstrual cycle length. Age, smoking, physical activity, nationality, and alcohol consumption were found to affect the follicular phase duration. Asian women exhibited significantly longer mean cycle length (+1.65 days) compared to Caucasian women, while alcohol consumption was associated with a shorter cycle (−1.26 days).

PCOD is fundamentally a disorder of hormonal imbalance, wherein dysregulation of the HPO axis, excess androgen production, and insulin resistance interact to produce the characteristic clinical and biochemical phenotype.

Jain L *et al*. (2006) demonstrated that oxidative stress and chronic low-grade inflammation play a pivotal role in PCOD pathophysiology. Elevated reactive oxygen species (ROS) impair ovarian steroidogenesis and follicular development, while concurrent inflammation exacerbates insulin resistance. Antioxidant and anti-inflammatory interventions were shown to have potential in ameliorating hormonal imbalance, improving ovulation, and restoring metabolic function.

Dunaif J *et al*. (2014) established that PCOS is not solely an endocrine disorder but is invariably associated with metabolic derangements. Fasting insulin levels were found to be approximately twice as high in obese women with PCOS compared to obese controls without PCOS, indicating a substantially increased risk of type 2 diabetes mellitus. Hypothalamic nuclei, including the arcuate (infundibular) nucleus, were implicated through the actions of appetite-regulating neuropeptides AgRP and NPY (pro-orexigenic) and POMC neurons (hypoglycaemic) in the metabolic pathogenesis of PCOS.

Lieu *et al*. (2016) investigated the role of insulin in ovarian steroidogenesis. Insulin was found to enhance the activity of 17α-hydroxylase and 17–20 lyase (components of the P450c17 enzyme system) and to stimulate 3β-hydroxysteroid dehydrogenase expression in granulosa cells. Insulin also increased pituitary sensitivity to GnRH and potentiated ovarian steroidogenic responses to LH, creating a self-reinforcing cycle of hyperinsulinemia, hyperandrogenism, and anovulation.

Mill J *et al*. (2016) corroborated the findings of Dunaif *et al*. (2014), confirming that women with PCOS exhibit approximately double the fasting insulin levels of obese non-PCOS controls. The study reinforced the association between PCOS and metabolic syndrome and highlighted the substantially elevated risk of type 2 diabetes in this population.

Singh S *et al*. (2012) provided the phytopharmacological basis for natural product interventions in PCOD. Flavonoids and phenolics were shown to modulate the HPO axis, reduce androgen excess, and improve FSH and progesterone levels. Saponins and phenolics demonstrated improvement in lipid metabolism and insulin sensitivity. Bioactive compounds were found to protect ovarian tissue from oxidative stress, thereby supporting follicular development and ovulation.

Grewia asiatica Linn. (Phalsa) is a traditionally valued medicinal plant of the family Tiliaceae. It is native to South and Southeast Asia and has been extensively used in Ayurvedic and Unani medicine for its diverse therapeutic properties. The following studies document its botanical characteristics, phytochemical composition, and pharmacological activities.

Tyson K *et al*. (2008) provided a detailed botanical description of Grewia asiatica. The plant is a small, deciduous tree or shaggy shrub growing up to 12–14 feet in height. Leaves are heart-shaped to oval (5–18 cm long, 9–14 cm broad). Small flowers are arranged in axillary fascicles, each with 2–10 peduncles. The fruit is a round drupe with a single seed, dark purple at full ripening, covered by a thin whitish bloom with massive symmetrical trichomes. The plant thrives in warm climates and is deciduous in winter.

Sharma K *et al*. (2007) documented the phytochemical profile and therapeutic rationale of Grewia asiatica in relation to PCOD. Phytochemical investigations revealed the presence of

flavonoids, phenolics, tannins, saponins, sterols, and glycosides in the fruit, leaves, and stem bark. These bioactive compounds are responsible for the plant's antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, and antidiabetic properties. The authors proposed that Grewia asiatica's multi-target pharmacological profile makes it a promising candidate for PCOD management.

Mimoh R *et al*. (2009) Systematically characterised the pharmacological properties of Phalsa. The fruit was identified as a rich source of flavonoids, polyphenols, and vitamins A, C, and E. These compounds exhibit potent free radical scavenging activity, reducing oxidative stress implicated in multiple chronic diseases. Phalsa extracts also demonstrated significant anti-inflammatory activity through inhibition of pro-inflammatory mediators, and possessed broad-spectrum antibacterial and antifungal properties.

Lal M *et al*. (2010) comprehensively reviewed the nutritional profile, bioactive components, and medicinal applications of Phalsa. Key activities documented include: antioxidant, anticancer, anti-inflammatory, radioprotective, anti-diabetic, anti-microbial, anti-malarial, hepatoprotective, anti-hyperlipidaemic, and analgesic activities. The fruit's high dietary fibre content supports gut health and acts as a natural laxative. Its cooling properties are used traditionally to treat heat-related illnesses in tropical regions.

Zhou K *et al*. (2011) investigated the amino acid composition of Phalsa fruit and seeds, identifying 18 amino acids. The predominant amino acids were aspartic acid (19.06%), glutamic acid (11%), and leucine (11.02%), highlighting the nutritional value of the fruit. In Phalsa juice, phosphoserine, taurine, serine, aspartic acid, threonine, tyrosine, and glycine were identified as significant free amino acids.

The development of clinically relevant animal models is essential for evaluating therapeutic agents targeting PCOD. The following studies document the comparative utility of various PCOD models, with a particular focus on the letrozole-induced rat model.

Damodar A *et al*. (2017) Reviewed and compared the major experimental models of PCOS in rats, including those induced by estradiol valerate, dehydroepiandrosterone (DHEA), androgens (DHT), 5α-dihydrotestosterone, neonatal androgenisation, and constant light exposure. Estrogen-induced models (e.g., estradiol valerate) fail to replicate the androgen-associated follicular dynamics of human PCOS. Among the models evaluated, letrozole (aromatase inhibitor) was identified as the most clinically relevant, best reproducing the hormonal, metabolic, and morphological features of human PCOS.

Grant K *et al*. (2017) reviewed the mechanisms of action and resistance profiles of letrozole as an aromatase inhibitor. Letrozole inhibits aromatase-mediated conversion of androgens to estrogens, resulting in androgen accumulation, pituitary feedback disruption, and gonadotropin dysregulation. Mechanisms of resistance were also discussed, including non-endocrine pathway activation and hormone-insensitive cell clone development, relevant considerations when interpreting the efficacy of letrozole-based models.

Lariono J *et al*. (2018) validated the letrozole-based PCOD model by linking it to tissue-level ovarian remodelling. Normal ovarian function depends on the precise regulation of cell division, differentiation, migration, and adhesion during the reproductive cycle. Letrozole disrupts these processes, producing follicular cysts that closely resemble the polycystic morphology seen in human PCOS patients. This model was confirmed to be suitable for studying the mechanism of cyst formation.

Baravalle *et al*. (2018) characterised the endocrine and metabolic disturbances induced by letrozole in rats, including elevated LH, FSH, and testosterone (reflecting aromatase blockade and androgen accumulation), irregular oestrus cycles, cystic ovarian morphology, increased body weight, adiposity, mesenteric fat accumulation, and peripheral insulin resistance. The authors confirmed that ovarian histological features closely resembled those of human PCOS patients.

Handelsman *et al*. (2019) summarised the multifactorial pathophysiology and management approaches for PCOS. Letrozole-induced elevation of LH, FSH, and testosterone was confirmed to produce metabolic disturbances, cystic ovarian morphology, and insulin resistance — all hallmarks of human PCOS. Management strategies reviewed included lifestyle modification, clomiphene citrate, thiazolidinediones, metformin, gonadotropins, and laparoscopic ovarian drilling.

Palomba *et al*. (2020) reviewed the clinical utility of Clomiphene Citrate (CC), the most widely used first-line ovulation induction agent in PCOS. Combined with metformin, CC achieves approximately 70% ovulation rate; however, pregnancy rates remain relatively low (~30%). Adverse effects of CC include endometrial and sub-endometrial vascular changes, mood swings, vasomotor flushes, and visual disturbances (blurred vision, scotomata, light sensitivity). Rare but serious complications, including optic neuropathy, have been reported.

Agrawal G *et al*. (2021) Assessed laparoscopic ovarian drilling (LOD) as a second-line surgical intervention for CC-resistant, anovulatory, infertile PCOS women. LOD was recommended as a safe, effective, and cost-effective alternative to gonadotropin therapy, without the risk of multiple gestations. However, its effects on insulin sensitivity, lipid and lipoprotein disturbances, acne, and hirsutism were noted to be inconclusive, and medication should remain the first-line approach.

Lebbi *et al*. (2022) reviewed the pharmacological profile and historical use of metformin (N, N-dimethylbiguanide) in PCOS management. Derived from Galega officinalis, metformin has been used in T2DM management for over 50 years. It demonstrates the best safety profile among biguanides (rate of lactic acidosis <3 per 100,000 patient-years). Metformin improves the lipid profile, restores ovarian function in PCOS, reduces hepatic steatosis, and reduces T2DM-related microvascular and macrovascular complications.

Takasaki *et al*. (2022) investigated the metabolic benefits of metformin in PCOS. Metformin restored ovarian cyclicity, improved lipid profile, and reduced fatty liver infiltration. The role of GLP-1 and GIP incretin hormones, secreted by enteroendocrine cells post-prandially, was highlighted as an

important determinant of glucose clearance and insulin sensitivity pathways modulated by metformin therapy.

Mathur D *et al*. (2023) documented the historical evolution of insulin-sensitising drugs (ISDs) in PCOS management. Metformin was the first ISD used in PCOS, demonstrating significant improvements in menstrual regularity and reductions in circulating androgen levels. Troglitazone (a thiazolidinedione), though since withdrawn, similarly improved cycle regularity and serum androgen concentrations, reinforcing the central role of insulin resistance in PCOS pathogenesis.

3. MATERIALS AND METHODS

3.1 Collection and Authentication of Fruits

Fresh *Grewia asiatica* fruits were collected from Sapullah Ganj, Rohtas, Bihar, India (Feb–Mar 2025). Authentication was done by Prof. Ravindra Kumar Pandey, Himalayan Institute of Pharmaceutical Sciences.

3.2 Preparation of Ethanolic Fruit Extract

Fruits were washed, deseeded, shade-dried (7–10 days), powdered (mesh #40), and extracted using 70% ethanol in a Soxhlet apparatus for 24–36 h. Extracts were filtered, concentrated using a rotary evaporator at 40–45 °C, and dried under vacuum to constant weight.

3.3 Inducer – Letrozole

Letrozole (Serum Institute, Pune) suspended in 0.5% CMC was administered orally at 0.01 mg/kg to induce PCOD for 21 days (except the control group).

3.4 Acute Oral Toxicity

Rats were fasted overnight and administered *G. asiatica* extract at 50, 250, 500, and 2000 mg/kg orally. No mortality or significant toxicity was observed. 500 mg/kg was selected for further studies.

3.5 Phytochemical Screening

Qualitative tests were performed for carbohydrates, glycosides, polysaccharides, amino acids, proteins, alkaloids, steroids, triterpenes, flavonoids, tannins, lipids, oils, and saponins using standard protocols.

3.6 Chemicals and Instruments

Key chemicals: Letrozole, Metformin, ethanol, CMC, ELISA kits, etc.

Instruments: Soxhlet apparatus, rotary evaporator, analytical balance, hot air oven, centrifuge, ELISA reader, refrigerator.

3.7 Experimental Design

36 female rats (6 per group) were divided as follows:

- **Group I:** Normal control (vehicle)
- **Group II:** Disease control (Letrozole 1 mg/kg)
- **Group III:** Standard (Letrozole + Metformin 300 mg/kg)
- **Group IV:** Letrozole + *G. asiatica* low dose (200 mg/kg)
- **Group V:** Letrozole + *G. asiatica* high dose (400 mg/kg)

3.8 Assessment Parameters

- **Oestrous cycle:** Vaginal smears observed daily.
- **Hormonal analysis:** Blood collected on day 22; LH, FSH, Testosterone, Insulin, Progesterone measured by ELISA; LH/FSH ratio calculated.
- **Metabolic parameters:** Body weight, fasting glucose, lipid profile, and HOMA-IR calculated.

3.9 Statistical Analysis

Data expressed as Mean ± SEM. One-way ANOVA followed by Tukey's post-hoc test; $p < 0.05$ considered significant (GraphPad Prism v9).

4. RESULTS AND DISCUSSION

4.1 Plant Extraction Yield

The ethanolic extract of *Grewia asiatica* fruits yielded 12–15% w/w of the dried fruit weight. The extract was dark green with a semi-hard, viscous texture.

4.2 Phytochemical Evaluation

Phytochemical screening (Table 7.1) revealed the presence of flavonoids, phenolics, tannins, saponins, glycosides, steroids, and alkaloids. These bioactive constituents contribute antioxidant, anti-inflammatory, hormone-modulating, and metabolic regulatory activities, providing a mechanistic basis for the therapeutic effects of *G. asiatica* in PCOD. Flavonoids and phenolics may improve folliculogenesis and HPO axis regulation, while saponins, tannins, and sterols aid in lipid metabolism, insulin sensitivity, and steroidogenesis.

4.3 Effect on Body Weight

Letrozole-induced PCOD rats exhibited significant weight gain (182 ± 2 g) compared to controls (160 ± 5 g). Treatment with *G. asiatica* extract showed dose-dependent reduction in weight gain: low dose (170 g), high dose (166 g), comparable to standard metformin (165 g) (Table 7.2, Fig. 7.1). This effect is attributed to flavonoids, saponins, and antioxidants improving lipid metabolism and glucose homeostasis.

4.4 Hormonal Profile

PCOD induction elevated LH (5.9 ± 0.4 mIU/mL) and testosterone (3.8 ± 0.3 ng/mL), with reduced FSH (2.1 ± 0.3 mIU/mL) and progesterone (2.5 ± 0.2 ng/mL). *G. asiatica* extract normalised these parameters dose-dependently, with high dose restoring levels close to normal and comparable to standard metformin (Table 7.3, Fig. 7.2). Restoration of oestrous cyclicity was also observed. Flavonoids, anthocyanins, and antioxidants likely mediated HPO axis modulation, reduced oxidative stress, and improved steroidogenesis.

4.5 Lipid Profile and Glucose Homeostasis

Letrozole-induced PCOD caused dyslipidemia (↑ total cholesterol, ↑ triglycerides, ↓ HDL) and hyperglycemia (↑ FBG). High-dose *G. asiatica* significantly improved lipid profile (total cholesterol: 140 ± 5 mg/dL, triglycerides: 110 ± 4 mg/dL, HDL: 50 ± 3 mg/dL) and fasting glucose (95 ± 4

mg/dL), comparable to standard treatment (Table 7.4, Fig. 7.3). These effects are attributed to flavonoids, saponins, tannins, and phenolics, which enhance insulin sensitivity, reduce oxidative stress, and regulate lipid metabolism.

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