EFFECT OF SIMVASTATIN AND METFORMIN ON PLATELET FUNCTION AND HISTOLOGY OF THE OVARY OF POLYCYSTIC OVARIAN SYNDROME INDUCED SPRAGUE DAWLEY RATS

BY:

ANETOR FAVOUR ESOHE

BMS2106349

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCE

UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this project titled "EFFECT OF SIMVASTATIN AND METFORMIN ON PLATELET FUNCTION AND HISTOLOGY OF THE OVARY OF POLYCYSTIC OVARIAN SYNDROME INDUCED SPRAGUE DAWLEY RATS" has been carried out by ANETOR FAVOUR ESOHE, with matriculation number BMS2106349 in partial fulfillment of the requirements for the award of Bachelor of Science Degree (B.Sc) in department of Physiology, at the School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin city, Edo state, Nigeria.

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ANETOR FAVOUR ESOHE

STUDENT DATE

……. ……

MR. W. J. SILAS

SUPERVISOR DATE:

…… ……

PROF. O. K. UCHE

HEAD OF DEPARTMENT DATE

…… ……

PROF. S. A. ONASANWO

EXTERNAL EXAMINER: DATE

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With profound gratitude and heartfelt appreciation, I acknowledge the individuals whose invaluable contributions have been pivotal to the successful completion of my research project, titled EFFECT OF SIMVASTATIN AND METFORMIN ON PLATELET FUNCTION AND HISTOLOGY OF THE OVARY OF POLYCYSTIC OVARIAN SYNDROME INDUCED SPRAGUE DAWLEY RATS **"**. Their steadfast support, guidance, and encouragement have played a crucial role in shaping the outcome of this work.

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I also extend sincere appreciation to all those who, in one way or another, contributed

DEDICATION

I dedicate this work to God Almighty, my Creator, my constant source of strength, and the wellspring of my inspiration, wisdom, and understanding. It is by His grace alone that I have been able to embark on and complete this journey. Through His guidance, I have found direction; on His wings, I have soared.

This work is also lovingly dedicated to my beloved family, whose unwavering support, encouragement, and belief in me have been the foundation of my perseverance. Their steadfast presence has fueled my determination and carried me through every challenge. May God's abundant blessings continue to be with them now and always.

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a prevalent endocrine disorder characterized by reproductive dysfunction, including anovulation and adverse ovarian remodeling, as well as systemic complications such as a prothrombotic state involving platelet dysfunction. This study was designed to investigate a dual-pronged therapeutic strategy targeting both the systemic metabolic and local ovarian aspects of the syndrome. This study aimed to evaluate the individual and combined effects of metformin and simvastatin on ovarian histology and surrogate markers of platelet function in a letrozole-induced Sprague Dawley rat model of PCOS. PCOS was induced in female Sprague Dawley rats via oral administration of letrozole (1 mg/kg) for 21 days. The rats were subsequently randomized into five groups: a healthy control group, an untreated PCOS group, and three treatment groups receiving simvastatin (20 mg/kg), metformin (300 mg/kg), or a combination of simvastatin and metformin for 28 days. At the end of the treatment period, ovarian tissues were collected for histopathological examination using Hematoxylin and Eosin (H&E) staining, and whole blood was analyzed for platelet indices, including Platelet Count, Mean Platelet Volume (MPV), and Platelet Distribution Width (PDW). Histopathological analysis revealed that the untreated PCOS group developed multiple cystic follicles and an absence of corpora lutea, confirming an anovulatory state. Metformin monotherapy significantly improved ovarian architecture and restored ovulation, evidenced by the presence of multiple corpora lutea. Simvastatin monotherapy showed partial amelioration, with a reduction in cystic follicles but scarce corpora lutea. The combination therapy demonstrated a potent synergistic effect, resulting in a near-complete normalization of ovarian histology that closely resembled the healthy control group, with numerous developing follicles and corpora lutea. Hematological analysis showed no significant differences in platelet count or MPV across the treated groups compared to the untreated PCOS group. However, metformin monotherapy led to a paradoxical and statistically significant increase in PDW and Platelet-Large Cell Ratio (P-LCR) ($P < 0.05$). In concluson, Metformin and simvastatin exert distinct effects on both hematological and ovarian parameters in PCOS-induced rats. Metformin monotherapy showed a significant impact on platelet morphology, increasing PDW and P-LCR. Furthermore, it was highly effective in restoring ovarian cytoarchitecture, evidenced by the absence of cysts. Simvastatin monotherapy also improved ovarian morphology, reducing cystic structures, but showed no significant effect on platelet indices. Critically, the combination therapy presented a complex interaction, showing limited efficacy in resolving ovarian cysts and no significant improvement in platelet parameters compared to monotherapies. These findings suggest that while both drugs are beneficial, their mechanisms may not be synergistic when combined in this model.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Polycystic ovary syndrome (PCOS), also known as the Stein-Leventhal syndrome (Bednarska and Siejka, 2017). Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting reproductive-aged women worldwide, with a global prevalence of approximately 9.2% using combined diagnostic criteria, ranging from 5.5% (NIH criteria) to 11.5% (Rotterdam criteria) (Salari, *et al.*, 2024) . This complex, multifactorial condition has emerged as a significant public health concern, affecting an estimated 15% of females of reproductive age and representing one of the leading causes of female infertility (Lin *et al*., 2025). The syndrome is characterized by a collection of reproductive, metabolic, and psychological alterations hat impose substantial burden on affected women throughout their lifespan (Lin *et al*., Chen, 2025).

The pathophysiology of PCOS involves intricate interactions between hormonal dysregulation, metabolic dysfunction, and inflammatory processes (Houston and Templeman, 2025). Central to the syndrome is hyperandrogenism, which underlines many of the clinical manifestations including hirsutism, acne, and irregular menstrual cycles. Concurrently, insulin resistance affects up to 70% of women with PCOS, creating a self-perpetuating cycle where hyperinsulinemia exacerbates ovarian androgen production (Houston and Templeman, 2025). This metabolic dysfunction extends beyond glucose homeostasis, encompassing dyslipidemia, increased cardiovascular risk, and systemic inflammatory responses (Das *et al*., 2023).

Recent research has highlighted the role of chronic low-grade inflammation in PCOS pathogenesis, with evidence pointing to systemic inflammatory markers such as elevated C-reactive protein, increased neutrophil-lymphocyte ratios, and enhanced platelet reactivity (Velez *et al*., 2021). These inflammatory processes

contribute not only to metabolic complications but also to thrombotic risk and cardiovascular morbidity, positioning PCOS as a systemic disorder rather than merely a reproductive condition (Rajendran *et al*., 2008).

Chronic low-grade inflammation has been increasingly implicated in the endocrine, metabolic, and reproductive dysfunctions characteristic of polycystic ovary syndrome (PCOS) (Velez *et al*., 2021). Abdominal adiposity and obesity frequently observed in individuals with PCOS play a significant role in this inflammatory state (Özay and Özay, 2021). Emerging evidence highlights the immunological activity of adipose tissue, which participates in both innate and adaptive immune responses (Özay and Özay, 2021). Persistent secretion of proinflammatory mediators including cytokines, acute-phase proteins, and adipokines sustains systemic inflammation in obese women with PCOS and may contribute to insulin resistance and elevated long-term cardiometabolic risk (Chen *et al*., 2020). Additionally, genetic polymorphisms in genes encoding inflammatory regulators have been associated with PCOS pathogenesis, and their interaction with environmental exposures may underlie the syndrome's clinical heterogeneity (Velez *et al*., 2021).

The ovarian morphology in PCOS is characterized by multiple small follicular cysts, thickened tunica albuginea, and stromal hyperplasia (Takahashi *et al*., 1994). These structural changes reflect underlying disruptions in folliculogenesis, with arrested follicular development leading to anovulation and the characteristic polycystic appearance on ultrasonography (Mason, 2000) . The histological features include increased numbers of atretic follicles, hypertrophied theca cells, and reduced or absent corpus luteum formation (Mason, 2000).

Metformin is an orally administered antihyperglycemic agent widely recognized for the treatment of type 2 diabetes mellitus (T2DM) (Nestler, 2008) . Beyond its glucose-lowering properties, metformin has demonstrated significant benefits in improving insulin sensitivity in women with polycystic ovary syndrome (PCOS), even in the absence of overt diabetes (Nestler, 2008) . Metformin use has been associated with

improved menstrual regularity, a reduction in circulating androgen levels, and enhanced ovulatory function (Nestler, 2008) . These effects are largely attributed to the drug's ability to lower insulin resistance, thereby disrupting the hyperinsulinemia-driven stimulation of ovarian androgen production that contributes to anovulation and hyperandrogenic symptoms. Consequently, metformin serves not only as a metabolic regulator but also as a reproductive modulator in the management of PCOS (Nestler, 2008) . Metformin remains one of the most widely utilized pharmacologic agents in the current management of polycystic ovary syndrome (PCOS) (Guan *et al*., 2020). It exerts therapeutic effects by modulating metabolic activity in hepatic, muscular, and adipose tissues, thereby improving insulin sensitivity and attenuating hyperandrogenic symptoms (Gao, *et al*., 2020).

In addition to metformin, statins have emerged as a complementary therapeutic option in the management of PCOS (Bednarska and Siejka, 2017). These lipid-lowering agents have demonstrated benefits in reducing cardiovascular morbidity and mortality, lowering circulating androgen levels, improving lipid profiles, and enhancing endothelial function (Bednarska and Siejka, 2017).. Furthermore, their anti-inflammatory and antioxidant properties confer additional cardiovascular protection in women with PCOS (Bednarska and Siejka, 2017).

The co-administration of metformin and simvastatin presents a synergistic approach to the treatment of polycystic ovary syndrome (PCOS), targeting its metabolic and endocrine abnormalities through complementary mechanisms (Liu *et al*., 2021). Metformin primarily enhances insulin sensitivity by inhibiting hepatic gluconeogenesis, promoting peripheral glucose uptake, and improving insulin signaling pathways (Banaszewska *et al*., 2019). These effects contribute to the attenuation of hyperinsulinemia, which in turn reduces ovarian androgen synthesis, a central pathogenic feature of PCOS. Simvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, lowers circulating low-density lipoprotein cholesterol (LDL-C) and has been shown to reduce serum androgen levels, likely through suppression of

ovarian theca cell steroidogenesis and improvement of insulin sensitivity(Liu *et al*., 2021). Additionally, both agents exert anti-inflammatory effects: metformin via activation of AMP-activated protein kinase (AMPK), and simvastatin through inhibition of proinflammatory cytokines and oxidative stress pathways. Together, this pharmacologic combination may provide additive or synergistic benefits in regulating glycemic control, lipid metabolism, and hormonal imbalances, thereby offering a comprehensive therapeutic strategy for improving clinical outcomes in women with PCOS (Banaszewska *et al*., 2019).

1.2 AIM

The aim of this study is to evaluate and compare the effects of simvastatin and metformin, as both monotherapies and in combination, on platelet function and ovarian histology in a letrozole-induced Sprague Dawley rat model of Polycystic Ovarian Syndrome.

1.3 RESEARCH QUESTION

What is the effect of simvastatin monotherapy on platelet function and ovarian histology in a rat model of PCOS?

What is the effect of metformin monotherapy on platelet function and ovarian histology in a rat model of PCOS?

Does the combination of simvastatin and metformin produce a greater therapeutic effect on platelet function and ovarian histology than either drug administered alone?

1.4 JUSTIFICATION OF STUDY

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age worldwide, with a substantial burden on their reproductive, metabolic, and psychological health. While traditional treatment focuses on symptoms like irregular periods and infertility, it is now clear that PCOS is a systemic condition with serious long-term health risks, particularly concerning cardiovascular health.

Women with PCOS have a significantly higher risk of developing cardiovascular diseases. A key factor in this increased risk is abnormal platelet function. Platelets, the blood cells responsible for clotting, become hyperactive in PCOS, leading to a greater tendency for blood clot formation and atherosclerosis. However, this aspect of PCOS is often overlooked in standard therapy. This study is justified by the urgent need to investigate how common PCOS medications can directly influence this platelet dysfunction to reduce future cardiovascular complications.

1.5 SPECIFIC OBJECTIVES

1. To analyze ovarian histological changes following individual and combined drug treatments, including assessment of follicular development, atresia patterns, corpus luteum formation, stromal changes, and tunica albuginea thickness.

2. To examine the correlation between platelet function improvements and ovarian histological restoration to understand the mechanistic relationships between cardiovascular and reproductive benefits of the treatments.
3. To evaluate the effect of simvastatin monotherapy on platelet function indices and the histological characteristics of the ovary in the PCOS rat model.

4. To evaluate the effect of metformin monotherapy on platelet function indices and the histological characteristics of the ovary in the PCOS rat model.

5. To determine if combined therapy with simvastatin and metformin has an ameliorative effect on ovary in the PCOS rat model.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition and Overview of Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome (PCOS) represents the most prevalent endocrine disorder affecting women of reproductive age worldwide, with significant implications for reproductive, metabolic, and cardiovascular health (Attia *et al*., 2023). The syndrome was first described in 1935 by Stein and Leventhal, who associated the presence of ovarian cysts with anovulation, obesity, and hirsutism (Zoppini *et al*., 2014). Since its initial description, understanding of PCOS has evolved considerably, transforming from a primarily reproductive disorder to recognition as a complex, systemic condition with far-reaching health consequences.

PCOS is fundamentally characterized by chronic anovulation, clinical or biochemical evidence of hyperandrogenism, and polycystic ovarian morphology on ultrasonography (Christ and Cedars, 2023). However, the syndrome encompasses a much broader spectrum of abnormalities, including insulin resistance, dyslipidemia, chronic inflammation, and increased cardiovascular risk (Das *et al*., 2023). This heterogeneous presentation has led to recognition of PCOS as a syndrome rather than a single disease entity, with multiple phenotypes exhibiting varying combinations of reproductive and metabolic features (Christ and Cedars, 2023).

The complexity of PCOS extends beyond its clinical manifestations to encompass its underlying pathophysiology. The syndrome involves dysregulation of the hypothalamic-pituitary-ovarian axis, peripheral insulin action, adipose tissue function, and inflammatory pathways (Velez *et al*., 2021). These interconnected abnormalities create self-perpetuating cycles that maintain and exacerbate the syndrome's features, making PCOS a chronic condition requiring long-term management strategies (Velez *et al*., 2021).

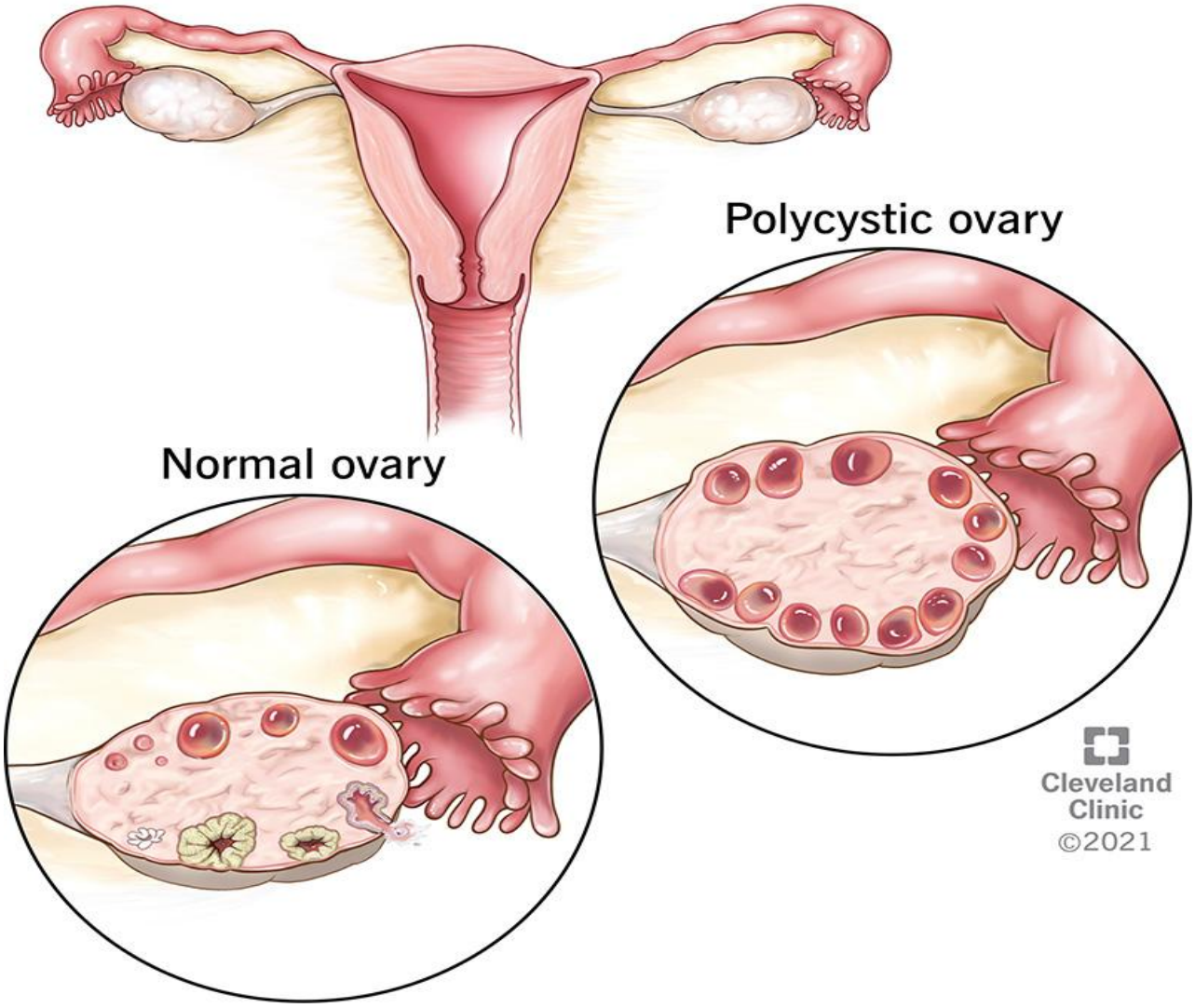

Table 2. 1: Polycystic ovary syndrome (PCOS).

Source: **ClevelandClinic.com**

2.1.1 Diagnostic Criteria And Classification of Polycystic ovary syndrome (PCOS)

The diagnostic approach to PCOS has evolved through several iterations, reflecting improved understanding of the syndrome's heterogeneous nature. The 1990 National Institutes of Health (NIH) criteria established the

foundation for PCOS diagnosis, requiring both oligo-ovulation or anovulation and clinical or biochemical evidence of hyperandrogenism, with exclusion of other disorders. These criteria emphasized the androgenic aspects of the syndrome but potentially missed patients with milder phenotypes. Polycystic ovary syndrome (PCOS) is estimated to affect approximately 3–15% of women globally, making it one of the most common endocrine disorders among females of reproductive age (Bednarska and Siejka, 2017).. While the primary etiological factor lies in ovarian dysfunction, the clinical manifestation of PCOS is influenced by a range of contributing factors, including obesity and environmental exposures. The most widely adopted diagnostic framework is the Rotterdam criteria (2003), which stipulates that a diagnosis of PCOS is confirmed when at least two of the following three criteria are met: (1) clinical and/or biochemical evidence of hyperandrogenism; (2) ovulatory dysfunction, including oligovulation or anovulation; and (3) polycystic ovarian morphology, defined as the presence of 12 or more follicles in a single ovary or an ovarian volume exceeding 10 mL (Bednarska and Siejka, 2017). According to the Rotterdam criteria, polycystic ovary syndrome (PCOS) can be classified into four distinct phenotypes based on the combination of clinical features present: (1) the classic phenotype (HOP), characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology on ultrasound; (2) the HO phenotype, involving hyperandrogenism and ovulatory dysfunction, but with normal ovarian morphology on ultrasound; (3) the HP phenotype, defined by the presence of hyperandrogenism and polycystic ovaries on ultrasound, in the absence of ovulatory irregularities; and (4) the OP phenotype, in which ovulatory dysfunction and polycystic ovarian morphology are present, but without clinical or biochemical evidence of hyperandrogenism (Milewicz, 2013) .The 2003 Rotterdam consensus expanded the diagnostic criteria significantly, requiring any two of three features: oligo-anovulation, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasonography (Christ and Cedars, 2023). This expansion increased the estimated prevalence from 4-6.6% to 21% among women of reproductive age, as it included women with ovulatory PCOS (hyperandrogenism plus polycystic ovaries) and non-hyperandrogenic

PCOS (oligo-ovulation plus polycystic ovaries) (Christ and Cedars, 2023). The Rotterdam criteria define polycystic ovaries as containing ≥12 follicles measuring 2-9 mm in diameter in each ovary or increased ovarian volume ≥10 mL (Christ and Cedars, 2023).. Recent updates have increased this threshold to ≥20 follicles per ovary using transvaginal ultrasonography with transducers ≥8 MHz, reflecting improvements in ultrasound technology (Christ and Cedars, 2023). Clinical hyperandrogenism is typically assessed using the modified Ferriman-Gallwey score, with thresholds varying by ethnicity (≥4-8 depending on population). The Androgen Excess and PCOS Society (AE-PCOS) proposed alternative criteria in 2006, emphasizing hyperandrogenism as a mandatory component while requiring evidence of ovarian dysfunction (oligo-ovulation and/or polycystic ovaries).

2.2 PATHOPHYSIOLOGY OF POLYCYSTIC OVARY SYNDROME (PCOS)

2.2.1 Endocrine Profile

The endocrine profile of polycystic ovary syndrome (PCOS) is marked by intricate disturbances across multiple hormonal axes, resulting in a collection of dysregulations that maintain and exacerbate the syndrome's clinical features (Houston and Templeman, 2025). Central to these abnormalities is disruption of the hypothalamic-pituitary-ovarian (HPO) axis, characterized by increased luteinizing hormone (LH) secretion relative to follicle-stimulating hormone (FSH), impaired ovarian steroidogenesis, and resistance to peripheral hormone signaling. These alterations collectively contribute to hyperandrogenism, anovulation, and the broader reproductive and metabolic phenotype observed in PCOS (Houston and Templeman, 2025).

A fundamental aspect of polycystic ovary syndrome (PCOS) pathophysiology is the disruption of normal folliculogenesis, leading to the accumulation of small antral follicles and impaired selection of a dominant follicle (Mason, 2000) . This aberrant process is mediated by complex interactions among gonadotropins (notably elevated LH and relatively low FSH), intraovarian growth factors, and metabolic cues such as insulin and androgens, regulating follicular development and atresia (Mason, 2000). The premature arrest of follicular maturation contributes both to the characteristic polycystic ovarian morphology and to chronic anovulation, perpetuating the hormonal dysfunction that define the syndrome (Mason, 2000).

The temporal development of endocrine abnormalities in PCOS suggests that hyperinsulinemia may represent an early defect that precedes and contributes to other hormonal disturbances (Houston and Templeman, 2025). This perspective challenges the traditional view of insulin resistance as a compensatory response to metabolic dysfunction, instead positioning hyperinsulinemia as a primary driver of PCOS pathogenesis. The bidirectional relationship between insulin and androgen levels creates amplifying cycles that progressively worsen both metabolic and reproductive dysfunction (Houston and Templeman, 2025).

Hyperandrogenism

Hyperandrogenism represents the hallmark endocrine feature of PCOS, present in the majority of affected women and directly responsible for many characteristic clinical manifestations (Mason, 2000) . Elevated androgen levels arise from multiple sources, including increased ovarian production, enhanced peripheral conversion from precursors, and reduced hepatic clearance through decreased sex hormone-binding globulin synthesis (Houston and Templeman, 2025). Ovarian androgen excess in PCOS primarily stems from theca cell dysfunction, characterized by increased expression of steroidogenic enzymes including CYP11A (cholesterol side-chain cleavage) and CYP17 (17α-hydroxylase/17,20-desmolase) (Mason, 2000) . These enzymatic abnormalities result in enhanced conversion of cholesterol to androgens, with individual theca cells from PCOS ovaries producing significantly greater androgen amounts compared to normal controls (Banaszewska *et al*., 2019). The increased enzyme expression appears to represent an intrinsic defect rather than solely a response to external stimuli (Mason, 2000) . Insulin plays a crucial role in amplifying ovarian androgen production through direct effects on theca cells (Houston and Templeman, 2025). Hyperinsulinemia enhances LH-stimulated androgen synthesis and may directly stimulate steroidogenic enzyme expression. Additionally, insulin reduces hepatic production of sex hormone-binding globulin, increasing the proportion of bioactive free androgens in circulation. This insulin-androgen interaction creates positive feedback loops that perpetuate hyperandrogenism even in the presence of appropriate therapeutic interventions (Houston and Templeman, 2025).

The peripheral effects of hyperandrogenism extend beyond cosmetic concerns to encompass metabolic and cardiovascular consequences (Gao, *et al*., 2023). Androgens promote visceral adiposity, insulin resistance, and dyslipidemia while also affecting vascular function and inflammatory status. These systemic effects position hyperandrogenism not merely as a reproductive abnormality but as a contributor to the increased cardiovascular risk observed in PCOS patients (Gao, *et al*., 2023).

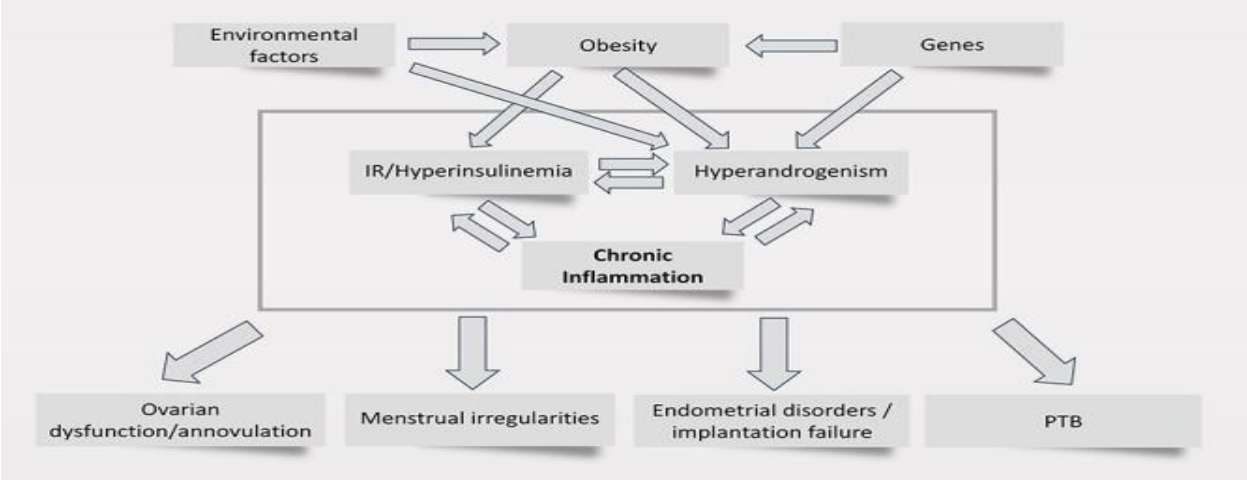

Table 2. 2: Proposed mechanisms of interactions between IR, hyperandrogenism and chronic inflammation in PCOS, and relation to reproductive outcomes. Environmental and genetic factors may predispose to obesity. These risk factors in turn favor the establishment of hyperandrogenism and IR/hyperinsulinemia. Cross-talk between IR and hyperandrogenism may recreate a welcoming environment to chronic inflammation. Finally, the combination and cross-talk of chronic inflammation, IR and hyperandrogenism may drive many of the pathological reproductive outcomes in PCOS (Velez *et al*., 2021).

LH/FSH Imbalance

Luteinizing hormone and follicle-stimulating hormone dysregulation represents a central feature of PCOS pathophysiology, with characteristic elevation of LH levels and relative FSH deficiency (Takahashi *et al*., 1994). This gonadotropin imbalance contributes significantly to ovarian dysfunction, hyperandrogenism, and anovulation. The elevated LH:FSH ratio, typically >2:1 in PCOS patients, reflects altered hypothalamic-pituitary sensitivity and feedback mechanisms (Takahashi *et al*., 1994). The mechanisms underlying LH hypersecretion in PCOS involve multiple factors including altered GnRH pulse frequency, enhanced pituitary responsiveness to GnRH, and disrupted feedback inhibition by ovarian hormones (Takahashi *et al*., 1994). Hyperinsulinemia may directly affect hypothalamic GnRH neurons, increasing pulse frequency and amplitude. Additionally, the lack of regular ovulation eliminates the normal progesterone-mediated negative feedback, allowing continued LH elevation (Takahashi *et al*., 1994). Elevated LH levels directly stimulate theca cell androgen production while simultaneously disrupting normal folliculogenesis (Takahashi *et al*., 1994). The inappropriate LH stimulation of small follicles leads to premature luteinization without ovulation, contributing to both hyperandrogenism and anovulation (Takahashi *et al*., 1994). Furthermore, high LH levels may impair oocyte quality through direct effects on granulosa cells and cumulus cell-oocyte communication (Takahashi *et al*., 1994).

The relative FSH deficiency in PCOS impairs follicular development and estrogen production (Mason, 2000). Insufficient FSH stimulation prevents normal granulosa cell proliferation and aromatase activity, limiting the conversion of androgens to estrogens within developing follicles (Mason, 2000) . This creates a local hyperandrogenic environment that further compromises follicular development and contributes to follicular atresia (Mason, 2000).

2.2.2 Metabolic Disturbances

PCOS is characterized by profound metabolic dysfunction affecting glucose homeostasis, lipid metabolism, and energy balance (Lin *et al*., 2025). These abnormalities contribute not only to immediate health concerns but also to long-term risks including type 2 diabetes, cardiovascular disease, and metabolic syndrome. The metabolic features of PCOS often appear early in the disease course and may precede reproductive abnormalities in some patients (Das *et al*., 2023).

Insulin Resistance

Insulin resistance affects approximately 65-70% of women with PCOS, regardless of body weight, though it is more severe in obese patients. The mechanisms underlying insulin resistance in PCOS are multifactorial, involving post-receptor signaling defects, chronic inflammation, and altered adipokine secretion. These abnormalities affect multiple tissues including skeletal muscle, liver, and adipose tissue, creating systemic metabolic dysfunction (Houston and Templeman, 2025).

The molecular basis of insulin resistance in PCOS involves defects in insulin signaling pathways, particularly at the level of insulin receptor substrate-1 (IRS-1) phosphorylation and downstream PI3K/Akt signaling (Houston and Templeman, 2025). Chronic inflammation and oxidative stress contribute to these signaling defects through activation of stress kinases and inflammatory pathways. Additionally, elevated androgen levels may directly impair insulin sensitivity through effects on muscle glucose uptake and adipose tissue function. Compensatory hyperinsulinemia develops in response to insulin resistance, creating additional pathophysiological consequences. Elevated insulin levels directly stimulate ovarian androgen production, enhance LH secretion, and reduce sex hormone-binding globulin synthesis. This hyperinsulinemia-hyperandrogenism cycle perpetuates both metabolic and reproductive dysfunction, making it a crucial target for therapeutic intervention (Houston and Templeman, 2025).

Dyslipidemia

Lipid abnormalities are present in 60-70% of women with PCOS, contributing significantly to cardiovascular risk (Liu *et al*., 2021). The characteristic dyslipidemic pattern includes elevated triglycerides, reduced HDL cholesterol, increased small dense LDL particles, and elevated total cholesterol levels. These abnormalities often occur independently of body weight and appear related to both insulin resistance and hyperandrogenism (Das *et al*., 2023).

The pathogenesis of dyslipidemia in PCOS involves multiple mechanisms including enhanced hepatic VLDL production, altered lipoprotein lipase activity, and changes in cholesterol metabolism (Das *et al*., 2023). Insulin resistance promotes hepatic triglyceride synthesis and VLDL secretion while simultaneously impairing peripheral triglyceride clearance. Hyperandrogenism further exacerbates these abnormalities through direct effects on hepatic lipid metabolism and lipoprotein synthesis (Das *et al*., 2023). The clinical significance of dyslipidemia in PCOS extends beyond traditional cardiovascular risk assessment to encompass effects on ovarian function and fertility (Das *et al*., 2023). Altered lipid metabolism may directly affect steroidogenesis, given that cholesterol serves as the precursor for all steroid hormones. Additionally, dyslipidemia contributes to systemic inflammation and oxidative stress, further exacerbating PCOS pathophysiology (Das *et al*., 2023).

2.3 **Ovarian Morphology**

The characteristic ovarian morphology in PCOS reflects the underlying disruptions in folliculogenesis and hormonal regulation (Takahashi *et al*., 1994). Polycystic ovaries typically demonstrate increased size, with volumes often exceeding 10 mL, and contain multiple small follicular cysts arranged peripherally beneath a thickened tunica albuginea. These structural changes represent the cumulative effects of chronic anovulation, altered gonadotropin stimulation, and local growth factor dysregulation (Takahashi *et al*., 1994).

The key feature of polycystic ovaries is the presence of multiple small follicles, typically 2-9 mm in diameter, arrested at various stages of development (Mason, 2000) . These cystic follicles represent arrested antral follicles that have failed to undergo either ovulation or normal atresia. The number of follicles typically exceeds 12 per ovary (or ≥20 with newer ultrasound technology), creating the characteristic "string of pearls" appearance on ultrasonography (Christ and Cedars, 2023).

Histological examination reveals that these cystic structures are primarily functional follicles rather than atretic cysts, particularly in anovulatory PCOS (Mason, 2000) . The granulosa cells often show signs of premature luteinization despite the absence of ovulation, suggesting inappropriate LH stimulation (Mason, 2000) . This premature luteinization contributes to altered steroidogenesis and may explain the characteristic hormonal profile observed in PCOS patients (Mason, 2000) . The tunica albuginea demonstrates significant thickening in PCOS ovaries, with capsular thickness often 2-5 times greater than normal (Takahashi *et al*., 1994). This thickening appears to result from increased collagen deposition and may contribute to mechanical impedance of ovulation. The relationship between capsular thickness and other PCOS features suggests that tunica albuginea changes may be both a consequence of chronic anovulation and a contributing factor to ongoing ovulatory dysfunction (Takahashi *et al*., 1994).

Stromal hyperplasia represents another characteristic feature of PCOS ovaries, with increased stromal cell density and enhanced androgen production capacity (Mason, 2000) . The hyperplastic stroma demonstrates increased vascularity and contains elevated numbers of luteinized theca cells, contributing to local hyperandrogenism (Mason, 2000) . This stromal hyperplasia may result from chronic LH stimulation and appears to correlate with the severity of biochemical hyperandrogenism (Mason, 2000).

2.4 **Simvastatin and Its Role in PCOS**

Simvastatin belongs to the class of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, which represent the cornerstone of lipid-lowering therapy (Banaszewska *et al.,* 2019). The drug undergoes hepatic metabolism to its active β-hydroxyacid form, which demonstrates potent and selective inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. This mechanism of action has implications beyond lipid reduction, affecting multiple cellular processes that may be relevant to PCOS pathophysiology.

The primary mechanism of simvastatin action involves competitive inhibition of HMG-CoA reductase through binding to the enzyme's active site. The activated form of simvastatin demonstrates high affinity for the substrate binding pocket, effectively blocking the conversion of HMG-CoA to mevalonate, the precursor for cholesterol synthesis. This inhibition occurs preferentially in hepatic tissue due to the drug's pharmacokinetic properties and first-pass metabolism (Banaszewska *et al.,* 2019). HMG-CoA reductase inhibition affects multiple cellular pathways beyond cholesterol synthesis, given that mevalonate serves as the precursor for several important cellular mediators. The mevalonate pathway produces isoprenoid intermediates including farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are essential for

protein prenylation. These post-translational modifications are crucial for the function of various proteins involved in cell signaling, proliferation, and inflammatory responses. In the context of PCOS, HMG-CoA reductase inhibition may affect ovarian steroidogenesis by limiting cholesterol availability for hormone synthesis. Since cholesterol serves as the precursor for all steroid hormones, including androgens, reducing cholesterol synthesis could theoretically decrease androgen production. However, the clinical relevance of this mechanism in vivo remains debated, as compensatory mechanisms may maintain cholesterol supply for steroidogenesis (Banaszewska *et al.,* 2019).

Simvastatin demonstrates significant anti-inflammatory properties that extend beyond its cholesterol-lowering effects. These pleiotropic effects involve multiple mechanisms including inhibition of inflammatory cytokine production, reduction of acute-phase reactants, and improvement of endothelial function. In PCOS patients, these anti-inflammatory effects may contribute to improvement in both metabolic and reproductive parameters (Barale *et al.,* 2018). The anti-inflammatory actions of simvastatin involve modulation of nuclear factor-κB (NF-κB) signaling pathways, leading to reduced expression of inflammatory genes. Additionally, statins affect the production of inflammatory mediators such as C-reactive protein, interleukin-6, and tumor necrosis factor-α. These effects may be particularly relevant in PCOS, given the chronic inflammatory state that characterizes the syndrome (Barale *et al.,* 2018). Direct anti-androgenic effects of simvastatin have been demonstrated both in vitro and in clinical studies. The mechanisms underlying these effects may involve direct inhibition of steroidogenic enzymes, reduction of LH-stimulated androgen production, or effects on androgen metabolism and clearance. Clinical studies consistently demonstrate reductions in total and free testosterone levels following simvastatin treatment in PCOS patients. The molecular mechanisms of simvastatin's anti-androgenic effects may involve inhibition of theca cell proliferation and steroidogenesis. In vitro studies using rat and human theca cells demonstrate that statins reduce cell proliferation, increase apoptosis, and inhibit

testosterone production. These effects appear to be independent of cholesterol depletion and may involve direct effects on steroidogenic enzyme activity or gene expression (Barale *et al.,* 2018).

2.4.1 Effects on Platelet Function

Simvastatin demonstrates significant antiplatelet effects that contribute to its cardiovascular protective properties. These effects involve multiple mechanisms including direct inhibition of platelet activation pathways, improvement of endothelial function, and reduction of inflammatory mediators that promote platelet reactivity. In PCOS patients, these antiplatelet effects may help address the platelet dysfunction that contributes to increased cardiovascular risk (Banaszewska *et al.,* 2019).

Studies demonstrate that simvastatin treatment significantly reduces platelet aggregation responses to various agonists including ADP, collagen, and arachidonic acid. These effects appear to be independent of cholesterol reduction and may involve direct effects on platelet membrane composition and signaling pathways. The antiplatelet effects of simvastatin develop relatively rapidly, often within 2-4 weeks of treatment initiation (Banaszewska *et al.,* 2019). The mechanisms underlying simvastatin's antiplatelet effects involve activation of peroxisome proliferator-activated receptors (PPARs) in platelets (Ali, *et al*., 2009). PPAR activation leads to inhibition of protein kinase C and increased cyclic adenosine monophosphate (cAMP) levels, both of which suppress platelet activation. Additionally, simvastatin may affect platelet membrane cholesterol content, altering membrane fluidity and receptor function (Ali, *et al*., 2009).

Thromboxane A2 synthesis is significantly reduced following simvastatin treatment, contributing to decreased platelet aggregation and vasoconstriction. This effect involves inhibition of cyclooxygenase activity and reduced arachidonic acid availability for prostaglandin synthesis. The reduction in thromboxane production

may be particularly beneficial in PCOS patients, who demonstrate elevated thromboxane levels and enhanced platelet reactivity (Banaszewska *et al.,* 2019). Simvastatin treatment also improves platelet sensitivity to antiplatelet agents such as aspirin. This enhanced sensitivity may result from improved endothelial function, reduced inflammatory mediators, or direct effects on platelet signaling pathways. The combination of intrinsic antiplatelet effects with enhanced sensitivity to other antiplatelet agents may provide superior cardiovascular protection (Ali, *et al.*, 2009).

2.4.2 Effects on Ovarian Histology

Clinical and experimental studies demonstrate that simvastatin treatment produces beneficial effects on ovarian morphology in PCOS patients and animal models. These effects include reduction in ovarian volume, improvement in follicular development, and restoration of more normal ovarian architecture. The histological improvements often correlate with biochemical improvements in hormonal profiles and metabolic parameters (Banaszewska *et al.,* 2019).

Simvastatin treatment leads to progressive reduction in ovarian volume and cystic features in PCOS patients. This improvement appears to result from reduced follicular arrest and improved follicular atresia, leading to resolution of accumulated cystic structures. The time course of these improvements suggests ongoing effects on follicular development rather than acute changes in existing structures (Banaszewska *et al.,* 2019). Histological studies in animal models demonstrate that simvastatin treatment reduces the number of cystic follicles and improves overall ovarian architecture. The mechanism involves effects on theca cell proliferation and function, with statins reducing theca cell hyperplasia and androgen production. Additionally, simvastatin may improve granulosa cell function and survival, promoting normal follicular development (Banaszewska *et al.,* 2019). The reduction in ovarian hyperplasia following simvastatin treatment appears to involve multiple

mechanisms including direct effects on cell proliferation, improved apoptotic pathways, and reduced inflammatory stimuli. Theca cell hyperplasia, a characteristic feature of PCOS ovaries, shows particular responsiveness to statin treatment. This may result from the dependence of theca cell proliferation on cholesterol synthesis and isoprenoid production (Banaszewska *et al.,* 2019).

2.5 METFORMIN AND ITS ROLE IN PCOS

Metformin, a biguanide derivative, represents the most widely studied and utilized therapeutic agent for PCOS management beyond its primary indication for type 2 diabetes (Attia *et al.,* 2023). The drug's mechanism of action in PCOS involves complex interactions with cellular energy metabolism, hormonal regulation, and inflammatory pathways. Understanding these mechanisms is crucial for optimizing its therapeutic application and identifying patients most likely to benefit from treatment (Attia *et al.,* 2023).

The primary mechanism underlying metformin's therapeutic effects involves activation of AMP-activated protein kinase (AMPK), a cellular energy sensor that responds to changes in cellular energy status. Metformin inhibits mitochondrial respiratory chain complex I, creating a state of cellular energy stress that mimics starvation conditions. This leads to decreased ATP production and increased AMP:ATP ratios, which directly activate AMPK through allosteric mechanisms (Attia *et al.,* 2023). AMPK activation triggers multiple downstream effects that contribute to metformin's therapeutic benefits in PCOS. These include enhanced glucose uptake through increased GLUT4 translocation, increased fatty acid oxidation, inhibition of gluconeogenesis, and suppression of lipogenesis. Additionally, AMPK activation affects gene expression through phosphorylation of transcriptional regulators, leading to long-term metabolic adaptations (Chen *et al.,* 2020). The relevance of AMPK activation to PCOS pathophysiology extends beyond metabolic effects to encompass reproductive and inflammatory functions. AMPK signaling affects ovarian function through

regulation of steroidogenesis, follicular development, and cell survival pathways. The enzyme is expressed in ovarian tissues and responds to metabolic signals, making it a potential mediator of the metabolic-reproductive interactions characteristic of PCOS (Attia *et al.,* 2023).

Metformin's insulin-sensitizing effects represent a cornerstone of its therapeutic utility in PCOS. The drug improves insulin sensitivity through multiple mechanisms including enhanced peripheral glucose uptake, reduced hepatic glucose production, and improved insulin signaling pathways (Attia *et al.,* 2023). These effects help break the cycle of hyperinsulinemia and hyperandrogenism that perpetuates PCOS pathophysiology. The molecular mechanisms of metformin's insulin-sensitizing effects involve both direct and indirect pathways (Chen *et al.,* 2020). Direct effects include AMPK-mediated enhancement of glucose transport and improvement of insulin receptor signaling. Indirect effects involve reduction of inflammatory mediators, improvement of endothelial function, and modulation of adipokine production from adipose tissue (Chen *et al.,* 2020). In PCOS patients, metformin's insulin-sensitizing effects typically result in 20-40% reductions in fasting insulin levels and significant improvements in insulin resistance indices. These metabolic improvements often translate into reproductive benefits including restoration of ovulation, improvement in menstrual regularity, and reduction in androgen levels. The dose-response relationship suggests that higher doses may provide superior benefits, though tolerability must be considered.

2.5.1 Effects on Platelet Function

Metformin demonstrates significant effects on platelet function and cardiovascular risk that may be particularly relevant for PCOS patients. These effects involve both direct actions on platelets and indirect effects through improvement of metabolic and inflammatory parameters. Understanding these platelet effects is important for assessing metformin's cardiovascular protective potential in PCOS (Chen *et al.,* 2020).

Metformin treatment leads to significant improvements in endothelial function through multiple mechanisms including enhanced nitric oxide bioavailability, reduced oxidative stress, and improved endothelial cell metabolism (Attia *et al.,* 2023). These effects are mediated partly through AMPK activation, which enhances endothelial nitric oxide synthase (eNOS) phosphorylation and activity. The resulting increase in nitric oxide production improves vascular function and provides antiplatelet effects The endothelial protective effects of metformin appear early in treatment and may precede metabolic improvements. These effects include improved flow-mediated vasodilation, reduced endothelial inflammatory marker expression, and enhanced endothelial progenitor cell function. In PCOS patients, who commonly demonstrate endothelial dysfunction, these effects may contribute to cardiovascular risk reduction (Di Pietro, *et al.*, 2015).

Metformin demonstrates significant antioxidant properties that contribute to reduced platelet reactivity and improved cardiovascular function. The drug reduces reactive oxygen species production through effects on mitochondrial metabolism and enhances cellular antioxidant defenses. These antioxidant effects help preserve platelet membrane integrity and reduce activation by oxidative stimuli. Clinical studies demonstrate that metformin treatment reduces platelet aggregation responses and improves platelet sensitivity to antiplatelet agents. These effects appear to be mediated through AMPK activation and may involve direct effects on platelet metabolism and signaling pathways. The antiplatelet effects of metformin may contribute to its cardiovascular protective benefits beyond glucose-lowering effects (Attia *et al.,* 2023).

2.5.2 Effects on Ovarian Morphology

Metformin treatment produces significant improvements in ovarian morphology in both PCOS patients and animal models (Di Pietro, *et al*., 2015) . These effects include reduction in cystic follicles, improvement in follicular development, and restoration of more normal ovarian architecture. The morphological

improvements often correlate with hormonal and metabolic improvements, suggesting mechanistic relationships between these effects (Di Pietro, *et al*., 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

- Oral gavage needles
- Syringes (5ml and 10ml)
- EDTA bottles
- Plastic cages
- Sawdust
- Feed
- Water
- Plates
- Latex gloves
- Chloroform
- Dissecting materiasl
- Saline
- Letrozole
- Simvastatin
- Metformin
- Weighing scale

3.2 Study Design

This study was an experimental animal study designed to investigate the effects of Simvastatin and Metformin on platelet function and ovarian histology in a rat model of Polycystic Ovary Syndrome (PCOS).

3.3 Experimental Animals

- **Species and Strain:** Twenty-five (25) healthy female **Sprague Dawley** rats were used.
- **Weight:** The rats weighed between **124g and 142g** at the beginning of the study.
- **Housing Conditions:** The animals were housed in standard laboratory cages under controlled conditions of temperature (22±2°C), humidity (50-60%), and a 12-hour light/dark cycle. They had free access to standard rat chow and water.
- **Acclimatization:** The rats were allowed to acclimatize to the laboratory environment for one week before the commencement of the experiment.

3.4 Induction of PCOS Model

Polycystic Ovary Syndrome was induced using Letrozole.

Procedure: A 10 mg Letrozole tablet was dissolved in 10 ml of normal saline (0.9% Sodium Chloride) to achieve a concentration of 1 mg/ml.

Dosage and Administration: The rats (excluding the negative control group) received Letrozole orally at a dose of **1 mg/kg body weight** daily for 28 consecutive days.

Weekly Weighing: All rats were weighed weekly to adjust the dosage according to any changes in body weight.

3.5 Experimental Grouping

The twenty-five rats were randomly divided into five (5) groups, with five (5) rats in each group (n=5).

1. **Group A (Negative Control):** Received normal saline only for 28 days.

2. **Group B (Positive Control - PCOS Untreated):** Received Letrozole (1 mg/kg) for 28 days to induce PCOS, but no treatment.
3. **Group C (PCOS + Simvastatin):** Received Letrozole for 28 days, followed by **Simvastatin monotherapy**.
4. **Group D (PCOS + Metformin):** Received Letrozole for 28 days, followed by **Metformin monotherapy**.
5. **Group E (PCOS + Combination):** Received Letrozole for 28 days, followed by a combination of **Simvastatin and Metformin**.

3.6 Sample Collection

At the end of the 28-day treatment period, samples were collected as follows:

Anaesthesia: Rats were humanely anaesthetized using chloroform vapor in a sealed chamber.

Blood Collection: Blood was collected via **cardiac puncture** and transferred into Serum Separator Tubes (SST) for biochemical analysis.

Ovary Excision: Immediately after blood collection, a midline abdominal incision was made. Both ovaries were carefully excised, trimmed of adhering fat, blotted dry, and weighed.

Tissue Preservation: The ovarian tissues were immediately fixed in **10% neutral buffered formalin** for histological processing.

3.7 Laboratory Analysis

3.7.1 Biochemical Analysis (Platelet Function)

- **Parameters Measured:** Platelet indices were analyzed within one hour of collection using an automated hematological analyzer. The indices included:

    a) Platelet Count (PLT)

b) Mean Platelet Volume (MPV)

c) Platelet Distribution Width (PDW)

d) Plateletcrit (PCT)

e) Platelet-Large Cell Ratio (P-LCR)

3.7.2 Histological Analysis (Ovarian Morphology)

The formalin-fixed ovarian tissues were processed for standard histological examination:

1. **Tissue Processing:** Tissues were dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax.
2. **Sectioning:** Serial sections of **5 µm thickness** were cut using a rotary microtome.
3. **Staining:** The sections were mounted on slides and stained with **Haematoxylin and Eosin (H&E)**.
4. **Examination:** The stained tissue sections were examined under a light microscope for histological changes such as cystic follicles, corpora lutea, and stromal hyperplasia.

3.8 Statistical Analysis

All data were analyzed using the **Statistical Package for the Social Sciences (SPSS)** software. Results were expressed as **Mean ± Standard Error of the Mean (SEM)**. A probability value of $\mathbf{p < 0.05}$ was considered statistically significant. The specific statistical test used, One-way ANOVA followed by a post-hoc test like Tukey, was employed to compare differences between the experimental groups.

CHAPTER FOUR

4.0 RESULTS

Table 4. 1: Comparing the mean values of platelets cell indices in polycystic ovarian syndromes (PCOS) induced rat following the treatment of simvastatin and metformin.

Parameters	Control	PCOS	PCOS + Simvastatin	PCOS + Metformin	PCOS + Simvastatin + Metformin
Platelets count ($x10^3/\mu l$)	549.4 ± 94.45	542.4 ± 40.17	514.8 ± 31.57	600.2 ± 16.95	548.6 ± 50.04
MPV (fL)	7.660 ± 0.18	7.580 ± 0.14	8.020 ± 0.18	7.940 ± 0.12	7.960 ± 0.25
PDW	9.780 ± 0.57	9.660 ± 0.29	10.28 ± 0.16	10.64 ± 0.18	10.56 ± 0.55
PCT (%)	0.4200 ± 0.08	0.4120 ± 0.03	0.4080 ± 0.03	0.4480 ± 0.02	0.4340 ± 0.05
P-LCR	6.340 ± 2.19	4.848 ± 1.43	8.300 ± 1.03	8.740 ± 0.79	8.520 ± 2.45

*$P < 0.05$ indicates significant difference treated group is compared with PCOS untreated group

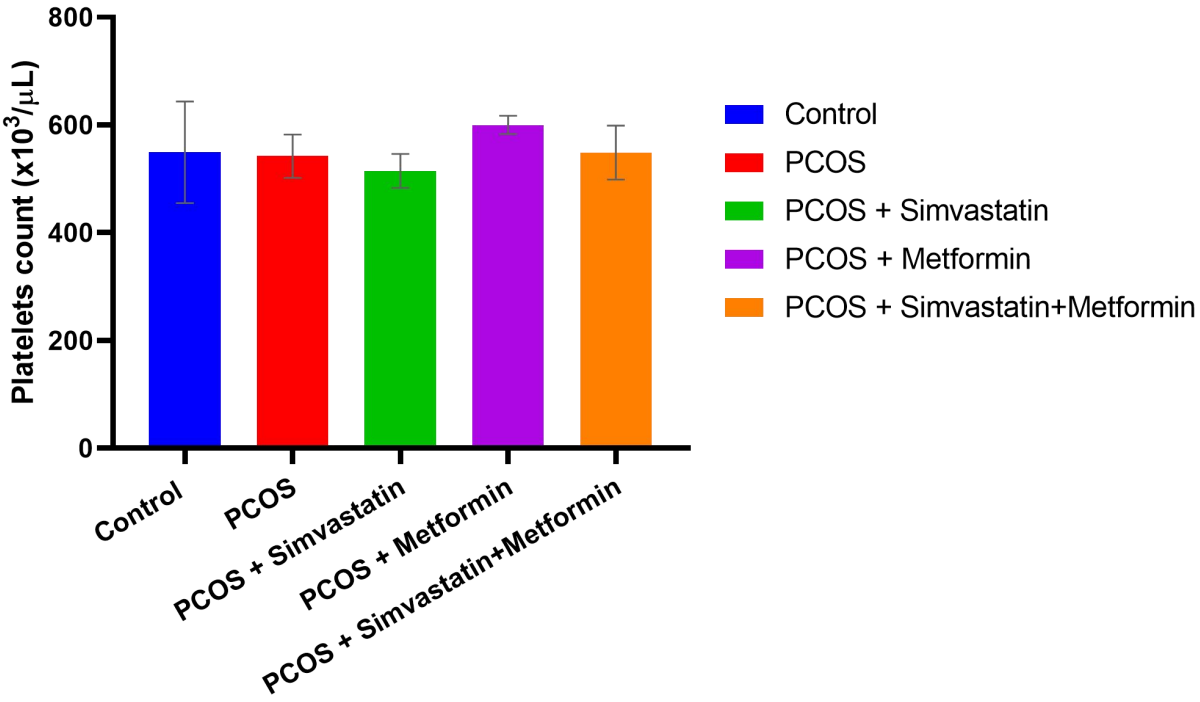

Figure 4. 1: Showing the platelets cell of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were no significant differences in PCOS treated groups compared with PCOS untreated group.

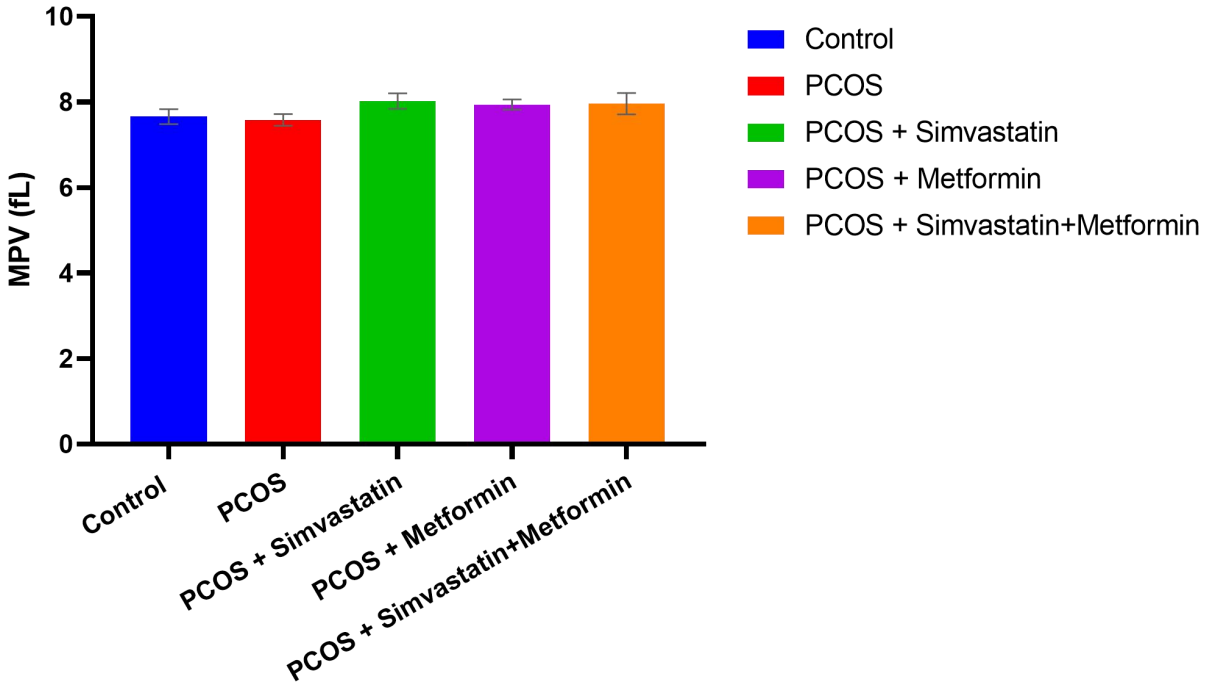

Figure 4. 2: Showing the mean platelets cell of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were no significant differences in PCOS treated groups compared with PCOS untreated group.

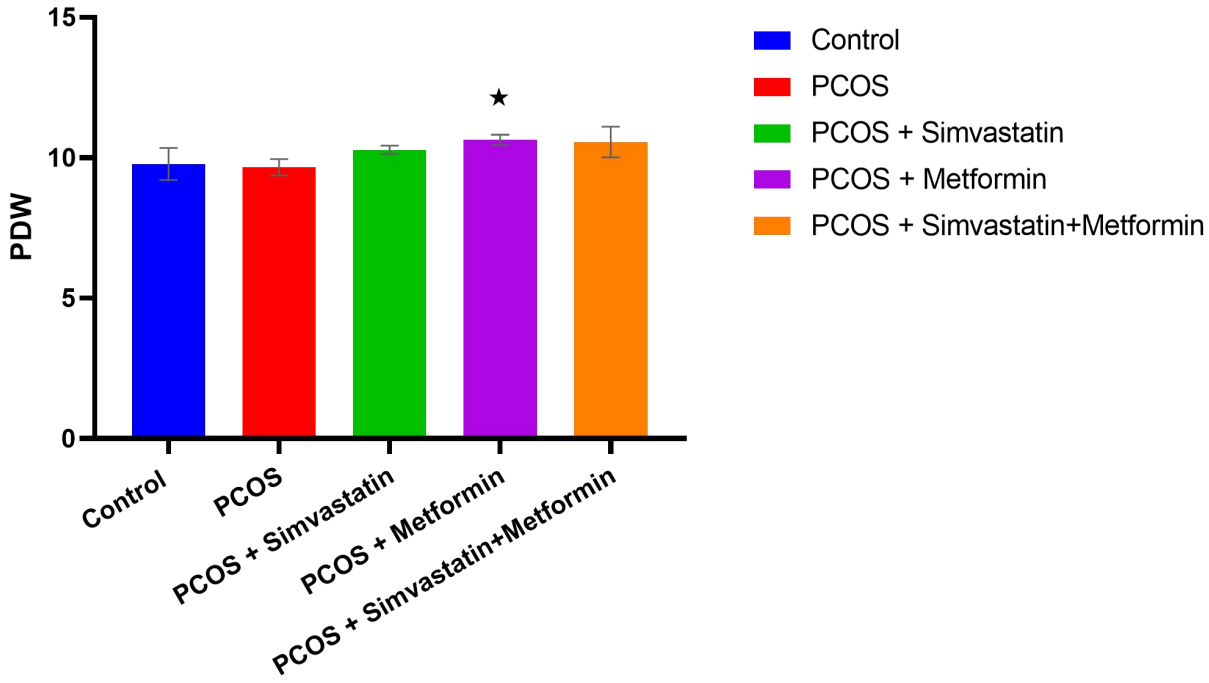

Figure 4. 3: Showing the PDW of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There was a significant increase PCOS + metformin compared with PCOS untreated, though, there were no significant differences in PCOS + simvastatin and PCOS + simvastatin + metformin treated groups compared with PCOS untreated group.

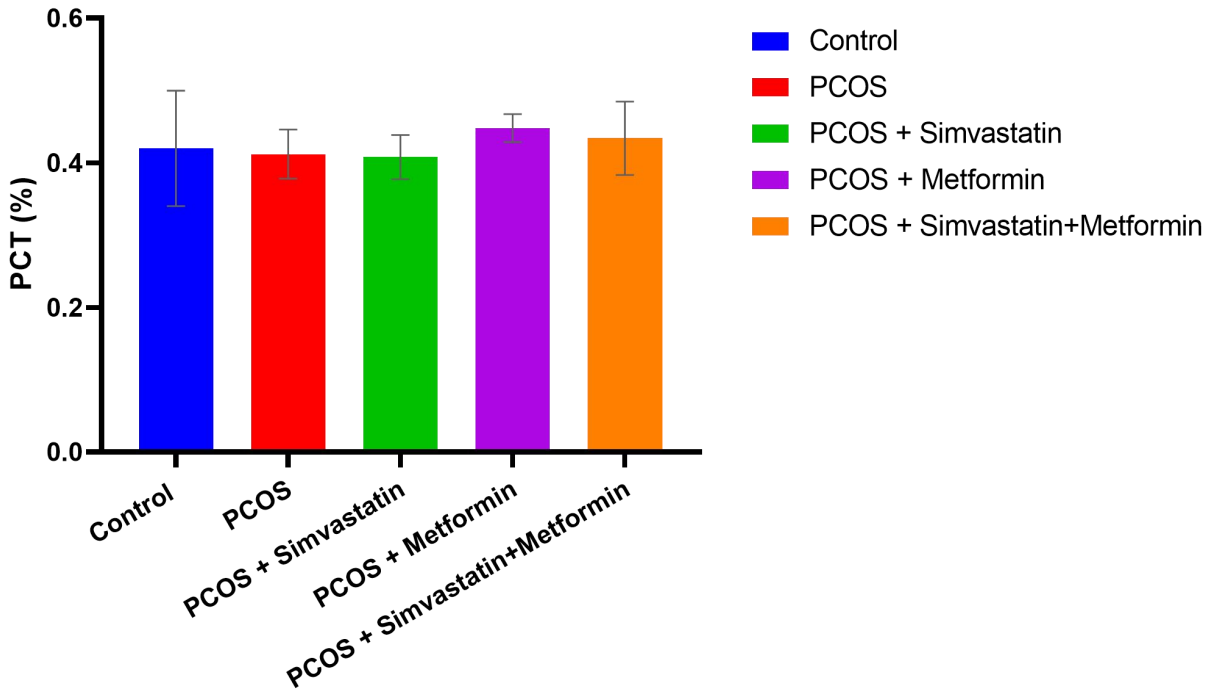

Figure 4. 4: Showing the PCT of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were no significant differences in PCOS treated groups compared with PCOS untreated group.

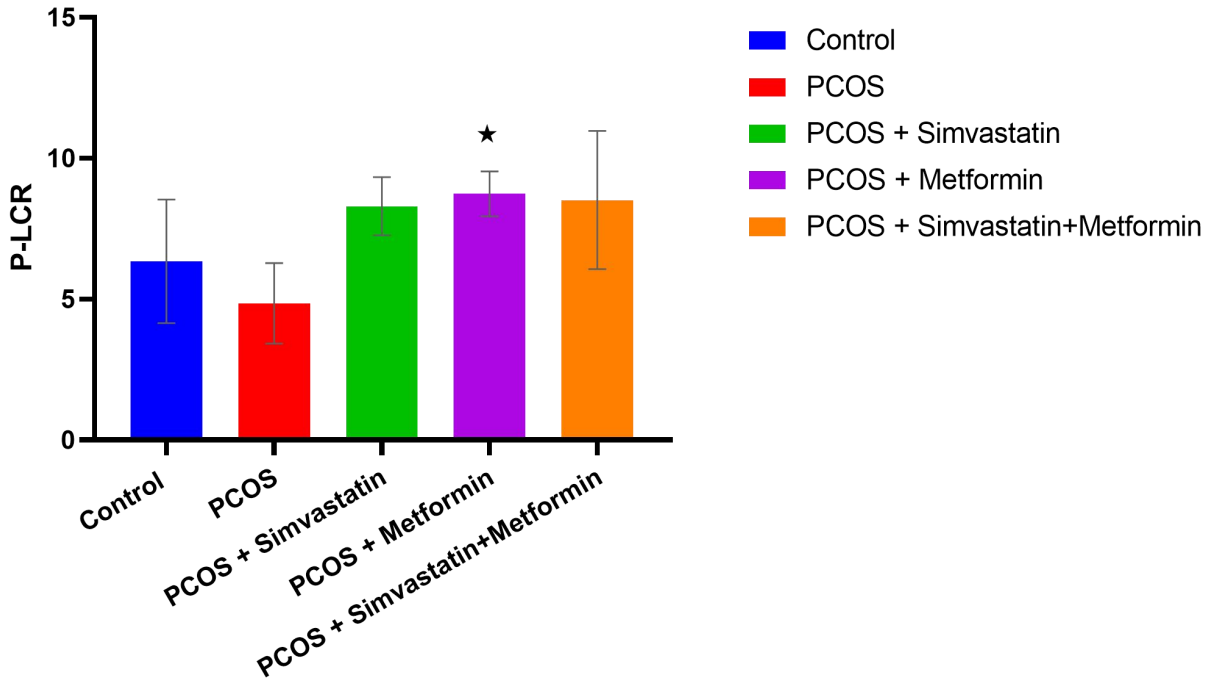

Figure 4. 5: Showing the P-LCR of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There was a significant increase PCOS + metformin compared with PCOS untreated, though, there were no significant differences in PCOS + simvastatin and PCOS + simvastatin + metformin treated groups compared with PCOS untreated group.

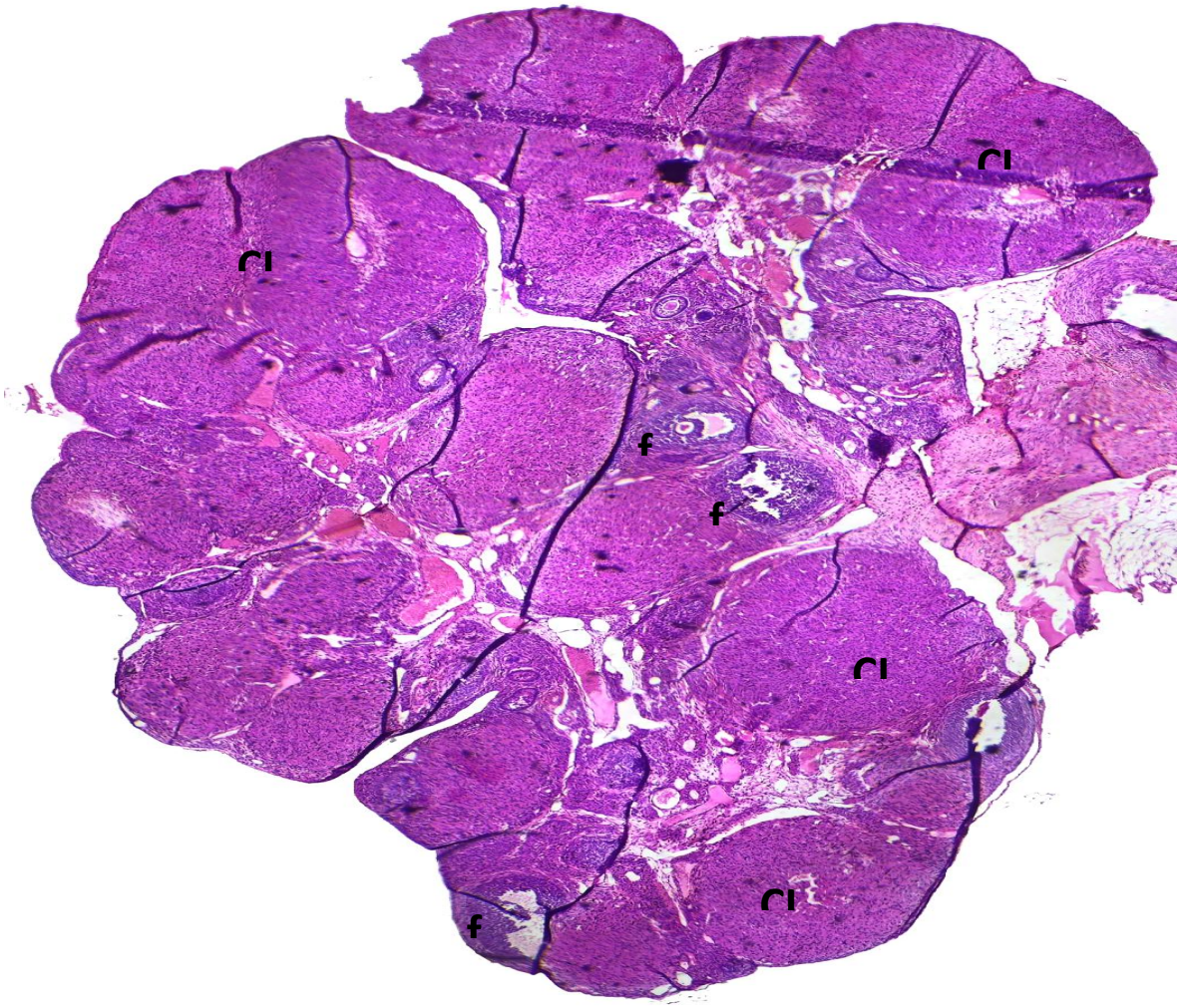

Figure 4. 6: Group A: Negative control
The ovary is normal with several developing follicles (f) and corpora lutea (CL) (H&E; 40x)

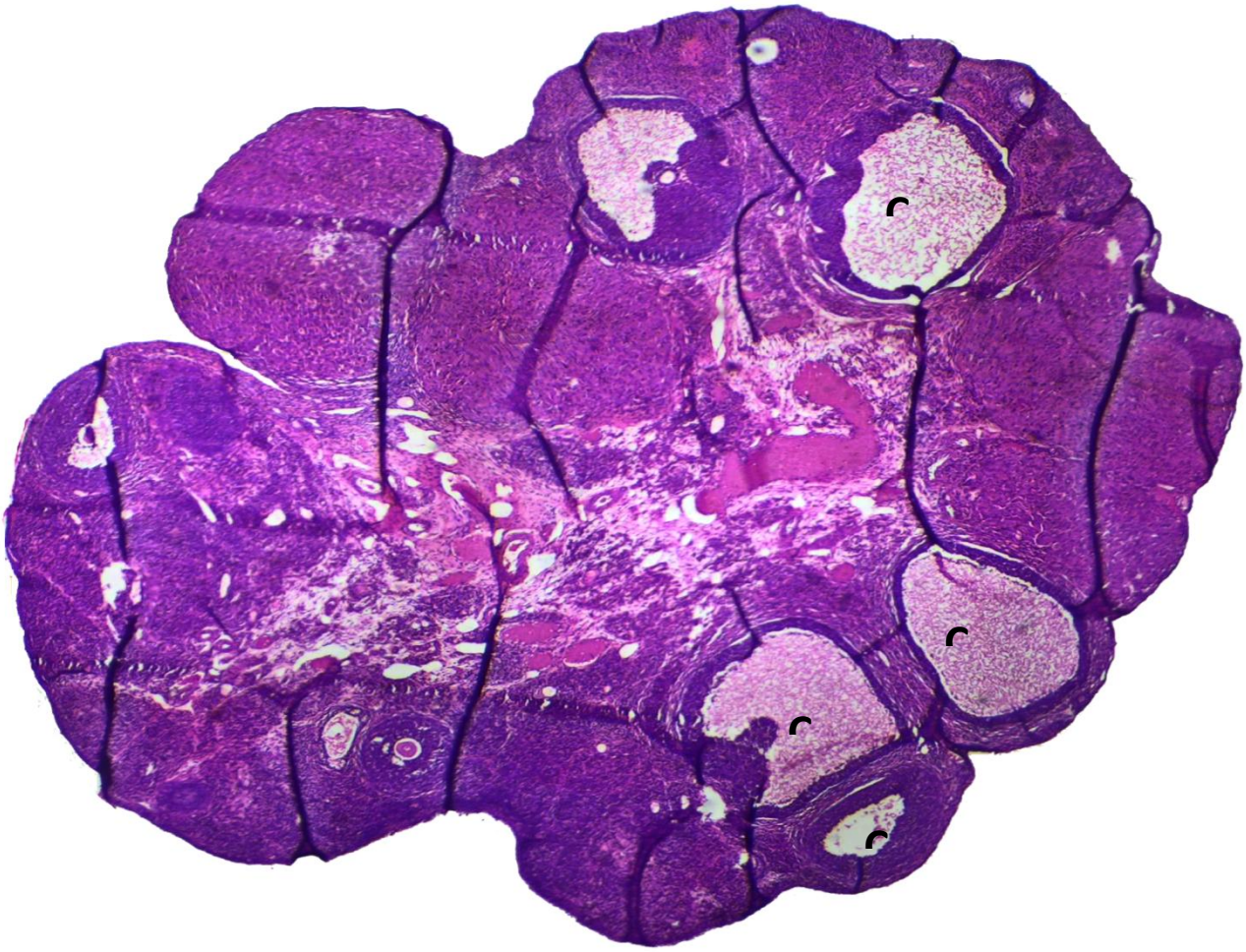

Figure 4. 7: Group B: Positive control
The ovary has several cysts (C) (H&E; 40x)

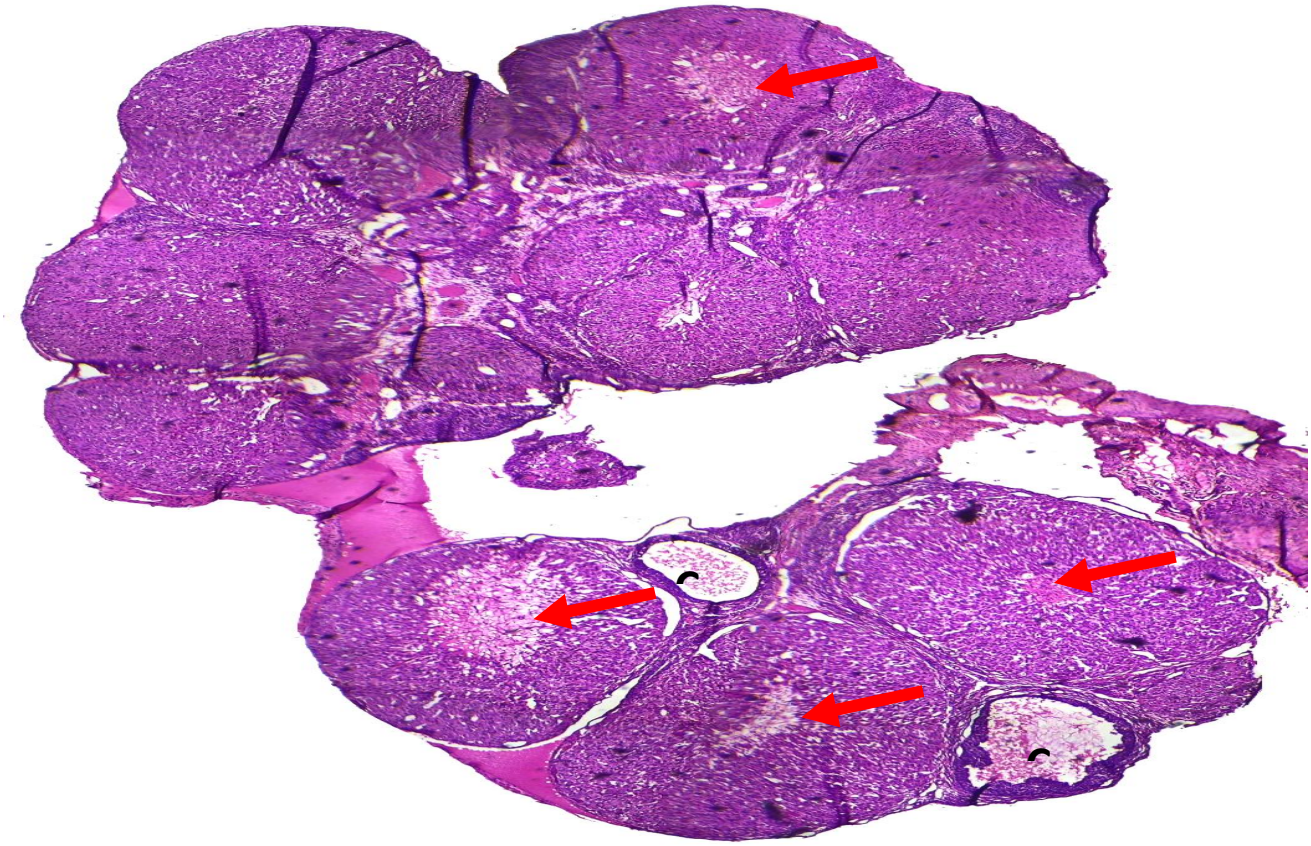

Figure 4. 8: Group C: Simvastatin
The ovary has reduced number of cysts (c) with luteolysis of the corpora lutea (red arrow) (H&E; 40x)

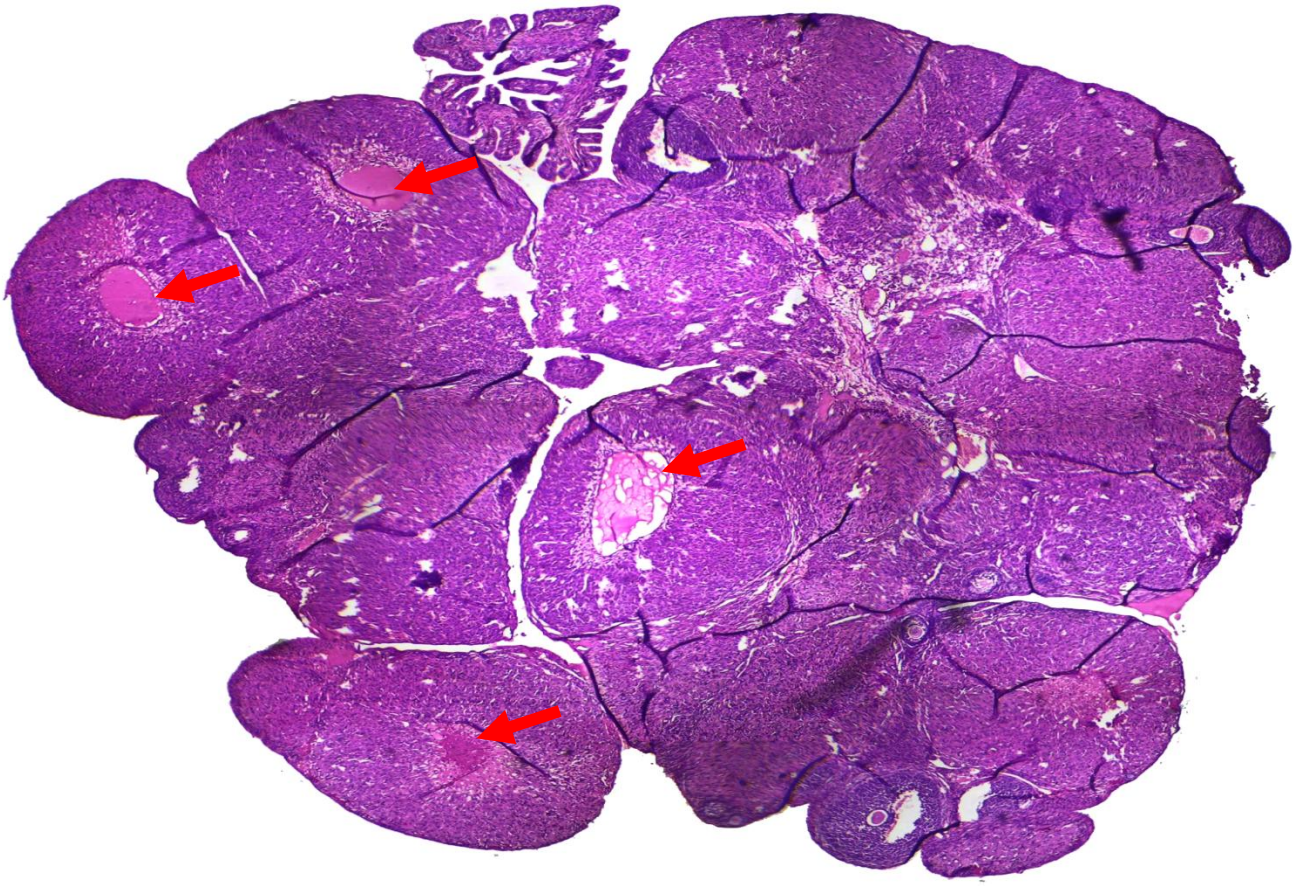

Figure 4. 9: Group D: Metformin
There is absence of cysts with several luteolysis of the corpora lutea

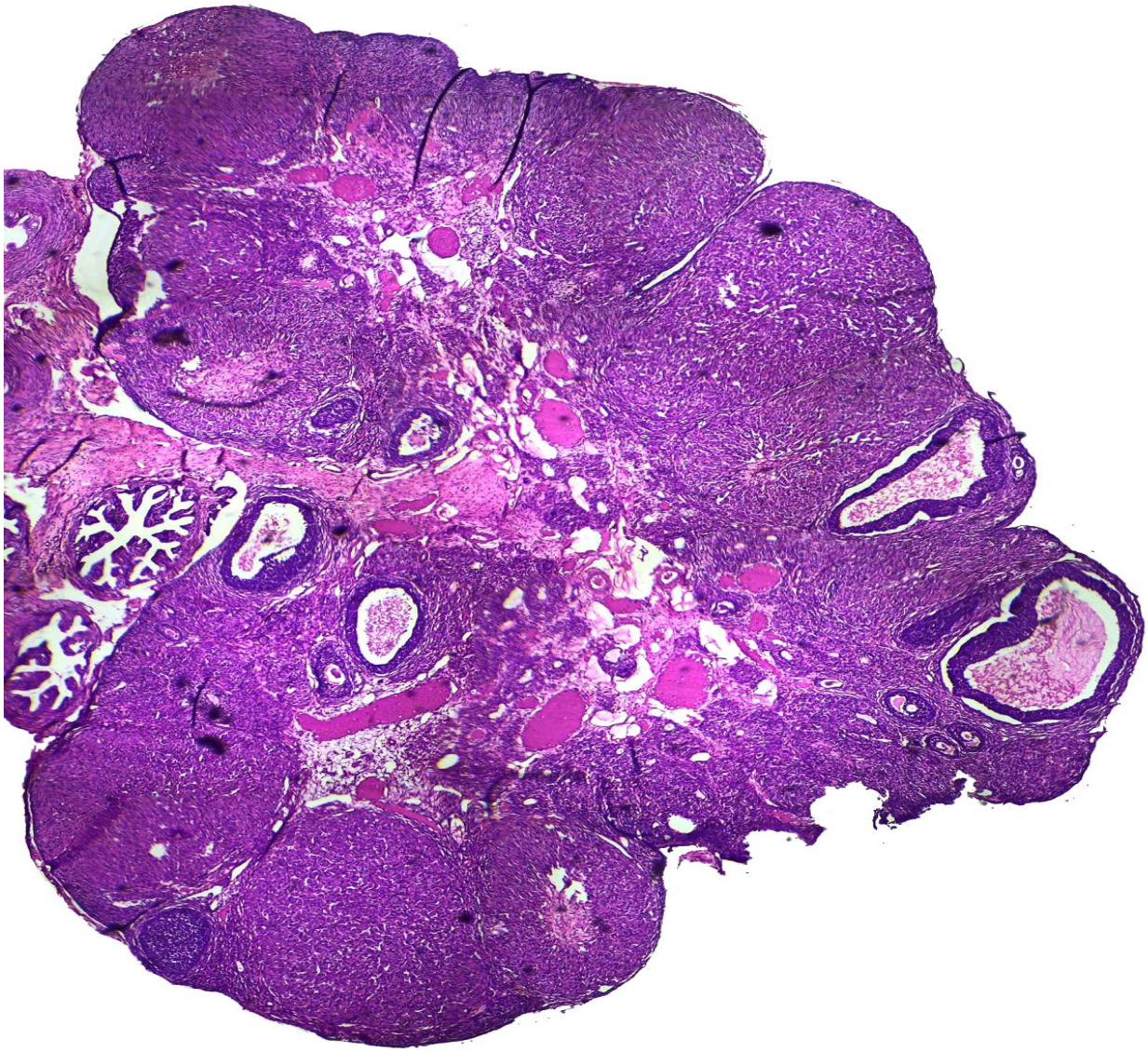

Figure 4. 10: Group E: Simvastatin
There are several cysts present with luteolysis of the corpora lutea (H&E; 40x)

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION OF RESULTS

This study investigated the individual and combined effects of Simvastatin and Metformin on platelet function and ovarian histology in a letrozole-induced PCOS rat model. The findings reveal a complex interaction between these two drugs, showing that while both are beneficial, their mechanisms are distinct and their combination may not be synergistic in this model.

The results on platelet function revealed that Metformin monotherapy significantly increased Platelet Distribution Width (PDW) and Platelet-Large Cell Ratio (P-LCR), suggesting a shift in the platelet population towards larger, more reactive platelets (Banaszewska *et al*., 2011) . This was an unexpected finding, as Metformin is widely recognized for its beneficial effects on cardiovascular health, including improving endothelial function (Van Rensburg *et al*., 2025).

In contrast, Simvastatin monotherapy showed no significant effect on any of the measured platelet indices. This is consistent with the known pharmacological profile of statins. While some studies indicate that statins can reduce platelet aggregation and thromboxane synthesis through anti-inflammatory and antioxidant properties, their primary effect is on improving the lipid profile and reducing systemic inflammation, which might not directly translate to changes in basic platelet indices like PDW or P-LCR over the studied period (Kolnikaj *et al*., 2024).

The combination therapy also showed no significant improvement in platelet parameters compared to the PCOS untreated group. This lack of additive effect further suggests that the drugs' mechanisms concerning platelet morphology and activity are independent and may not reinforce each other in the context of PCOS.

The histological results of this study demonstrate a clear therapeutic benefit of both Simvastatin and Metformin on ovarian morphology, albeit through different pathways.

Metformin monotherapy was highly effective in restoring ovarian cytoarchitecture, evidenced by the absence of cysts and the presence of luteolysis. Luteolysis, the regression of the corpus luteum, is a key indicator of a completed ovulatory cycle. This finding aligns with extensive literature on Metformin's role in PCOS. Metformin improves insulin sensitivity, which in turn reduces the hyperinsulinemia-driven stimulation of ovarian androgen production. Furthermore, recent reviews highlight that Metformin promotes follicular development and ovulation by enhancing autophagy in follicular granulosa cells and modulating the PI3K/AKT/mTOR signaling pathway, crucial for normal folliculogenesis (Zeng *et al*., 2025)

Simvastatin monotherapy also showed a beneficial effect, reducing the number of cystic structures and inducing luteolysis. The mechanism here is likely different. Statins directly target the ovarian theca cells. By inhibiting the enzyme HMG-CoA reductase, Simvastatin reduces the availability of cholesterol, a fundamental building block for androgen synthesis. This action directly counteracts the excessive androgen production that is a hallmark of PCOS pathology (Banaszewska *et al*., 2011; Kolnikaj *et al*., 2024). The reduction in theca cell hyperplasia and androgen output leads to an improved follicular environment.

5.2 CONCLUSION

In conclusion, this study demonstrates that both Metformin and Simvastatin monotherapies are effective in reversing the pathological ovarian features in a PCOS rat model, with Metformin showing a particularly strong restorative effect. However, their impacts on platelet function differ, with Metformin monotherapy significantly altering platelet morphology. The critical finding of this research is that the combination of Simvastatin and Metformin did not provide a synergistic effect. Instead, it showed limited efficacy on ovarian histology and no significant improvement in platelet parameters. This suggests that while both drugs are beneficial alone, their mechanisms may not be complementary and could even be antagonistic when co-administered in this model.

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