INFLUENCE OF SIMVASTATIN AND METFORMIN ON SOME HEMATOLOGICAL PARAMETERS OF LETROZOLE INDUCED POLYCYSTIC OVARIAN SYNDROME IN FEMALE SPRAGUE DAWLEY RATS

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OCTOBER, 2025.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICFAL SCIENCES, UNIVERSITY OF BENIN IN PARTIAL FULFILMENT OF THE AWARD OF THE BACHELOR OF SCIENCE (B.Sc) DEGREE IN PHYSIOLOGY

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work on "INFLUENCE OF SIMVASTATIN AND METFORMIN ON SOME HEMATOLOGICAL PARAMETERS OF LETROZOLE INDUCED POLYCYSTIC OVARIAN SYNDROME IN FEMALE SPRAGE DAWLEY RATS" was carried out by **AFUBA EMMANUEL MUNACHIMSOAGA**, with matriculation number, **BMS2101600**, in partial fulfillment of the requirement for the award of Bachelor of Science Degree (B.Sc) in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

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(Head of Department) DATE:

……. ……

PROF. DATE

(External Examinar)

DEDICATION

This project work is dedicate to God Almighty, my parents and siblings and every single person who has been rooting for me.

ACKNOWLEDGMENT

With immense gratitude and appreciation, I extend my heartfelt acknowledgement to every single person who have played a vital role in the completion of my research work, their unwavering support, guidance, and encouragement have been instrumental in shaping this endeavor.

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a multifactorial endocrine disorder that primarily affects women of reproductive age and is characterized by a group of symptoms, including chronic anovulation, hyperandrogenism, and polycystic ovarian morphology. Beyond its metabolic and reproductive abnormalities, PCOS has also been shown to influence hematological parameters.This study investigated the influence of simvastatin and metformin on some hematological parameters in PCOS induced rats; red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Twenty five (25) female Sprague-Dawley rats, were used for this study. The rats were acclimatized for a total of two (2) weeks, then weighed and randomly assigned into five (5) groups of five (5) rats each (n=5). Group 1:(-ve control) without PCOS induction, Group 2:(+ve control) induced with PCOS without treatment, Group 3:PCOS + simvastatin, Group 4:PCOS + metformin, Group 5:PCOS + simvastatin +metformin. PCOS was induced by administration of letrozole at 1mg/kg body weight for 28 days after which, the PCOS animals were treated with simvastatin and metformin for a period of 28 days. All drugs were administered using oral gavage. At the end of the experiment, rats were anesthetized using chloroform, and blood was obtained through cardiac puncture, placed in a EDTA bottle, and analyzed for hematological parameters using an automated hematology analyzer. Statistical analysis was conducted using GraphPad Prism version 10.0. Data obtained were expressed as mean ± standard error of mean (SEM). Statistical comparisons among experimental groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine significance between groups. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. Results showed that PCOS caused a slight,

non-significant increase in RBC, Hb, and HCT levels compared to the control group. Metformin treatment increased hemoglobin and hematocrit values, while simvastatin mainly increased MCV and MCH. The combination of both drugs produced the best overall improvement, suggesting a stronger positive effects when used together. MCHC values remained stable in all groups. The present study demonstrates that letrozole induced PCOS in female Sprague-Dawley rats produced alterations in some hematological indices, which were significantly improved following treatment with simvastatin and metformin, either alone or in combination. In conclusion, simvastatin and metformin maybe described as a reasonable therapeutic strategy in the management of PCOS.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Polycystic Ovary Syndrome (PCOS) is a complex and metabolic disorder that primarily affects women of reproductive age. It is characterized by hyperandrogenism, anovulation, and the presence of multiple ovarian cycts (Teede *et al.,* 2018) The syndrome is one of the most common causes of infertility and is frequently associated with metabolic disturbances such as insulin resistance, obesity, and dyslipidemia (Teede *et al.*, 2018). According to the Rotterdam criteria adopted in 2003 and widely accepted since, a diagnosis of PCOS requires at least two of the following: oligo- or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound, with the exclusion of other etiologies (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

The exact etiology of PCOS remains unclear, but it is believed to result from a complex interaction between genetic predispositions and environmental or lifestyle factors. Insulin resistance plays a central role in the pathophysiology, contributing to both metabolic and reproductive dysfunctions by enhancing androgen production in the ovaries and decreasing sex hormone-binding globulin (SHBG) levels (Dunaif, 2016). Furthermore, low-grade chronic inflammation and alterations in the hypothalamic-pituitary-ovarian (HPO) axis are also implicated (Lizneva *et al.*, 2016).

PCOS manifests with a variety of clinical symptoms, including irregular menstrual cycles, hirsutism, acne, alopecia, and difficulty conceiving (Smith and Lee, 2018). Polycystic ovaries, as seen on ultrasound, appear enlarged with mult32iple small follicles peripherally located ("string of pearls" appearance). Moreover, women with PCOS are at an increased risk for long-term complications such as type 2 diabetes

mellitus, cardiovascular disease, endometrial cancer, and mood disorders like anxiety and depression (Teede *et al.*, 2018).

Beyond its metabolic and reproductive abnormaliuties, PCOS has also been shown to influence hematological parameters. Hematological indicies such as red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), meaan corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) serve as essential indicators of oxygen-carrying capacity, erythropoietic activity, and general physiological status (Tarkun *et al.*, 2020).

Simvastatin is a lipid-lowering agent that belongs to the class of medications known as HMG-CoA reductase inhibitors, or statins. It functions by competitively inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, which plays a critical role in the biosynthesis of cholesterol in the liver. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, a precursor of cholesterol; by inhibiting this process, simvastatin effectively reduces low-density lipoprotein cholesterol (LDL-C), total cholesterol, and triglycerides while modestly increasing high-density lipoprotein cholesterol (HDL-C) (Alonso *et al.,* 2008).

Simvastatin is administered orally as an inactive lactone prodrug, which is hydrolyzed in the liver to its active β-hydroxyacid form. The drug exhibits its maximal effect on serum lipids within 4–6 weeks of initiation and is primarily metabolized by the cytochrome P450 3A4 (CYP3A4) enzyme system (Neuvonen *et al.*, 2006). Due to this metabolic pathway, simvastatin has the potential for numerous drug-drug interactions, especially with other agents that inhibit or compete for CYP3A4, such as certain antifungals, macrolide antibiotics, and calcium channel blockers (Bellosta *et al.,* 2004).

Metformin is an oral antihyperglycemic agent belonging to the biguanide class and is considered the first-line pharmacological treatment for type 2 diabetes mellitus (T2DM), particularly in overweight and obese individuals (Nathan *et al.,* 2009). Chemically known as 1,1-dimethylbiguanide hydrochloride, metformin acts primarily by decreasing hepatic glucose production through inhibition of gluconeogenesis, increasing insulin sensitivity, and enhancing peripheral glucose uptake in skeletal muscle (Rena *et al.*, 2013). Unlike insulin or sulfonylureas, metformin does not stimulate insulin secretion, thereby minimizing the risk of hypoglycemia (Viollet *et al.,* 2012).

Metformin's glucose-lowering effect is mediated largely by activation of the AMP-activated protein kinase (AMPK) pathway in the liver, a crucial cellular energy sensor that regulates glucose and lipid metabolism (Zhou *et al.*, 2001). Additionally, it reduces intestinal glucose absorption and improves insulin action, particularly in hepatic and muscle tissue. These multifaceted actions make metformin effective not only in glycemic control but also in improving metabolic profiles, such as reducing triglyceride and low-density lipoprotein (LDL) cholesterol levels (Hundal *et al.*, 2000).

Letrozole, an aromatase inhibitor, is frequently used to induce exdperimentqal PCOS in rodents. It inhibits the conversion of androgens to estrogens, leading to elevated androgen levels and cystic ovarian morphology that mimic human PCOS (Kafali *et al.,* 2019). The letrozole-induced PCOS also reproduces metabolic abnormalities such as hyperinsulinemia, dyslipidemia, and oxidative stress, making it suitable for evaluating therapeutic agaents like simvastatin and metformin.

1.2 JUSTIFICATION OF STUDY

The hematological alterations associated with PCOS remain understudied compared to its hormonal and metabolic aspects. Evaluating parameters such as red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), meaan corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) provides important insights into the systemic effects of PCOS on erythropoiesis and oxygen transport. Since oxidative stress and inflammation are key componenyts of PCOS, its essential to assess how pharmacologiocal agents that target these pathways influence blood physiology.

1.3 AIM OF STUDY

To investigate the influence of simvastatin and metformin on some hematological parameters of letrozole-induced polycystic ovarian syndrome in female Sprague Dawley rats.

1.4 RESEARCH PROBLEM

Studies have shown that metformin and simvastatin, individually, have shown potential in managing PCOS symptoms due to their metabolic, anti-inflammatory, and insulin-sensitizing properties. However, limited studies have explored their comparative or combined influence on hematological parameters, such as red blood cell counts, hemoglobin levels, hematocrit levels, which are often altered in PCOS. This study will provide insights into the hematological profiles affected by PCOS and the modulating role of simvastatin and metformin.

1.5 RESEARCH QUESTIONS

1. Does letrozole-induced polycystic ovarian syndrome alter hematological parameteters such as RBC, Hb, HCT, MCV, MCH, and MCHC in female Sprague Dawley rats?
2. What is the effect of simvastatin treatment on hematological parameters in letrozole-induced PCOS rats?
3. How does metformin treatment influence hematological parameters in letrozole-induced PCOS rats?
4. Does combined administration of simvastatin and metformin produce enhanced effect on hematological indicies compared to single treatments?

1.6 SPECIFIC OBJECTIVES

1. To evaluate the effect of letrozole-induced polycystic ovarian syndrome on selected hematological parameters in female Sprague Dawley rats.
2. To determine the influence of simvastatin on hematological parameters in letrozole-induced PCOS rats.
3. To assess the effect of metformin on hematological parameters in letrozole-induced PCOS rats.
4. To compare the combined the effects of simvastatin and metformin on hematological indicies with those of simvastatin only and metformin only.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 POLYCYSTIC OVARIAN SYNDROME

PCOS is an endocrine and metabolic disorder, defined by oligo-ovulation, hyperandrogenism, and polycystic ovaries per Rotterdam criteria. PCOS affects 6–15% of reproductive-age women and is strongly linked to insulin resistance and dyslipidemia (Peng *et al.,* 2021). These metabolic disturbances are often accompanied by chronic low-grade inflammation, evident in elevated levels of hs-CRP, TNF-α, and IL-6 (Smith and Lee, 2018). The convergence of metabolic and inflammatory pathways may disrupt hematopoiesis, yielding leukocytosis, aberrant erythropoiesis, and platelet activation, making it essential to study blood parameters comprehensively in rodent PCOS models (Peng *et al.,* 2021).

Polycystic Ovary Syndrome (PCOS) typically begins in adolescence or early adulthood, but its development is gradual and multifactorial. The condition arises from a complex interplay of genetic, hormonal, metabolic, and environmental influences (Escobar-Morreale, 2018).

2.1.1 GENETIC PREDISPOSITION

There is strong evidence that genetic factors contribute significantly to the development of PCOS. Family studies have shown that first-degree female relatives of women with PCOS have a higher risk of developing the condition themselves, suggesting a heritable component (Goodarzi *et al.,* 2011). Multiple gene variants related to insulin signaling, androgen biosynthesis, and inflammatory pathways have been identified, though the exact genetic etiology remains polygenic and complex (Escobar-Morreale, 2018).

2.1.2 HORMONAL IMBALANCE

One of the earliest and most critical changes in PCOS is the disruption of the hypothalamic-pituitary-ovarian (HPO) axis. Women with PCOS often exhibit an increased luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio, which stimulates theca cells in the ovaries to overproduce androgens such as testosterone (Diamanti-Kandarakis and Dunaif, 2012). These excess androgens interfere with the maturation of ovarian follicles, preventing regular ovulation and leading to the formation of multiple immature ovarian cysts (Escobar-Morreale, 2018).

2.1.3 INSULIN RESISTANCE AND HYPERINSULINEMIA

Insulin resistance is a central feature of PCOS and is present in both lean and obese individuals, although it's more severe in the latter (Diamanti-Kandarakis and Dunaif, 2012). Compensatory hyperinsulinemia worsens androgen excess by stimulating ovarian androgen production and suppressing sex hormone-binding globulin (SHBG), thereby increasing free testosterone levels (Goodarzi *et al.,* 2011). This insulin resistance does not only affect reproductive hormones but also contributes to the metabolic syndrome frequently seen in PCOS, including abnormal glucose tolerance, dyslipidemia, and increased visceral fat (Escobar-Morreale, 2018).

2.1.4 CHRONIC LOW GRADE INFLAMMATION

PCOS is now recognized as a state of chronic low-grade inflammation. Elevated levels of C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) have been observed in women with PCOS (Escobar-Morreale, 2018). This inflammation may stem from adipose tissue dysfunction, oxidative stress, or even gut dysbiosis (Ali *et al.,* 2020). Inflammation in PCOS not only contributes to insulin resistance but may also impact hematopoiesis (blood cell production), leading to abnormalities in white blood cell counts and possibly in platelet function, though this

area remains underexplored (Ali *et al.,* 2020).

2.1.5 ENVIRONMENTAL AND LIFESTYLE TRIGGERS

Environmental factors and lifestyle choices can exacerbate the expression of PCOS in genetically predisposed individuals. Key influences include: obesity, particularly central obesity, which worsens insulin resistance, sedentary lifestyle, high-glycemic diets, exposure to endocrine disrupting chemicals (e.g., BPA).

Obesity, in particular, amplifies nearly all the core features of PCOS, including anovulation, androgen excess, and insulin resistance (Escobar-Morreale, 2018).

2.2 ANIMAL MODELS OF PCOS

Animal models have been instrumental in elucidating PCOS pathogenesis, particularly the role of androgen excess and insulin resistance (Franks *et al.,* 2018). They also enable preclinical testing of pharmacological agents such as metformin and simvastatin on reproductive, metabolic, and hematological parameters (Li *et al.,* 2020). For example, letrozole-induced PCOS rats have been widely used to evaluate the efficacy of insulin sensitizers and anti-androgens (Kafali *et al.,* 2019).

2.2.1 RODENT MODELS (RATS AND MICE)

Rodents are the most commonly used species for PCOS modeling due to their short reproductive cycles, ease of handling, and genetic manipulability.

Androgen-Induced Models: Administration of androgens such as testosterone propionate or dihydrotestosterone (DHT) to neonatal or adult female rats or mice induces PCOS-like features including persistent estrus, cystic ovaries, elevated serum androgens, and disrupted folliculogenesis (Liu *et al.,* 2017). This model closely mimics the hyperandrogenic state of PCOS.

Letrozole-Induced Models: Letrozole, an aromatase inhibitor, is used to block estrogen synthesis, causing increased LH secretion and hyperandrogenism in rodents

(Sahmay *et al.,* 2018). Letrozole-treated rats display acyclic estrous cycles, polycystic ovaries, and metabolic disturbances, replicating many PCOS characteristics (Sahmay *et al.,* 2018).

Dehydroepiandrosterone (DHEA)-Induced Models: DHEA administration in rodents leads to ovarian cyst formation, elevated androgens, insulin resistance, and altered glucose metabolism (Schoenfeld *et al.,* 2019). This model is useful for studying metabolic and reproductive aspects of PCOS.

High-Fat Diet (HFD) Models: Feeding rodents a high-fat diet induces obesity and insulin resistance, which can exacerbate or mimic PCOS phenotypes when combined with androgen exposure (Huang *et al.,* 2018). These models help explore the metabolic components of PCOS.

2.2.2 NON-RODENT MODELS

Primate Models: Non-human primates, such as rhesus monkeys, exhibit natural menstrual cycles and ovarian physiology similar to humans. Prenatal androgen exposure in monkeys induces PCOS-like traits, including anovulation, hyperandrogenism, and metabolic dysfunction (Abbott *et al.,* 2017). While physiologically relevant, primate models are costly and ethically complex.

Sheep Models: Prenatal androgenization in sheep results in adult females with reproductive and metabolic features of PCOS, including follicular cysts and insulin resistance (Padmanabhan *et al.,* 2018). Sheep models allow long-term studies of developmental origins of PCOS.

2.3 HEMATOLOGICAL ALTERATIONS IN PCOS

While PCOS is primarily characterized by reproductive and hormonal abnormalities, increasing evidence highlights its impact on various hematological parameters (Kaya *et al.,* 2014). These alterations are largely attributed to chronic low-grade inflammation,

hyperandrogenism, oxidative stress, and insulin resistance (Escobar-Morreale, 2018; Diamanti-Kandarakis and Dunaif, 2012).

2.3.1 RED CELL DISTRIBUTION WIDTH (RDW)

RDW is a measure of variability in red blood cell (RBC) size. In women with PCOS, RDW is frequently elevated, suggesting red cell anisocytosis and abnormal erythropoiesis. This has been attributed to systemic inflammation and oxidative stress, which can impair the structural integrity and lifespan of erythrocytes (Zhu *et al.,* 2022). Elevated RDW values in PCOS patients have been positively correlated with markers such as high-sensitivity C-reactive protein (hs-CRP), insulin resistance (HOMA-IR), low-density lipoprotein (LDL), and serum testosterone (Zhu *et al.,* 2022; Kaya *et al.,* 2014). This makes RDW a potentially valuable, low-cost indicator of cardiovascular and metabolic risk in these patients.

2.3.2 WHITE BLOOD CELL (WBC) COUNT AND DIFFERENTIAL

Elevated total WBC count is a commonly observed feature in women with PCOS, which reflects the underlying state of chronic systemic inflammation (Verit and Verit, 2016). Among WBC subtypes, neutrophils are often increased, while lymphocyte counts may remain unchanged or slightly reduced, leading to an elevated neutrophil-to-lymphocyte ratio (NLR). NLR is a sensitive marker of subclinical inflammation and has been strongly associated with insulin resistance, dyslipidemia, and androgen excess in PCOS (Kaya *et al.,* 2014; Seow *et al.,* 2015). Monocytes and basophils have also been reported as elevated in some cohorts of PCOS women, indicating a broad activation of the immune system (Alhabardi *et al.,* 2021). These hematological changes emphasize the immunometabolic nature of the disorder.

2.3.3 RED BLOOD CELL (RBC), HEMOGLOBIN (HB), AND HEMATOCRIT (HCT)

Although findings are mixed, several studies have shown that women with PCOS may have increased RBC counts, hemoglobin levels, and hematocrit values (Karabulut *et al.,* 2014). These increases may be driven by hyperandrogenism, as androgens like testosterone can stimulate erythropoiesis. Another contributing factor is reduced menstrual blood loss due to anovulatory cycles, which can preserve iron and enhance erythropoietic activity (Escobar-Morreale, 2018). However, other studies have noted lower mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in PCOS patients, suggesting a trend toward microcytic anemia, possibly related to nutrient deficiencies or subclinical inflammation affecting iron metabolism (Alhabardi *et al.,* 2021).

2.3.4 INFLAMMATORY HEMATOLOGIC RATIOS (NLR, PLR, SII)

Composite hematological indices such as NLR and PLR have gained prominence as surrogate markers of inflammation in PCOS. NLR is often significantly elevated and has been associated with both reproductive and metabolic severity of the disorder (Verit and Verit, 2016). Similarly, PLR reflects increased platelet activity alongside decreased lymphocyte-mediated immune regulation. More recently, the systemic immune-inflammation index (SII), which integrates platelet, neutrophil, and lymphocyte counts, has been shown to be elevated in PCOS, further substantiating the inflammatory basis of the condition (Zhu *et al.,* 2022).

2.4 ROLE OF SIMVASTATIN AND METFORMIN IN PCOS AND THEIR MECHANISM

Polycystic Ovary Syndrome (PCOS) is characterized by hyperandrogenism, insulin resistance, dyslipidemia, and chronic low-grade inflammation, which contribute to its reproductive and metabolic complications (Diamanti-Kandarakis and Dunaif, 2012;

Teede *et al.,* 2018). Simvastatin, a member of the statin class of lipid-lowering drugs, has gained attention as an adjunct therapy in PCOS due to its pleiotropic effects beyond cholesterol reduction, including anti-inflammatory, anti-androgenic, and insulin-sensitizing properties (Kumar *et al.,* 2020).

2.4.1 THERAPEUTIC ROLE OF SIMVASTATIN IN PCOS

Simvastatin is primarily used to correct the dyslipidemic profile commonly observed in PCOS, particularly elevated low-density lipoprotein cholesterol (LDL-C) and triglycerides (Moghetti, 2016). Beyond lipid-lowering, simvastatin demonstrates several beneficial effects in PCOS patients:

Reduction of hyperandrogenism: Statins decrease serum testosterone and other androgen levels, improving clinical signs such as hirsutism and acne (Badawy *et al.,* 2018).

Improvement of inflammatory status: Simvastatin downregulates pro-inflammatory cytokines, reducing systemic inflammation associated with PCOS (Glintborg *et al.,* 2016).

Enhancement of endothelial function: It improves vascular health by reducing oxidative stress and enhancing nitric oxide bioavailability, which is important given the increased cardiovascular risk in PCOS (Housa *et al.,* 2015).

Insulin sensitization: Some studies report modest improvements in insulin sensitivity and glucose metabolism with statin therapy, although this remains controversial due to potential diabetogenic effects in some populations (Kumar *et al.,* 2020).

Potential normalization of menstrual cycles and ovulation: By reducing androgen levels and improving metabolic parameters, simvastatin may contribute to improved reproductive function (Badawy *et al.,* 2018).

2.4.2 MECHANISMS OF ACTION OF SIMVASTATIN IN PCOS

The beneficial effects of simvastatin in PCOS are mediated through multiple biochemical and molecular pathways:

Inhibition of HMG-CoA Reductase and Cholesterol Synthesis: Simvastatin inhibits 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. This lowers circulating cholesterol levels, particularly LDL-C, thereby improving lipid profiles frequently deranged in PCOS (Moghetti, 2016).

Reduction of Androgen Biosynthesis: Cholesterol is the precursor for steroid hormone synthesis. By limiting cholesterol availability, simvastatin indirectly reduces the substrate for androgen production in the ovaries and adrenal glands (Badawy *et al.,* 2018). Moreover, statins may inhibit the expression of enzymes involved in steroidogenesis, such as CYP17, further reducing androgen synthesis (Glintborg *et al.,* 2016).

Anti-Inflammatory Effects: Statins exert pleiotropic anti-inflammatory actions by down-regulating nuclear factor kappa B (NF-κB) signaling and reducing pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Housa *et al.,* 2015). This contributes to ameliorative effect on chronic low-grade inflammation characteristic of PCOS.

Improvement of Endothelial and Oxidative Stress Markers: Simvastatin increases endothelial nitric oxide synthase (eNOS) activity and reduces reactive oxygen species (ROS) production, leading to enhanced endothelial function and vascular health (Kumar *et al.,* 2020). This is especially important since endothelial dysfunction is common in PCOS and predisposes to cardiovascular disease.

Modulation of Insulin Signaling Pathways: Though the evidence is mixed, some data suggest that simvastatin may improve insulin receptor sensitivity and glucose uptake

via upregulation of insulin receptor substrate (IRS) proteins and glucose transporter 4 (GLUT4) expression in peripheral tissues (Badawy *et al.,* 2018). However, statins have also been reported to impair insulin secretion and increase diabetes risk in certain contexts, warranting cautious use (Kumar *et al.,* 2020).

2.4.3 THERAPEUTIC ROLE OF METFORMIN IN PCOS

Metformin primarily targets insulin resistance, a central pathophysiological feature in the majority of PCOS patients, regardless of obesity status (Moghetti, 2016). Clinical benefits of metformin in PCOS include:

Improvement in insulin sensitivity: Metformin reduces hepatic glucose production and enhances peripheral glucose uptake, thereby lowering circulating insulin levels (Diamanti-Kandarakis and Dunaif, 2012).

Reduction in hyperinsulinemia: Lower insulin levels decrease ovarian androgen production, leading to improved ovulatory function (Nestler *et al.,* 2018).

Restoration of menstrual cyclicity and ovulation: Metformin use has been shown to increase rates of spontaneous ovulation and improve menstrual regularity (Legro *et al.,* 2019).

Weight management: Although metformin's effect on body weight is modest, it may aid in weight stabilization or modest loss, which further contributes to metabolic improvements (Palomba *et al.,* 2020).

Cardio-metabolic risk reduction: Metformin improves lipid profiles, reduces inflammatory markers, and lowers risk factors associated with cardiovascular disease in PCOS (Miller *et al.,* 2017).

2.4.4 MECHANISMS OF ACTION OF METFORMIN IN PCOS

The therapeutic effects of metformin in PCOS are mediated through multiple interconnected mechanisms:

Activation of AMP-activated protein kinase (AMPK): Metformin activates AMPK, a cellular energy sensor that regulates glucose and lipid metabolism. AMPK activation inhibits hepatic gluconeogenesis, reduces lipogenesis, and enhances glucose uptake in skeletal muscle and adipose tissue (Viollet *et al.,* 2012). This leads to decreased blood glucose and insulin levels, which reduce ovarian androgen synthesis stimulated by hyperinsulinemia (Moghetti, 2016).

Improvement of insulin receptor signaling: Metformin enhances insulin receptor sensitivity and downstream signaling pathways in peripheral tissues, thereby alleviating insulin resistance (Diamanti-Kandarakis and Dunaif, 2012). Improved insulin signaling reduces compensatory hyperinsulinemia, mitigating its stimulatory effect on ovarian theca cells that produce androgens.

Reduction of hyperandrogenemia: By lowering insulin levels, metformin indirectly reduces ovarian androgen production. It may also have direct effects on the ovaries by modulating steroidogenic enzymes and decreasing androgen biosynthesis (Nestler *et al.,* 2018).

Modulation of adipokines and inflammatory cytokines: Metformin reduces circulating pro-inflammatory cytokines such as TNF-α and IL-6, and improves adipokine profiles (increased adiponectin and decreased leptin), which collectively ameliorate insulin resistance and systemic inflammation commonly seen in PCOS (Miller *et al.,* 2017).

Effect on ovarian folliculogenesis: Through insulin and androgen modulation, metformin improves follicular development and reduces the incidence of anovulation and cyst formation (Palomba *et al.,* 2020). This improves fertility outcomes in women with PCOS.

2.4.5 COMBINED THERAPY: METFORMIN + SIMVASTATIN

Anti-Inflammatory Effects: The combined administration of metformin and simvastatin has been shown to significantly reduce inflammatory markers, particularly C-reactive protein (CRP), which reflects systemic inflammation (Foda *et al.,* 2019). This is relevant to hematological outcomes because systemic inflammation is known to elevate white blood cell (WBC) counts, neutrophils, and NLR, while also increasing platelet reactivity (Moini *et al.,* 2020). By lowering inflammatory cytokines such as TNF-α and IL-6, the drug combination may normalize elevated hematological indices commonly seen in PCOS.

Lipid Profile and Hematological Link: Dyslipidemia, a hallmark of PCOS, contributes to oxidative stress and endothelial dysfunction, further exacerbating abnormalities in hematological parameters such as mean platelet volume (MPV) and platelet-to-lymphocyte ratio (PLR). Simvastatin has a potent lipid-lowering effect, while metformin supports modest lipid improvements through enhanced insulin sensitivity. Studies in women with PCOS have demonstrated that combined therapy leads to more pronounced reductions in LDL-C, triglycerides, and total cholesterol, indirectly improving hematological markers linked to cardiovascular risk (Ramezani Tehrani *et al.,* 2014; Jalilian *et al.,* 2015).

Androgen Suppression and Hematopoiesis: Hyperandrogenism in PCOS has been linked to increased erythropoiesis and altered leukocyte distribution. Simvastatin reduces androgen levels by inhibiting ovarian steroidogenesis, while metformin improves hyperandrogenemia through insulin-sensitization mechanisms (Glintborg *et al.,* 2016). Reduction in androgens can stabilize red blood cell (RBC) production and hemoglobin (Hb) levels and may normalize variations in red cell distribution width (RDW), a parameter associated with oxidative stress and inflammation (Kara *et al.,* 2021).

Evidence from Animal Models: Although direct studies focusing on the hematological effects of combined simvastatin and metformin in PCOS-induced rats are limited, related research provides insights. In rodent models induced with letrozole or dehydroepiandrosterone (DHEA), metformin alone has been shown to restore estrous cyclicity, reduce insulin resistance, and decrease inflammation (Kafali *et al.,* 2004). Similarly, simvastatin reduces ovarian volume and androgen levels in rodent models, indicating systemic hormonal balance, which may influence hematological profiles (Maliqueo *et al.,* 2017).

CHAPTER THREE

3.0 RESEARCH DESIGN AND METHODOLOGY

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 EXPERIMENTAL ANIMALS

Twenty five (25) female Sprague-Dawley rats, obtained from Ibadan, were used for this study. The rats were acclimatized for a total of two (2) weeks in the new environment (The animal house) before the commencement of the study. Ethical approval was obtained from the College of Medical Sciences ethics board and the rats were kept under standard animal husbandry conditions; standard temperature, at 25-29 ºC, under

natural light/dark cycle and were allowed normal rat chow and water. They were fed with rodent pellets and water daily *ad libitum*, in accordance with the recommendation of the 1996 guide for the care and use of the laboratory animals, published by the National Research Council (NRC) (Mansour *et al.,* 2017).

3.3 STUDY AREA

This study was carried out in the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State.

3.4 EXPERIMENTAL DESIGNS

After acclimatization, the rats were weighed and randomly assigned into five (5) groups of five (5) rats each (n=5).

Group 1: (-ve control) without PCOS induction

Group 2: (+ve control) induced with PCOS without treatment

Group 3: PCOS + simvastatin

Group 4: PCOS + metformin

Group 5: PCOS + simvastatin +metformin

All treatments were administered using oral gavage.

3.4.1 SOURCES OF LETROZOLE, SIMVASTATIN AND METFROMIN

The drugs were obtained from Success Pharmacy, Benin city, Edo state.

3.4.2 LETROZOLE PREPARATION

10mg of Letrozole tablet was dissolved in 10ml of normal saline to achieve a dosage of 1mg/kg body weight. The rats were weighed weekly to calculate their doses throughout the 28 days.

3.4.3 SIMVASTATIN PREPARATION

10mg of Simvastatin tablet was dissolved in 10ml of normal saline to achieve a solution

concentration of 1mg/mL. Target dose = 10mg/kg body weight/day.

Dose per group (mL) = [Target dose (mg/kg) × Average body weight (kg)] ÷ Solution concentration (mL)

The rats were weighed weekly to calculate their doses throughout the 28 days.

3.3.4 METFORMIN PREPARATION

500mg of Metformin tablet was dissolved in 10ml of normal saline to achieve a solution concentration of 50mg/mL. Target dose = 200mg/kg body weight.

Dose per group (mL) = [Target dose (mg/kg)× average body weight (kg)] ÷ Solution concentration (mg/mL)

The rats were weighed weekly to calculate their doses throughout the 28 days.

3.4 STUDY DURATION

The experiment lasted for a period of eight (8) weeks, Letrozole was administered for 28 days to induce PCOS, after which a conformational test was carried out to ascertain if the rats had PCOS. The rats were then treated with Simvastatin and Metformin for 28 days, after which the animals were sacrificed, and samples were collected.

3.5 SAMPLE COLLECTION

At the conclusion of the experiment, the rats' final weights were recorded using a digital scale calibrated in grams prior to their sacrifice. The rats were then placed in a sealed container containing cotton wool saturated with chloroform to induce anesthesia. After few minutes, the rats were taken out of the container and positioned on their backs on a dissection table, where an incision was made in the abdomen and thorax with scissors to access the heart. Blood was obtained through cardiac puncture and placed in a EDTA bottle.

3.6 SAMPLE ANALYSIS

Test for the following Hematological Parameters

i. RBC

ii. Hb

iii. HCT

iv. MCV

v. MCH

vi. MCHC

3.7 METHOD FOR SAMPLE ANALYSIS

Whole blood sasmples were collected from each experimental rat via cardiac puncture at the end of the treatment period into labeled ethylenediaminetetra-acetic acid (EDTA) bottles to prevent coagulation. The samples were gentely mixed by inversion to ensure uniform distribution and analyzed within two (2) hours of collection to maintain accuracy. Hematological analysis was conducted using an automated hematology analyzer (Sysmex KX- 21N, Japan), which was calibrated for small laboratory animals. All analyses were carried out in duplicate for precision, and the mean values were recorded for statistical analysis.

3.8 STATISTICAL ANALYSIS

Data obtained were expressed as mean ± standard error of mean (SEM). Statistical comparisons among experimental grouos were perfomed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to determine significance between groups. Statistical analysis was conducted using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, USA). A p-value less than 0,05 ($p < 0,05$) was considered statistically signifiocant.

CHAPTER FOUR

4.0 RESULTS

Table 1: Comparing the mean values of some red blood 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
RBC	6.042 ± 0.172	6.152 ± 0.123	6.048 ± 0.082	6.300 ± 0.073	6.240 ± 0.134
Haemoglobin Concentration (g/dl)	14.82 ± 0.508	15.16 ± 0.277	15.50 ± 0.29	15.94 ± 0.16*	15.78 ± 0.19
Haematocrit (%)	38.90 ± 1.07	39.16 ± 0.77	40.14 ± 0.92	41.58 ± 0.37*	41.78 ± 0.42*
MCV (fL)	64.48 ± 0.64	63.74 ± 0.40	66.40 ± 0.74*	66.12 ± 0.29*	67.14 ± 0.98*
MCH (g/dl)	24.48 ± 0.23	24.60 ± 0.21	25.58 ± 0.26*	25.24 ± 0.21	25.26 ± 0.48
MCHC (mg/dl)	38.02 ± 0.32	38.68 ± 0.28	38.62 ± 0.67	38.28 ± 0.20	37.74 ± 0.37

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

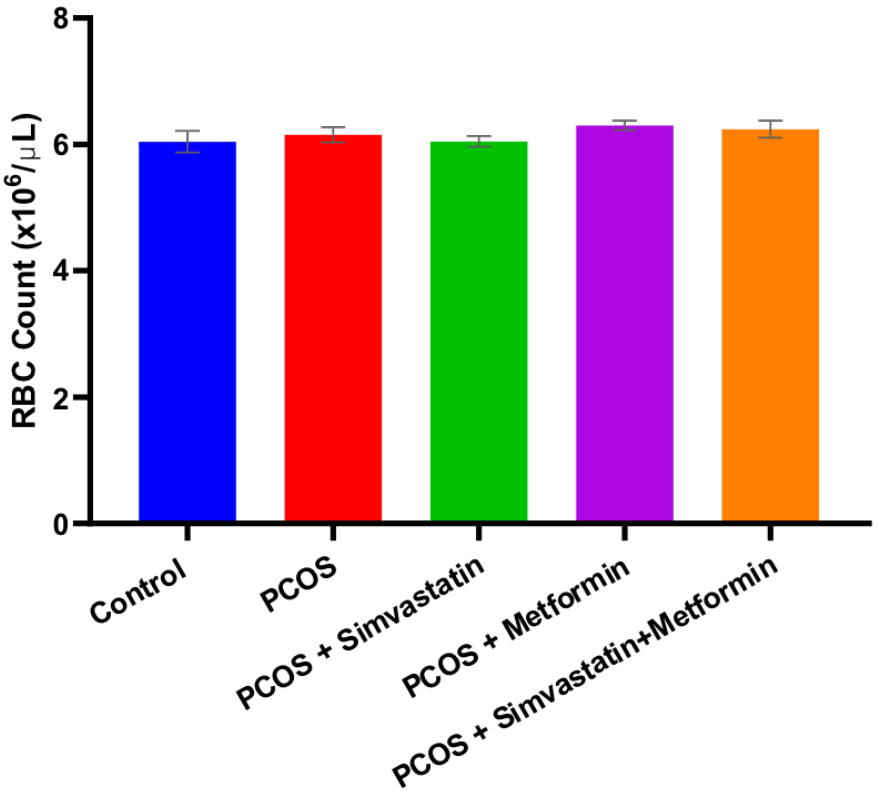

Fig I: Showing the red blood cells of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were no significant differences in PCOS treated groups compared with the PCOS untreated group. However, when compared with the control group, all PCOS and treated group showed slightly higher RBC count.

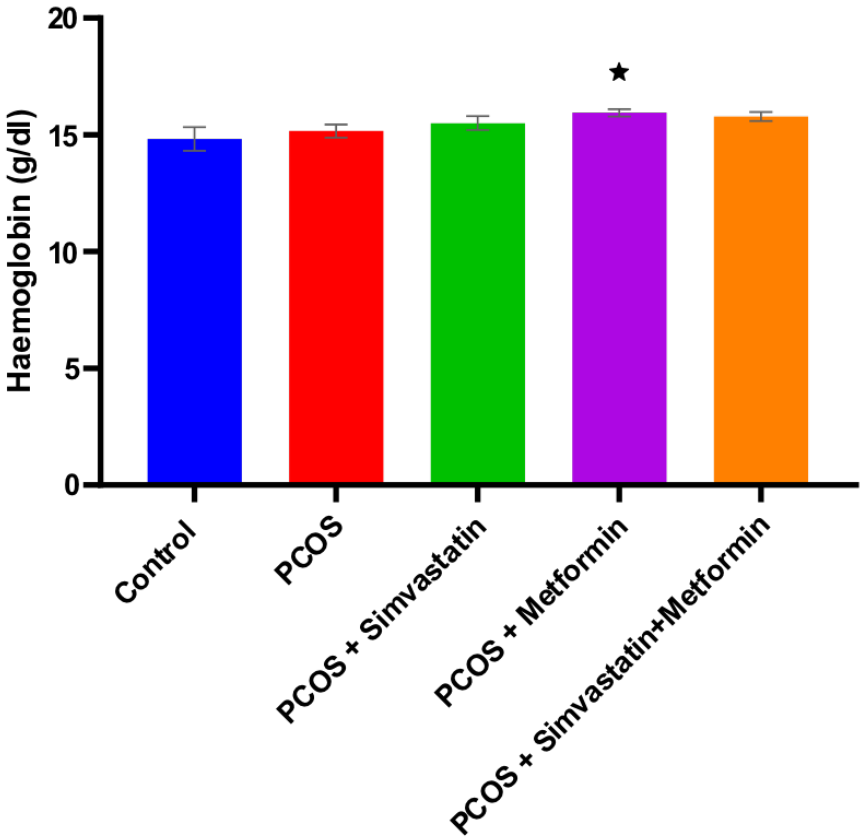

Fig II: Showing the haemoglobin concentration of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There was a significant increase PCOS + metformin compared with PCOS untreated group, though there were no significant differences in PCOS + simvastatin and PCOS + simvastatin + metformin treated groups compared with PCOS untreated group. When compared with the control group, both PCOS and treated groups demonstrated slightly higher haemoglobin concentration.

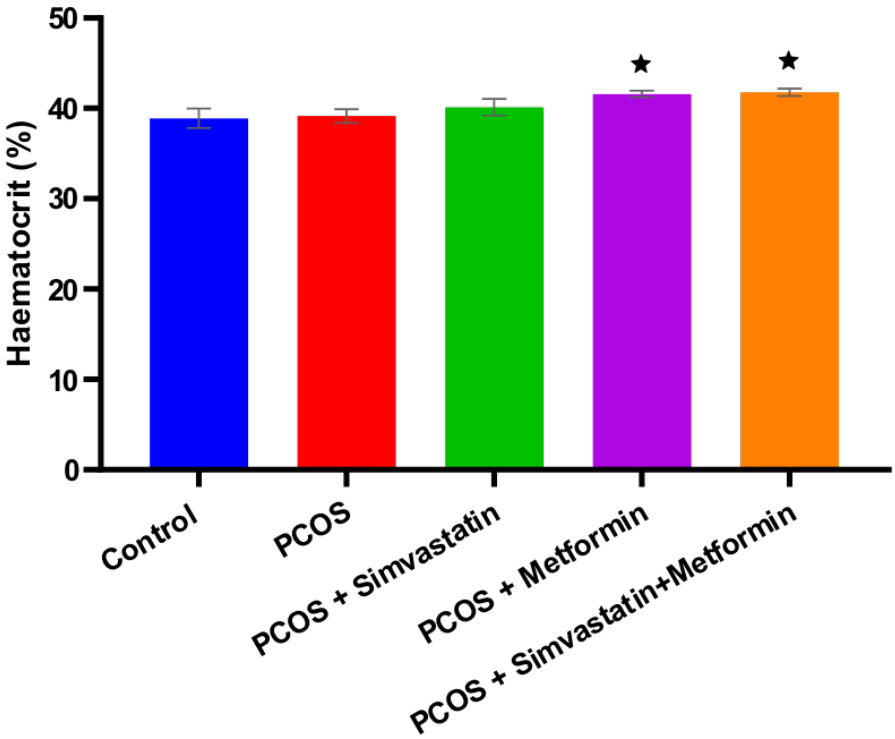

Fig III: Showing the haematocrit level of polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were significant increases PCOS + metformin and the combined treatment of simvastatin + metformin compared with PCOS untreated, though, there was no significant differences in PCOS + simvastatin treated group compared with PCOS untreated group. Relative to the control group, PCOS and treated groups exhibited moderately elevated haematocrit levels.

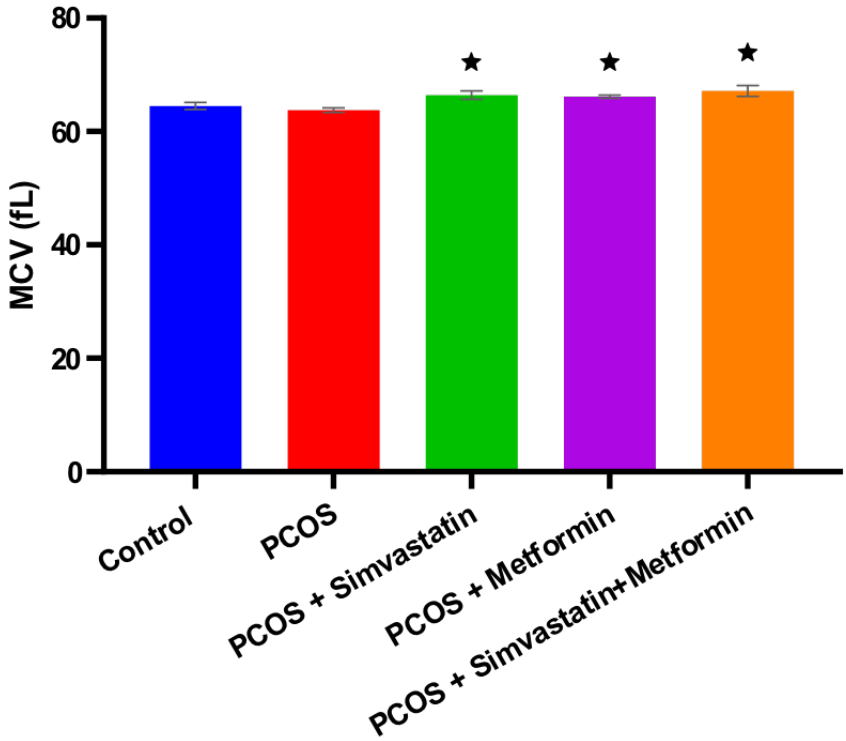

Fig IV: Showing the MCV in polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were significant increases in the PCOS + simvastatin, PCOS + metformin and the combined treatment of simvastatin + metformin compared with PCOS untreated. When compared with the control group, MCV values were slightly higher across PCOS and treated groups.

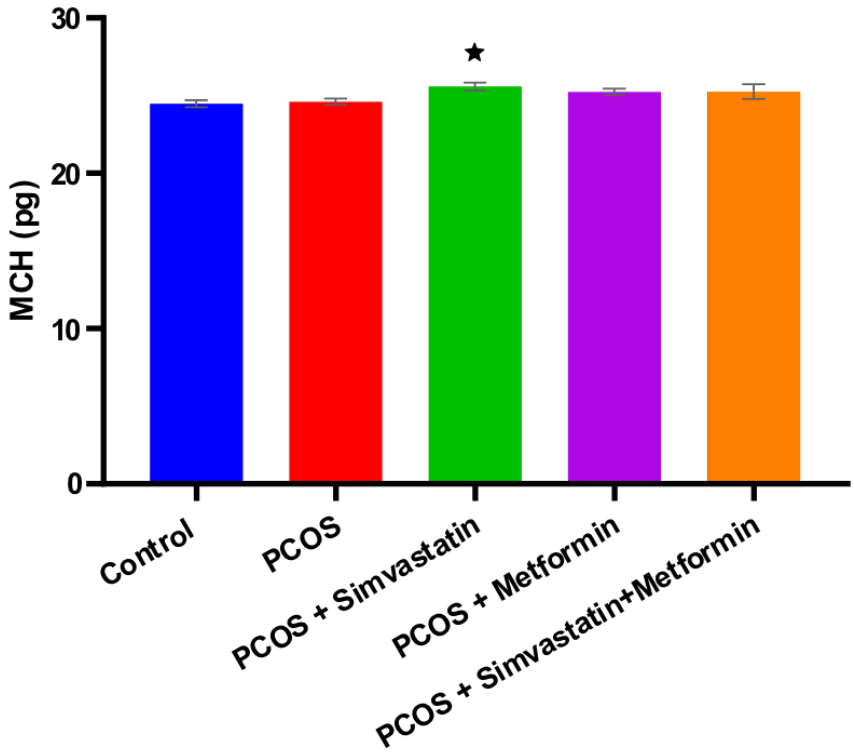

Fig V: Showing the MCH in polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There was a significant increase in the PCOS + simvastatin group compared with PCOS untreated, though there were no significant differences in PCOS + metformin and PCOS + simvastatin + metformin treated groups compared with PCOS untreated group. When compared with the control group, all PCOS related groups had slightly elevated MCH values.

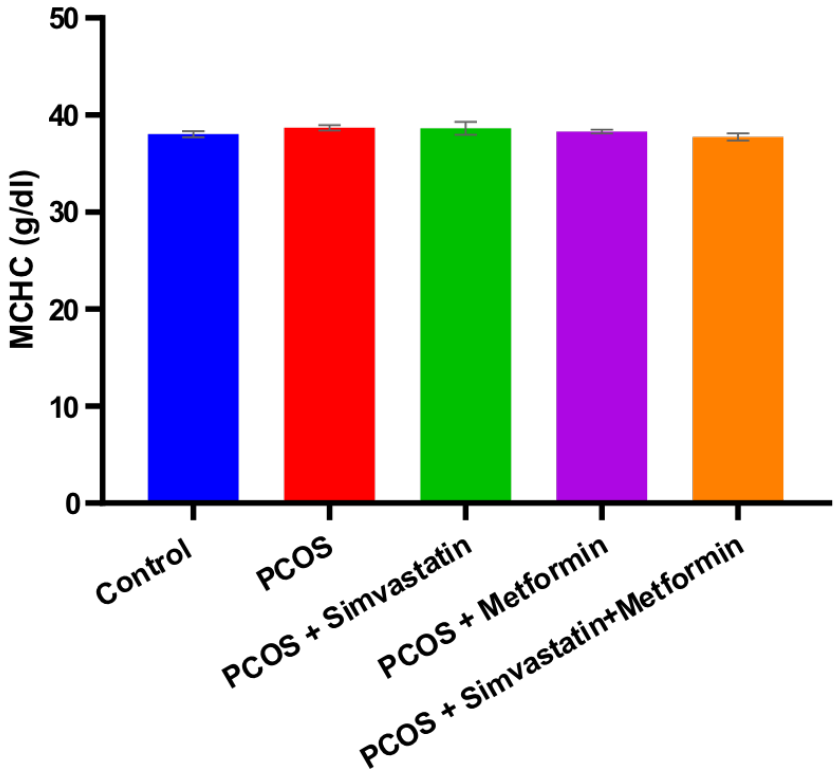

Fig VI: Showing the MCHC in polycystic ovarian syndromes induced rats treated with Simvastatin and metformin

There were no significant differences in PCOS treated groups compared with PCOS untreated group. When compared to the control group, MCHC values remained largely within normal range across all groups.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

This study investigated the influence of simvastatin and metformin on selected hematological parameters in letrozole-induced polycystic ovarian syndrome (PCOS) using female Sprague Dawley rats as the experimental model. The hematological indices evaluated included red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The aim was to assess whether treatment with simvastatin, metformin, or their combination could modify the hematological alterations associated with PCOS, a condition characterized by chronic endocrine and metabolic disturbances.

The findings revealed that PCOS induction by letrozole produced mild, non-significant elevations in RBC, hemoglobin, and hematocrit levels compared to the control group. This observation agrees with earlier reports suggesting that hyperandrogenism and hyperinsulinemia in PCOS may enhance erythropoiesis through androgen-stimulated erythropoietin production (Tarkun *et al.,* 2020). The non-significant change, however, indicates that the degree of hematological alteration may depend on the duration and severity of PCOS induction. Letrozole, an aromatase inhibitor, is known to increase androgen levels by suppressing estrogen synthesis, thereby mimicking the hormonal imbalance characteristic of PCOS (Kafali *et al.,* 2004). Although elevated androgens have been associated with increased red cell production, the mild change seen in this study suggests a moderate PCOS state that may not have produced severe systemic hematological effects.

Treatment with metformin produced a significant increase in hemoglobin concentration and hematocrit compared with the untreated PCOS group. Metformin is a well-established insulin sensitizer that improves glucose uptake and reduces hyperinsulinemia, which in turn decreases systemic inflammation and oxidative stress (Palomba *et al.,* 2021). These metabolic improvements may have enhanced the hematopoietic function and oxygen-carrying capacity of the blood. The increase in hemoglobin and hematocrit suggests that metformin may promote effective erythropoiesis, possibly by improving tissue oxygenation and reducing inflammatory inhibition of red blood cell production. This finding aligns with Banaszewska *et al.* (2021), who reported that metformin improves both reproductive and hematological outcomes in women with PCOS by stabilizing the metabolic milieu. The absence of significant changes in RBC and MCHC following metformin treatment further indicates that while red cell production was enhanced, cellular morphology and hemoglobin concentration relative to cell size remained stable, reflecting balanced erythropoiesis without cytotoxic or hemolytic effects.

Similarly, simvastatin administration produced significant increases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), indicating improved red cell size and hemoglobin content per cell. Statins, such as simvastatin, are HMG-CoA reductase inhibitors that not only lower cholesterol but also exert anti-inflammatory and antioxidant effects (Sirtori, 2021). These pleiotropic actions may improve endothelial function and blood flow, enhancing erythrocyte deformability and survival. The observed elevation in MCV and MCH may therefore reflect improved red cell quality and hemoglobin synthesis efficiency, possibly due to reduced oxidative stress in the bone marrow microenvironment. Statins have also been shown to restore

normal red blood cell rheology and protect against lipid peroxidation of the erythrocyte membrane in metabolic disorders (Kavalipati *et al.,* 2015). The absence of a significant increase in total hemoglobin concentration in the simvastatin-only group implies that its primary effect may be structural rather than stimulatory on erythropoiesis.

The combination of simvastatin and metformin produced a more pronounced improvement in several hematological indices, including hemoglobin concentration, hematocrit, and mean corpuscular volume, suggesting a synergistic or complementary effect of both drugs. This outcome is consistent with previous findings that the combination of statins and metformin enhances cardiometabolic health in insulin-resistant states by targeting distinct but overlapping metabolic pathways (Athyros *et al.,* 2018). Metformin primarily improves glucose utilization and insulin sensitivity, while simvastatin reduces lipid synthesis and oxidative stress. Their combined use may therefore provide a favorable internal environment that supports optimal erythropoiesis and red blood cell function. This combination also appeared to normalize hematological indices toward negative control values, which may indicate restoration of systemic metabolic and oxidative balance. González *et al.,* (2020) have similarly suggested that reduced oxidative stress and inflammation following combination therapy in metabolic disorders improve blood cell stability and function. Interestingly, mean corpuscular hemoglobin concentration (MCHC) remained unchanged across all treatment groups. The stability of MCHC suggests that neither simvastatin nor metformin altered the hemoglobin concentration relative to red cell size, maintaining normal osmotic equilibrium and erythrocyte hydration. This finding further supports the hematological safety of both agents, indicating that they do not adversely affect red blood cell morphology or membrane integrity (Al-Biate *et al.,*

2022). Thus, the overall hematological profile across groups reflects beneficial modulation without deleterious effects on red cell homeostasis.

The hematological findings of this study can be interpreted within the broader physiological context of PCOS, which is not only a reproductive but also a systemic metabolic disorder (Azziz *et al.,* 2016). The chronic low-grade inflammation and oxidative stress characteristic of PCOS can impair erythropoiesis and alter red cell function (Mohammadi *et al.,* 2018). Therefore, improvements in red cell indices following treatment reflect not just local effects but systemic physiological normalization. Enhanced hemoglobin and hematocrit levels may also indicate improved oxygen transport capacity and tissue perfusion, both essential for maintaining cellular metabolism and reproductive function.

The results obtained demonstrate that both simvastatin and metformin have the potential to ameliorate hematological disturbances associated with PCOS. The beneficial effects observed are likely due to their combined antioxidant, anti-inflammatory, and metabolic regulatory properties. These findings are in agreement with previous studies that emphasized the therapeutic role of metformin and statins in reducing metabolic and cardiovascular risks in PCOS (Harrison *et al.,* 2020; Zhou *et al.,* 2022). Importantly, the combined administration of both drugs produced the most notable improvements, highlighting a possible therapeutic advantage of combination therapy in addressing the multifactorial nature of PCOS-related hematological and metabolic dysfunctions.

5.2 CONCLUSION

In conclusion, the present study demonstrates that letrozole-induced PCOS in female

Sprague Dawley rats produced alterations in hematological indices, which were significantly improved following treatment with simvastatin and metformin, either alone or in combination. Metformin improved hemoglobin concentration and hematocrit, while simvastatin enhanced red cell morphology as reflected by increases in MCV and MCH. The combination of both drugs resulted in the most comprehensive hematological improvement, suggesting synergistic benefits through metabolic and vascular modulation. The stability of MCHC across all groups indicates that neither drug exerted adverse effects on erythrocyte integrity. Collectively, these findings suggest that simvastatin and metformin can effectively ameliorate hematological disturbances associated with PCOS by improving systemic metabolism, reducing oxidative stress, and supporting healthy erythropoiesis. Further studies employing molecular and histological analyses are recommended to elucidate the precise cellular mechanisms underlying these hematological modulations and to explore the potential clinical implications of combining statins and metformin in the management of PCOS-related hematological and cardiovascular abnormalities.

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