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Effects of Aqueous Extract of Hunteria umbellata on Hormonal, Metabolic, and Hematological Parameters of Polycystic Ovarian Syndrome in Sprague-Dawley Rat Model

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Effects of Aqueous Extract of *Hunteria umbellata* on Hormonal, Metabolic, and Hematological Parameters of Polycystic Ovarian Syndrome in Sprague-Dawley Rat Model

By

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Abstract

Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder, recognized as a leading cause of female infertility. It is characterized by hyperandrogenism, anovulation, and ovarian cysts, affecting multiple organ systems. Despite its impact, PCOS is poorly understood and lacks a cure. Current treatments primarily target associated issues such as insulin resistance but are often limited in efficacy and may have side effects, necessitating the exploration of new approaches like complementary medicine. This study investigated the benefits of Hunteria umbellata (HUE), a traditional antidiabetic herbal medicine, in a rat model of PCOS induced by a high-fat diet and testosterone propionate. Evaluations at 14, 28, and 56 days post-PCOS induction revealed dose- and time-dependent effects of HUE. PCOS was successfully induced in rats, exhibiting diagnostic characteristics such as hyperandrogenism, ovulation issues, and ovarian cysts. HUE treatment reduced ovarian cyst incidence, especially with prolonged high-dosage treatment. While metabolic disturbances were observed in all PCOS-induced groups, HUE effectively improved insulin resistance regardless of treatment duration or dosage. Evaluation of renal function showed an increase in kidney weights following PCOS induction, although other renal function parameters remained within normal ranges. Our findings highlight the potential therapeutic effect of HUE in managing PCOS, underscoring the need for further research to elucidate its mechanisms of action and explore its clinical applications in this condition.

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List of Abbreviations

PCOS: Polycystic Ovarian Syndrome.

HUE: Aqueous extract of *Hunteria umbellate.*

HU: *Hunteria umbellata*

LH: Luteinizing hormone.

FSH: Follicle stimulating hormone.

GnRH: Gonadotropin-releasing hormone.

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Chapter I: Introduction

A great proportion of individuals all around the world are incapable of conceiving children resulting in psychological and emotional disadvantages. With one-third of infertility cases being associated with women individually. The most common causes of infertility amongst women include tubal disorders, uterine abnormalities, endocrine disorders, and ovulatory disorders. Ovulatory disorders such as polycystic ovarian syndrome (PCOS) are the cause of up to 25% of female infertility cases (Cunha & Póvoa, 2021).

PCOS is one of the most common infertility disorders, responsible for 70% of anovulatory cycles amongst adult women (Nautiyal et al., 2022). Up to 5 million women in the United States are affected by this heterogeneous syndrome according to CDC (2022). Depending on the diagnostic criterion, PCOS prevalence is between 6 to 20% in women of childbearing age (Sanchez-Garrido & Tena-Sempere, 2020). This syndrome is characterized by both endocrine and metabolic abnormalities. The resulting clinical symptoms include amenorrhea, oligomenorrhea, anovulation, hirsutism, acne, obesity, insulin resistance, and infertility (Witchel et al., 2019). PCOS is a risk factor for diabetes mellitus type II, hypertension, myocardial infarction, dyslipidemia, and other conditions (Ndefo et al., 2013).

In individuals with PCOS, disruptions in the hypothalamic-pituitary-gonadal (HPG) axis lead to symptoms associated with infertility. In typical mammalian reproduction, the HPG axis plays a pivotal role. Within this axis, Gonadotropin Releasing Hormone (GnRH) is released by neurons in the hypothalamus. This hormone serves as the main driver of the reproductive system. The frequency and amplitude of GnRH release dictate the synthesis of gonadotropins, specifically Follicle Stimulating Hormone (FSH) and Luteinizing hormone (LH), in the anterior pituitary

gland. GnRH secretion occurs in pulses in response to positive or negative feedback from estrogen and progesterone.

For normal follicular development and ovulation to occur in healthy women during their reproductive age, the pulsatile pattern of GnRH release changes following steroid feedback. Estrogen is low in the early follicular phase and increases halfway through the follicular phase of the cycle. In the mid-follicular phase, estradiol positive feedback increases GnRH pulse frequency, which in turn increases the release of LH, triggering ovulation. After the ovulatory phase, increased progesterone from the corpus luteum decreases GnRH pulse frequency through progesterone-negative feedback (Blair et al., 2015; Holesh et al., 2023).

This disruption in hormones contributes to the hallmark feature of anovulation or oligoovulation in women with PCOS. Anovulation, the absence of regular ovulatory cycles, or oligoovulation, irregular ovulation, are prevalent manifestations of the reproductive dysfunction associated with PCOS. The heightened levels of androgens, such as testosterone, and the presence of insulin resistance further exacerbate the imbalance within the Hypothalamus-Pituitary-Gonad (HPG) axis. These disturbances interfere with the regulation of GnRH pulsatility, disrupting the coordinated release of FSH and LH. As a result, the normal cyclic changes in ovarian follicular development and the subsequent release of mature eggs are hindered, resulting in the observed challenges to ovulation experienced by women with PCOS.

Due to these disruptions, women with PCOS usually have irregular or abnormal menstrual cycles. Other models of PCOS such as non-primate mammals also have these disrupted cycles which are referred to as estrous cycle disruptions. These disruptions are indicative of abnormal ovulation as seen in women with PCOS.

To address the pathogenesis of PCOS, current treatment strategies aim to alleviate symptoms of the syndrome using insulin sensitizers, anti-androgen agents, and hormonal contraceptives (Dong & Rees, 2023). *Hunteria umbellata* (HU) is a plant that is commonly used in folkloric medicine to improve insulin sensitivity (Ajiboye et al., 2017). Since insulin sensitizers such as metformin are used in managing PCOS, an ethnobotanical that has hypoglycemic actions might have effects comparable to those of insulin sensitizers used in the treatment of PCOS. Therefore, this study aims to investigate whether treatment with *Hunteria umbellata* aqueous extract (HUE) could provide therapeutic benefits and ameliorate PCOS in rats.

Chapter II: Literature Review

Pathogenesis of PCOS

Hypothalamic neuroendocrine dysfunctions define the pathogenesis PCOS. Patients diagnosed with PCOS manifest increased or faster GnRH pulse frequency. This subsequently increases LH and reduces FSH secretions. The imbalance in the ratios of LH and FSH production is because LH-secreting cells are more responsive to a faster GnRH pulsatile frequency (Witchel et al., 2019). As FSH is relatively low in women with PCOS, the maturation of follicles does not occur, resulting in anovulation. These immature follicles then aggregate on the ovaries, developing what is referred to as cysts in PCOS. Lowered levels of FSH hinder the normal conversion of androgens to estrogen and elevated LH levels stimulate the overproduction of androgens, resulting in increased levels of testosterone in women with the condition (Ding et al., 2021). Hyperandrogenism, the clinical hallmark of PCOS is observed in up to 90% of patients with PCOS (Singh et al., 2023).

Metabolic dysfunction in PCOS

In addition to neuroendocrine dysfunctions, PCOS is associated with metabolic disruptions. These metabolic dysfunctions result from excess androgen production, the main feature of PCOS. Hyperandrogenism has been shown to impact various metabolic tissues such as adipose tissue, liver, pancreas, and muscle (Sanchez-Garrido & Tena-Sempere, 2020). Studies suggest that hyperandrogenism is a contributor to metabolic complications, including obesity and insulin resistance, which are common in PCOS (Barber et al., 2019). Notably, insulin resistance is the most prevalent metabolic disruption in PCOS, affecting up to 70% of all patients (Sanchez-Garrido & Tena-Sempere, 2020). This highlights the significant role of hyperandrogenism in the development of metabolic disturbances observed in PCOS patients.

Hyperandrogenism leads to increased visceral fat accumulation compared to women with normal androgen levels, who predominantly store fat in subcutaneous adipose tissues. This condition also induces adipocyte hypertrophy, observed in both women and rodents with elevated androgen levels, potentially leading to adipose tissue dysfunction. This dysfunction, characterized by impaired adipocyte function, can contribute to metabolic disturbances such as insulin resistance (Hammarstedt et al., 2018; Sanchez-Garrido & Tena-Sempere, 2020). Additionally, adipose tissue plays a crucial role in fat sequestration and the regulation of adipokine secretion, including leptin and adiponectin, essential for maintaining insulin sensitivity and glucose homeostasis. Dysfunction or absence of adipose tissue, as observed in women with hyperandrogenism, can elevate circulating triglycerides and fatty acids, thus exacerbating insulin resistance and disrupting metabolic balance (Guilherme et al., 2008). Furthermore, hyperandrogenism has been associated with decreased levels of insulin-sensitizing adipokines such as adiponectin and omentin-1 in patients and animal models of PCOS (Sanchez-Garrido & Tena-Sempere, 2020).

Previous studies have demonstrated that hyperandrogenism is associated with non-alcoholic fatty liver disease (NAFLD) and other hepatic pathologies. Increased Alanine aminotransferase (ALT), an indicator of hepatic damage has been shown to be positively associated with increased levels of androgens (Hong et al., 2023). Other studies that induced hyperandrogenism in rodents resulted in elevated liver fat accumulation (Zhang et al., 2018).

Skeletal muscles are affected by hyperandrogenism, primarily insulin sensitivity and glucose uptake. Insulin-stimulated glucose uptake is reduced in women with PCOS, and this has been associated with skeletal muscle insulin resistance (Dunaif, 1999). Alterations of insulin receptors and intracellular insulin pathway phosphorylation patterns have been shown to contribute to skeletal muscle insulin resistance (Boucher et al., 2014). Additionally, insulin-mediated glucose

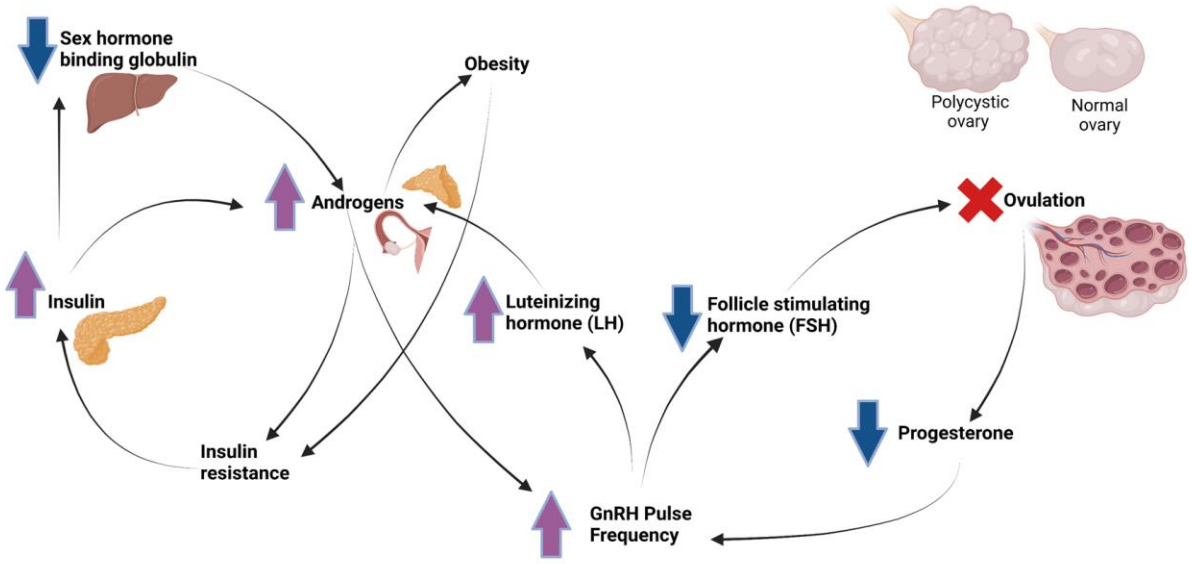
transport in muscles has been shown to be reduced, leading to compensatory hyperinsulinemia (another symptom of PCOS) (Zhao et al., 2023).

In the pancreas, excessive androgens are thought to mediate β-cell dysfunction directly and indirectly as these cells have androgen receptors. Hyperandrogenism results in the hyperactivation of androgen receptors in β pancreatic cells which may stimulate mitochondrial dysfunction, oxidative damage, and insulin hypersecretion (Unluhizarci et al., 2021).

As such, humans or animal models of PCOS present with clinical features of metabolic syndrome including but not limited to obesity, dyslipidemia, and insulin resistance. PCOS is a risk factor for diabetes mellitus type II (T2DM), hypertension, myocardial infarction, and other conditions (Ndefo et al., 2013). Of all symptoms, insulin resistance and hyperandrogenism are conjoined in the pathogenesis of PCOS and they both contribute to the development of other symptoms in PCOS (Chen & Pang 2021). Current literature suggests that elevated levels of (compensatory) insulin further increase ovarian steroidogenesis, impacting the endocrine system (Baillargeon & Nestler, 2006). *Figure* 2.1 depicts the interplay of different systems and comorbidities in PCOS, illustrating its heterogeneous nature and complex pathogenesis.

Figure 2.1

PCOS Pathogenesis



Note. Interplay of different systems and comorbidities in PCOS, illustrating its self-perpetuating nature and highlighting its heterogeneous nature and complex pathogenesis.

The many metabolic disturbances associated increase the risk of developing other complications such as chronic kidney disease (CKD). Insulin resistance, in particular, plays a key role in the development of metabolic complications in PCOS, including those affecting the kidneys. Insulin resistance and oxidative stress has been found to potentially lead to renal injury. Insulin resistance is often associated with chronic low-grade inflammation and increased oxidative stress, which can damage kidney cells and contribute to the development of kidney disease (Kosmas et al., 2018).Increased production of proinflammatory cytokines, connective tissue growth factors, and profibrotic factors, along with microvascular injury and renal ischemia, are possible mechanisms of this kidney damage (Prasad, 2014). Additionally, Elevated insulin levels (another comorbidity of PCOS) can have direct effects on the kidneys, promoting sodium retention

and increasing the production of certain growth factors that can contribute to kidney damage (Pina et al., 2020). Androgen excess in PCOS also contributes to kidney dysfunction by causing increased visceral fat deposition. Additionally, hyperandrogenism is linked to endothelial dysfunction, which can further exacerbate kidney damage (Lau et al., 2022).

Being a metabolic and reproductive endocrine disorder, PCOS treatments target symptoms of these systems individually or in combination. Currently appreciated therapies employ the use of insulin sensitizers such as metformin or clomiphene citrate. In a previous clinical study, metformin was effective in restoring and inducting ovulation in patients with PCOS in addition to lowering androgen production (Attia et al., 2023). Other treatment approaches include the use of estrogen-progestin compounds, anti-androgen receptor blockers, and lifestyle changes such as exercise for weight loss in cases of obesity (Akre et al., 2022).

Phenotypes of PCOS

As a multifactorial disorder, PCOS predisposition has been shown to be related to individual genes or gene to gene interactions as well as gene-environment interactions (Khan et al., 2019). Twin studies strengthened these findings by showing that 72% of the variance observed in PCOS is due to genetic factors. Phenotypes of PCOS have been classified as A, B, C, and D. Phenotype A consists of women with cystic ovaries, oligo- or ano-ovulation, and hyperandrogenism. Phenotype B consists of women with normal ovaries, oligo- or ano-ovulation and hyperandrogenism. Phenotype C consist of women with cystic ovaries, normal ovulatory cycles, and hyperandrogenism, Phenotype D consists of women with cystic ovaries, oligo- or ano-ovulation, and normal androgen levels. Phenotype A and B are classified as classic PCOS and they are the most frequently observed with the most severe symptoms including increased risks of metabolic syndrome, insulin resistance, and compensatory hyperinsulinemia with more menstrual

disruptions observed. Phenotype C is characterized as the ovulatory PCOS and it results in higher insulin resistance and androgen levels with more hirsutism observed. This phenotype is mostly observed in high socioeconomic groups in comparison to the other phenotypes. Phenotype D or non-hyperandrogenic PCOS results in the lowest metabolic dysfunctions and better or close to normal ovulatory cycles. The frequency of PCOS phenotypes differs with the most common one being Phenotype A consisting of 45-66% of PCOS cases, followed by Phenotype B affecting 8-33%, Phenotype C affecting 3-29%, and Phenotype D only 0-23% (Khan et al., 2019).

Genotypes of PCOS

Since PCOS is a disruption of normal ovarian morphology and function as well as androgen synthesis, several genes that are involved in ovarian and adrenal steroidogenesis can be attributed to PCOS. *Cyp11A* is a gene that encodes for an enzyme required for the conversion of cholesterol to progesterone in the ovaries. *Cyp21* is another gene that codes for an enzyme involved in steroidogenesis in the ovaries that is involved in PCOS. *Cyp17*, another gene that encodes for an enzyme involved in steroidogenesis has been associated with elevated levels of androgens while polymorphisms at the promoter region of this gene are associated with PCOS patients. CYP19 codes for the aromatase enzyme, aromatase is involved in estrogen conversion, and it has been shown to have lower activity in women who have PCOS.

Other genes that are involved in the effects of steroid hormones such as the Androgen Receptor (AR) Gene and Sex Hormone Binding Globulin gene (SHBG) can be associated with PCOS. The AR gene which is found on the arm of an X chromosome has been linked to PCOS. Any mutation in the AR gene in addition to the inactivation of X chromosome has been shown to cause PCOS. SHBG gene is responsible for the synthesis of SHBG protein in the liver. SHBG is significantly lower in women with PCOS and this produces hyperandrogenism because SHBG

controls androgen levels in the blood by binding to androgens. It has been shown that the lowered levels of SHBG are associated with single nucleotide polymorphism in the SHBG gene.

Genes that are involved in gonadotropin regulation and function such as the LH receptor gene, the follicle-stimulating hormone receptor (FSHR) gene and the AMH gene are associated with PCOS pathogenesis. It has been found that polymorphisms of the LH gene are associated with PCOS. The AMH gene usually encodes for a protein that results in infertility and once exon-sequencing and GWAS were done, several variants in the AMH genes were indicators or predictors of PCOS development in women.

Because insulin is involved in androgen production, and insulin resistance is very common with women who have PCOS, genes that are involved in insulin action and production are associated with PCOS. elevated frequencies of insulin receptor substrate genes that produce insulin receptors have been observed in women with PCOS. Another gene, *Calpain10*, associated with Type I diabetes, is also associated with PCOS. Mutations of the Calpain10 gene have been shown to cause PCOS. Other genes involved with conditions like obesity are also linked to PCOS. For example, the Fat mass obesity gene is associated with type II diabetes, and obesity is also linked to PCOS when polymorphisms of this gene happen (Khan et al., 2019).

Hunteria umbellata

Ethnobotanicals remain a widely used form of medicine in various parts of the world. Many medicinal plants have been shown to have actions comparable to that of pharmaceuticals (Karimi et al., 2015). Folk medicine in several countries relies on ethnobotanicals for the treatment of several metabolic diseases. *Hunteria umbellata* (HU), (K. Schum.) is a medicinal plant used as such. HU, belonging to the family Apocynaceae is a tropical rainforest tree commonly found in the western sub-Saharan countries of Africa (Adeneye et al., 2012). The ethnomedicinal uses of

HU are seen in the management of diabetes mellitus, treatment of sexually transmissible diseases, dysmenorrhea (Adeneye et al., 2012) obesity, and anemia (Adeneye & Crooks, 2015). Past literature indicates that dose-dependent treatment with HU solution exerts hypoglycemic effects. (Adeneye et al., 2012) Other studies show the antihyperglycemic effects of HU in rats and suggested that their effects occur by inhibiting intestinal glucose uptake, which is known in plants containing high saponins like HU. HU may also mediate its effects through other pathways to increase hepatic glucose uptake (Adeneye & Adeyemi, 2009).

Studies investigated the phytochemical composition of HU and it was found that the crude plant extract contained saponins, glycosides, steroids, tannins, phenols, and alkaloids, (Dubale et al., 2023). Erinidine, a bisindole alkaloid isolated from HU may be the antihyperglycemic agent resulting in hypoglycemic effects of HU by inhibiting intestinal glucose uptake (Adeneye Adejuwon et al., 2012).

Hunteria umbellata **and PCOS**

As previously mentioned, current therapeutic approaches to PCOS employed the use of insulin sensitizers such as metformin to control insulin resistance and reverse other symptoms of PCOS, bringing about ovulation. Insulin sensitizers or euglycemic drugs reduce blood glucose by increasing peripheral sensitivity to insulin as well as inhibiting hepatic glucose production (Li et al., 2022). Recently, herbal medicine has emerged as a promising approach in PCOS management, by targeting such symptoms. For instance, *Cinnamomum verum* has been demonstrated to be effective in the management of PCOS by alleviating insulin resistance (Rashid et al., 2022). Other studies have shown that a combination of flaxseed and spearmint improved ovarian and endocrine profiles in PCOS rat models (Mehraban et al., 2020). Another herbal approach in managing PCOS, utilizing Berberine, an active constituent of *Rhizoma coptidis*, was found to be more effective

compared to the commonly used insulin sensitizer metformin by enhancing insulin sensitivity and reducing androgen levels and LH/FSH ratios (Xie et al., 2019). Although the use of HU in the treatment of diabetes has been previously investigated, there are no known studies that were performed to investigate the effects of HU on PCOS. This forms the basis for this study to investigate the metabolic and hematological alterations resulting from oral administration of HU in testosterone propionate induced PCOS rats to determine whether its hypoglycemic actions are effective in the management of PCOS.

Chapter III: Hypotheses

Research Hypothesis 1

Oral administration of an aqueous extract of HU will reverse hormonal, metabolic, and hematological symptoms of PCOS, leading to a reduction in the manifestation of PCOS indicators across all parameters, ultimately resulting in the resolution of PCOS profiles in rats.

Null Hypothesis 1

There will be no significant difference in the hormonal, metabolic, and hematological symptoms of PCOS, nor in the manifestation of PCOS indicators across all parameters, following oral administration of an aqueous extract of HU, thus no resolution of PCOS profiles will be observed in rats.

Research Hypothesis 2

Increased dosages and prolonged oral administration of an aqueous extract of HU will lead to more reductions in hormonal, metabolic, and hematological symptoms of PCOS, resulting in a greater diminishment of PCOS indicators across all parameters, ultimately leading to a more significant resolution of PCOS profiles in rats.

Null Hypothesis 2

There will be no significant difference in the reduction of hormonal, metabolic, and hematological symptoms of PCOS, nor in the diminishment of PCOS indicators across all parameters, following increased dosages and prolonged oral administration of an aqueous extract of HU. Ultimately, there will be no notable difference in the resolution of PCOS profiles in rats due to dosage and timing of treatment.

Chapter IV: Materials and Methods

Approval by the Institutional Animal Care and Usage Committee of SCSU, St. Cloud, MN was obtained for all the research protocols utilized in this study. All experiments were carried out in the vivarium and WSB-235 at SCSU, St. Cloud, MN. All animals used in this study were bred in-house at SCSU.

Animal Model

The animal model chosen for this study was the Sprague Dawley rat (*Rattus norvegicus*), a widely employed model in PCOS research due to its ability to replicate both metabolic and reproductive abnormalities seen in humans with PCOS. Three-week-old female Sprague Dawley rats weighting 60-80 grams were used in this study to capture key developmental stages relevant to PCOS pathogenesis. PCOS was induced in the rats to generate features such as anovulation and cystic ovarian morphology. This model has been extensively used in past research investigating the effects of HU aqueous extract.

Plant Acquisition and Preparation

Dried HU seeds were obtained from Falana Farms, Ibadan, Oyo State, Nigeria. Authentication of the seeds was performed at the University of Lagos in Lagos, Nigeria by Dr. Taofikat Adesalu in the Department of Botany. Before the preparation of the aqueous solutions, the seeds were stored at room temperature in WSB-235.

Aqueous Extraction of HU Seeds

To prepare the extract, a blender was used to grind the dried seeds to a fine powder-colored beige brown. 25 grams of the powder was boiled in 500 mL of distilled water for 1 hour while being stirred. This mixture was then left to cool while still being stirred for 6 hours and then filtered using a clean muslin cloth after which the filtrate was left to dry and solidify on a petri dish in an

aerated oven at 37°C for 24 hours. The residue was scraped off and mixed in distilled water at a concentration of 100 mg/ml. The fresh stock was recreated daily based on changes in body weight and dosage of the treatment group.

Preliminary Study

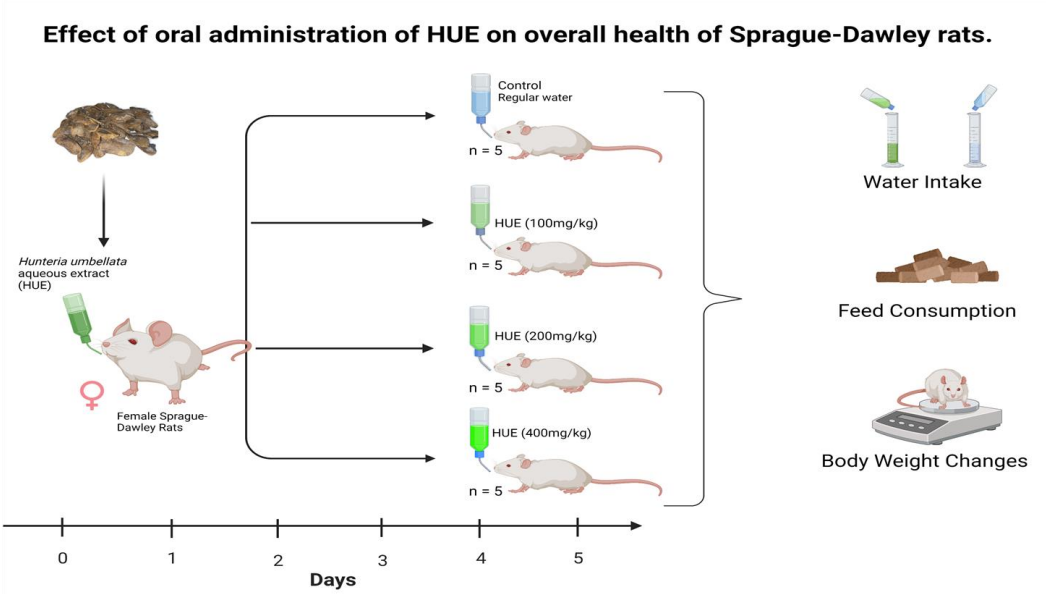
Experiment 1: The effect of administration of Hunteria umbellata aqueous seed extract on feed intake and body weight in Sprague-Dawley rats.

The main objective of this experiment was to investigate the effects of oral administration of aqueous extract of HU on the water and feed intake as well as body weight of Sprague-Dawley Rats. The results of this study will be useful in determining the feasibility of the second and main study. Most importantly, this experiment focused on determining inappropriate outcomes that may occur with the oral administration of HU.

Methodology of preliminary study

Twenty-three weeks old Sprague-Dawley rats weighing 36 grams on average were used in this pilot study. On day one of the study, the ad-lib water intake of all rats was determined for 10 rats that would be used in the study. Following this, 20 rats were randomly assigned into four treatment groups: Control group (n=5), Low dose group (n=5), Mid dose group (n=5), and High dose group (n=5). The control group was given deionized water, the low-dose group was given 100 mg/kg BW, the mid-dose group 200 mg/kg BW, and the high-dose group 400 mg/kg BW of the aqueous extract of HU. The rats were individually caged and maintained at standard conditions in the vivarium. Daily water intake and body weight were recorded as volume (mL) and mass (grams), respectively. A 10% increase in grams of the extract used per solution was made in regard to the increasing body weight. The methodology of this preliminary experiment is depicted in Figure 3.1.

Figure 3.1



Note. Illustration of timeline and methodological approaches used in the preliminary study. TP:Testosterone propionate, HUE:*Hunteria umbellata* aqueous extract.

Statistical analysis of preliminary study

Microsoft Excel was used to analyze data by performing descriptive statistics to determine differences in body weight changes and absolute water intake among all groups during the three-day period of the study.

Main Study

Experiment 2: The effect of oral administration of aqueous extracts of Hunteria umbellata on metabolic indices and reproductive hormones in PCOS-induced Sprague-Dawley rats.

Pre-treatment with aqueous extract of HU

86 rats, each weighing approximately 60-80 grams, were randomly divided into two groups as PCOS (n=30) and non-PCOS (n=60). These were further randomly assigned into six treatment groups, namely regular water nonPCOS (n=13), regular water PCOS (n=13), low dose HU water

(100mg/kg BW; n= 14), mid-dose HU water nonPCOS (200 mg/kg BW; n=14), mid-dose HU water PCOS (200 mg/kg BW; n=14) and high dose HU water (400 mg/kg BW; n=15).

All the rats grouped as HU water, received HU aqueous extract solution and the dosage was given as 100, 200, and 300 mg per kg of body weight for the low dose, mid-dose, and high dose groups, respectively. The volume of the water and solution given to all the rats varied from 75mL to 100mL, exceeding the 24-hour water consumption of the rats. All rats in the regular water groups received 75mL to 100mL of deionized water in the same manner. The water and extract solutions given to the rats were made readily available in the caging system of each rat. During this time, each rat was weighed every 3 days until the end of the study.

Induction of PCOS

To induce PCOS, at the fourth week of life, each rat in the PCOS group was subcutaneously injected with testosterone propionate (1mg per 100 gram of body weight) dissolved in sesame seed over the shoulders into the loose folds of skin on the back of the neck oil, and fed a high-fat diet consisting 29% fat, 26% proteins, and 30% carbohydrates (Envigo Teklad Diets). By contrast, the rats in the non-PCOS group were fed the regular Teklad Global rodent diet containing 4% fat, 16% protein, and 49% carbohydrates and received sesame seed oil injections at the same volume. This is done daily for 28 days or until the endpoint when rats were euthanized.

Vaginal Smears

Simultaneously, at the fourth week of life, vaginal smears from 3 randomly picked rats out of every group were obtained for 21 consecutive days and preserved for cytology. This is important to demonstrate whether there was disruption of the estrous cycles of the rats(Ajayi and Akhigbe, 2020),

Post-induction procedures

At the second, fourth, and eighth-week post-induction, euthanasia was performed on four to five rats in each group. A week prior to the rat's euthanasia, each rat that would be sacrificed underwent an oral glucose tolerance test. Rats were fasted eight hours before-hand and then restrained in a rat restraint device, after which a lancet was used to nick the lateral vein on the tail of the rat. An Accu-check glucose monitoring kit was used to collect the blood sample and read the blood glucose levels. After recording the baseline blood glucose values, the oral gavage administration of glucose at 2g per kg of body weight was performed on each rat. 30, 60, and 120 minutes after gavage, tail blood samples were obtained using the technique described above and read using an Accu-chek kit. Reading collected at each time point was recorded for analysis of glucose tolerance.

Euthanasia

Four to five rats per treatment group were euthanized at three-time points: 2 weeks of age, 6 weeks of age, and 12 weeks of age. Euthanasia was performed by cervical dislocation whereby the base of the skull was compressed with a rod, then the cervical vertebra was separated from the skull by promptly pulling the base of the tail. The cervical dislocation was followed by decapitation which was achieved by pressing a guillotine blade between the skull and neck below the styloid process to achieve trunk and head separation. Trunk blood was immediately collected in EDTA-coated and plain tubes which were then kept for analysis. The ovaries, uterus, kidneys, lungs, and heart were surgically removed after decapitation, photographed, and weighed for analysis.

Blood Collection and storage

Blood without EDTA was left to coagulate for approximately 20 minutes and then spun at 3000 rpm for 15 minutes. The serum was then stored in a -80°C freezer until shipping to Idexx laboratories where analyses were made.

Blood collected in EDTA-coated tubes was inverted several times and then placed on ice for hours until all tubes were stored in a 4°C refrigerator. Whole blood was spun 24-36 hours after storage at 2400 rpm for 15 minutes. Plasma was then stored in a -20 °C freezer until analyses were performed.

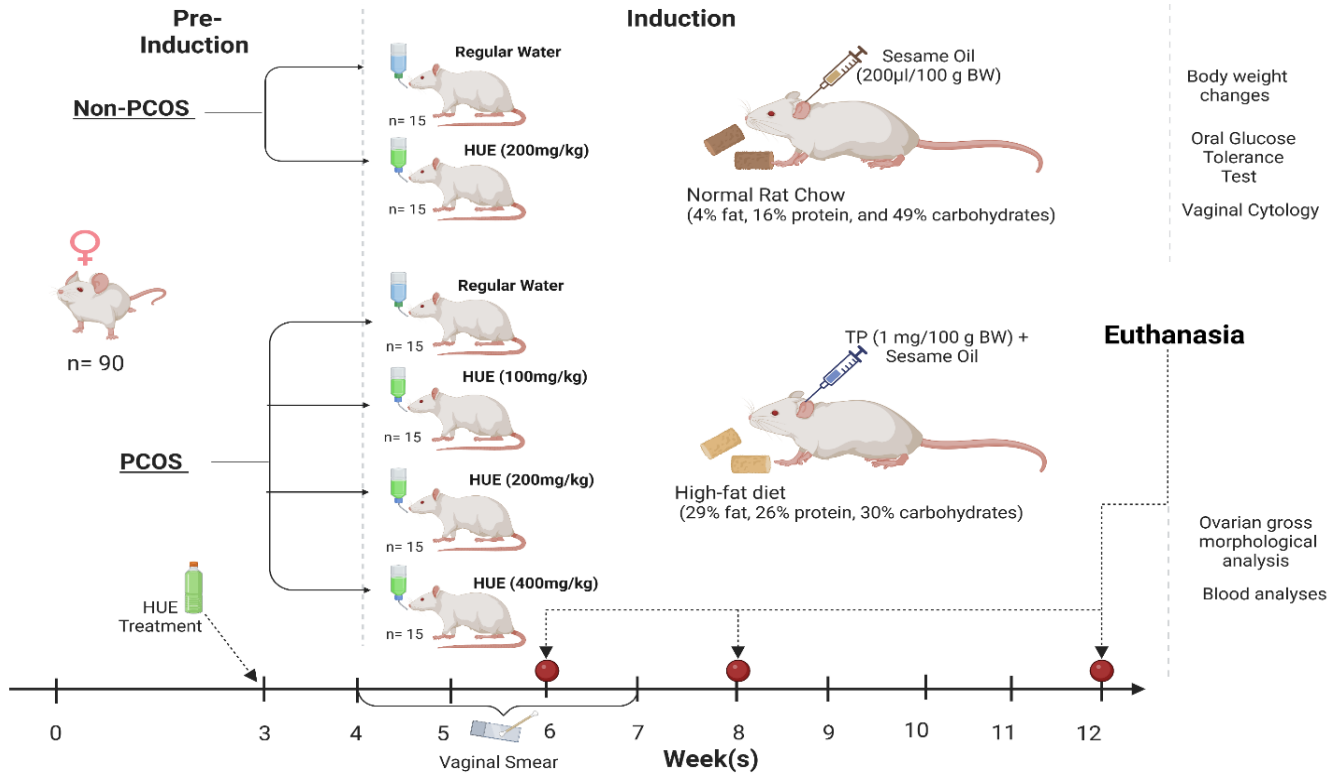
Hormone Assays

Enzyme-linked immunosorbent assays (ELISAs) were used to measure levels of FSH, LH, testosterone, and insulin in rat serum samples. FSH was quantified using the Rat FSH ELISA Kit from Elabscience (catalog #EL-R0391). The Elabscience Rat LH ELISA Kit (catalog #E-EL-R0026) was used to measure LH. Testosterone was quantified with the QuicKey Pro Rat Testosterone ELISA from Elabscience (catalog #E-OSEL-R0003). Insulin was measured using the Rat Insulin ELISA from Mercodia. ELISA procedures provided by the manufacturers were followed. Hormone concentrations were determined by spectrophotometric detection and generating standard curves. Refer to kit manuals for detailed protocols. (Elabscience. (2022)

The study design, methodology and timeline of this experiment is summarized and depicted in *Figure 3.2*.

Figure 3.2

Methodology and Design



Note. Illustration of timeline and key methodological approaches used in the main study. TP:Testosterone propionate, HUE:*Hunteria umbellata* aqueous extract.

Statistical analysis of main study

A Two-way Analysis of Variance (ANOVA) using the SAS GLM procedure was used to assess the effects of different dosages and durations of oral administration of an aqueous extract of HU on hormonal, metabolic, and hematological symptoms of PCOS in rats. The Tukey Honest Significant Difference test was used to determine the significance of differences between specific groups. Results were considered significant at $p < 0.05$ in both tests.

Table 1

List of Chemicals, Reagents, and Equipment Used in the Study

Item	Vendor	Use
HU seeds	Falana Farms, Nigeria	Utilized to prepare an aqueous extract for oral administration at varying doses.
Testosterone propionate	Sigma aldridge	Employed in injections to induce Polycystic Ovary Syndrome (PCOS) in Sprague-Dawley (SD) rats.
Pure Sesame seed oil	Coborns, St. Cloud, MN	Utilized as a solvent for dissolving Testosterone propionate and administered in control groups.
LH Elisa kit	Elabscience	Utilized for the quantification of Luteinizing Hormone (LH) levels.
FSH Elisa kit	Elabscience	Utilized for the quantification of Follicle Stimulating Hormone (FSH) levels.
Accu-chek glucose monitoring kit	Byerleys	Utilized for the measurement of blood glucose levels in rats during Oral Glucose Tolerance Test (OGTT).
Normal Rat Chow	Envigo Telkat Diets	Fed to rats in the control groups.
High Fat Rat Chow	Envigo Telkat Diets	Administered to rats for the induction of PCOS.

Note. List of chemicals, reagents, and equipment used in the preliminary and main study, including vendors and uses.

Chapter V: Results

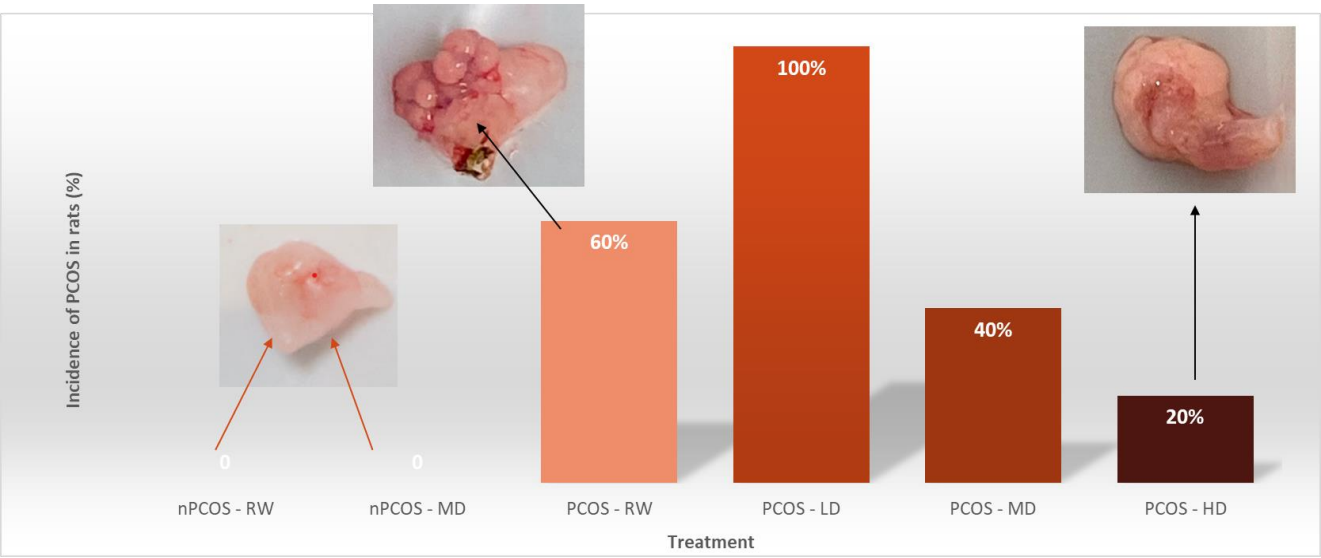
Indices of PCOS

I. Polycystic ovarian morphology

The incidence of ovarian cysts decreased over time in all PCOS groups. At 14 days post-PCOS induction, 60% of rats in the PCOS-RW group and 100% of rats in the low-dose treatment group exhibited cysts, while the mid and high-dose groups had lower incidences. At 28 days, 75% of rats in the PCOS-RW group had cysts, with varied percentages in the treatment groups. By 56 days, none of the high-dose treatment group rats exhibited ovarian cysts. These results are depicted in Figures 5.1, 5.2, and 5.3 for day 14, day 28, and 56, respectively.

Figure 5.1

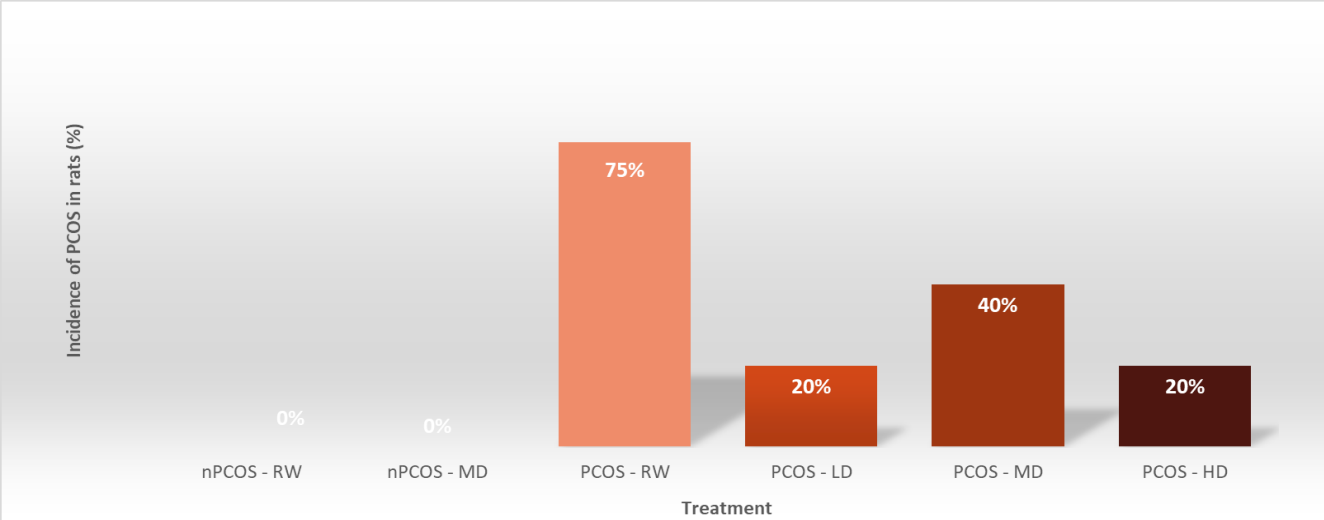
The effect of Hunteria umbellata treatment on the incidence of cystic ovaries in rats 14 days post PCOS induction.



Note. Data are presented as percentages of rats with cystic ovaries per group.

Figure 5.2.

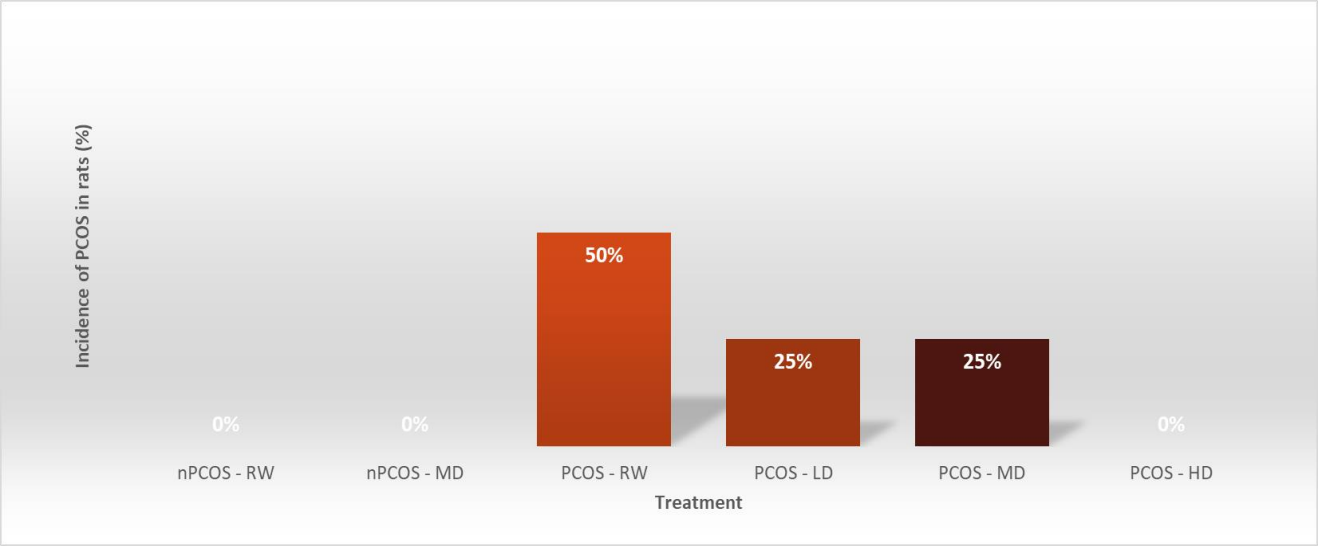
The effect of Hunteria umbellata treatment on the incidence of cystic ovaries in rats 28 days post PCOS induction.



Note. Data are presented as percentages of rats with cystic ovaries per group.

Figure 5.3.

The effect of *Hunteria umbellata* treatment on the incidence of cystic ovaries in rats 56 days post PCOS induction.



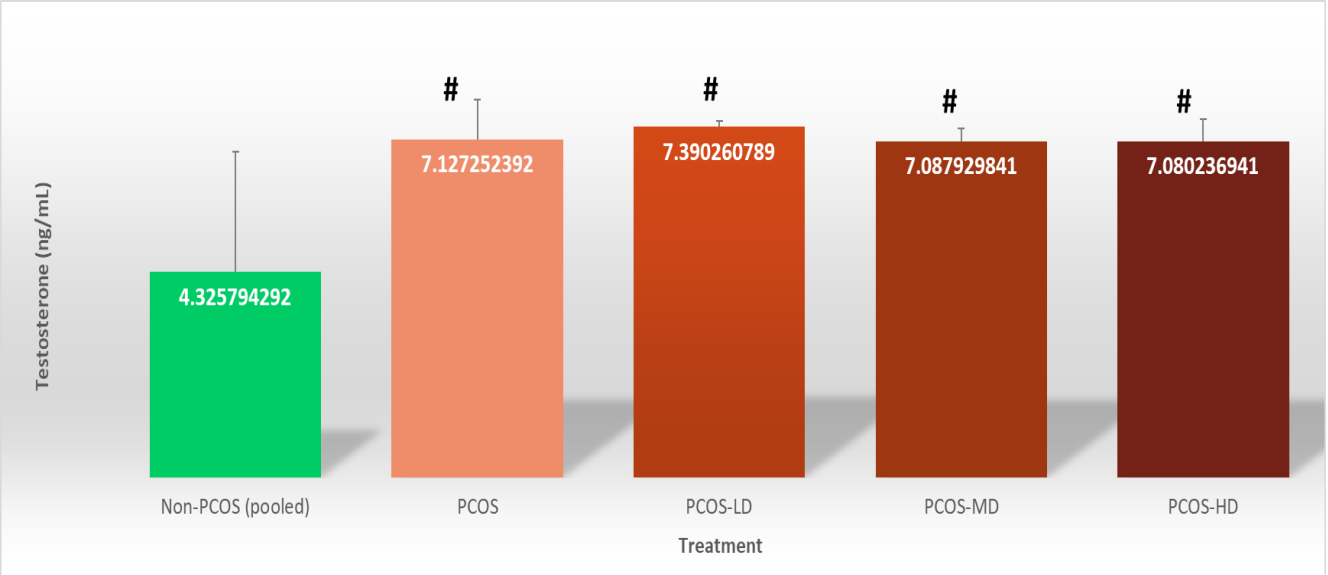
Note. Data are presented as percentages of rats with cystic ovaries per group.

II. Serum Testosterone Concentrations

Significant variations were observed between non-PCOS and PCOS groups. The non-PCOS groups had an average testosterone concentration of 4.326 ng/ml, while the PCOS-RW group had 7.127 ng/ml. The low and mid-dose treatment groups showed similar concentrations (7.390 ng/ml and 7.089 ng/ml, respectively), while the high-dose group had 7.080 ng/ml (Figure 5.4).

Figure 5.4.

Overall effect of Hunteria umbellata on serum testosterone concentration (ng/ml) in rats.



Note. Data are shown as averages versus standard deviation, with significant differences measured at $P<0.05$.

#: Significantly different compared with Non-PCOS control.

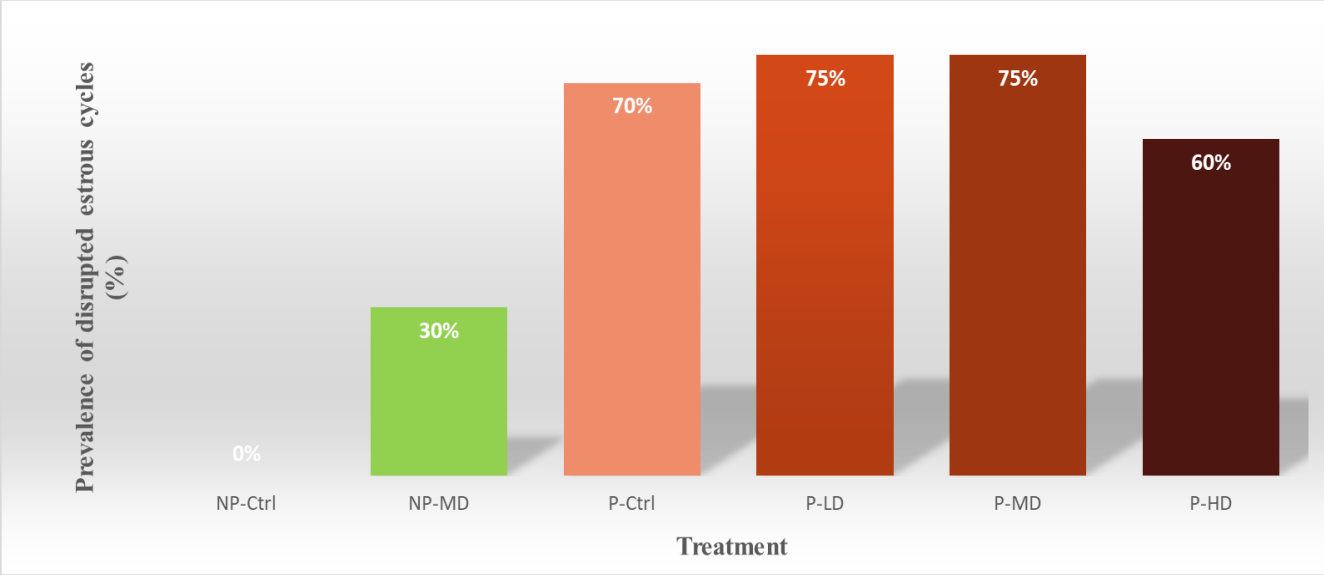
III. Estrous Cycle Disruptions

In the non-PCOS group, untreated rats exhibited no disruptions, while the other non-PCOS group had 30% with disruptions. In the PCOS groups, the untreated group had 70% with disrupted

cycles, and the low and mid-dose treatment groups had 75% each. The high-dose treatment group showed 60% with disrupted cycles as depicted in Figure 5.5.

Figure 5.5

The overall effect of Hunteria umbellata on estrous cycle disruption in rats, determined through vaginal cytology.



Note. Data is presented as the percentage of rats showing estrous cycle disruption over a 21-day cycle.

PCOS Comorbidities

Insulin Resistance – Oral Glucose Tolerance Tests (OGTT)

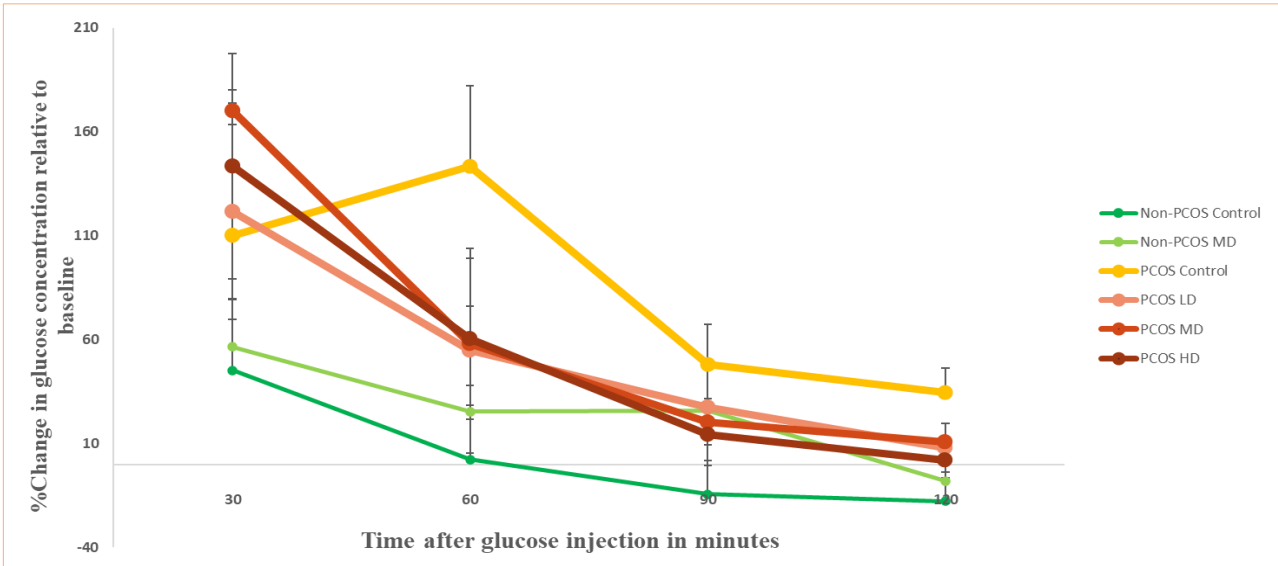
To assess insulin resistance, oral glucose tolerance tests (OGTT) were performed. Initial observations, recorded 30 minutes post-glucose injection, revealed elevated glucose levels within the PCOS groups. The corresponding changes in glucose concentration, illustrated in Figure 6, demonstrated an increase ranging from 110% to 170% when compared to the non-PCOS groups. Specifically, the non-PCOS group without treatment showed a 45.2% increase, while the HUE treatment group exhibited a 56.6% increase.

60-minute post-glucose injection, the PCOS group without treatment exhibited the highest percentage change in glucose concentration at 143%, while the treatment groups displayed changes ranging from 57% to 60%. In contrast, the non-PCOS groups maintained lower levels at 2% and 25.3%. Ninety minutes after glucose injection, blood glucose concentrations significantly decreased in the non-PCOS groups, ranging from -14.4% to 27.5%. PCOS groups receiving various doses of *Hunteria umbellata* exhibited changes between 14.3% and 27.5%, with the untreated PCOS group having the highest change at 48%.

After the 120-minute mark post-glucose injection, the change in glucose concentration remained lower for non-PCOS groups, ranging from -17.5% to 8%, while in the PCOS groups, those receiving HUE treatments, demonstrated higher changes, varying from 2% to 10.6%. The untreated PCOS group had the highest change with a 34.5% increase. A statistically significant difference ($P<0.05$) was observed between the non-PCOS control and PCOS control groups at all time points as illustrated in figure 5.6.

Figure 5.6

Impact of Hunteria umbellata on glucose tolerance.



Note. Data represents changes in blood glucose levels over time, presented as averages versus standard deviations. A significant difference ($P<0.05$) is observed between the Non-PCOS control and PCOS control group.

Body Weight Gain

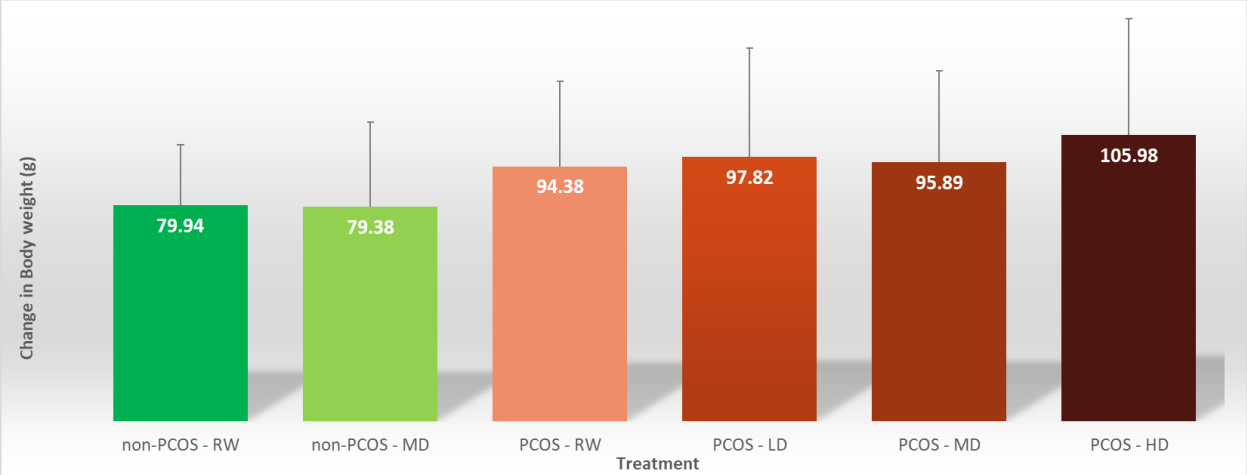
Changes in body weight were evaluated across all treatment groups throughout the experiment and a trend was observed, where all PCOS groups exhibited higher changes in body weight, although statistical significance was not reached. The non-PCOS groups showed changes of 79.94g for the non-PCOS control and 79.38g for the non-PCOS group that received a mid-dose treatment.

In contrast, the PCOS groups displayed variations in body weight changes, measuring 94.38g, 97.82g, 95.89g, and 105.98g for the PCOS control group without treatment, the low-dose treatment group, the mid-dose treatment group, and the high-dose treatment group, respectively.

These observations suggest a consistent trend in increased body weight gain among PCOS groups compared to non-PCOS groups, as illustrated in figure 5.7.

Figure 5.7

The overall effect of Hunteria umbellata treatment on body weight changes in body weight in rats.



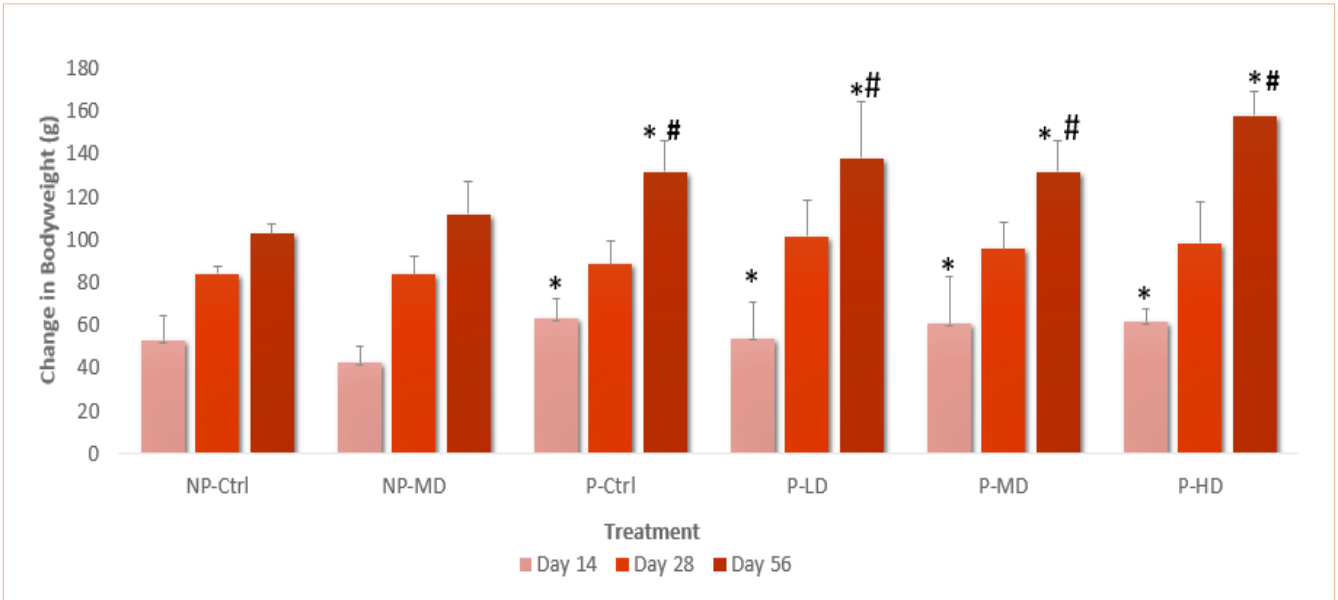
Note. Data are shown as averages versus standard deviation.

When examining changes in body weight at three specific time points—14 days, 28 days, and 56 days after PCOS induction, the same trend was observed, as shown in figure 5.8. 14 days after PCOS induction, all PCOS groups exhibited statistically significant increases in body weight compared to the non-PCOS group that received a mid-dose treatment. The corresponding values were 53.1g, 42.6g, 62.9g, 54g, 60.7g, and 61.6g for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. 28 days after PCOS induction, all groups exhibited no statistically significant amongst all groups. The corresponding values were 84.2g, 83.6g, 88.6g, 101.6g, 95.4g, and 98.5g for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. days after PCOS induction, all PCOS groups exhibited statistically significant increases in body weight compared to the non-PCOS groups. The corresponding values

were 102.6g, 112g, 131.7g, 137.9g, 131.6g, and 157.9g for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively.

Figure 5.8

Effect of Hunteria umbellata treatment on body weight changes in rats 14-, 28-, and 56-days post PCOS induction.



Note. Data are shown as averages versus standard deviation, with significant differences measured at $P<0.05$.

#: Significantly different compared with Non-PCOS control, *: Significantly different compared with Non-PCOS MD.

Organ changes

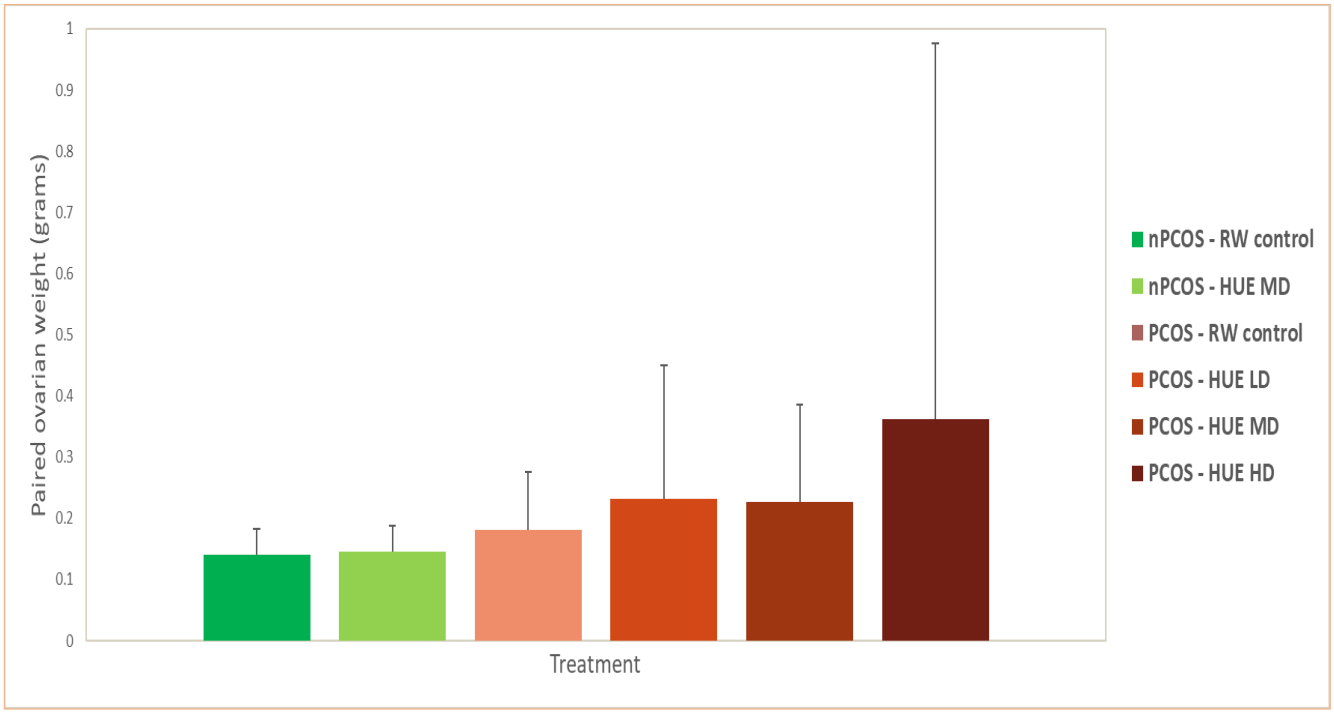
Ovarian Weight

Ovarian weights were measured for each group and results are depicted in figure 5.9. The results of ovarian weights also followed a similar trend whereby PCOS groups tended to have increased paired ovarian weights in comparison to non-PCOS groups. Weights in grams were 0.14,

0.145, 0.181, 0.231, 0.227, 0.362 for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively.

Figure 5.9

Overall effect of Hunteria umbellata treatment on paired ovarian weights in rats.



Note. Data are shown as averages versus standard deviation.

Kidney Weight

Gross kidney morphology was done after euthanasia, and relative kidney weights for each group were assessed at three distinct time points—14, 28, and 56 days post-PCOS, as depicted in Figure 5.10. A consistent trend emerged where non-PCOS groups exhibited lower kidney weights compared to PCOS groups, which demonstrated an increase in kidney weights. Statistically significant differences were observed only 14 and 56 days after PCOS induction.

The PCOS control group, receiving no treatment, displayed statistically significantly higher increased kidney weights in comparison to both non-PCOS groups on day 28 (12.535 mg/g)

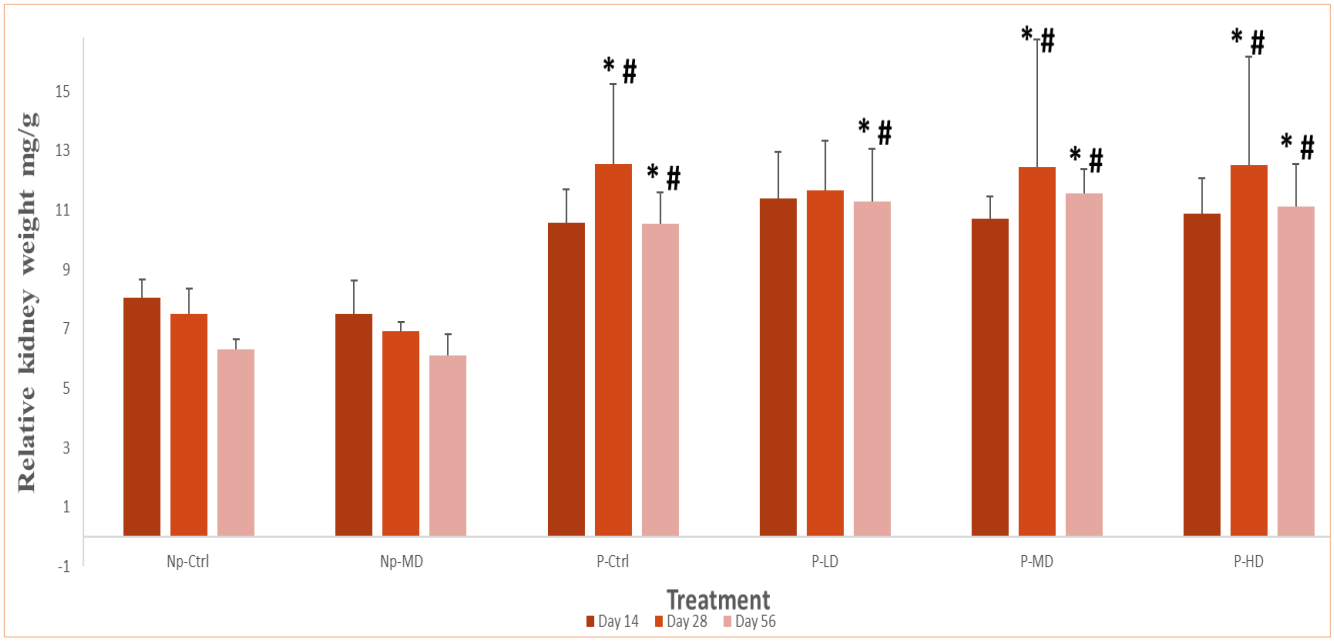
and day 56 (10.527 mg/g) post-PCOS induction. On day 14, the value for this group was 10.581 mg/g. The PCOS low-dose group exhibited kidney weights of 11.399 mg/g, 11.648 mg/g, and 11.278 mg/g on days 14, 28, and 56 post-PCOS induction. Significantly higher kidney weights were observed on day 56 for this group compared to the non-PCOS groups.

Similarly, the PCOS mid-dose group displayed kidney weights of 10.707 mg/g, 12.433 mg/g, and 11.553 mg/g on days 14, 28, and 56 post-PCOS induction, respectively. Both days 28 and 56 showed statistically significant increases compared to the non-PCOS groups. For the PCOS-high dose group, kidney weights were 10.872 mg/g, 12.51 mg/g, and 11.106 mg/g on days 14, 28, and 56 post-PCOS induction, with significant increases on days 28 and 56 compared to the non-PCOS groups.

In the non-PCOS control group that received regular water, relative kidney weights were 8.034 mg/g, 7.489 mg/g, and 6.308 mg/g on days 14, 28, and 56 post-PCOS induction, respectively. The non-PCOS group that received a mid-dose of HUE exhibited kidney weights of 7.508 mg/g, 6.919 mg/g, and 6.114 mg/g on days 14, 28, and 56 post-PCOS induction, respectively.

Figure 5.10

Effect of Hunteria umbellata treatment on relative kidney weight in rats 14-, 28-, and 56-days post PCOS induction.



Note. Data are shown as averages versus standard deviation, with significant differences measured at P<0.05. #: Significantly different compared with Non-PCOS control, *: Significantly different compared with Non-PCOS MD.

Hormones

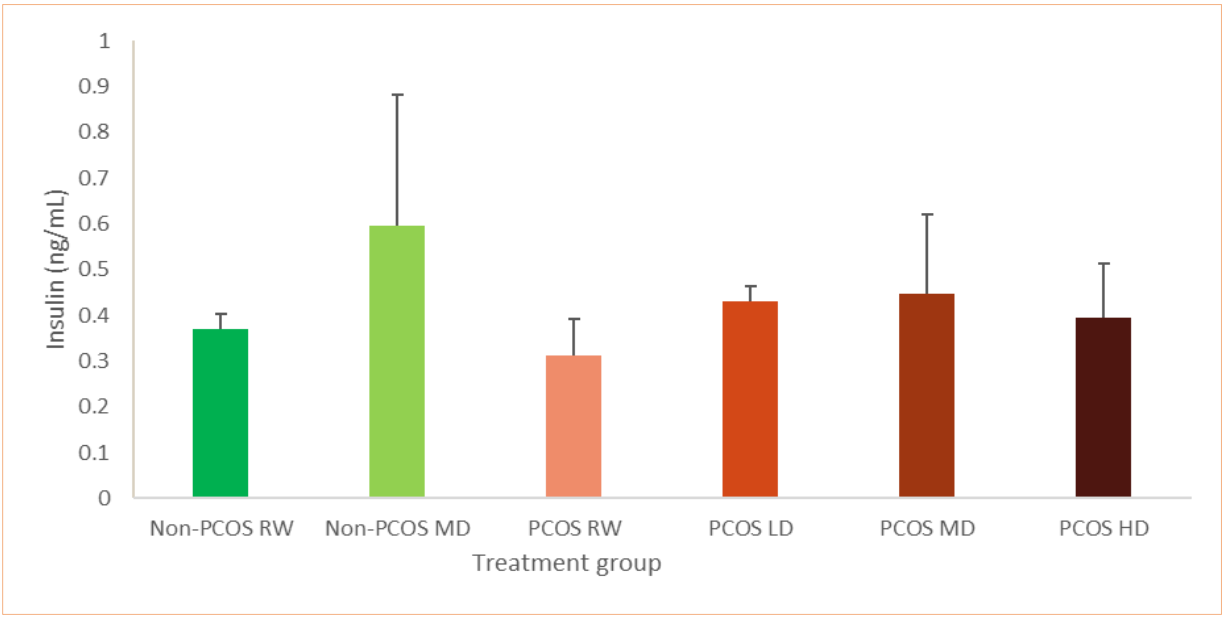
Insulin

Serum insulin concentrations were assessed for all groups at two time points, namely, 28 and 56 days post-PCOS induction. 28-days post-PCOS induction, insulin concentrations measured 0.371 ng/mL, 0.597 ng/mL, 0.312 ng/mL, 0.43 ng/mL, 0.446 ng/mL, and 0.396 ng/mL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. Similarly, 56-days post-PCOS induction, insulin concentrations

were recorded as 0.358 ng/mL, 0.35 ng/mL, 0.368 ng/mL, 0.341 ng/mL, 0.406 ng/mL, and 0.425 ng/mL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. These results are shown in Figures 5.11 and 5.12 for both time points. However, no statistically significant differences were observed, and no discernible trends were identified amongst the groups at both time points.

Figure 5.11

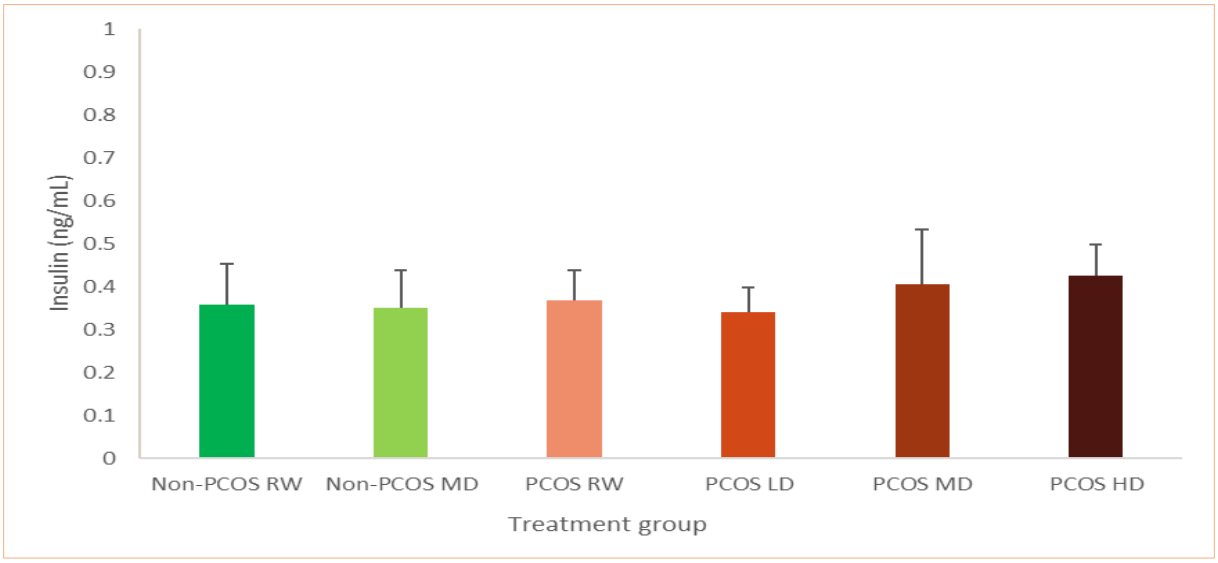
Effect of Hunteria umbellate treatment on insulin concentrations in rats 28 days post-PCOS Induction.



Note. Data are presented as means versus standard deviations.

Figure 5.12

Effect of Hunteria umbellate treatment on insulin concentrations in rats 28 days post-PCOS Induction.



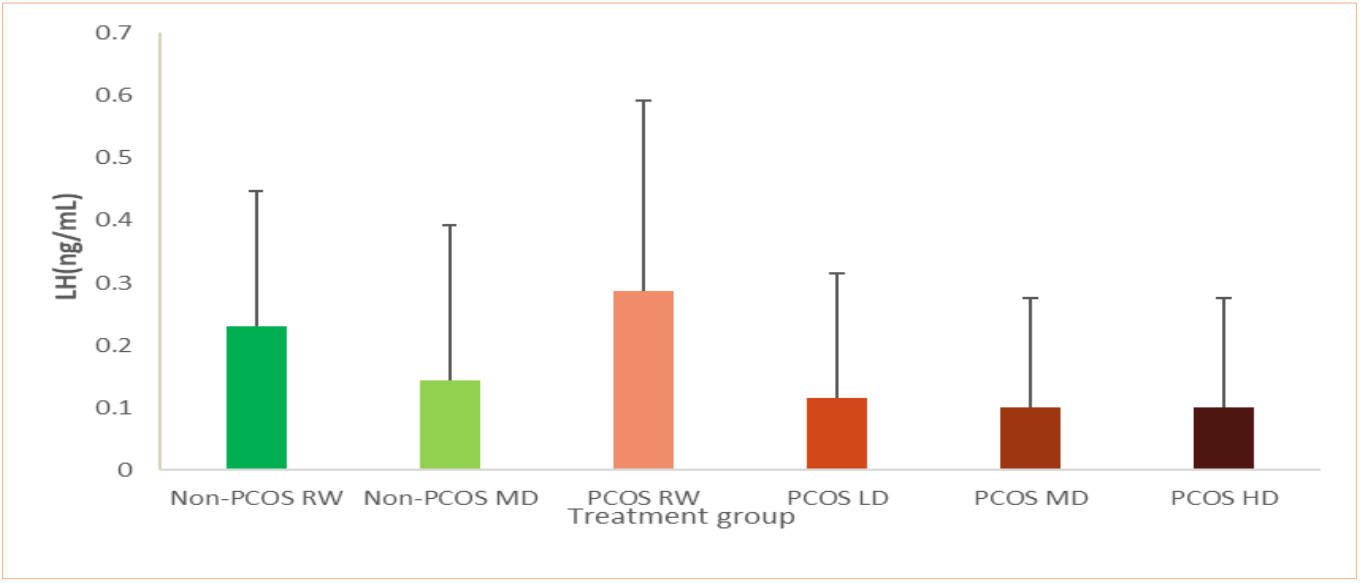
Note. Data are presented as means versus standard deviations.

Luteinizing Hormone

Serum luteinizing hormone concentrations were assessed for all groups at two-time points, namely, 28 and 56 days post-PCOS induction. 28-days post-PCOS induction, LH concentrations measured 0.2307 ng/mL, 0.1443 ng/mL, 0.287 ng/mL, 0.1159 ng/mL, 0.1009 ng/mL, 0.1009 ng/mL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. Similarly, 56-days post-PCOS induction, LH concentrations were recorded as 0.258 ng/mL, 0.173 ng/mL, 0.0433 ng/mL, 0.173 ng/mL, 0.173 ng/mL, 0.109 ng/mL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. These results are shown in Figures 5.13 and 5.14 for day 28, and 56, respectively. However, no statistically significant differences were observed, and no discernible trends were identified amongst the groups at both time points.

Figure 5.13

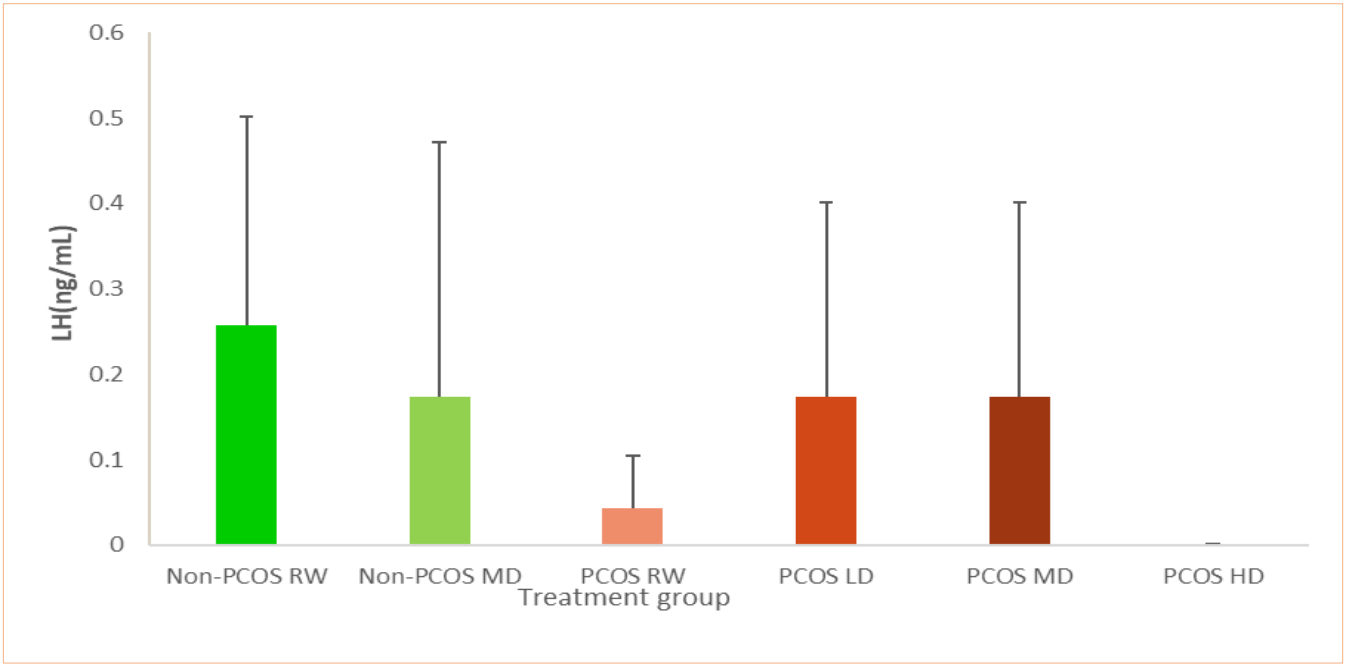
Effect of Hunteria umbellata treatment on luteinizing hormone concentrations in rats 28 days post-PCOS Induction



Note. Data are presented as means versus standard deviations.

Figure 5.14

Effect of *Hunteria umbellata* treatment on luteinizing hormone concentrations in rats 56 days post-PCOS Induction.



Note. Data are presented as means versus standard deviations.

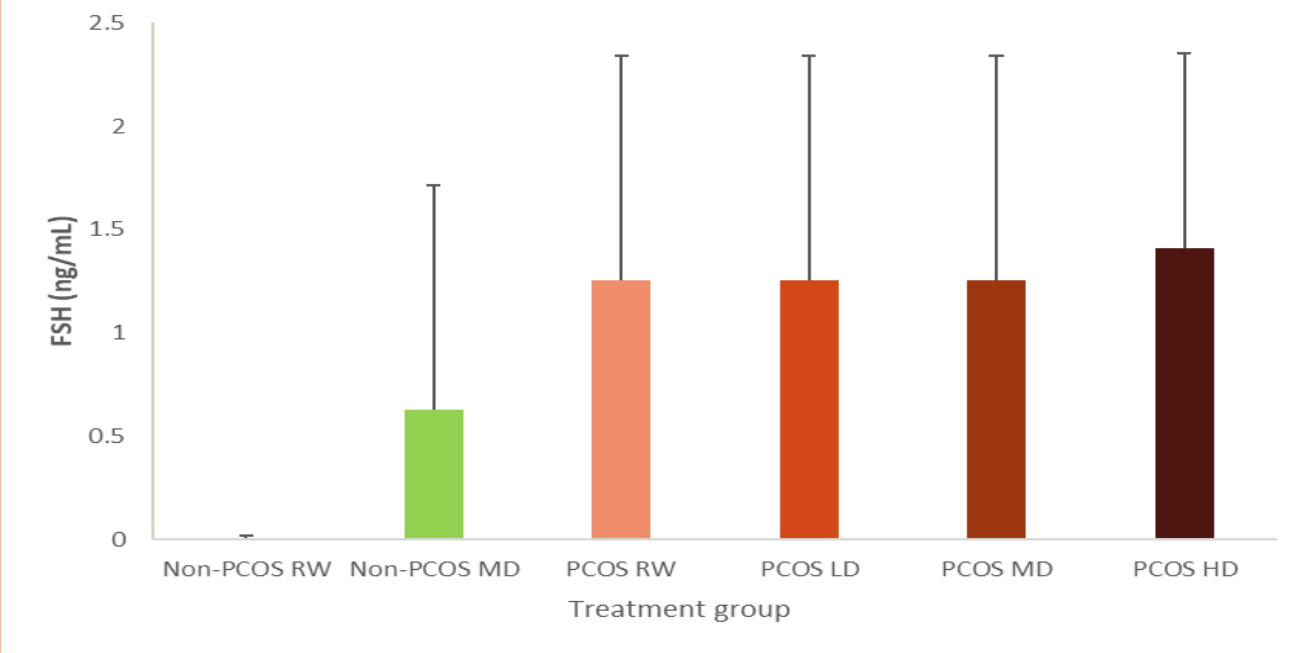
Follicle Stimulating Hormone

Serum FSH concentrations were assessed for all groups at two-time points, namely, 28 and 56 days post-PCOS induction. 28-days post-PCOS induction, FSH concentrations measured 0.008 ng/mL, 0.629 ng/mL, 1.254 ng/mL, 1.253 ng/mL, 1.254 ng/mL, 1.411 ng/mL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. Similarly, 56-days post-PCOS induction, FSH concentrations were recorded as 1.88ng/mL, 0.942 ng/mL, 1.88ng/mL, 0.63ng/mL, 0.63ng/mL for the non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. These results are shown in Figures 5.15 and 5.16 for day 28, and 56, respectively. However, no

statistically significant differences were observed, and no discernible trends were identified amongst the groups at both time points.

Figure 5.15

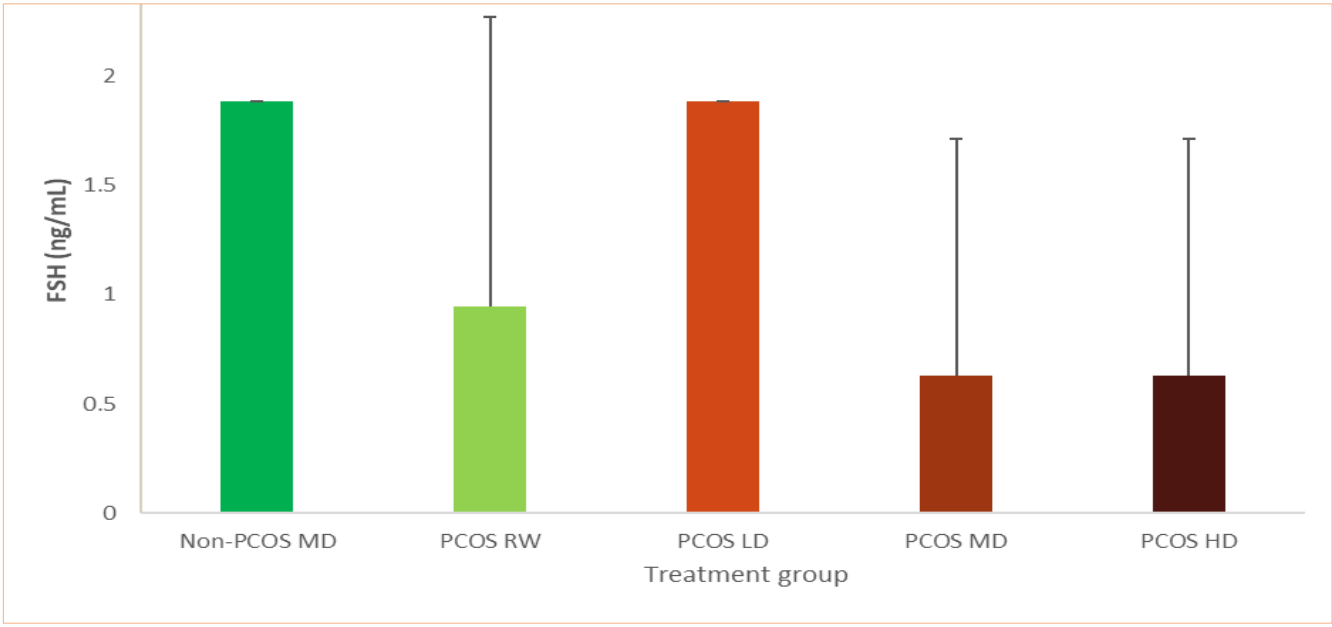
Effect of Hunteria umbellata treatment on follicle- stimulating hormone concentrations in rats 28 days post-PCOS Induction.



Note. Data are presented as means versus standard deviations.

Figure 5.16

Effect of *Hunteria umbellata* treatment on follicle-stimulating hormone concentrations in rats 58 days post-PCOS Induction.



Note. Data are presented as means versus standard deviations.

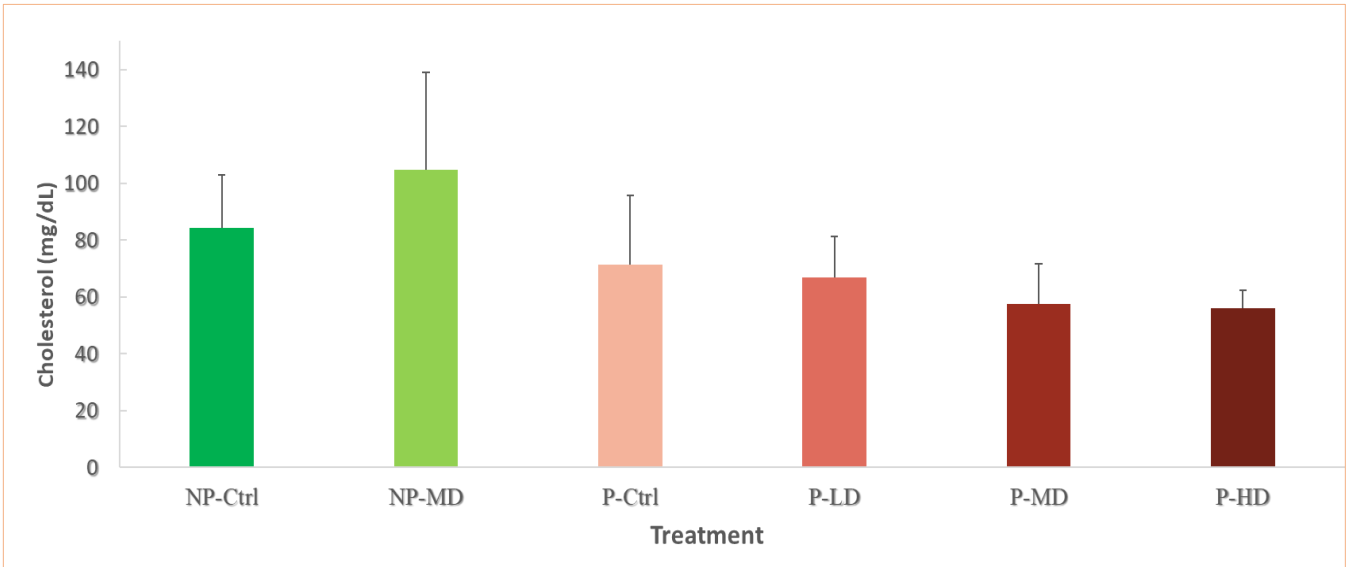
Other Parameters

Cholesterol

Cholesterol concentrations were measured for each group and results are depicted in figure 17. The results of cholesterol concentrations had no statistically significant differences observed, the results follow a trend whereby PCOS groups tended to have decreased body cholesterol concentrations in comparison to non-PCOS groups. 84.4mg/dL, 104.7mg/dL, 71.4mg/dL, 66.7mg/dL, 57.4mg/dL, 55.9mg/dL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. These results are depicted in Figure 5.17.

Figure 5.17

Overall effect of Hunteria umbellata treatment on serum cholesterol concentration in rats.



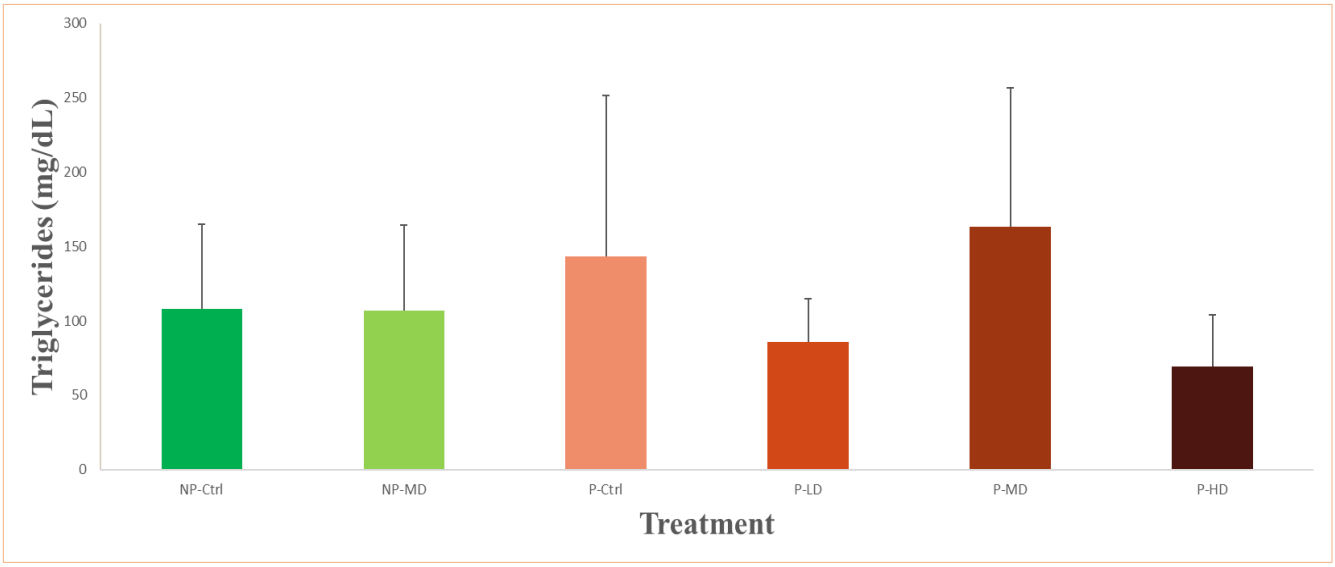
Note. Data are shown as averages versus standard deviation.

Triglycerides

Triglyceride concentrations were assessed for each group, and the findings are presented in Figure 5.18. The results indicated no statistically significant differences in triglyceride levels among the groups and exhibited no identifiable trend or pattern. Specifically, the concentrations were 108 mg/dL, 107 mg/dL, 143.5 mg/dL, 85.7 mg/dL, 163.4 mg/dL, and 69.2 mg/dL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively.

Figure 5.18

Overall effect of Hunteria umbellate treatment on triglycerides in rats.



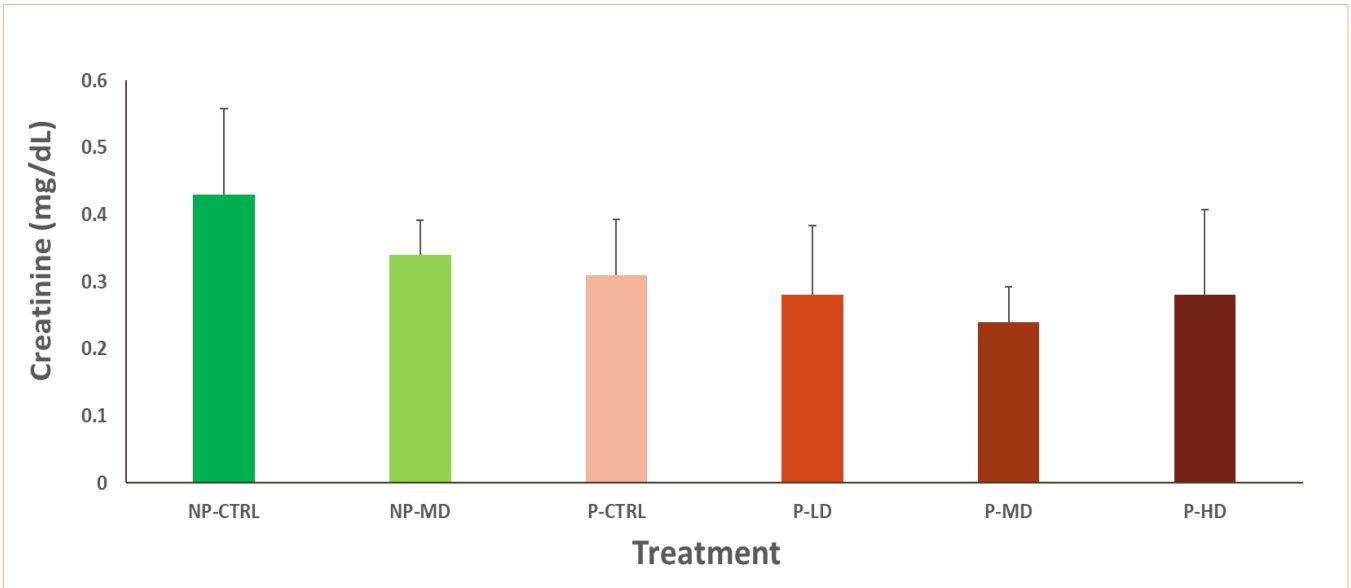
Note. Data are shown as averages versus standard deviation.

Serum Creatinine concentrations

Serum creatinine concentrations were quantified for each group, and the results are depicted in Figure 5.19. Although no statistically significant differences were found among the groups, a trend where the non-PCOS groups receiving treatment tended to exhibited lower levels of serum creatinine concentrations was observed. The concentrations were recorded as 0.43 mg/dL, 0.34 mg/dL, 0.31 mg/dL, 0.28 mg/dL, 0.24 mg/dL, and 0.28 mg/dL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively.

Figure 5.19

Overall effect of Hunteria umbellata treatment on serum creatinine concentration in rats.



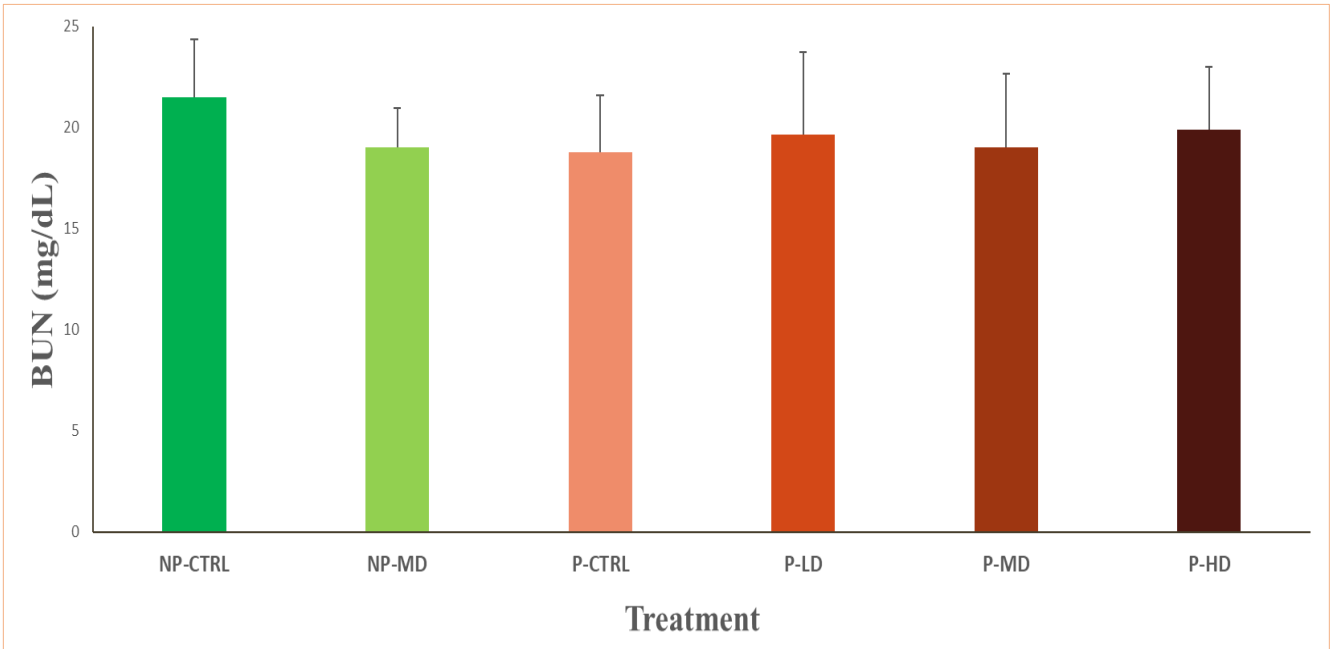
Note. Data are shown as averages versus standard deviation.

Blood Urea Nitrogen

Blood urea nitrogen (BUN) concentrations in plasma were assessed, with values of 21.5 mg/dL, 19 mg/dL, 18.75 mg/dL, 19.625 mg/dL, 19 mg/dL, and 19.875 mg/dL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. Figure 5.20 presents these results and no statistically significant differences or discernible trends were observed for this parameter.

Figure 5.20

Overall effect of Hunteria umbellata treatment on Blood urea nitrogen (BUN) in rats.



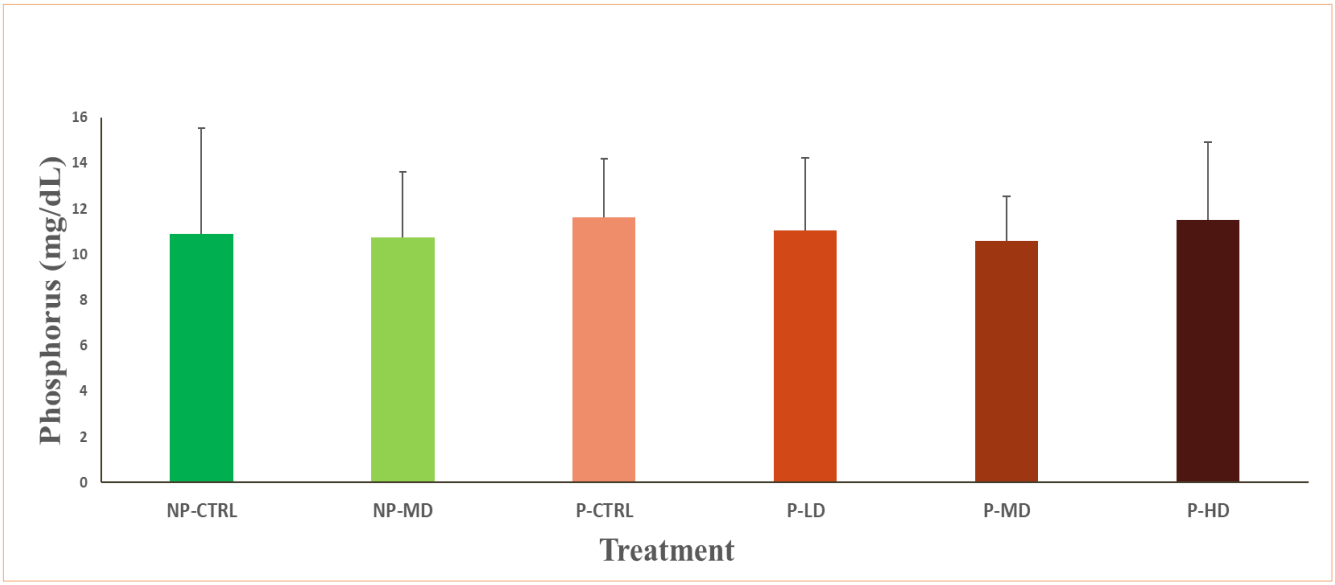
Note. Data are shown as averages versus standard deviation.

Phosphorus concentration

Figure 5.21 illustrates the phosphorus concentration values, where no apparent patterns or statistically significant differences were observed among the groups. The recorded concentrations for phosphorus were 10.883 mg/dL, 10.75 mg/dL, 11.625 mg/dL, 11.05 mg/dL, 10.575 mg/dL, and 11.513 mg/dL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively.

Figure 5.21

Overall effect of Hunteria umbellata treatment on phosphorus in rats.



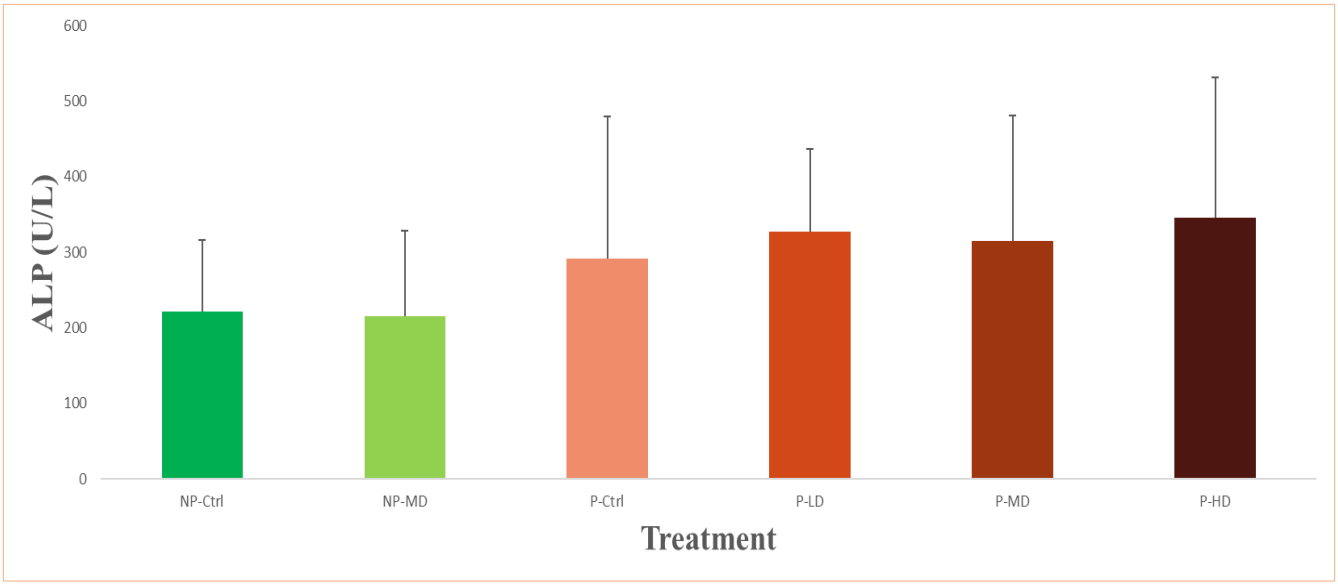
Note. Data are shown as averages versus standard deviation.

Alkaline phosphatase

Alkaline phosphatase (ALP) levels in the blood were observed, finding concentrations of 221.1 U/L, 215.4 U/L, 291.625 U/L, 326.8 U/L, 314.6 U/L, and 346.2 U/L for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. Even though we didn't find any statistically significant differences, the results exhibit a trend where PCOS groups show higher ALP levels in their blood. These results are depicted in figure 5.22.

Figure 5.22

Overall effect of Hunteria umbellate treatment on alkaline phosphatase in rats.



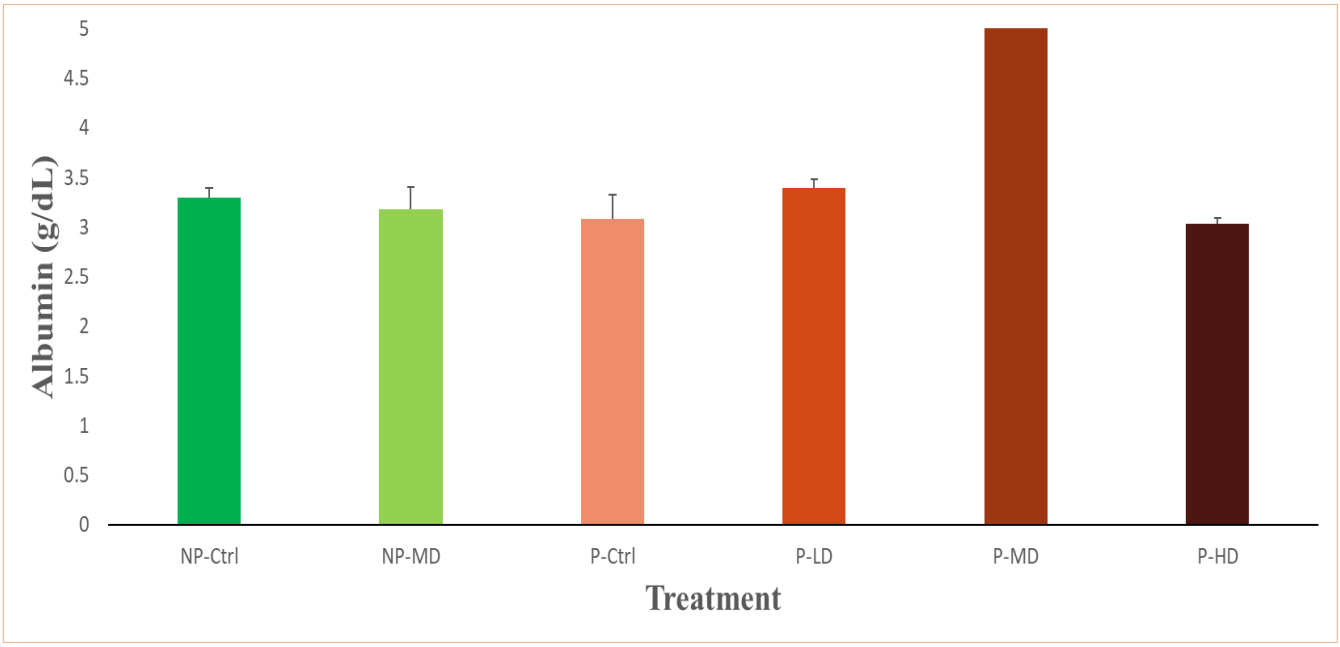
Note. Data are shown as averages versus standard deviation.

Albumin

Blood albumin concentrations were assessed, and no statistically significant differences were found among the groups and no discernible pattern was observed. The recorded concentrations were 3.3 g/dL, 3.18 g/dL, 3.08 g/dL, 3.4 g/dL, 5.18 g/dL, and 3.03 g/dL for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. These results are illustrated in Figure 5.23.

Figure 5.23

Overall effect of Hunteria umbellata treatment on albumin in rats.



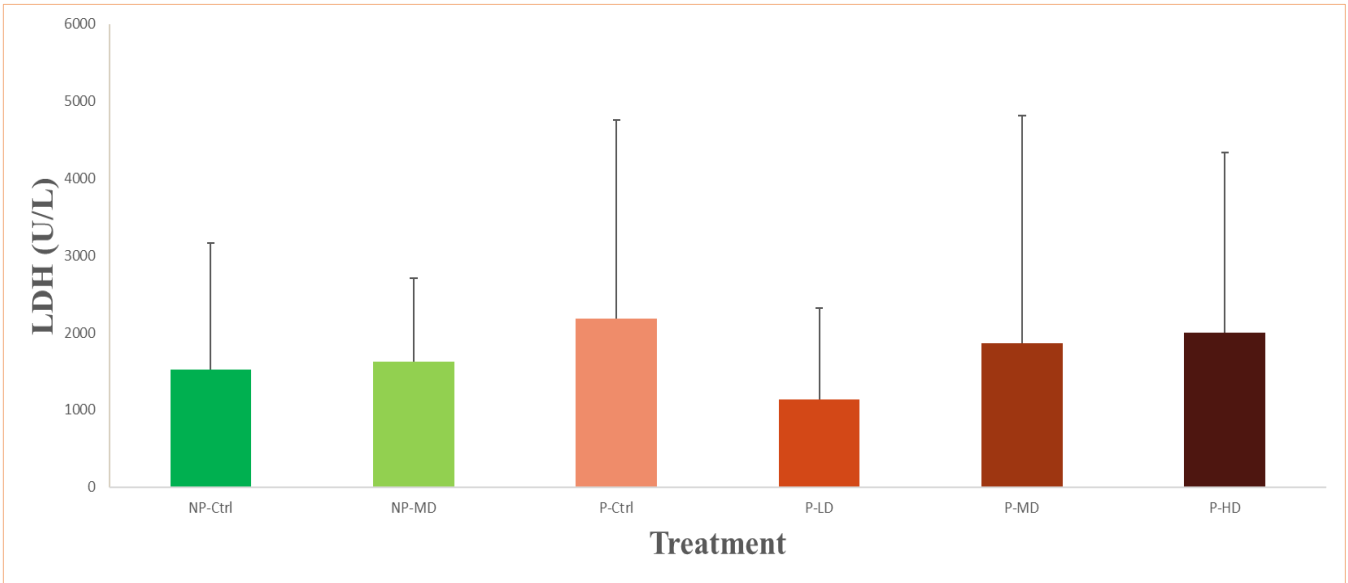
Note. Data are shown as averages versus standard deviation.

Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) concentrations in the blood were assessed, and similarly, there was no statistically significant difference among the groups, with no observable pattern in the recorded values. The concentrations of LDH were 1523.6 U/L, 1626 U/L, 2187.2 U/L, 1133.75 U/L, 1861.18 U/L, and 1997 U/L for the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, respectively. These results are illustrated in Figure 5.24.

Figure 5.24

Overall effect of *Hunteria umbellata* treatment on lactate dehydrogenase in rats.



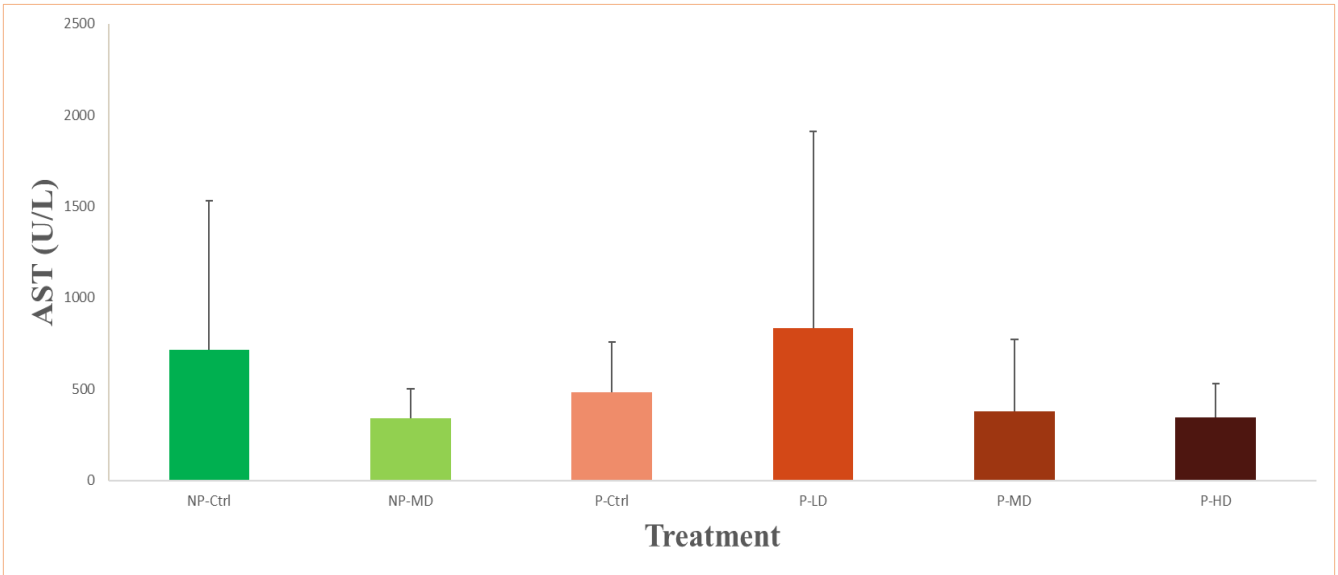
Note. Data are shown as averages versus standard deviation.

Aspartate aminotransferase

The blood concentrations of aspartate aminotransferase (AST) were evaluated, and similarly, no statistically significant differences were found among the groups, and the recorded values showed no discernible pattern. For the non-PCOS control, non-PCOS mid-dose, PCOS control, PCOS low dose, PCOS mid-dose, and PCOS high-dose groups, the AST concentrations stood at 715.2 U/L, 339.8 U/L, 482.3 U/L, 834.6 U/L, 378.7 U/L, and 346.2 U/L, respectively. These results are depicted in figure 5.25.

Figure 5.25

Overall effect of Hunteria umbellata treatment on lactate dehydrogenase in rats.



Note. Data are shown as averages versus standard deviation.

Chapter VI: Discussion

PCOS is a complex disorder associated with several complications, notably infertility, and has become increasingly prevalent. A key challenge in understanding and managing PCOS lies in its varied clinical presentations and associated comorbidities. Diagnostic criteria for PCOS vary, leading to diverse treatment approaches targeting the different aspects of the syndrome. However, current pharmacological therapies are only moderately effective, with reported success rates of approximately 60% (Sadeghi et al., 2022). Complementary and alternative medicine (CAM) strategies, including dietary interventions such as herbal foods, have shown promise in alleviating the severity of PCOS (Jia et al., 2021).

Our study employed a CAM approach using HUE to assess its impact on PCOS symptoms and severity. The results of this study will be essential for future research on the treatment of the syndrome using ethnobotanicals, known contributors in the development of some pharmaceuticals (Iwu, 2002). By integrating CAM approaches by using HUE for PCOS management, we contribute to the expanding repertoire of treatment options and potentially address the limitations of current pharmacological therapies. Our findings underscore the importance of considering alternative therapeutic modalities in the holistic management of PCOS, offering new avenues for improving patient outcomes and quality of life.

Clinical Indicators of PCOS

Our results suggest that oral HUE treatment may lead to a dose- and time-dependent reduction in PCOS morphology, with complete resolution observed after prolonged treatment at the highest dosage. However, it's important to note that spontaneous resolution of follicular cysts occurs in 70-80% of PCOS patients, which could also contribute to the observed resolution in our

study (Mobeen, 2023). Control groups not induced with PCOS did not exhibit ovarian cysts, confirming the successful induction of PCOS in the testosterone-induced groups.

Hyperandrogenism, characterized by significantly elevated serum testosterone concentrations, was observed in all PCOS-induced groups, consistent with previous animal models of PCOS, in which induction of PCOS with testosterone administration resulted in hyperandrogenism (Osuka et al., 2018). Additionally, the high-fat diet may have contributed to increased testosterone levels, as reported in other studies which demonstrated a positive correlation between a high fat diet and testosterone concentrations (Whittaker & Wu, 2021).

Rats with induced PCOS exhibited irregular estrous cycles, indicative of ovulatory dysfunction . Interestingly, a small percentage of non-PCOS rats treated with HUE also showed irregular cycles, although the reason for this is unclear.

Overall, our PCOS models accurately reflected the three diagnostic clinical features of the syndrome; the presence of follicular cysts, hyperandrogenism, and ano-ovulation or oligo-ovulation.

Metabolic Features in PCOS

Induction of PCOS in rats resulted in metabolic features akin to those seen in PCOS patients, including insulin resistance. Treatment with HUE led to a reduction in plasma glucose levels, suggesting a potential improvement in insulin sensitivity. While all PCOS groups showed higher blood glucose concentrations compared to non-PCOS rats, those treated with HUE exhibited lower levels, indicating that the PCOS condition caused some extent of insulin resistance and that HUE mitigated the severity of that insulin resistance. These findings align with previous research demonstrating the hypoglycemic effects of *Hunteria umbellata* in rodent models with

insulin resistance (Ajiboye et al., 2017). Interestingly, no significant differences in serum insulin concentrations were observed among groups.

Kidney Function

The assessment of kidney size revealed a significant increase in kidney size in all PCOS-induced groups compared to healthy controls. This observation aligns with previous studies demonstrating that testosterone injections, a key component of our PCOS model, can lead to an increase in kidney weight (Jones et al., 1998); (Shortliffe et al., 2014). Additionally, the administration of a high-fat diet, another component of our model, may have contributed to this increase in kidney size. However, despite the enlargement, no signs of renal dysfunction were observed, indicating that the changes in kidney size were not accompanied by impaired kidney function. The observed increase in kidney size in our PCOS-induced groups is particularly relevant given that individuals with PCOS are known to be at an increased risk of developing kidney disease (Patil et al., 2017). This suggests that the structural changes observed in the kidneys of our PCOS model rats may have implications for long-term kidney health. While our study did not find evidence of renal dysfunction at the administered doses, further investigation is warranted to understand the potential long-term effects of PCOS.

Hormonal Changes

Insulin concentrations did not show significant differences across all treatment groups and between PCOS and non-PCOS groups. Similarly, LH and FSH plasma concentrations were not significantly different between groups. While women with PCOS typically exhibit low FSH and high LH levels, our results showed normal serum FSH and LH levels in both PCOS and non-PCOS groups, consistent with some studies where normal FSH levels were observed in PCOS cohorts (Christodoulopoulou et al., 2016). Our results are inconsistent with the most common findings as

elevated LH levels and LH/FSH ratios are observed in 35 - 77% of PCOS patients (Atoum et al., 2022). It would be expected that most PCOS models would fit that criterion of hormonal imbalance as the neuroendocrine feature of PCOS is increased LH/FSH levels (Blank et al., 2006). This discrepancy in hormonal profiles of PCOS models and patients highlights the complexity of PCOS and the variability in its presentation.

Other Clinical Features

Contrary to our expectations, PCOS rats tended to have lower cholesterol concentrations compared to non-PCOS groups, despite receiving testosterone injections. This finding is not consistent with past research that has shown that women with PCOS usually present with decreased high-density lipoprotein cholesterol and increased levels of high-density lipoprotein cholesterol. This is also consistent with individuals in insulin resistant states which goes hand in hand with PCOS (Kim & Choi, 2013). Other studies have shown that dyslipidemia is usually associated with PCOS. Our model showing lowered levels of cholesterol might possibly be attributed to high lipid administration which can lead to negative feedback mechanisms that regulate lipid synthesis in the body. Additionally, our results might be explained by other studies which propose that metabolic reactions in high lipid states can result in lowered lipids by converting excess lipid deposits into energy (Littlejohn et al., 2020). Triglycerides levels were also similar in all groups. From our results, our PCOS models showed no signs of dyslipidemia. Although dyslipidemia is usually observed in patients with PCOS, some individuals diagnosed with the condition present with normal lipid profiles, as seen in (Christodoulopoulou et al., 2016). The mechanisms behind these varying lipid changes in PCOS warrant further investigation.

Elemental Analyses in PCOS

Contrary to previous studies suggesting abnormalities in calcium homeostasis in PCOS patients, our results did not show significant differences in serum calcium levels between PCOS and non-PCOS groups (De Felici et al., 1991; Thys-Jacobs et al., 1999). Similarly, potassium levels were not significantly different between groups, despite reports of hyperkalemia in some women with PCOS (deOliveira et al., 2024). These results suggest that the mechanisms underlying calcium and potassium dysregulation in PCOS are complex and may not be universal among all individuals with the condition.

Other elemental analyses did not reveal significant differences between PCOS and non-PCOS groups.

Hematological Analyses

Hematological parameters, including hemoglobin, red blood cell count, white blood cell count, and platelet count, did not show significant differences between PCOS and non-PCOS groups. This is consistent with findings in other studies, indicating no significant differences in hematological parameters between women with PCOS and healthy controls (ALhabardi et al., 2020).

Chapter VII: Conclusion

In conclusion, our study sheds light on the complex nature of polycystic ovarian syndrome (PCOS) and the potential therapeutic effects of *Hunteria umbellata* in the rat model. Our PCOS model demonstrated clinical manifestations of the syndrome, including ovarian cysts, hyperandrogenism, ovulatory dysfunction, and metabolic abnormalities, mirroring aspects of the human condition. Our findings suggest that HUE treatment may lead to a reduction in PCOS morphology, with the greatest therapeutic effect observed at longer treatment durations and higher dosages.

Despite inducing metabolic features similar to those seen in PCOS patients, such as insulin resistance and altered lipid profiles, our model did not fully replicate the dyslipidemia typically associated with PCOS. This discrepancy underscores the need for further research to elucidate the mechanisms underlying these metabolic changes in the PCOS condition.

Additionally, our study provides insights into the renal effects of PCOS induction, with enlarged kidneys observed in PCOS-induced rats. However, no significant renal dysfunction was detected, suggesting that the administered doses of testosterone and high-fat diet did not induce kidney injury.

Overall, our study contributes to the current knowledge on the pathophysiology of PCOS and highlights the potential of HUE as a complementary therapy for managing symptoms and the severity of the syndrome. Further research is warranted to elucidate the precise mechanisms of action of HUE and its potential clinical applications in PCOS management.

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