

Ethanollic Leaf Extract of *Parquetina nigrescens* Ameliorates Reproductive Dysfunction, Oxidative Stress, Inflammation, and Ovarian Histopathology in a Rat Model of Polyendocrine Metabolic Ovarian Syndrome

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Abstract

Polyendocrine Metabolic Ovarian Syndrome (PMOS) is a prevalent endocrine–metabolic disorder and a leading cause of anovulatory infertility among women of reproductive age. It is characterized by hyperandrogenism, ovulatory dysfunction, chronic inflammation, oxidative stress, and metabolic disturbances. Although pharmacological therapies are available, their effectiveness may be limited by adverse effects, treatment resistance, and accessibility challenges. *Parquetina nigrescens* is widely used in African traditional medicine for managing reproductive disorders, but its therapeutic potential in PMOS remains poorly understood. This study evaluated the effects of ethanollic leaf extract of *Parquetina nigrescens* in a testosterone enanthate-induced rat model of PMOS. Female Wistar rats were assigned to normal control, extract-only (200 and 400 mg/kg), PMOS control, extract-treated PMOS (200 and 400 mg/kg), clomiphene–letrozole-treated PMOS, and vehicle control groups. Reproductive hormones, oxidative stress biomarkers, inflammatory cytokines, liver enzymes, ovarian weight, and histomorphological changes in ovarian and hepatic tissues were assessed following treatment. Testosterone enanthate successfully induced PMOS-like features, including ovarian enlargement, hormonal imbalance, oxidative stress, inflammation, and histopathological abnormalities. Treatment with *P. nigrescens* significantly improved follicle-stimulating hormone, luteinizing hormone, oestradiol, and progesterone levels. The extract reduced ovarian enlargement, restored follicular architecture, decreased cystic follicles, and enhanced corpus luteum formation. It also lowered malondialdehyde concentrations, improved antioxidant status, reduced tumour necrosis factor- α and interleukin-1 β levels, increased interleukin-10 concentrations, and ameliorated hepatic dysfunction and tissue damage. The 400 mg/kg dose produced the greatest therapeutic effects. *Parquetina nigrescens* exhibits reproductive-modulatory, antioxidant, anti-inflammatory, and hepatoprotective properties in experimental PMOS, supporting its potential as a complementary therapeutic agent for PMOS-associated infertility and endocrine dysfunction.

Keywords: Aromatase Inhibitors, Hyperandrogenism, Infertility, Oxidative Stress, Oestrogen receptor Modulator, Polycystic Ovarian syndrome

1. Introduction

Polyendocrine Metabolic Ovarian Syndrome (PMOS) is a multifaceted endocrine–metabolic disorder affecting an estimated 6–20% of women of reproductive age and remains one of the most common causes of anovulatory infertility worldwide [1-3]. The syndrome is characterised by a heterogeneous

constellation of clinical manifestations, including hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology, often accompanied by insulin resistance, obesity, chronic low-grade inflammation, oxidative stress, and psychological disturbances [2, 4]. Increasing evidence indicates that PMOS extends far beyond reproductive dysfunction, contributing substantially to long-term metabolic and cardiovascular complications such as type 2 diabetes mellitus, dyslipidaemia, hypertension, and cardiovascular disease [5]. As a result, PMOS is increasingly recognised as a systemic disorder with profound implications for women's reproductive, metabolic, and overall health.

Infertility represents a major global public health concern, affecting nearly one in six individuals during their reproductive years [6]. Among female infertility disorders, PMOS accounts for a substantial proportion of cases owing to its disruptive effects on follicular development, ovulation, and endocrine homeostasis [7]. The pathogenesis of PMOS is complex and multifactorial, involving intricate interactions among genetic susceptibility, hormonal dysregulation, metabolic disturbances, and environmental influences [7]. Central to this pathophysiology is hyperandrogenism, which interferes with normal folliculogenesis, promotes follicular arrest, and culminates in chronic anovulation and impaired fertility [8].

Beyond endocrine abnormalities, oxidative stress and chronic inflammation have emerged as critical contributors to the initiation, progression, and persistence of PMOS [9]. Oxidative stress occurs when the production of reactive oxygen species overwhelms endogenous antioxidant defense systems, resulting in lipid peroxidation, protein modification, DNA damage, and cellular dysfunction [10]. Ovarian tissues are particularly susceptible to oxidative injury because of their high metabolic demands and the continuous processes of follicular growth, maturation, ovulation, and regression. Excessive oxidative stress has been implicated in impaired oocyte competence, abnormal steroidogenesis, follicular atresia, and reduced reproductive potential [11].

Similarly, chronic low-grade inflammation is now regarded as a defining feature of PMOS pathophysiology. Elevated circulating concentrations of pro-inflammatory mediators, including tumour necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), have consistently been reported in women with PMOS and are closely associated with reproductive dysfunction, insulin resistance, and metabolic abnormalities [12, 13]. Persistent inflammatory activation adversely affects ovarian function by disrupting steroidogenic pathways, impairing follicular maturation, and amplifying oxidative damage within reproductive tissues [14]. Consequently, therapeutic strategies capable of simultaneously mitigating oxidative stress and inflammatory responses may offer significant advantages in the management of PMOS and its associated infertility.

Current treatment approaches for PMOS-related infertility include lifestyle modification, insulin-sensitising agents, ovulation induction therapies, and assisted reproductive technologies [15]. Among pharmacological interventions, clomiphene citrate and letrozole remain the most widely prescribed agents for ovulation induction and fertility enhancement [16]. While these therapies have improved reproductive outcomes for many patients, their clinical utility may be constrained by adverse effects, treatment resistance, ovarian hyperstimulation, multiple gestations, and financial barriers, particularly in low- and middle-income countries [17]. These limitations have fueled growing interest in complementary and alternative therapeutic options, especially medicinal plants with established ethnopharmacological relevance and favourable safety profiles.

Medicinal plants have served as the foundation of traditional healthcare systems for centuries and continue to represent an important source of bioactive compounds with therapeutic potential [18-21]. A wide range of phytochemicals possess antioxidant, anti-inflammatory, endocrine-modulating, antimicrobial, and cytoprotective properties capable of influencing reproductive physiology and restoring biological homeostasis [18-21]. Consequently, the scientific validation of medicinal plants traditionally employed in fertility management may facilitate the identification of novel therapeutic agents for reproductive disorders.

Parquetina nigrescens (Afzel.) Bullock, a perennial climbing shrub belonging to the family Apocynaceae, is widely distributed across tropical Africa and occupies a prominent position in indigenous medicinal practice [5, 22]. Traditionally, the plant has been used in the management of anaemia, gastrointestinal disorders, infectious diseases, inflammatory conditions, diabetes mellitus, and reproductive ailments [5, 23]. Ethnobotanical surveys conducted in several West African communities further indicate its widespread use as a fertility-enhancing remedy and as a treatment for female reproductive disorders, suggesting potential modulatory effects on endocrine and reproductive functions [23].

Phytochemical investigations have revealed that *Parquetina nigrescens* contains a rich diversity of biologically active constituents, including flavonoids, alkaloids, saponins, tannins, glycosides, phenolic compounds, and terpenoids [5, 24]. These phytoconstituents are well known for their antioxidant and anti-inflammatory activities and may contribute to cellular protection and restoration of physiological balance. Moreover, gas chromatography-mass spectrometry analyses have identified additional bioactive compounds such as friedelan-3-one, neophytadiene, palmitic acid, oleic acid derivatives, stearic acid, and linoleic acid derivatives, many of which exhibit antioxidant, anti-inflammatory, hepatoprotective, and endocrine-modulating properties [25, 26]. Such phytochemical diversity provides a

plausible mechanistic basis for the traditional therapeutic applications of the plant.

Previous experimental studies have documented a broad spectrum of pharmacological activities of *Parquetina nigrescens*, including haematinic, antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and hepatoprotective effects [25, 26]. Despite these promising biological activities and its widespread ethnomedicinal use, scientific evidence regarding its specific effects on female reproductive health remains limited. In particular, there is a paucity of data concerning its influence on reproductive hormone regulation, ovarian architecture, inflammatory mediators, oxidative stress biomarkers, and associated hepatic alterations under conditions of endocrine and metabolic dysfunction.

Animal models remain indispensable for elucidating the mechanisms underlying reproductive disorders and for evaluating the efficacy of emerging therapeutic interventions [5]. The testosterone enanthate-induced PMOS model closely reproduces several key features of the human condition, including hyperandrogenism, ovarian cyst formation, endocrine imbalance, oxidative stress, chronic inflammation, and disrupted folliculogenesis [27, 28]. Consequently, this model provides a robust experimental platform for investigating potential therapeutic agents aimed at restoring reproductive and metabolic homeostasis.

Therefore, the present study investigated the effects of ethanolic leaf extract of *Parquetina nigrescens* on reproductive function, oxidative stress, inflammation, and ovarian histomorphology in female Wistar rats with testosterone enanthate-induced PMOS. By evaluating endocrine, biochemical, inflammatory, and histological outcomes, this study sought to provide mechanistic evidence supporting the traditional use of *P. nigrescens* in the management of female reproductive disorders and to explore its potential as a complementary therapeutic candidate for PMOS-associated infertility.

2. Materials and Methods

2.1 Study Design

This study adopted a controlled laboratory-based experimental design to investigate the reproductive, biochemical, and histomorphological effects of the ethanolic leaf extract of *Parquetina nigrescens* in female Wistar rats with testosterone enanthate-induced Polyendocrine Metabolic Ovarian Syndrome (PMOS). The experimental approach was designed to evaluate the capacity of the extract to modulate reproductive endocrine function, oxidative stress, inflammatory responses, hepatic integrity, and ovarian morphology. Outcome measures included body weight, food intake, ovarian weight, reproductive hormone profiles, inflammatory cytokines, oxidative stress biomarkers, liver function indices, and histopathological changes in ovarian and hepatic tissues.

2.2 Plant Collection, Identification, and Authentication

Fresh leaves of *Parquetina nigrescens* (Afzel.) Bullock were collected from Ogbomoso, Oyo State, Nigeria. Botanical authentication was performed by a plant taxonomist in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. Authentication was based on established taxonomic keys and morphological characteristics consistent with published descriptions of the species. A voucher specimen was prepared and deposited in the departmental herbarium for future reference and verification.

2.3 Preparation of Ethanolic Extract of *Parquetina nigrescens* Leaf

The freshly collected *Parquetina nigrescens* leaves were thoroughly rinsed with distilled water to remove dust, soil particles, and other contaminants. The plant material was subsequently air-dried under shade at ambient temperature to minimize degradation of heat-sensitive phytoconstituents. Upon complete drying, the leaves were pulverized into a coarse powder using an electric grinder.

Extraction was carried out using the cold maceration technique. Briefly, the powdered plant material was soaked in absolute ethanol for 72 hours with intermittent agitation to enhance solvent penetration and extraction efficiency. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further dried in a temperature-controlled water bath to obtain a semi-solid residue. The final extract was transferred into airtight containers and stored at 4°C until required for experimental administration.

2.4 Experimental Animals and Husbandry

Adult female Wistar rats weighing between 100 and 130 g were obtained from the Animal House Facility of the Behavioural Neuroscience Research Group, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were housed in well-ventilated polypropylene cages under standard laboratory conditions, with ambient temperature maintained between 22 and 25°C, relative humidity of approximately 50–60%, and a 12-hour light/dark cycle. Standard laboratory pellet diet and clean drinking water were provided *ad libitum* throughout the study.

Prior to commencement of the experiment, the animals were allowed to acclimatize to the laboratory environment for two weeks. Animal handling, care, and experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and complied with the ARRIVE guidelines for reporting animal research.

2.5 Acute Oral Toxicity Assessment

The acute oral toxicity profile of the ethanolic leaf extract of *Parquetina nigrescens* was evaluated in accordance with the

Organisation for Economic Co-operation and Development (OECD) Guideline 423 for Acute Toxic Class Testing. Female

Wistar rats received graded oral doses of the extract ranging from 100 to 4000 mg/kg body weight via oral gavage [29, 30]. Following administration, animals were closely monitored during the first four hours and subsequently observed daily for fourteen days for signs of toxicity. Parameters assessed included mortality, behavioural alterations, locomotor activity, grooming behaviour, food and water intake, respiratory distress, neurological manifestations, and changes in body weight. The findings from this preliminary assessment guided dose selection for the main study.

2.6 Induction of Polyendocrine Metabolic Ovarian Syndrome

Experimental PMOS was induced using testosterone enanthate, an established androgen-induced model that reproduces several endocrine, metabolic, and ovarian features observed in human PMOS. Testosterone enanthate was dissolved in corn oil and administered subcutaneously at a dose of 10 mg/kg body weight once daily for seven consecutive days. This protocol has been shown to induce hyperandrogenism, ovarian cyst formation, hormonal imbalance, oxidative stress, inflammation, and disrupted folliculogenesis [3, 4].

2.7 Experimental Design and Treatment Regimen

Following acclimatization, animals were randomly allocated into eight experimental groups consisting of ten animals per group. The normal control group received normal saline at a dose of 10 mL/kg body weight orally. Two extract-only groups received *Parquetina nigrescens* extract at doses of 200 mg/kg and 400 mg/kg body weight, respectively. The PMOS control group underwent testosterone enanthate induction and subsequently received dimethyl sulfoxide (DMSO) vehicle treatment at 10 mL/kg body weight. Two treatment groups received testosterone enanthate induction followed by administration of *Parquetina nigrescens* extract at doses of 200 mg/kg and 400 mg/kg body weight, respectively. A positive control group received testosterone enanthate induction followed by clomiphene citrate (100 µg/kg) and letrozole (5 mg/kg) [3, 4]. An additional vehicle control group received DMSO only.

Following successful induction of PMOS, treatment administration commenced and continued once daily for fourteen consecutive days through the oral route.

2.8 Measurement of Body Weight and Feed Intake

Body weight measurements were obtained at baseline and subsequently recorded weekly throughout the experimental period using a calibrated digital weighing balance. Food consumption was monitored daily by subtracting the quantity of feed remaining from the amount initially supplied. Weekly average feed intake was calculated for each experimental group

and used as an index of feeding behaviour and metabolic status [3,4].

2.9 Determination of Ovarian Weight

At the end of the treatment period, animals were anaesthetized and euthanized humanely. Both ovaries were carefully excised, cleaned of surrounding connective tissues, and weighed immediately using a precision analytical balance. Relative ovarian weight was calculated by expressing ovarian weight as a percentage of final body weight according to the formula:

$$\text{Relative ovarian weight (\%)} = (\text{Ovarian weight} \div \text{Final body weight}) \times 100$$

2.10 Blood Collection and Serum Preparation

Blood samples were collected by cardiac puncture immediately following sacrifice. The samples were allowed to clot at room temperature and subsequently centrifuged at 3000 rpm for 10 minutes to obtain serum. The separated serum was aliquoted and stored at -20°C until biochemical and hormonal analyses were performed.

2.11 Assessment of Reproductive Hormones

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, and progesterone were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers' instructions. Absorbance readings were obtained using a microplate reader, and hormone concentrations were determined from corresponding standard calibration curves [3, 4].

2.12 Determination of Inflammatory Cytokines

Inflammatory status was evaluated by measuring serum concentrations of interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α), and interleukin-10 (IL-10). Quantification was performed using ELISA-based immunoassays following standardized protocols based on antigen-antibody interactions and colorimetric detection [31].

2.13 Assessment of Oxidative Stress Biomarkers

Oxidative stress status was evaluated using established biochemical markers. Lipid peroxidation was assessed by determining malondialdehyde (MDA) concentrations using the thiobarbituric acid reactive substances (TBARS) assay [32]. Superoxide dismutase (SOD) activity was measured spectrophotometrically based on its ability to inhibit superoxide-mediated reactions. Total antioxidant capacity (TAC) was determined using commercially available assay kits and expressed as Trolox equivalent antioxidant capacity [31, 32].

2.14 Evaluation of Hepatic Function

To assess hepatic integrity and potential hepatoprotective effects of the extract, serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined using standard enzymatic colorimetric methods as previously described [33].

2.15 Gas Chromatography–Mass Spectrometry Analysis

The phytochemical composition of the ethanolic leaf extract of *Parquetina nigrescens* was characterized using gas chromatography–mass spectrometry (GC–MS). The extract was injected into a GC–MS system equipped with a capillary column operating under optimized chromatographic conditions. Identification of phytoconstituents was achieved by comparing retention times and mass spectral fragmentation patterns with entries in the National Institute of Standards and Technology (NIST) spectral library. Relative abundance of detected compounds was calculated from peak area percentages [34].

2.16 Histopathological Evaluation

Following sacrifice, ovarian and hepatic tissues were harvested and fixed in 10% neutral-buffered formalin for 48 hours. The tissues were subsequently processed through graded ethanol dehydration, xylene clearing, and paraffin wax embedding. Serial sections measuring 5 µm in thickness were prepared using a rotary microtome and mounted on glass slides. Ovarian tissue sections were stained with haematoxylin and eosin (H&E) and Van Gieson stains to evaluate follicular development, oocyte integrity, corpus luteum formation, cystic follicle occurrence, and stromal architecture. Liver sections were similarly stained and examined for evidence of hepatocellular degeneration, inflammatory cell infiltration, vascular congestion, fibrosis, and restoration of normal hepatic architecture. Histological observations were documented using a digital light microscopy imaging system.

2.17 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism software. Comparisons among experimental groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Where assumptions of homogeneity of variance were not satisfied, Welch's ANOVA was applied. Statistical significance was accepted at a probability level of $p < 0.05$.

3. Results

3.1 Acute Toxicity Evaluation

Acute oral toxicity assessment demonstrated that the ethanolic leaf extract of *Parquetina nigrescens* was well tolerated at doses up to 4000 mg/kg body weight as shown in **Table 1**. Throughout the 14-day observation period, no mortality or treatment-related signs of toxicity were observed. Experimental animals maintained normal grooming behaviour, locomotor activity, feeding patterns, and physiological responses, with no evidence of behavioural abnormalities or significant alterations in body weight attributable to the extract. These findings indicate a favourable safety profile and suggest that the oral median lethal dose (LD₅₀) of the extract exceeds 4000 mg/kg body weight.

3.2 GC–MS Characterisation of the Ethanolic Leaf Extract

Gas chromatography–mass spectrometry (GC–MS) analysis identified thirteen major phytochemical constituents in the ethanolic leaf extract of *Parquetina nigrescens* as shown in **figure 1**. The chromatographic profile was dominated by fatty acids, fatty acid esters, terpenoids, and triterpenoid compounds, reflecting the chemical complexity of the extract. Friedelan-3-one was the predominant constituent, accounting for 26.77% of the total peak area, followed by *n*-hexadecenoic acid (23.51%), *cis*-13-octadecenoic acid (14.01%), tetradecanoic acid (9.81%), octadecanoic acid (4.98%), dodecanoic acid (3.58%), hexadecanoic acid ethyl ester (3.16%), tetramethylheptadecanolide (2.73%), neophytadiene (2.30%), and linoleic acid ethyl ester (2.30%). Several of these compounds have been reported to possess antioxidant, anti-inflammatory, hepatoprotective, and endocrine-modulatory properties, suggesting that they may collectively contribute to the pharmacological activities observed in the present study.

3.3 Effect of *Parquetina nigrescens* on Body Weight

Progressive increases in body weight were observed in all experimental groups throughout the study period (**Figure 2**). However, rats with testosterone enanthate-induced polycystic ovary syndrome (PMOS) exhibited significantly greater weight gain than the normal control group, indicating the successful induction of metabolic alterations associated with hyperandrogenism. Treatment with the ethanolic leaf extract of *Parquetina nigrescens* attenuated this excessive weight gain in a dose-dependent manner. Animals receiving the extract following PMOS induction showed significantly lower body weight gain than untreated PMOS controls, with the effect being more pronounced at the higher dose. Similarly, the clomiphene–letrozole-treated group demonstrated reduced weight gain relative to the untreated PMOS group. These findings suggest that *P. nigrescens* ameliorates androgen-induced metabolic disturbances and may improve metabolic homeostasis in experimental PMOS.

3.4 Effect of *Parquetina nigrescens* on Feed Intake

Feed intake increased progressively throughout the experimental period in all groups. Nevertheless, testosterone enanthate-induced PMOS was associated with a greater increase in food consumption compared with normal controls, consistent with the metabolic dysregulation accompanying hyperandrogenism. Administration of *Parquetina nigrescens* moderated this increase in feed intake, with animals receiving the extract consuming less feed than untreated PMOS controls. The reduction was more pronounced in the 400 mg/kg treatment group than in the 200 mg/kg group, indicating a dose-dependent effect. Comparable reductions were observed in animals treated with the clomiphene–letrozole combination. These findings suggest that *P. nigrescens* may mitigate hyperphagia associated with androgen-induced ovarian dysfunction, thereby

contributing to its beneficial effects on body weight regulation and metabolic function.

Table 1: Phytochemical constituents identified in ethanol leaf extract of *Parquetina nigrescens*

Peak	Retention time (min)	Peak Area (%)	Name of compound	Molecular formula	Molecular Weight (g/mol)	Quality
1	10.005	3.58	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200.32	99
2	11.853	9.81	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37	99
3	12.357	2.30	Neophytadiene	C ₂₀ H ₃₈	278.50	94
4	13.553	23.51	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	99
5	13.662	3.16	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.50	99
6	14.629	2.09	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	C ₁₀ H ₁₈	138.25	80
7	14.903	14.01	cis-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282.50	99
8	14.983	2.30	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308.50	99
9	15.018	1.62	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310.50	99
10	15.058	4.98	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.50	99
11	16.494	3.14	Friedelan-3-one	C ₃₀ H ₅₀ O	426.70	95
12	16.603	2.73	4,8,12,16-Tetramethylheptadecan-4-olide	C ₂₁ H ₄₀ O ₂	324.50	53
13	17.221	26.77	Friedelan-3-one	C ₃₀ H ₅₀ O	426.70	99

3.5 Effect of *Parquetina nigrescens* on Ovarian Weight

Figure 4 shows the effect of *P. nigrescens* on absolute and relative ovarian weights. Rats in the untreated PMOS group exhibited marked ovarian enlargement, as evidenced by significantly higher absolute and relative ovarian weights than the normal control group. This increase is consistent with the ovarian hypertrophy and stromal hyperplasia characteristic of androgen-induced polycystic ovary syndrome. Treatment with the ethanolic leaf extract of *Parquetina nigrescens* significantly reduced both absolute and relative ovarian weights in PMOS-induced rats. The effect was dose-dependent, with the 400 mg/kg dose producing greater reductions than the 200 mg/kg dose. Ovarian weights in extract-treated animals approached those observed in the normal control and clomiphene–letrozole-treated groups, suggesting that the extract effectively attenuated testosterone-induced ovarian hypertrophy and promoted restoration of normal ovarian morphology.

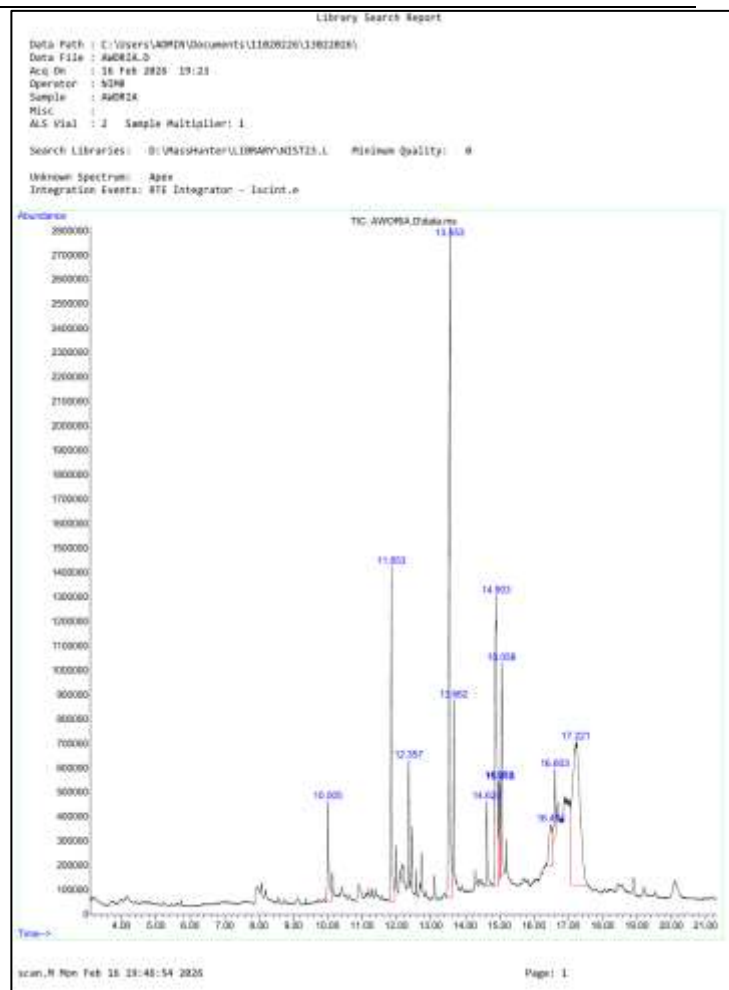


Figure 1: GC–MS Total Ion Chromatogram (TIC) of the ethanolic leaf extract of *Parquetina nigrescens* showing the distribution of identified phytochemical constituents.

3.6 Effect of *Parquetina nigrescens* on Reproductive Hormones

Figure 5 shows the effect of *P. nigrescens* on reproductive hormones including follicle stimulating hormone, luteinising hormone, oestradiol (oestrogen) and progesterone. Induction of PMOS with testosterone enanthate resulted in a significant reduction in serum follicle-stimulating hormone (FSH) compared with the normal control group, indicating disruption of hypothalamic–pituitary–ovarian axis function. Treatment with *Parquetina nigrescens* significantly increased FSH concentrations in both treatment groups, with values comparable to those observed following clomiphene–letrozole administration, suggesting improved follicular function and endocrine regulation.

Serum luteinising hormone (LH) concentrations were significantly reduced in untreated PMOS animals relative to normal controls. Administration of *Parquetina nigrescens* restored LH concentrations in a dose-dependent manner, with the higher dose producing values that closely approximated those of the normal control group. These findings indicate partial restoration of pituitary–ovarian communication following treatment.

Testosterone enanthate-induced PMOS was associated with a significant decline in circulating oestradiol levels. Treatment with *Parquetina nigrescens* significantly increased serum oestradiol concentrations compared with untreated PMOS animals, with the 400 mg/kg dose demonstrating the greatest effect. Oestradiol concentrations in the high-dose group were comparable to those observed in animals receiving clomiphene-letrozole, indicating improved follicular steroidogenesis. Serum progesterone concentrations were markedly reduced following PMOS induction, reflecting impaired ovulation and reduced corpus luteum function. Administration of *Parquetina nigrescens* significantly increased progesterone concentrations, with a more pronounced effect at 400 mg/kg. The hormonal improvement corresponded with histological evidence of enhanced corpus luteum formation, suggesting restoration of ovarian cyclicity and ovulatory function. Collectively, these findings demonstrate that *Parquetina nigrescens* ameliorates testosterone-induced endocrine dysfunction and promotes recovery of reproductive hormonal homeostasis.

3.7 Effect of *Parquetina nigrescens* on Inflammatory Cytokines

Figure 6 shows the effect of *P. nigrescens* on inflammatory cytokines including Interleukin-1 β (IL-1 β), Tumour Necrosis Factor- α (TNF- α), Interleukin-10 (IL-10). Serum interleukin-1 β concentrations were significantly elevated in untreated PMOS rats, indicating activation of systemic inflammatory pathways. Treatment with *Parquetina nigrescens* significantly reduced IL-1 β concentrations in a dose-dependent manner, with the higher dose producing the greatest suppression of the pro-inflammatory response.

Untreated PMOS animals exhibited significantly increased serum tumour necrosis factor- α concentrations compared with normal controls. Administration of *Parquetina nigrescens* significantly attenuated this increase, although TNF- α levels remained modestly higher than those of healthy animals. These findings indicate that the extract mitigates androgen-induced inflammatory activation.

Induction of PMOS significantly reduced serum concentrations of the anti-inflammatory cytokine interleukin-10. Treatment with *Parquetina nigrescens* significantly increased IL-10 levels relative to untreated PMOS animals, with the greatest improvement observed in the 400 mg/kg treatment group. The response was comparable to that achieved with clomiphene-letrozole, suggesting restoration of anti-inflammatory regulatory mechanisms. Overall, the cytokine profile demonstrates that *Parquetina nigrescens* exerts significant immunomodulatory effects by suppressing pro-inflammatory mediators while enhancing anti-inflammatory responses.

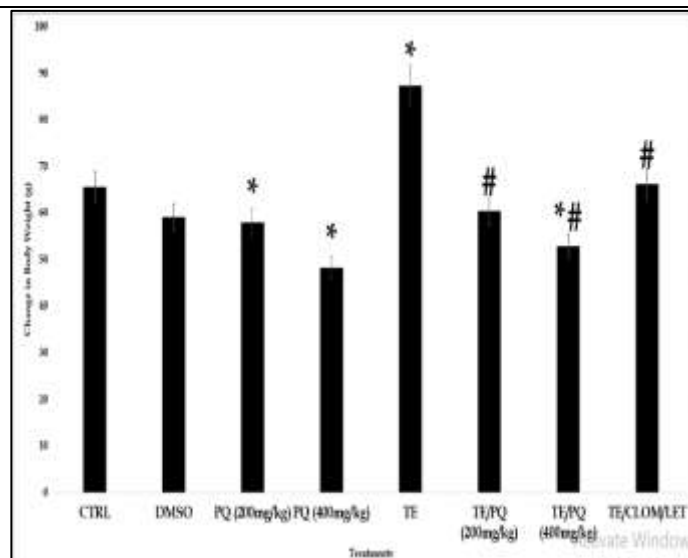


Figure 2: Effect of *Parquetina nigrescens* and clomiphene-letrozole on change in body weight of female wistar rats. Each bar represents Mean $\pm$ S.E.M, number of rats per treatment group=10. CTRL: Normal TE: Testosterone control, PQ (200mg/kg): Rats treated with 200 mg/kg body weight of *Parquetina nigrescens* TE/CLOM/LET: Rats treated with combination of clomiphene/letrozole *p<0.05 vs control, #p<0.05 vs PCOS control

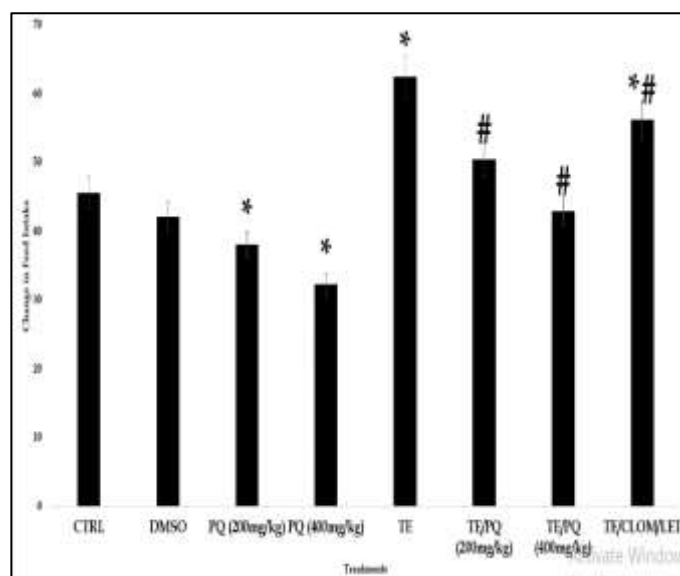


Figure 3: Effect of *Parquetina nigrescens* and clomiphene-letrozole on change in feed intake of female wistar rats. Each bar represents Mean $\pm$ S.E.M, number of rats per treatment group =10. CTRL: Normal control, TE: Testosterone control, PQ (200mg/kg): Rats treated with 200mg/kg body weight of *Parquetina nigrescens* PQ (400mg/kg): Rats treated with 400mg/kg body weight of *Parquetina nigrescens* TE/CLOM/LET: Rats treated with combination of clomiphene/letrozole *p<0.05 vs control, #p<0.05 vs PCOS control

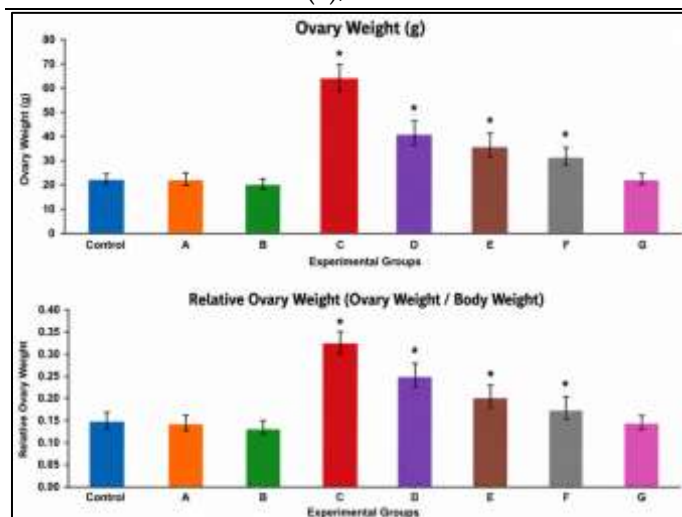


Figure 4: Effects of *Parquetina nigrescens* leaf extract on absolute and relative ovary weight in female Wistar rats. The experimental groups comprised of the Control group, *Parquetina nigrescens* leaf extract at 200 mg/kg (A), *Parquetina nigrescens* leaf extract at 400 mg/kg (B), PCOS control group (C), PCOS plus *P. nigrescens* at 200 mg/kg (D), PCOS plus *P. nigrescens* at 400 mg/kg (E), PCOS plus clomiphene citrate and letrozole (F), and DMSO control (G).

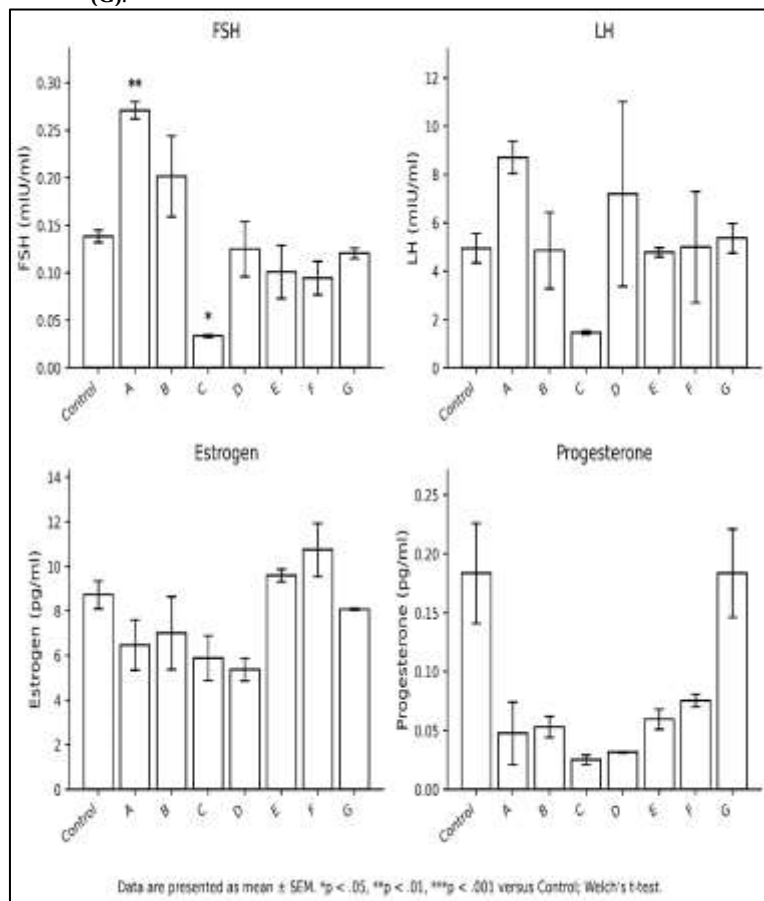


Figure 5: Effects of *Parquetina nigrescens* leaf extract on serum reproductive hormone concentrations. The experimental groups comprised of the Control group, *Parquetina nigrescens* leaf extract at 200 mg/kg (A), *Parquetina nigrescens* leaf extract at 400 mg/kg (B), PCOS control group (C), PCOS plus *P. nigrescens* at 200 mg/kg (D), PCOS plus *P. nigrescens* at 400 mg/kg (E), PCOS plus clomiphene citrate and letrozole (F), and DMSO control (G).

3.8 Effect of *Parquetina nigrescens* on Oxidative Stress Markers

Figure 7 shows the effect of *P. nigrescens* on oxidative stress markers including Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Total Antioxidant Capacity (TAC). Untreated PMOS animals exhibited significantly elevated MDA concentrations, indicative of enhanced lipid peroxidation and oxidative tissue injury. Treatment with *Parquetina nigrescens* significantly reduced MDA concentrations, with the 400 mg/kg dose producing the greatest reduction, suggesting effective attenuation of oxidative stress.

PMOS induction was associated with impaired SOD activity, reflecting compromised antioxidant defence. Administration of *Parquetina nigrescens* significantly increased SOD activity compared with untreated PMOS animals, with the higher dose producing the greatest improvement. These findings indicate enhanced enzymatic antioxidant capacity following treatment. A significant reduction in TAC was observed in untreated PMOS animals, demonstrating depletion of endogenous antioxidant reserves. Treatment with *Parquetina nigrescens* significantly restored TAC in a dose-dependent manner. Animals receiving 400 mg/kg exhibited antioxidant capacity comparable to that of the clomiphene–letrozole-treated group, indicating substantial recovery of systemic antioxidant status. Together, these findings demonstrate that *Parquetina nigrescens* protects against testosterone-induced oxidative stress by reducing lipid peroxidation while enhancing endogenous antioxidant defense mechanisms.

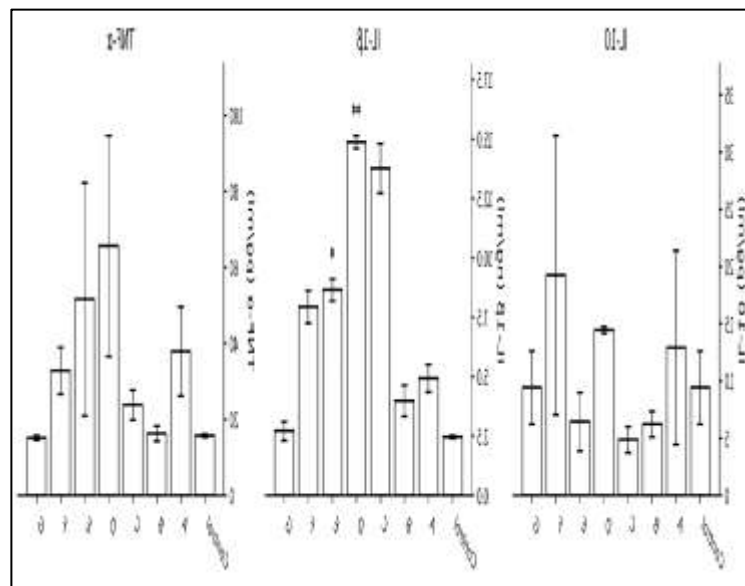


Figure 6: Effect of *Parquetina nigrescens* leaf extract on inflammatory markers in female Wistar rats. The experimental groups comprised of the Control group, *Parquetina nigrescens* leaf extract at 200 mg/kg (A), *Parquetina nigrescens* leaf extract at 400 mg/kg (B), PCOS control group (C), PCOS plus *P. nigrescens* at 200 mg/kg (D), PCOS plus *P. nigrescens* at 400 mg/kg (E), PCOS plus clomiphene citrate and letrozole (F), and DMSO control (G).

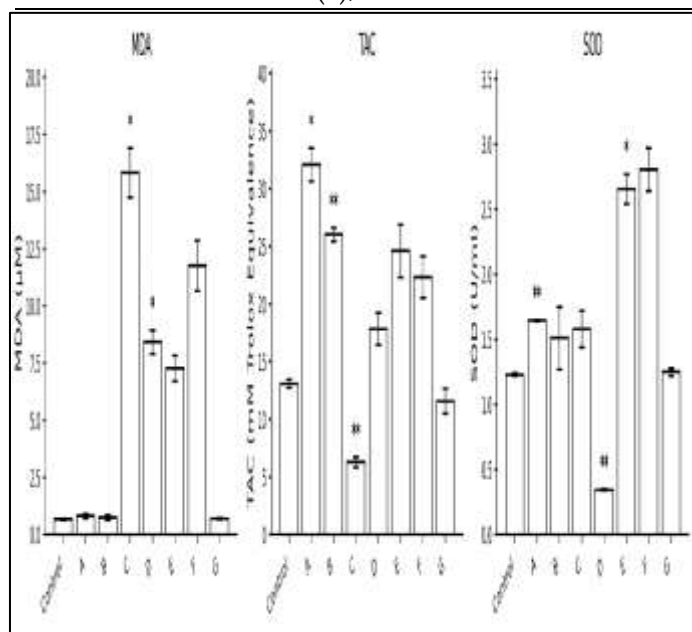


Figure 7: Effect of *Parquetina nigrescens* leaf extract on oxidative stress markers in female Wistar rats. The experimental groups comprised of the Control group, *Parquetina nigrescens* leaf extract at 200 mg/kg (A), *Parquetina nigrescens* leaf extract at 400 mg/kg (B), PCOS control group (C), PCOS plus *P. nigrescens* at 200 mg/kg (D), PCOS plus *P. nigrescens* at 400 mg/kg (E), PCOS plus clomiphene citrate and letrozole (F), and DMSO control (G).

3.9 Effect of *Parquetina nigrescens* on Liver Function Enzymes

Figure 8 shows the effect of *P. nigrescens* on liver function enzymes. Testosterone enanthate-induced PMOS was associated with significant elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, indicating hepatic injury secondary to endocrine and metabolic disturbances. Treatment with *Parquetina nigrescens* significantly reduced the activities of all three enzymes compared with untreated PMOS animals. The hepatoprotective effect was dose-dependent, with the 400 mg/kg dose producing greater reductions and enzyme activities approaching those of the normal control group. These findings suggest that *Parquetina nigrescens* preserves hepatic integrity and mitigates liver dysfunction associated with experimental PMOS.

3.10 Histopathological Evaluation of Ovarian Tissue

Representative photomicrographs of ovarian sections stained with Haematoxylin and Eosin stains (Plate 1A-H) and Van Gieson's stain (Plate 2 A-H). Histological examination of the ovaries revealed marked differences in follicular architecture, oocyte integrity, and follicular organization among the treatment groups. The ovaries of animals in the control group (Plate 1A, 2A) exhibited normal histoarchitecture characterized by well-developed follicles containing centrally located oocytes surrounded by intact zona pellucida and regularly arranged follicular cells. Similar histological features were observed in animals treated with *Parquetina nigrescens* alone at doses of 200

mg/kg (Plate 1B, 2B) and 400 mg/kg (Plate 1C, 2C), indicating that administration of the extract did not adversely affect normal ovarian morphology. The follicles in these groups demonstrated preserved oocyte morphology, distinct zona pellucida, and normal follicular cell organization comparable to those observed in the control animals. In contrast, the PMOS control group (Plate 1D, 2D) exhibited pronounced ovarian abnormalities consistent with androgen-induced ovarian dysfunction. Histological alterations included enlarged cystic follicles, distortion of follicular architecture, disruption of normal follicular development, and evidence of impaired oocyte maturation. These findings are characteristic of follicular arrest and cystic degeneration associated with PMOS and confirm the successful induction of the experimental model. Treatment of PMOS-induced animals with *P. nigrescens* at 200 mg/kg (Plate 1E, 2E) resulted in moderate histological improvement, evidenced by partial restoration of follicular organization and improved preservation of oocyte structure compared with the untreated PMOS group. However, some residual cystic changes and follicular disorganization remained evident. More pronounced therapeutic effects were observed in PMOS animals treated with 400 mg/kg of *P. nigrescens* (Plate 1F, 2F). Ovarian sections from this group demonstrated substantial restoration of normal follicular architecture, with improved follicular maturation, preservation of oocyte integrity, reduced cystic degeneration, and better organization of follicular cell layers. These findings suggest a dose-dependent ovarian protective effect of the extract. Similarly, the clomiphene citrate–letrozole-treated group (Plate 1G, 2G) exhibited marked recovery of ovarian morphology characterized by healthy developing follicles, intact oocytes, well-defined zona pellucida, and restoration of normal follicular architecture. The degree of histological improvement in this group was comparable to that observed in animals treated with the higher dose of *P. nigrescens*. The DMSO control group (Plate 1H, 2H) showed normal ovarian histology with intact follicles, normal oocytes, and preserved follicular organization, indicating that the vehicle had no observable adverse effects on ovarian structure.

3.11 Histopathological Evaluation of Hepatic Tissue

Haematoxylin and eosin stained (Plate 3 A-H) liver sections from normal controls (3A) and *P. nigrescens* controls (3B, and 3C) exhibited normal hepatic architecture with intact hepatocytes, centrally-located nuclei, and well-defined hepatic cords. Untreated PMOS (3D) animals demonstrated hepatocellular degeneration, vascular congestion, inflammatory infiltration, and disruption of hepatic architecture. Treatment with *Parquetina nigrescens* (3E and 3F) ameliorated these pathological changes. Improvement was characterised by restoration of hepatocyte morphology, reduced inflammatory infiltration, and preservation of hepatic structural integrity. Van Gieson staining (Plate 4A-H) further confirmed hepatic

protection by demonstrating preservation of connective tissue architecture and reduction of pathological tissue alterations in extract-treated animals. The 400 mg/kg extract group (4F) exhibited a more-pronounced histological recovery and closely-resembled normal control tissues.

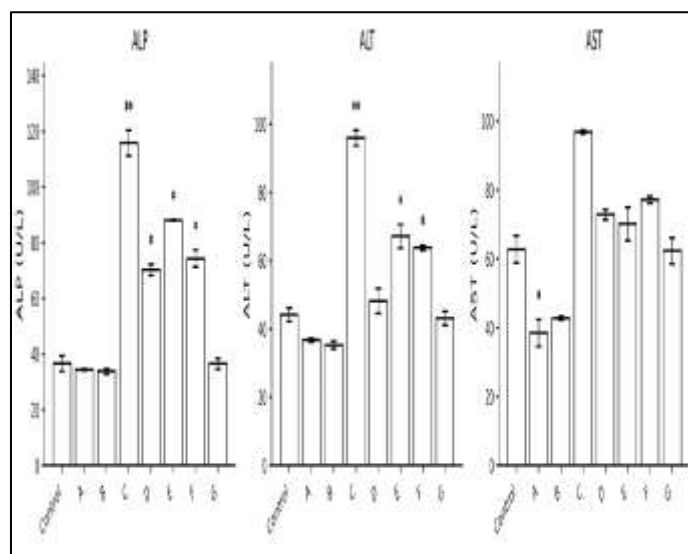


Figure 8: Effect of *Parquetina nigrescens* leaf extract on liver enzymes in female Wistar rats. The experimental groups comprised of the Control group, *Parquetina nigrescens* leaf extract at 200 mg/kg (A), *Parquetina nigrescens* leaf extract at 400 mg/kg (B), PCOS control group (C), PCOS plus *P. nigrescens* at 200 mg/kg (D), PCOS plus *P. nigrescens* at 400 mg/kg (E), PCOS plus clomiphene citrate and letrozole (F), and DMSO control (G).

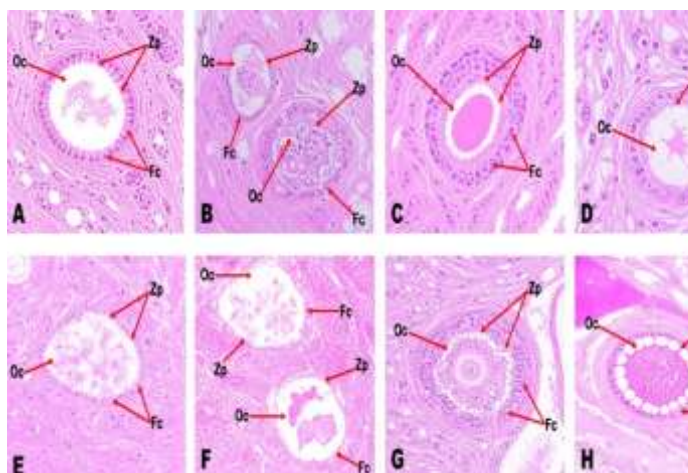


Plate 1: Representative photomicrographs of ovarian sections from control and experimental groups (H&E and Van Gieson staining, ×160). The control (A), *Parquetina nigrescens* 200 mg/kg (B), *Parquetina nigrescens* 400 mg/kg (C), clomiphene-letrozole-treated PMOS (G), and DMSO control (H) groups demonstrated normal follicular architecture with preserved oocytes (Oc), intact zona pellucida (Zp), and organized follicular cells (Fc). The PMOS control group (D) exhibited cystic follicular changes, disrupted follicular development, and impaired oocyte morphology. Treatment with *P. nigrescens* at 200 mg/kg (E) and 400 mg/kg (F) progressively restored ovarian architecture, reduced cystic changes, and improved follicular maturation, with the higher dose producing near-normal ovarian morphology. Oc = oocyte; Fc = follicular cells; Zp = zona pellucida.

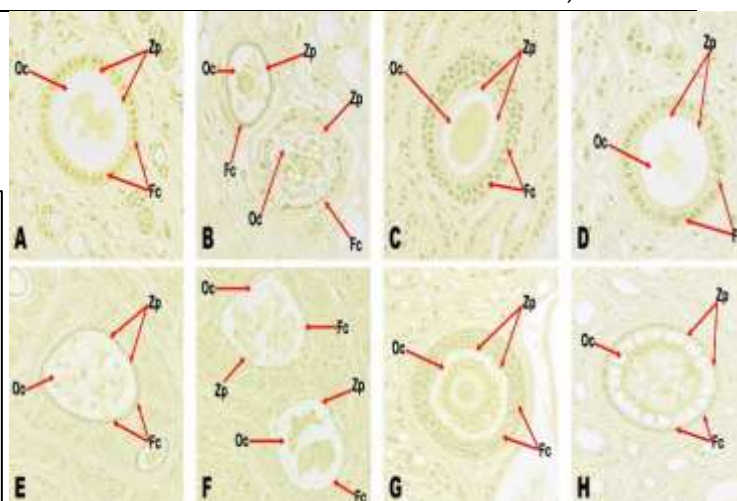


Plate 2: Effect of *Parquetina nigrescens* on histomorphology of the ovaries (Van Gieson Stain) Panel A- 'Control group', Panel B- 'Group of the rats that had 200mg/kg of *Parquetina nigrescens* leaf extract only', Panel C- 'Group of the rats that had 400mg/kg of *Parquetina nigrescens* leaf extract only' Panel D- 'the PCOS control group' Panel E- 'PCOS rats that were continued on 200mg/kg of *Parquetina nigrescens* leaf extract' Panel F- 'PCOS rats that were continued on 400mg/kg of *Parquetina nigrescens* extract' Panel G- 'PCOS rats that were continued on a combination of clomiphene citrate and letrozole' Panel H- 'the group of rats that had DMSO only'. Oc: Oocytes Fc: Follicles, ZP: Zona pellucida (magnification, 160×)

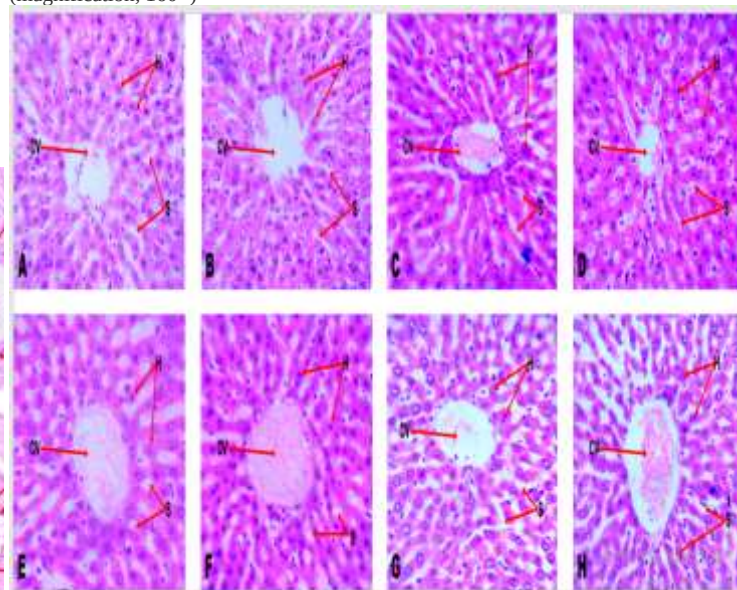


Plate 3: Effect of *Parquetina nigrescens* on histomorphology of the liver (Hematoxylin and eosin stain) Panel A- 'Control group', Panel B- 'Group of the rats that had 200mg/kg of *Parquetina nigrescens* leaf extract only', Panel C- 'Group of the rats that had 400mg/kg of *Parquetina nigrescens* leaf extract only' Panel D- 'the PCOS control group' Panel E- 'PCOS rats that were continued on 200mg/kg of *Parquetina nigrescens* leaf extract' Panel F- 'PCOS rats that were continued on 400mg/kg of *Parquetina nigrescens* extract' Panel G- 'PCOS rats that were continued on a combination of clomiphene citrate and letrozole' Panel H- 'the group of rats that had DMSO only'. CV: Central vein H: Hepatocytes S: Sinusoids (Magnification, 160×)

Discussion

The present study demonstrates that the ethanolic leaf extract of *Parquetina nigrescens* exerts significant protective effects against testosterone enanthate-induced polycystic ovary syndrome (PCOS) in rats. Treatment with the extract attenuated

ovarian hypertrophy, restored reproductive hormonal balance, improved ovarian histoarchitecture, reduced oxidative stress and inflammatory responses, and preserved hepatic function. Collectively, these findings suggest that *P. nigrescens* ameliorates multiple pathological processes associated with experimental PCOS and support its potential as a multi-target therapeutic agent.

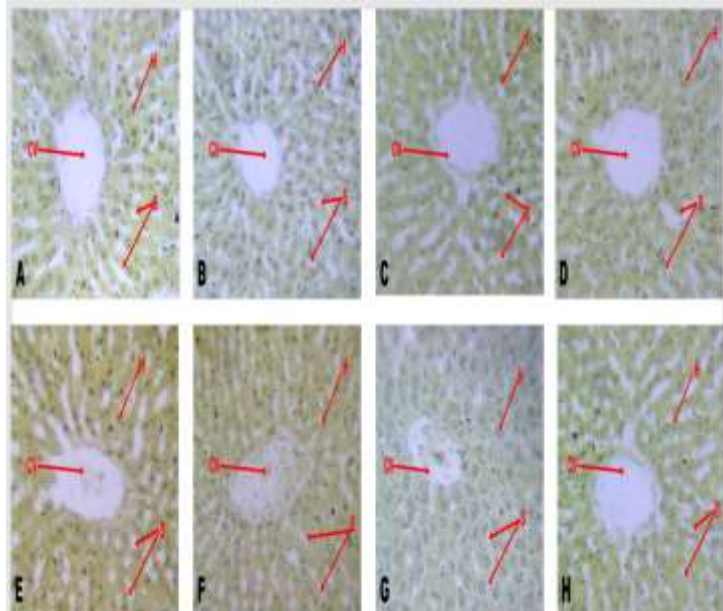


Plate 4: Effect of *Parquetina nigrescens* on histomorphology of the liver (Van Gieson stain). Panel A- 'Control group', Panel B- 'Group of the rats that had 200mg/kg of *Parquetina nigrescens* leaf extract only', Panel C- 'Group of the rats that had 400mg/kg of *Parquetina nigrescens* leaf extract only' Panel D- 'the PCOS control group' Panel E- 'PCOS rats that were continued on 200mg/kg of *Parquetina nigrescens* leaf extract' Panel F- 'PCOS rats that were continued on 400mg/kg of *Parquetina nigrescens* extract' Panel G- 'PCOS rats that were continued on a combination of clomiphene citrate and letrozole' Panel H- 'the group of rats that had DMSO only'. CV: Central vein H: Hepatocytes S: Sinusoids (Magnification, 160×)

Successful induction of the experimental PCOS model was evidenced by ovarian enlargement, disrupted reproductive hormone profiles, increased oxidative stress, elevated pro-inflammatory cytokines, hepatic dysfunction, and characteristic histopathological changes, including cystic follicular development and reduced corpus luteum formation [3, 4]. These findings are consistent with previous studies demonstrating that chronic testosterone administration reproduces many of the endocrine, metabolic, and morphological features of human PCOS, making it a reliable experimental model for investigating potential therapeutic interventions [35-38]. Hyperandrogenism is recognized as a key driver of PCOS pathogenesis, promoting follicular arrest, anovulation, stromal hyperplasia, and ovarian cyst formation through disruption of hypothalamic–pituitary–ovarian (HPO) axis function [3, 4, 39].

One of the notable findings of this study was the attenuation of excessive body weight gain and increased feed intake following administration of *P. nigrescens*. Hyperandrogenism is frequently

associated with obesity, insulin resistance, altered appetite regulation, and metabolic dysfunction, all of which contribute to the progression of PCOS [3, 4, 39]. Increased adiposity further exacerbates oxidative stress and chronic low-grade inflammation, creating a self-perpetuating cycle of endocrine and metabolic dysfunction [39]. The reduction in body weight gain and normalization of feed intake observed in extract-treated animals suggest that *P. nigrescens* may improve metabolic homeostasis in addition to its reproductive effects, although the underlying metabolic mechanisms require further investigation. The reduction in ovarian weight following treatment was accompanied by marked improvements in ovarian histology. Untreated PCOS animals exhibited enlarged ovaries characterized by numerous cystic follicles, attenuated granulosa cell layers, and diminished corpus luteum formation, whereas treatment with *P. nigrescens* restored follicular architecture, increased the number of developing follicles and corpora lutea, and reduced cystic degeneration. These observations indicate recovery of folliculogenesis and ovulatory activity and are consistent with previous reports describing the beneficial effects of phytotherapeutic agents on ovarian morphology in experimental PCOS [9,12, 40]. The histological restoration further supports the ethnomedicinal use of *P. nigrescens* in the management of female reproductive disorders.

The extract also produced significant improvements in reproductive endocrine function. Testosterone-induced suppression of follicle-stimulating hormone (FSH), luteinising hormone (LH), oestradiol, and progesterone was reversed following treatment, with the higher dose producing responses comparable to those observed with clomiphene–letrozole therapy. Restoration of these hormones is indicative of improved HPO axis function, enhanced follicular maturation, and resumption of ovulatory activity [15–17]. The concurrent increase in progesterone concentrations and histological evidence of corpus luteum formation strongly suggest that the extract promotes restoration of ovarian cyclicity, an essential prerequisite for fertility.

Inflammation is increasingly recognised as a central component of PCOS pathophysiology, contributing to ovarian dysfunction, insulin resistance, and impaired follicular development [13, 14]. In the present study, untreated PCOS animals exhibited significant elevations in the pro-inflammatory cytokines, interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α), together with reduced concentrations of the anti-inflammatory cytokine interleukin-10 (IL-10). Administration of *P. nigrescens* significantly suppressed IL-1 β and TNF- α while restoring IL-10 concentrations, demonstrating a shift towards an anti-inflammatory cytokine profile. These findings are consistent with previous reports describing the anti-inflammatory activities of *P. nigrescens* and other phytochemical-rich medicinal plants [41, 42].

Oxidative stress represents another important mechanism underlying ovarian dysfunction in PCOS, where excessive generation of reactive oxygen species impairs follicular development and steroidogenesis [10–12]. The significant reduction in malondialdehyde (MDA) concentrations, together with increased superoxide dismutase (SOD) activity and total antioxidant capacity (TAC), indicates that *P. nigrescens* effectively enhances endogenous antioxidant defense systems. Similar antioxidant effects have been reported for medicinal plants rich in flavonoids, phenolic compounds, and terpenoids, which scavenge reactive oxygen species, inhibit lipid peroxidation, and preserve cellular integrity [41–43]. The antioxidant activity observed in the present study likely contributed to the restoration of ovarian structure and function. In addition to its reproductive benefits, *P. nigrescens* demonstrated marked hepatoprotective activity. Elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities in untreated PCOS animals indicated hepatic injury associated with androgen-induced metabolic dysfunction. Treatment significantly reduced these enzyme activities and improved hepatic histoarchitecture, suggesting preservation of hepatocellular integrity. These findings are clinically relevant because women with PCOS are at increased risk of developing metabolic-associated fatty liver disease owing to obesity, insulin resistance, oxidative stress, and chronic inflammation [44, 45]. The hepatoprotective effects observed in this study corroborate previous experimental evidence demonstrating hepatic protective activity of *P. nigrescens* and related medicinal plants [46, 47].

The broad therapeutic effects of *P. nigrescens* are likely attributable to the synergistic actions of its diverse phytochemical constituents. GC–MS analysis identified several bioactive compounds, including friedelan-3-one, fatty acids, fatty acid esters, and terpenoids, many of which possess well-documented antioxidant, anti-inflammatory, cytoprotective, and membrane-stabilising properties [48, 49]. Previous phytochemical investigations have also identified flavonoids, phenolic compounds, alkaloids, tannins, and saponins in *P. nigrescens*, all of which may contribute to modulation of oxidative stress, inflammatory signalling, and endocrine function [42]. It is therefore plausible that the pharmacological activity of the extract results from synergistic interactions among multiple phytoconstituents rather than the action of a single bioactive compound.

Overall, the findings of the present study indicate that *Parquetina nigrescens* simultaneously targets several key mechanisms involved in PCOS pathogenesis, including endocrine dysregulation, oxidative stress, inflammation, ovarian structural abnormalities, and hepatic dysfunction. Although caution should be exercised when extrapolating findings from

animal models to humans, these results provide scientific support for the traditional use of *P. nigrescens* in reproductive health and justify further mechanistic studies, pharmacokinetic investigations, and well-designed clinical trials to establish its therapeutic potential in women with PCOS.

Conclusion

The present study demonstrates that the ethanolic leaf extract of *Parquetina nigrescens* confers significant protective effects in a testosterone enanthate-induced rat model of polycystic ovary syndrome. Treatment with the extract restored reproductive hormonal homeostasis, reduced ovarian hypertrophy, improved follicular development and corpus luteum formation, attenuated oxidative stress and inflammatory responses, and preserved hepatic structure and function.

The observed therapeutic effects were dose dependent, with the 400 mg/kg dose consistently producing greater improvements across endocrine, biochemical, histopathological, and oxidative stress parameters. These findings suggest that *P. nigrescens* exerts its beneficial effects through complementary mechanisms involving endocrine regulation, antioxidant activity, anti-inflammatory actions, and tissue protection.

Together, this study provides experimental evidence supporting the ethnopharmacological use of *Parquetina nigrescens* in the management of female reproductive disorders. The extract represents a promising source of bioactive compounds for the development of adjunctive therapies for PCOS. Nevertheless, further investigations are required to elucidate its molecular mechanisms of action, establish long-term safety, and evaluate its efficacy in well-designed clinical studies.

Strengths of the Study

This study possesses several important strengths. First, it employed a comprehensive experimental approach by simultaneously evaluating endocrine, biochemical, inflammatory, oxidative stress, morphological, and histopathological parameters, thereby providing an integrated assessment of the therapeutic effects of *Parquetina nigrescens*. Second, GC–MS characterization of the extract enabled the correlation of biological activity with its phytochemical composition, strengthening the mechanistic basis of the observed pharmacological effects. Third, the inclusion of a standard therapeutic comparator (clomiphene–letrozole) provided an appropriate benchmark against which the efficacy of the plant extract could be evaluated. Finally, the use of complementary biochemical and histological endpoints enhanced the robustness and internal validity of the findings.

Limitations of the Study

Despite these strengths, certain limitations should be acknowledged. The study was conducted in an experimental

animal model, which may not fully recapitulate the complex endocrine, metabolic, and genetic features of human PCOS. Molecular pathways involved in steroidogenesis, inflammatory signalling, oxidative stress regulation, apoptosis, and insulin signalling were not investigated and therefore remain to be elucidated. In addition, functional reproductive outcomes such as oestrous cyclicity, ovulation rate, fertility, implantation success, pregnancy outcome, and live birth rate were not evaluated. Long-term toxicity studies, chronic dosing experiments, and pharmacokinetic analyses were also beyond the scope of the present investigation. Future studies addressing these limitations will provide a more comprehensive understanding of the therapeutic potential of *P. nigrescens*.

Clinical Relevance

Polycystic ovary syndrome is one of the most common endocrine disorders affecting women of reproductive age and remains a major cause of infertility, metabolic dysfunction, and reduced quality of life worldwide. The multifactorial nature of the disorder often necessitates combination therapy targeting endocrine abnormalities, oxidative stress, inflammation, and metabolic disturbances. The ability of *Parquetina nigrescens* to modulate these interconnected pathological processes simultaneously highlights its potential as a complementary therapeutic candidate. Although clinical translation requires rigorous pharmacological evaluation and controlled clinical trials, the present findings provide a strong preclinical foundation for further development of *P. nigrescens* as a novel phytotherapeutic intervention for the management of PCOS and its associated reproductive complications.

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Author Contributions

OA conceived and designed the study, conducted the experiments, analysed the data, interpreted the findings, and drafted the manuscript. OO and YO supervised the study, contributed to study design, interpretation of findings, and critical revision of the manuscript. FM and AA analysed the study. All authors reviewed and approved the final manuscript.

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Availability of data and materials

Data generated and analysed during the course of this study are available from the corresponding author on request.

Competing interests

All authors of this paper declare that there is no conflict of interest related to the content of this manuscript.

Ethical Approval

All animal procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and complied with internationally accepted principles for animal research.

Declaration of Generative AI and AI-Assisted Technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT for Language editing. After using this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

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References

- Teede HJ, Khomami MB, Morman R, Laven JSE, Joham AE, Costello MF, Patil M, Rees DA, Berry L, Cree MG, Zhao H, Norman RJ, Dokras A, Piltonen T; Global Name Change Consortium. Polyendocrine metabolic ovarian syndrome, the new name for polycystic ovary syndrome: a multistep global consensus process. *Lancet*. 2026 Jun 6;407(10545):2329-2339. doi: 10.1016/S0140-6736(26)00717-8.
- Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum Reprod*. 2018;33(9):1602-1618.
- Aworinde OO, Adebayo A, Olufemi-Aworinde KJ, Adeyemi A, Onaolapo AY, Onaolapo OJ. Synergistic Modulation of Hormonal Balance and Ovarian Architecture by Clomiphene–Letrozole Co-Administration in a Rodent Model of Hyperandrogenism. *Acta Bioscientia* 2026;2(3):377-390. <https://doi.org/10.71181/actabioscientia12550>
- Olofinnade AT, Onaolapo OJ, Onaolapo AY. Clomiphene/letrozole administration can modulate neurobehavioural and neurochemical indices in hyperandrogenic female rats. *Acta Bioscientia* 2025;1(3):111-122. <https://doi.org/10.71181/actabioscientia12260>
- Aworinde OO, Fijabiyi M, Ajao JA, Adeyemi A, Onaolapo AY, Onaolapo OJ. *Parquetina nigrescens* in the Management of Polyendocrine Metabolic Ovarian Syndrome: Therapeutic Potential, Mechanistic Insights, and Future Perspectives. *Acta Bioscientia* 2026;2(4):445-457. <https://doi.org/10.71181/actabioscientia12595>
- Lim SS, Kakoly NS, Tan JWJ, Fitzgerald G, Bahri Khomami M, Joham AE, et al. Metabolic syndrome in polycystic ovary syndrome. *Endocr Rev*. 2019;40(2):421–459.
- World Health Organization. Infertility fact sheet. Geneva: World Health Organization; 2023.
- Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol*. 2011;7(4):219–231.
- Franks S. Polycystic ovary syndrome. *N Engl J Med*. 1995;333(13):853–861.
- Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Oxidative stress and polycystic ovary syndrome. *Hum Reprod Update*. 2013;19(3):268–288.
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Int J Biochem Cell Biol*. 2007;39(1):44–84.

12. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. 2005;3:28.
13. Duleba AJ, Dokras A. Is PCOS an inflammatory process? *Fertil Steril*. 2012;97(1):7–12.
14. Escobar-Morreale HF, Luque-Ramirez M, Gonzalez F. Circulating inflammatory markers in polycystic ovary syndrome. *Fertil Steril*. 2011;95(3):1048–1058.
15. González F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids*. 2012;77(4):300–305.
16. Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2013;98(12):4565–4592.
17. Franik S, Kremer JA, Nelen WJDM, Farquhar C. Aromatase inhibitors for subfertile women with polycystic ovary syndrome. *Cochrane Database Syst Rev*. 2018;5:CD010287.
18. Onaolapo, A.Y. and Onaolapo, O.J., 2019. Herbal beverages and brain function in health and disease. In *Functional and medicinal beverages* (pp. 313-349). Academic Press. Newman DJ,
19. Cragg GM. Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. *J Nat Prod*. 2020;83(3):770–803.
20. de Oliveira, M.R. ed., 2023. *Natural molecules in neuroprotection and neurotoxicity*. Elsevier.
21. Ravishankar, Gokare A., and Akula Ramakrishna, eds. *Serotonin and melatonin: their functional role in plants, food, phytochemistry, and human health*. CRC press, 2016.
22. Owolabi JO, Omogbai EKI, Obasuyi O. Pharmacological and therapeutic properties of *Parquetina nigrescens*: a review. *Afr J Tradit Complement Altern Med*. 2010;7(1):1–8.
23. Soladoye MO, Sonibare MA. Ethnobotanical survey of medicinal plants used in southwestern Nigeria. *J Med Plants Res*. 2013;7(5):261–271.
24. Yemele MD, Telefo PB, Lienou LL, Tagne SR, Fodouop CS, Goka CS, Lemfack MC, Moundipa FP. Ethnobotanical survey of medicinal plants used for pregnant women's health conditions in Menoua division-West Cameroon. *J Ethnopharmacol*. 2015 Feb 3;160:14-31. doi: 10.1016/j.jep.2014.11.017.
25. Ojuade FI, Olorundare OE, Akanbi OB, Afolabi SO, Njan AA. Antidiabetic and antihyperlipidemic effects of aqueous extract of *Parquetina nigrescens* in streptozotocin-nicotinamide induced type 2 diabetic rats. *Heliyon*. 2021 Jun 21;7(6):e07363. doi: 10.1016/j.heliyon.2021.e07363.
26. Femi-Olabisi JF, Ishola AA, Olujimi FO. Effect of *Parquetina nigrescens* (Afzel.) leaves on letrozole-induced PCOS in rats: a molecular insight into its phytoconstituents. *Appl Biochem Biotechnol*. 2023.
27. Ryu Y, Kim SW, Kim YY, Ku SY. Animal Models for Human Polycystic Ovary Syndrome (PCOS) Focused on the Use of Indirect Hormonal Perturbations: A Review of the Literature. *Int J Mol Sci*. 2019 Jun 3;20(11):2720. doi: 10.3390/ijms20112720.
28. Kalhori Z, Soleimani Mehranjan M, Azadbakht M, Shariatzadeh MA. Ovary stereological features and serum biochemical factors following induction of polycystic ovary syndrome with testosterone enanthate in mice: An experimental study. *Int J Reprod Biomed*. 2018 Apr;16(4):267–274.
29. Mollica, A., Zengin, G., Locatelli, M., Stefanucci, A., Macedonio, G., Bellagamba, G., Onaolapo, O., Onaolapo, A., Azeez, F., Ayileka, A. and Novellino, E., 2017. An assessment of the nutraceutical potential of *Juglans regia* L. leaf powder in diabetic rats. *Food and Chemical Toxicology*, 107, pp.554-564.
30. Mollica, A., Zengin, G., Locatelli, M., Stefanucci, A., Mocan, A., Macedonio, G., Carradori, S., Onaolapo, O., Onaolapo, A., Adegoke, J. and Olaniyan, M., 2017. Anti-diabetic and anti-hyperlipidemic properties of *Capparis spinosa* L.: in vivo and in vitro evaluation of its nutraceutical potential. *Journal of functional foods*, 35, pp.32-42.
31. Alade SO, Arojoye AM, Ajao JA, Akinsehinwa AF, Famuyide OO, Onaolapo OJ, Onaolapo AY. Cerebellar Neurotoxicity from Ketamine Exposure: Protective Impact of N-Acetylcysteine in a Rodent Model. *Acta Bioscientia*. 2025;1(5);265-273
<https://doi.org/10.71181/actabioscientia12430>
32. Stefanucci, A., Marinaccio, L., Llorent-Martínez, E.J., Zengin, G., Bender, O., Dogan, R., Atalay, A., Adegbite, O., Ojo, F.O., Onaolapo, A.Y. and Onaolapo, O.J., 2024. Assessment of the in-vitro toxicity and in-vivo therapeutic capabilities of *Juglans regia* on human prostate cancer and prostatic hyperplasia in rats. *Food Bioscience*, 57, p.103539.
33. Olofinnade, A.T., Onaolapo, A.Y., Onaolapo, O.J., Olowe, O.A. and Adeyeba, O.A., 2021. Food-added azodicarbonamide alters haematological parameters, antioxidant status and biochemical/histomorphological indices of liver and kidney injury in rats. *Journal of Basic and Clinical Physiology and Pharmacology*, 32(2), pp.39-50.
34. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th ed. Carol Stream: Allured Publishing; 2007.
35. Caldwell ASL, Edwards MC, Desai R, Jimenez M, Gilchrist RB, Handelsman DJ, Walters KA. Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *Proc Natl Acad Sci U S A*. 2017;114(16):E3334-E3343. doi:10.1073/pnas.1616467114.
36. Walters KA, Allan CM, Handelsman DJ. Rodent models for human polycystic ovary syndrome. *Biol Reprod*. 2012;86(5):149.
37. Abbott DH, Dumesic DA, Franks S. Developmental origin of polycystic ovary syndrome. *J Endocrinol*. 2002;174(1):1–5.
38. Stener-Victorin E, Padmanabhan V, Walters KA, Campbell RE, Benrick A, Giacobini P, et al. Animal models to understand the etiology and pathophysiology of PCOS. *Endocr Rev*. 2020;41(4):538–576.
39. Barber TM, Hanson P, Weickert MO, Franks S. Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2019;90(2):251–263.
40. Akpantah AO, Oremosu AA, Ajala MO, Noronha CC, Okanlawon AO. Effects of medicinal plant extracts on reproductive parameters in experimental animals. *Niger J Physiol Sci*. 2010;25(1):29–34.
41. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, et al. Phytochemical screening and antioxidant activities of selected medicinal plants. *Afr J Biotechnol*. 2008;7(17):2877–2881.
42. Walters KA. Role of steroid hormones in ovarian dysfunction. *Mol Cell Endocrinol*. 2018;465:52–60.
43. Ruder EH, Hartman TJ, Blumberg J, Goldman MB. Oxidative stress and infertility. *Hum Reprod Update*. 2008;14(4):345–357.
44. Rocha ALL, Faria LC, Guimarães TCM, Moreira GV, Candido AL, Couto CA, et al. Non-alcoholic fatty liver disease in women with PCOS. *Endocr Connect*. 2017;6(3):115–125.
45. Macut D, Božić-Antić I, Bjekić-Macut J, Tziomalos K. Management of endocrine disease: polycystic ovary syndrome and non-alcoholic fatty liver disease. *Eur J Endocrinol*. 2017;177(5):R145–R158.
46. Ayeleso AO, Oguntibeju OO, Brooks NL. Effects of dietary intake of medicinal plants on liver function. *Evid Based Complement Alternat Med*. 2014;2014:436139.
47. Ezejindu DN, Nwafor A, Udemazue OO. Hepatoprotective potential of *Parquetina nigrescens* in experimental animals. *Int J Biol Chem Sci*. 2014;8(4):1710–1718.
48. Mahato SB, Kundu AP. 13C NMR spectra of pentacyclic triterpenoids—a compilation and review. *Phytochemistry*. 1994;37(6):1517–1575.
49. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory properties of palmitic acid and related compounds. *J Pharm Res*. 2012;5(2):863–866.

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