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Full Length Research Article

EFFECT OF *Psidium guajava* LEAF ON CAFFEINE-INDUCED OVARIAN TISSUE DAMAGE IN WISTAR RATS

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ABSTRACT
Psidium guajava is a widely consumed tropical fruit known for its medicinal properties. Caffeine, a natural stimulant found in coffee, tea, and other sources, is known to affect reproductive function in both humans and experimental models. This study examines the effect of methanol leaf extract of *Psidium guajava* (MLEPG) and caffeine on female hormonal markers and ovarian histomorphology for 21 days. Twenty-five rats were divided into five groups (n=5): a normal control [NC], a 100 mg/kg body weight (BW) caffeine-alone [CA] group, and the groups that received caffeine plus doses of MLEPG (125, 250, and 500 mg/kg BW. CA alone resulted in a significant *(p<0.05)* elevation in FSH and reductions in LH, estradiol, and progesterone levels. Co-treatment with MLEPG produced dose-dependent hormonal restoration, with higher doses significantly *(p<0.05)* reducing FSH and increasing LH, estradiol, and progesterone concentrations. The CA group showed severe ovarian damage with atretic follicles and no follicular development. The three doses of MLEPG showed better ovarian restoration, with some follicular growth, signs of follicular regeneration and an almost normal ovarian histology, similar to the NC group. The results suggest that MLEPG may restore caffeine-induced hormonal alterations and ovarian damage and could be a potential natural remedy for protecting reproductive health.

Keywords: *Psidium guajava*, Caffeine, Hormonal biomarkers, Ovarian damage, Atretic follicles, Estradiol.

INTRODUCTION

The ovary is a vital component of the female reproductive system, responsible for producing oocytes and secreting sex hormones like estrogen and progesterone, which regulate fertility and reproductive health (Wani *et al*., 2020). Anatomically, the ovaries are paired, almond-shaped organs located in the pelvic cavity and supported by the ovarian and broad ligaments (Crawford & Hillman, 2021). They receive blood supply from the ovarian artery and drain via the ovarian vein, reflecting their active metabolic function (Cruz-Topete & Cidlowski, 2020). Any disruption in ovarian physiology can impair hormonal balance and fertility, underscoring the importance of exploring modifiable factors that may impact ovarian health (Meraiyebu *et al*., 2022).

Caffeine, a natural methylxanthine alkaloid, is among the most widely consumed psychoactive substances worldwide. It is found in coffee, tea, cocoa, kola nuts, energy drinks, and various medications (Dewailly *et al*., 2020). Acting primarily as an adenosine receptor antagonist, caffeine increases neuronal activity, neurotransmitter release, and central nervous system stimulation (Fraser & Duncan, 2020). It is metabolized primarily in the liver by cytochrome P_{450} enzymes, yielding metabolites including paraxanthine, theobromine, and theophylline (Groome & Knight, 2019). Although moderate intake is generally regarded as safe, high caffeine consumption has been associated with insomnia, anxiety, gastrointestinal irritation, and elevated cardiovascular risk (Kauffman & Navarro, 2021).

Of particular concern is caffeine's impact on reproductive health. Studies have linked high caffeine intake to hormonal imbalances, including elevated follicle-stimulating hormone (FSH), reduced luteinizing hormone (LH), estradiol, and progesterone, as well as impaired follicular development (Rasul *et al*., 2025). These changes may compromise ovulation and fertility. The mechanisms proposed include oxidative stress, disruption of steroidogenesis, and altered ovarian blood flow (Liu *et al*., 2025). Considering the high prevalence of caffeine consumption among women of reproductive age, such findings warrant closer investigation.

In recent years, interest has grown in natural remedies capable of mitigating reproductive toxicity. Phytochemicals, bioactive compounds from plants, are known for their antioxidant, anti-inflammatory, and hormone-modulating effects (Kumar *et al*., 2025). Within reproductive biology, certain extracts have been shown to counteract oxidative stress and toxicant-induced ovarian damage. Antioxidants in particular may play a protective role by neutralizing reactive oxygen species and preserving follicular integrity.

Psidium guajava (guava), a tropical fruit of the *Myrtaceae* family, is widely consumed and valued for its nutritional and medicinal properties. The fruit contains abundant amounts of vitamins A, C, and E, as well as minerals such as potassium, magnesium, and calcium (Kumar *et al*., 2021). Its leaves are rich in flavonoids, tannins, and polyphenols, which contribute to antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic activities (Naseer *et al*., 2018). Traditionally used in folk medicine for gastrointestinal disorders, wound healing, and infections, guava leaf extract has recently attracted scientific attention for its pharmacological potential (Khandagale *et al*., 2026).

Specifically, guava leaves contain compounds such as quercetin and catechin, which possess strong free radical-scavenging activity and anti-inflammatory effects (Huynh *et al*., 2025). These bioactive constituents can inhibit pro-inflammatory cytokines and enzymes, reducing tissue damage (Jang *et al*., 2014). By boosting antioxidant defense and modulating metabolic processes, guava leaf extract may also support reproductive health. Notably, its reported ability to regulate blood glucose and enhance insulin sensitivity (Bhattacharjee *et al*., 2025) further strengthens its potential role in fertility preservation.

Despite increasing recognition of caffeine's detrimental effects on ovarian function and the bioactivity of *P. guajava* leaves (Ogunwole *et al*., 2024; Butt *et al*., 2025; Adeleye *et al*., 2026), limited research has examined their interactions. While caffeine has been shown to disrupt ovarian histology and hormone levels, the capacity of guava leaf extract to counteract these effects remains underexplored. This represents a critical gap, particularly in light of rising infertility rates and the widespread consumption of caffeine-containing products. By evaluating markers such as FSH, LH, estradiol, and progesterone, alongside ovarian tissue histoarchitecture, this research seeks to clarify whether guava extract can mitigate caffeine-induced reproductive dysfunction. The findings may contribute to developing plant-based therapeutic strategies for safeguarding ovarian health in women with high caffeine exposure.

MATERIALS AND METHODS

Plant collection and identification

Fresh *Psidium guajava* leaves were collected in March 2024 from the Nile University Female Hostel in Abuja, Nigeria. Identification and authentication were performed by a botanist at the Baze University Herbarium in Nigeria, where a voucher specimen (BUH10000106) was deposited. Leaves were sorted to remove debris, air-dried for two weeks, and ground into fine powder using a Silver Crest blender.

Experimental animals

Twenty-five adult female albino Wistar rats (130–215 g) were obtained from the Animal House of Nile University of Nigeria, Abuja. Animals were housed in plastic cages with wood-shaving bedding under controlled conditions (25 ± 2 °C; 45–55% relative humidity; 12 h light/dark cycle). They were fed Chukun grower pellets and provided distilled water (DW) *ad libitum*. All animals were acclimatized for one week before experimentation.

Ethical approval

All procedures followed the guidelines of the Nile University Committee on Animal Use and Care. Ethical clearance was obtained in line with institutional policies and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Caffeine preparation

Nescafe Classic coffee (50 g) was purchased from Maxcare Stores, Abuja. Based on its composition, 20 g of caffeine was estimated per 50 g of coffee. Experimental rats were administered caffeine at 100 mg/kg BW once daily for 21 days (Adelakun *et al*., 2024). This dose was chosen with reference to the reported LD_{50} of caffeine (2000 mg/kg BW) (Olanrewaju *et al*., 2017).

Preparation of methanol leaf extract of *Psidium guajava*

Fresh leaves of *Psidium guajava* were air-dried until a reasonable weight was obtained. The dried leaves were ground into powder with a blender. The powdered sample (200 g) was soaked in 1 L of methanol for 48 hours, with intermittent shaking. The mixture was filtered with Whatman No. 1 filter paper to obtain the filtrate. The filtrate was dried in a rotatory evaporator to yield a semi-solid matter (extract). The extract was redissolved in DW to obtain the required doses (125, 250 and 500 mg/kg BW) for the study.

Animal arrangement and extract administration

The rats were randomly assigned to five groups as detailed below:
Group 1 (Normal control): 1 mL DW
Group 2 (Caffeine control): 100 mg/kg BW caffeine
Group 3: 100 mg/kg BW caffeine + 125 mg/kg BW MLEPG
Group 4: 100 mg/kg BW caffeine + 250 mg/kg BW MLEPG
Group 5: 100 mg/kg BW caffeine + 500 mg/kg BW MLEPG

The method of administration was once daily via an oral cannula for 21 days.

Collection of ovary supernatant

At the end of the treatment period, the animals were anesthetized using chloroform. The animals were opened to harvest the ovaries, one of which was fixed in 10% buffered formalin for histological examination. The other ovary was homogenized using a Teflon Homogenizer. The resulting mixture was centrifuged at 3000 rpm for 15 minutes. Thereafter, the ovary supernatant was decanted into a separate plain sample bottle for the determination of hormonal biomarkers (FSH, LH, estradiol and progesterone).

Determination of follicle-stimulating hormone (FSH) concentration

Principle: A competitive enzyme immunoassay protocol was used. The microtiter plate had been pre-coated. The kit used was a goat anti-rabbit antibody kit.
Assay procedure: The procedure previously used by Scriver et al. (2010) was adopted for this assay. All chemicals and reagents were stored at room temperature for at least 1 hour before use in the assay. The reagents were carefully mixed before use. The holder was filled with the required number of coated strips. To the selected wells, 50 µL of FSH standards, control, and samples (supernatant) were introduced. A known volume of enzyme conjugate (100 µL) was pipetted into each well. The plate was covered and incubated at room temperature (23–26 ℃) for 1 hour. The liquid contained in all the wells was removed. A known volume (300 µL) of 1X wash buffer was used to wash three times. Paper towels were used to blot the blood. To all wells, 100 µL of TMB substrate was added. The mixture was incubated at room temperature for 10 minutes. About 50 µL of the stop solution was added to all wells. The plate was carefully mixed, and after 10 minutes of applying the stop solution, the absorbance at 450nm was read using a Hiwell Diatek ELISA Plate Reader (Model: DR-200Bc, Hiwell Diatek Instruments Co., Ltd., Wuxi, China).

Determination of luteinizing hormone (LH) concentration

Principle: The competitive inhibition enzyme immunoassay technique was used for the assay. This involved using a goat anti-rabbit antibody kit pre-coated on a microtiter plate.
Assay procedure: The protocol previously described by Makela et al. (2020) was used to determine LH concentration. All the reagents were kept at room temperature before the commencement of the assay. The reagents were gently mixed before use. The required number of coated strips was placed into the holder, and 25 µL of LH control, standards and serum sample was pipetted into the plates. A known volume (100 µL) of the Conjugate Reagent was added to all wells, and the plates were mixed on a plate shaker for 25 seconds at 800 rpm, then incubated at room temperature (23-27°C) for 60 minutes. Immediately after incubation, the liquid was removed from the wells. To wash all the wells, a known volume (200 µL) of 1X wash buffer was blotted onto paper towels. Then,

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100 µL of TMB substrate was added to all wells, followed by incubation for 15 minutes at 25 °C. About 40 µl of stop solution was added to all the wells after incubation. The plates were gently shaken to ensure complete mixing. The absorbance was measured at 450 nm after adding the stop solution using a Hiwell Diatek ELISA Plate Reader (Model: DR-200Bc, Hiwell Diatek Instruments Co., Ltd., Wuxi, China) within 15 minutes.

Determination of progesterone concentration
The procedure of Tietz (1995) was used for this assay. Exactly 1 mL of whole blood was centrifuged at 2000 rpm for 15 minutes. The obtained supernatant was carefully transferred into test tubes and subsequently frozen at –10 °C for storage until analysis. To prepare the plasma samples for analysis, a 0.3 mL aliquot of each sample was transferred to screw-capped tubes. An estriol at a concentration of 1.0 µg/mL was added to the samples. The samples were alkalinized after thorough mixing by adding 1 µL of NaOH solution. Thereafter, a mix of diethyl ether and hexane (in a ratio of 1:1, v/v) in a volume of 7 mL was added to the test samples. The samples were vortexed for 1 minute to ensure adequate mixing and were then centrifuged at 3000 rpm for 10 minutes. The spinning procedure was to allow for complete separation, resulting in a clear supernatant free of any remaining particulate matter. The supernatant derived from the spinning process was carefully separated and allowed to evaporate to dryness under a nitrogen stream at 27 °C. The residue obtained was dissolved in ethanol to prepare the samples for further analysis.

Determination of estradiol concentration
The Enzyme-Linked Immunosorbent Assay (ELISA) method, previously described by Haisenleder et al. (2011), was used to estimate serum estradiol concentration. A known amount (0.5 mL) of the sample was measured into a test tube. This was followed by running an estradiol standard of known concentration on the sample. The absorbance of the mixture was measured with a BioBase Absorbance Microplate Reader (Model: BK-EL-10 Series); Shandong, China. The absorbance of the unknown sample was extrapolated from the standard curve to determine the corresponding estradiol concentration.

Histological examination of the ovaries
The procedure proposed by Rocha-Pereira et al. (2019) was employed to examine ovarian histoarchitecture. Briefly, the ovarian tissues were excised, fixed in 10% buffered formalin, and processed using standard histological procedures. Briefly, tissue samples were dehydrated in a graded ethanol series, cleared with xylene, and embedded in paraffin wax. Sections of 5 µm thickness were obtained using a rotary microtome, mounted on slides, and stained with hematoxylin and eosin (H&E) for light microscopic examination.

Statistical analysis
Data were expressed as mean ± standard error of the mean (SEM) of five determinations. Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and RStudio. One-way ANOVA followed by appropriate post hoc tests was used for multiple comparisons. A *p*-value of <0.05 was considered statistically significant.

RESULTS

Hormonal profile
The administration of caffeine alone significantly *(p<0.05)* lowered estradiol levels (30.38% decrease vs. control). Co-administration of *P. guajava* extract resulted in a dose-dependent increase, with the highest dose (500 mg/kg BW) producing a 19.48% increase relative to the distilled water-treated caffeine-induced animals (DWTCIA). The 125 mg/kg BW of the MLEPG produced an estradiol value that compared favourably *(p>0.05)* with that of the distilled water-treated control animals (DWTCA) (Table 1).
The induction of animals with caffeine significantly *(p<0.05)* reduced progesterone levels (26.85% decrease vs. control). *P. guajava* extract restored progesterone concentration in a dose-dependent manner, with the highest dose increasing levels by 13.65% when compared with the DWTCIA. Administration of the extract at 125 and 250 mg/kg BW produced progesterone values that were not significantly different *(p>0.05)* from those of the distilled water-treated control animals (DWTCA) (Table 1).
The exposure to caffeine alone *(p<0.05)* elevated FSH concentration by 23.14% compared with the DWTCA. *P. guajava* extract reversed this effect, producing dose-dependent reductions, with the highest dose showing a 34.05% decrease relative to the DWTCIA. It is noteworthy that all the doses of the extract (125, 250 and 500 mg/kg BW) produced FSH values that were comparable *(p>0.05)* to the DWTCA (Table 1).
Caffeine administration substantially reduced LH concentration by 4.27% *(p<0.05)* compared with the DWTCA. Extract supplementation increased LH levels in a dose-dependent manner, with the highest dose showing a 12.88% increase compared with the DWTCIA. The extract-treated groups also showed LH values that were not significantly different *(p>0.05)* from the DWTCA (Table 1).

Table 1: Effect of methanol leaf extract of *Psidium guajava* on hormone biomarkers in caffeine-induced rats

Parameter	Control	Caffeine (100 mg/kg)	Caffeine + Extract (125 mg/kg)	Caffeine + Extract (250 mg/kg)	Caffeine + Extract (500 mg/kg)
FSH (mIU/mL)	4.58±0.75^{a}	5.64±0.59^{a}	4.01±0.33^{a}	3.62±0.69^{a}	3.02±0.14^{a}
LH (mIU/mL)	6.60±0.79^{a}	3.12±0.04^{b}	6.76±0.04^{a}	7.15±1.07^{a}	7.45±1.14^{a}
Progesterone (pg/mL)	41.00±3.62^{a}	29.99±2.97^{b}	41.68±3.38^{a}	42.43±3.21^{a}	46.59±3.91^{c}
Estradiol (ng/mL)	101.12±7.05^{a}	70.41±4.11^{b}	102.61±7.38^{a}	113.16±7.38^{c}	120.82±7.83^{d}

Values are expressed as Mean ± SEM of five determinants. Differences were considered significant at $p<0.05$. Values superscripted with letters (b, c, d) that differ from their respective control (a) across the row are significantly different.

FSH = Follicle-stimulating hormone; **LH =** Luteinizing hormone; **SEM =** Standard Error of Mean

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Histoarchitecture of the ovary

Figures 1(a-e) show the histoarchitecture of the ovary of the animals. Figure 1a shows normal ovarian histology in the control group, with follicles at different stages of development (short red arrow) and containing oocytes (long red arrow). Figure 1b shows atretic follicles with no follicular development in the group that received caffeine alone: the negative control group (long red arrow). Figure 1c shows the group of animals that received a 125 mg/kg BW dose of MLEPG, with follicular growth (long red arrow) and a mild ovarian cyst (long yellow arrow). Figure 1d shows the group that received 250 mg/kg BW of the MLEPG with scanty follicles (two small red arrows). Figure 1e shows the group that received 500 mg/kg BW of MLEPG, depicting different stages of follicular development (long red arrow) and exhibiting features similar to those in Figure 1a, with follicular regeneration and an almost normal ovarian histology.

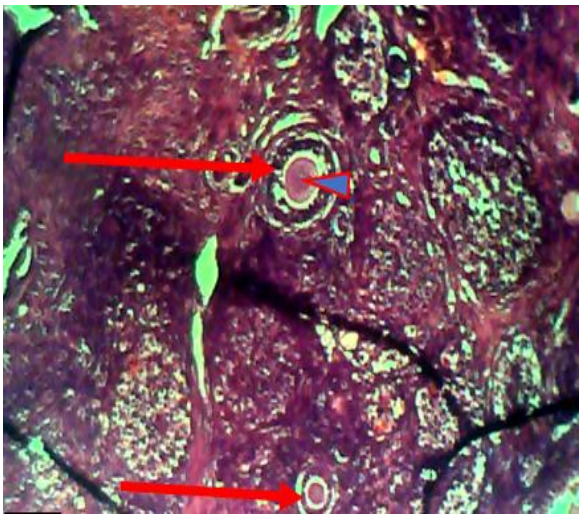

Figure 1a:: Photomicrograph of ovary section a rat that received distilled water; stained with H&E (x100)

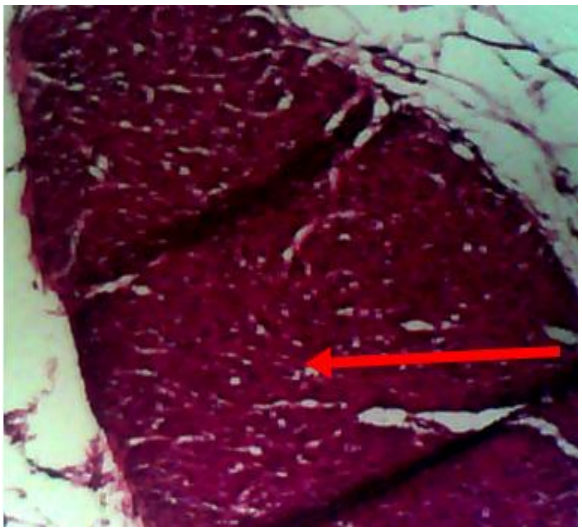

Figure 1b:: Cross section of the ovary of that received caffeine and distilled water; stained with H&E (x100)

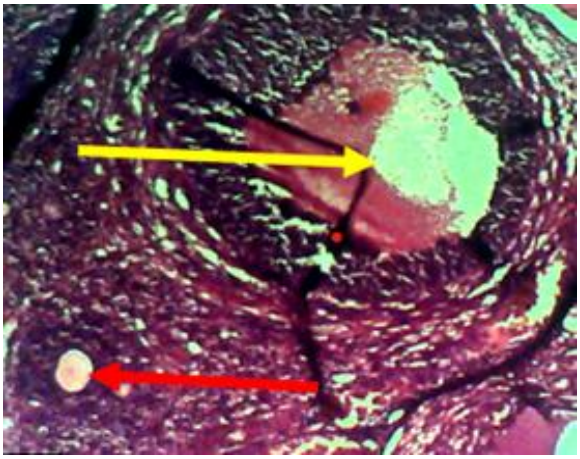

Figure 1c: Pictograph of the ovary of rats that received 125 mg/kg body weight of the P. guajava methanol leaf extract; stained with H&E (x100)

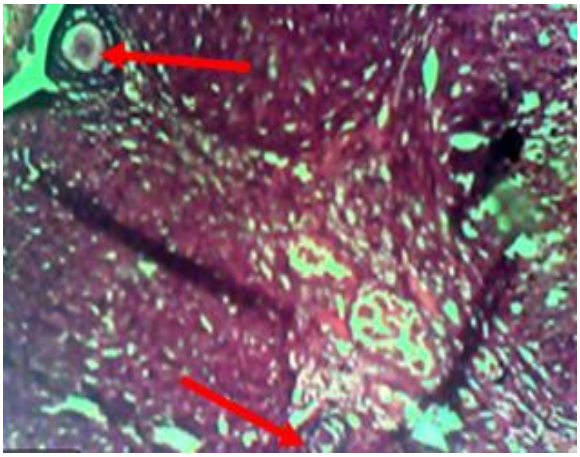

Figure 1d: Section of the ovary of a rat took 250 mg/kg body weight of P. guajava methanol leaf extract; stained with H&E (x100)

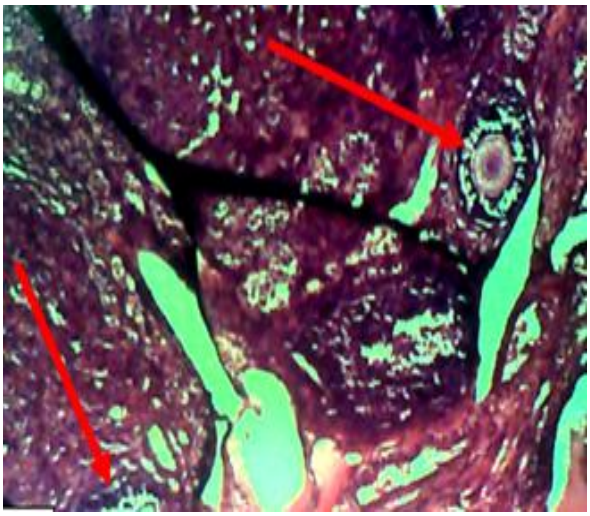

Figure 1e: Pictograph of the ovary of a rat that received 500 mg/kg body weight of the *P. guajava* methanol leaf extract; stained with H&E (x100)

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DISCUSSION

Plant-based extract could provide useful information on the protective capacity of natural products against hormonal and ovarian disruption in experimental models (Adams *et al*., 2025). Estradiol, the most active form of estrogen, is the primary and most potent estrogen steroid hormone of the female reproductive function. It is responsible for regulating the female reproductive system and developing secondary sexual characteristics. As a medication, it is widely used in hormone replacement therapy (HRT) and gender-affirming care. It orchestrates the menstrual cycle through follicular maturation, triggers ovulation, and prepares the uterus for pregnancy. It acts systemically to develop secondary sexual characteristics, maintain bone density, and support cardiovascular health (Yang *et al*., 2024). The reduction in estradiol concentration due to caffeine indicates a decline in ovarian function or a disruption in the hormonal communication between the brain and ovaries. Caffeine significantly reduced estradiol levels, which is consistent with earlier reports linking caffeine consumption to estrogen suppression and impaired follicular development (Goswami *et al*., 2019). In contrast, the increased estradiol concentration in the extract observed in this study could serve as the primary signal for the onset of maturation and the release of a healthy egg, while preparing the uterine lining for potential pregnancy. Also, *P. guajava* extract restored and enhanced estradiol levels, suggesting phytoestrogenic or antioxidative mechanisms. This is consistent with evidence showing that plant-derived polyphenols can improve ovarian steroidogenesis (Moore *et al*., 2016).

Progesterone is a vital steroid hormone produced primarily in the ovaries, adrenal glands, and placenta. It is essential for regulating the menstrual cycle, preparing the uterus for pregnancy, and supporting foetal development. It also acts in the brain to promote calming and sleep in pregnancy. The reduction in progesterone levels by caffeine could indicate an endocrine imbalance, primarily by disrupting the hypothalamo-pituitary-gonadal (HPG) axis, resulting in significantly lower serum progesterone levels. This reduction has profound systemic effects on reproductive health, the estrous cycle, and behavior (Dorostghoal *et al*., 2011). Progesterone levels, which were markedly suppressed by caffeine in this study, correlate with previous findings that caffeine interferes with luteal function and corpus luteum stability (Standring, 2016). The increase in progesterone levels induced by MLEPG could support uterine lining development, enhance female reproductive capacity, and exert antioxidant, anti-inflammatory, and neuroprotective effects in animals. The reversal observed with *P. guajava* supplementation suggests a luteotrophic effect, likely mediated through its antioxidant and anti-inflammatory phytoconstituents such as flavonoids and tannins (Fredholm *et al*., 1999).

Follicle-stimulating hormone (FSH), which is produced by the pituitary gland, is important for female reproductive health. It plays a major role in stimulating the growth and maturation of ovarian follicles before ovulation and in driving ovarian estrogen production (Atsukwei *et al*., 2015; Morton *et al*., 2023). The high FSH levels following caffeine induction could indicate impaired reproductive function, altered organ weights, and oxidative stress in the animals' reproductive tissues (Park *et al*., 2022). Also, the caffeine-induced elevation of FSH may represent a compensatory pituitary response to estrogen deficiency. The reduction in elevated FSH levels across all MLEPG doses might exert beneficial, hormone-regulating effects on the animals. High FSH levels usually indicate depleted ovarian reserve or reproductive aging. However, lowering elevated FSH levels may help correct hormonal imbalances, restore a regular estrous cycle, and protect the reproductive organs from toxicity (Ladele *et al*., 2025). Furthermore, the administration of MLEPG, which resulted in significant dose-dependent reductions in FSH, could imply restored estrogen feedback regulation. A similar modulatory effect of plant-based intervention on gonadotropins was reported by Fisone *et al*. (2004).

Luteinizing hormone (LH) is a vital reproductive hormone produced by the pituitary gland. In females, it drives the menstrual cycle by stimulating the ovaries to produce estrogen, triggering the release of a mature egg during ovulation, and forming the corpus luteum to produce progesterone for potential pregnancy (Al-Ameen *et al*., 2023). The low LH level induced by caffeine might indicate disruption of normal hypothalamic-pituitary-gonadal (HPG) axis function (Grosso *et al*., 2017). This hormonal imbalance, caused by the disruption, could lead to detrimental reproductive, structural, and systemic defects that can affect overall physiological health and estrous cycling. The increased LH levels across all MLEPG doses would enhance reproductive health by triggering the mid-cycle LH surge, promoting healthy ovarian follicular development, and stimulating the corpus luteum to produce progesterone (Cedrin-Durnerin *et al*., 2025). These elevated LH levels are primarily used in studies to correct anovulation and restore normal estrous cycles. Furthermore, supplementation of the caffeine-induced rats with *P. guajava* significantly increased LH levels in a dose-dependent manner, suggesting that its bioactive compounds may support hypothalamic GnRH release and pituitary LH secretion, thereby enhancing ovulatory potential.

Histopathological analysis of the ovary is the microscopic gold standard for diagnosing ovarian diseases (Rihal *et al*., 2021). It is critical for accurately differentiating benign from malignant tumors, staging ovarian cancer, assessing fertility using follicle counts, and guiding targeted, life-saving treatments (Dhatrak *et al*., 2025). Ovary histoarchitecture gives insight into the differences between benign functional cysts and aggressive malignancies, especially in late-stage presentations and rising mortality rates. It presents the gold standard for guiding appropriate surgical or chemotherapeutic interventions. The observed atretic follicles with no follicular development induced by caffeine in this study indicate a state of reproductive arrest or ovarian failure, in which growing follicles fail to mature and instead undergo programmed cell death (apoptosis). Caffeine induction could also have caused the ovarian follicles to die prematurely, halting the normal maturation process (Hahn *et al*., 2019). Follicular development describes the process by which immature egg cells grow and mature into viable follicles ready for ovulation; atresia is simply the biological term for cell death in ovarian follicles (Charel *et al*., 2020). Exposure of animals to caffeine can severely impair folliculogenesis, leading to DNA damage and apoptosis (programmed cell death) in ovarian cells. This toxic environment forces developing ovarian follicles to undergo atresia (premature degeneration) rather than progress to mature stages, ultimately leading to halted follicular development and an exhausted ovarian reserve (Massin *et al*., 2004).

The observation of follicular growth, follicular regeneration and normal ovarian histology, mediated by the MLEPG, in the present study, may be adduced to the extract's ability to promote follicular growth and regeneration by modulating oxidative stress (Eze *et al*., 2018), reducing granulosa cell apoptosis, and balancing hormones via the Hypothalamic-Pituitary-Ovarian (HPO) axis. These botanicals help restore normal ovarian histology and

folliculogenesis by providing phytoestrogens and antioxidant properties. The MLEPG could have restored and supported normal female reproductive health by acting as an antioxidant and a hormonal regulator. This might have been mediated by protecting ovarian cells from oxidative stress, reducing granulosa cell apoptosis, and creating a suitable environment for follicular development, regeneration, and healthy ovarian histology (Solomon *et al*., 2010; Chalana *et al*., 2025).

General Implication and Future Direction

Taken together, these findings suggest that while caffeine disrupts ovarian hormonal balance, *P. guajava* extract restores reproductive hormone profiles and may preserve fertility. The plant's protective role is likely attributable to its rich phytochemical composition, particularly flavonoids, tannins, and polyphenols, which have known antioxidant properties. Investigation of the plant extract's phytoconstituents, along with a positive control group (standard medication) for comparison, should be considered.

Conclusion

Caffeine significantly alters ovarian hormone profiles in female Wistar rats. *Psidium guajava* leaf extract mitigates these effects in a dose-dependent manner, restoring estradiol, progesterone, and LH levels while reducing elevated FSH. These findings highlight the therapeutic potential of *P. guajava* in counteracting caffeine-induced reproductive dysfunction.

Conflict of interest

The authors declare no conflict of interest in this study.

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