

Robusta peaberry coffee attenuates inflammation, regulates apoptosis, and improves testicular cell integrity in diabetic rats

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ABSTRACT

Background: Diabetes mellitus (DM) is a chronic metabolic disorder that can impair male reproductive function through oxidative stress, inflammation, and apoptosis. Robusta peaberry coffee contains chlorogenic acid (CGA) and other polyphenols with antioxidant and anti-inflammatory properties that may protect against diabetes-related testicular injury.

Objectives: This study aimed to evaluate the effects of robusta peaberry coffee on inflammation, apoptosis-related markers, reproductive hormones, and testicular cell integrity in streptozotocin (STZ)-induced diabetic male rats.

Materials and methods: Male Wistar rats (N=28) were randomly assigned to four groups: diabetic control (KN), diabetic rats treated with metformin (KP; 500 mg/kg body weight [BW]/day), diabetic rats treated with robusta peaberry coffee at 100 mg/kg BW/day (P1), and diabetic rats treated with robusta peaberry coffee at 200 mg/kg BW/day (P2). Experimental diabetic rats were induced by a single intraperitoneal injection of STZ (40 mg/kg BW). Rats with fasting blood glucose (FBG) ≥ 200 mg/dL were included. Treatments were administered orally for 14 consecutive days. At the end of the intervention, serum nitric oxide (NO), tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), caspase-3, testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the numbers of Sertoli and Leydig cells were evaluated.

Results: Testosterone, LH, FSH, Sertoli cell count, and Leydig cell count were significantly lower in the KN group than in the KP, P1, and P2 groups ($p < 0.05$). In contrast, NO, TNF- α , IL-1 β , and caspase-3 were significantly higher in the KN group than in the treatment groups ($p < 0.05$). The P2 group showed the greatest improvement, with higher reproductive hormone levels and testicular cell counts and lower inflammatory and apoptosis-related markers than P1 ($p < 0.05$).

Conclusion: Robusta peaberry coffee attenuates inflammation, modulates apoptosis-related responses, and improves testicular cell integrity in diabetic rats. These findings suggest that robusta peaberry coffee may serve as a promising complementary natural intervention for mitigating diabetes-induced male reproductive dysfunction.

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that can impair male reproductive function through persistent hyperglycemia, oxidative stress, inflammation, endocrine disruption, and apoptosis.^{1,2} In diabetic testes, excessive reactive oxygen species activate inflammatory pathways, including nuclear factor kappa B (NF- κ B), thereby increasing nitric oxide (NO), tumor necrosis factor-alpha (TNF- α), and interleukin-1 beta (IL-1 β). These mediators disturb the testicular microenvironment, damage Sertoli and Leydig cells, and reduce steroidogenesis and spermatogenesis.^{3,4}

Although coffee and coffee-derived polyphenols have been studied for their metabolic and antioxidant effects in diabetes, evidence on robusta peaberry coffee and testicular function remains limited.⁵⁻⁷ This gap is important because diabetic reproductive injury involves interconnected inflammatory, apoptotic, hormonal, and histological changes.^{8,9} Therefore, an intervention should not be evaluated only through a single marker, but through multiple parameters that reflect the inflammatory burden, apoptosis-related response, reproductive hormone status, and testicular cell integrity.

Robusta peaberry coffee was selected because peaberry beans contain a single seed within the coffee cherry and may have a denser and more concentrated bioactive profile than regular beans.^{10,11} Robusta coffee is also recognized for its relatively high chlorogenic acid (CGA) content compared with several other coffee types.^{12,13} CGA and other phenolic compounds can scavenge free radicals, suppress inflammatory signaling, and reduce cytokine expression. These properties provide a biological rationale for testing robusta peaberry coffee as a potential protective agent against diabetes-induced testicular injury.^{6,14,15}

The use of whole coffee extract may also introduce components such as caffeine and other methylxanthines that can cause adverse effects when consumed excessively, including increased sympathetic activity, gastrointestinal discomfort, and sleep disturbance.^{16,17} For this reason, the intervention dose should be justified and standardized. In the present study, the 100 and 200 mg/kg BW doses were selected based on previous experimental coffee-extract studies showing antioxidant and metabolic effects in diabetic models, while allowing comparison between a lower and a higher dose.^{18,19}

The novelty of this study lies in the simultaneous evaluation of robusta peaberry coffee on inflammatory biomarkers, an apoptosis-related marker, reproductive hormones, and histological indicators of testicular cell integrity in diabetic rats. This integrated approach provides a clearer understanding of whether robusta peaberry coffee can protect testicular function under diabetic conditions.

Materials and methods

Study design

This study was a real experimental laboratory study using a randomized post-test-only control-group design.

Experimental animals, diabetes induction, and materials

Twenty-eight male Wistar rats (*Rattus norvegicus*), aged 8-10 weeks and weighing approximately 250 gm, were obtained from a certified laboratory animal facility. Animals were acclimatized for one week before experimentation and were housed individually in stainless steel cages under controlled conditions (24 $\pm$ 2 °C, 50-60% relative humidity, and a 12-hour light/dark cycle). Standard pellet diet and water were provided *ad libitum*.

All procedures were approved by the Health Research Ethics Committee of the Polytechnic of Health, Ministry of Health, Semarang (No. 837/EA/F. XXIII.38/2025).

After acclimatization, experimental diabetic rats were induced by a single intraperitoneal injection of streptozotocin (STZ) at 40 mg/kg BW dissolved in 0.1M citrate buffer (pH 4.5). Rats with fasting blood glucose $\geq$ 200 mg/dL were considered diabetic and included in the study. Seventy-two hours after STZ administration, FBG was measured from tail-vein blood samples using a glucometer (EasyTouch®, GCUModel: ET-301, Biopik Technology, Taiwan). The device was checked using manufacturer-recommended control solution before measurement, and test strips were used within the expiration date and stored according to the manufacturer's instructions. Rats with FBG $\geq$ 200 mg/dL were considered diabetic and included in the study. Animals that did not reach this threshold were excluded to ensure homogeneity of diabetic status.

Robusta peaberry coffee beans were obtained from a local supplier in Banyuwangi, Indonesia. The beans were ground into fine powder using a dry electric grinder (Yongkang Tianqi Shengshi Industry and Trade Co., Ltd.). STZ, metformin hydrochloride (99.60%; PT Phapros Tbk, Indonesia), ELISA kits for NO, TNF- α , IL-1 β , caspase-3, testosterone, LH, and FSH (Wuhan Fine Biotech Co., Ltd. [FineTest], Wuhan, China), and hematoxylin-eosin reagents were used in this study.

Preparation and standardization of robusta peaberry coffee

An aqueous robusta peaberry coffee extract was freshly prepared each day from roasted coffee beans obtained from the same production batch. Coffee powder was mixed with distilled water at a fixed ratio of 1 gm:10 mL (w/v) and extracted by stirring at 90 °C for 15 minutes, followed by filtration through Whatman No. 1 filter paper to remove insoluble residues. The filtrate was diluted to obtain a working concentration

of 25 mg/mL, allowing accurate dose administration according to body weight. Preparation consistency was maintained by using the same coffee batch, extraction temperature, extraction time, powder-to-solvent ratio, filtration procedure, and freshly prepared extract before each administration. Although quantitative phytochemical standardization (e.g., chlorogenic acid or caffeine determination) was not performed for each daily preparation, previous studies have demonstrated that robusta coffee is naturally rich in chlorogenic acid, which is considered one of its principal bioactive constituents.

Experimental groups and treatment

Diabetic rats were randomly allocated into four groups (N=7 per group): 1) diabetic control (KN), 2) diabetic rats treated with metformin at 500 mg/kg BW/day (KP), 3) diabetic rats treated with robusta peaberry coffee at 100 mg/kg BW/day (P1), and 4) diabetic rats treated with robusta peaberry coffee at 200 mg/kg BW/day (P2). Based on an average body weight of 0.25 kg, the daily coffee doses were 25 mg (P1) and 50 mg (P2), corresponding to 1 mL/day and 2 mL/day, respectively, using the 25 mg/mL working solution. All interventions were administered orally by gavage once daily for 14 consecutive days. The KN group received an equivalent volume of distilled water by oral gavage as the vehicle control.

Sample collection

At the end of the treatment period, rats were fasted overnight and anesthetized using ketamine. Blood samples were collected through retro-orbital puncture using a capillary glass tube. Blood was allowed to clot at room temperature and was centrifuged at 3,000 rpm for 15 minutes to obtain serum. Testicular tissues were excised, rinsed with physiological saline, and processed for histopathological analysis.

Biochemical and hormonal analysis

Serum nitric oxide (NO) levels were measured using a commercial ELISA kit (FineTest catalog No. ER0150, Wuhan, China) according to the manufacturer's instructions. Absorbance was recorded at 450 nm, and concentrations were calculated from the standard curve. Serum TNF- α and IL-1 β were quantified using sandwich ELISA kits (FineTest catalog No. ER1393 for TNF- α and ER1094 for IL-1 β). Absorbance was measured at 450 nm, and cytokine concentrations were interpolated from calibration curves. Serum caspase-3 was measured using a quantitative ELISA kit (FineTest catalog No. ER0143).

In this study, serum caspase-3 was interpreted as an apoptosis-related systemic marker associated with diabetic testicular injury, not as a direct cell-specific measurement of apoptosis within Sertoli or Leydig cells.

Serum testosterone, LH, and FSH were measured with specific ELISA kits (FineTest catalog Nos. EU0400 for testosterone, ER1123 for LH, and ER0960 for FSH) and calculated using standard reference curves generated for each assay.

Histopathological examination

Testicular tissues were fixed in 10% neutral buffered formalin for 24 hours, dehydrated through graded ethanol, cleared in xylene, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin. Microscopic examination was performed at 400 $\times$ magnification. Sertoli and Leydig cells were counted using a systematic random field approach in five non-overlapping fields per section. Cells were identified based on typical morphology and clearly visible nuclei, and the mean value was calculated for each animal. Counting was performed in a blinded manner to reduce observer bias.

Statistical analysis

Data are presented as mean $\pm$ SD. Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test for multiple comparisons. Statistical analysis was conducted using SPSS version 25 (IBM Corp., USA). A $p < 0.05$ was considered statistically significant. Graphs were generated using GraphPad Prism version 9 (GraphPad Software, USA).

Results

Effects of robusta peaberry coffee on serum reproductive hormone levels

Serum testosterone levels were lowest in the KN group and were significantly lower than those in all other groups ($p < 0.05$). Serum testosterone levels were significantly higher in the KP, P1, and P2 groups than in the KN group ($p < 0.05$). The P2 group demonstrated a significantly higher testosterone level than the P1 group ($p < 0.05$) (Figure 1A).

Serum luteinizing hormone (LH) levels were lowest in the diabetic control group (KN). Treatment with metformin (KP) and robusta peaberry coffee (P1 and P2) resulted in significantly higher LH levels than those in the KN group ($p < 0.05$). The increase in LH levels was greater with higher coffee doses. The P2 group showed the highest LH level among all experimental groups ($p < 0.05$) (Figure 1B).

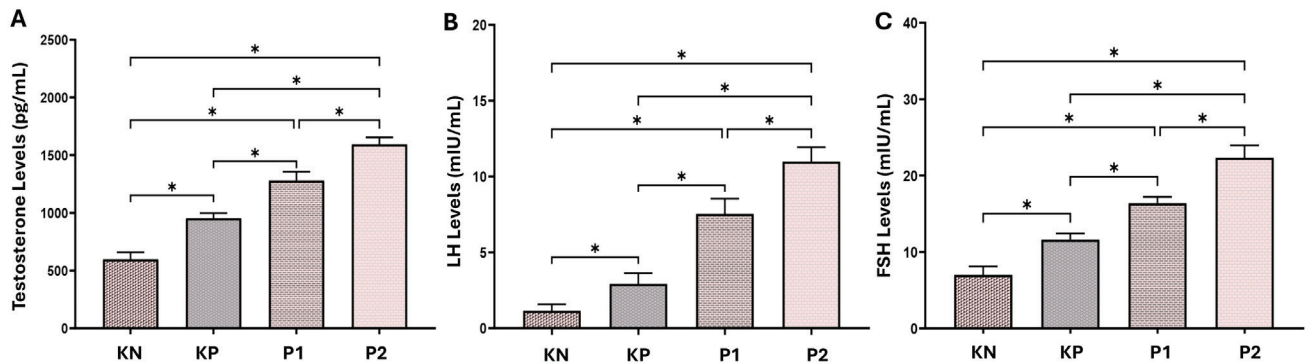


Figure 1. Reproductive hormone parameters. A: testosterone levels, B: luteinizing hormone (LH) levels, C: follicle-stimulating hormone (FSH) levels, KN: diabetic control, KP: diabetic rats treated with metformin, P1: diabetic rats treated with robusta peaberry coffee at 100 mg/kg BW/day, P2: diabetic rats treated with robusta peaberry coffee at 200 mg/kg BW/day. * $p < 0.05$.

A similar trend was observed for serum follicle-stimulating hormone (FSH) levels across the experimental groups. The KN group had the lowest FSH level, whereas significantly higher values were found in the KP, P1, and P2 groups ($p < 0.05$). FSH levels increased gradually from KP to P1, peaking in the P2 group. Significant differences were also observed between the coffee-treated groups and the diabetic control group ($p < 0.05$). Compared with the lower coffee dose, administration of 200 mg/kg BW/day produced a greater increase in FSH levels.

Effects of robusta peaberry coffee on Sertoli and Leydig cell integrity

Figure 2A shows the mean Sertoli cell count in the KN, KP, P1, and P2 groups. The KN group had the lowest Sertoli cell count, followed by progressively higher counts in the KP and P1 groups, with the highest count observed in the P2 group ($p < 0.05$). These quantitative findings were supported by the histopathological images in Figure 3, which showed reduced testicular cellular integrity in the KN group and progressive improvement in the KP, P1, and P2 groups, with the most pronounced improvement observed in the P2 group.

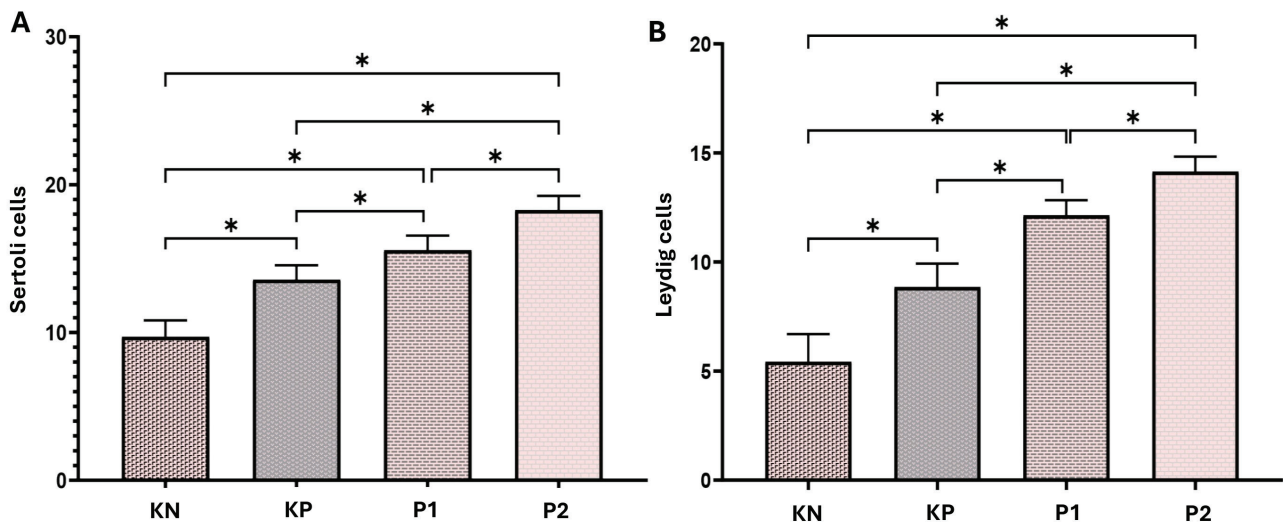


Figure 2. Testicular cell integrity after treatment. A: Sertoli cell count, B: Leydig cell counts in each group, KN: diabetic control, KP: diabetic rats treated with metformin, P1: diabetic rats treated with robusta peaberry coffee at 100 mg/kg BW/day, P2: diabetic rats treated with robusta peaberry coffee at 200 mg/kg BW/day. * $p < 0.05$.

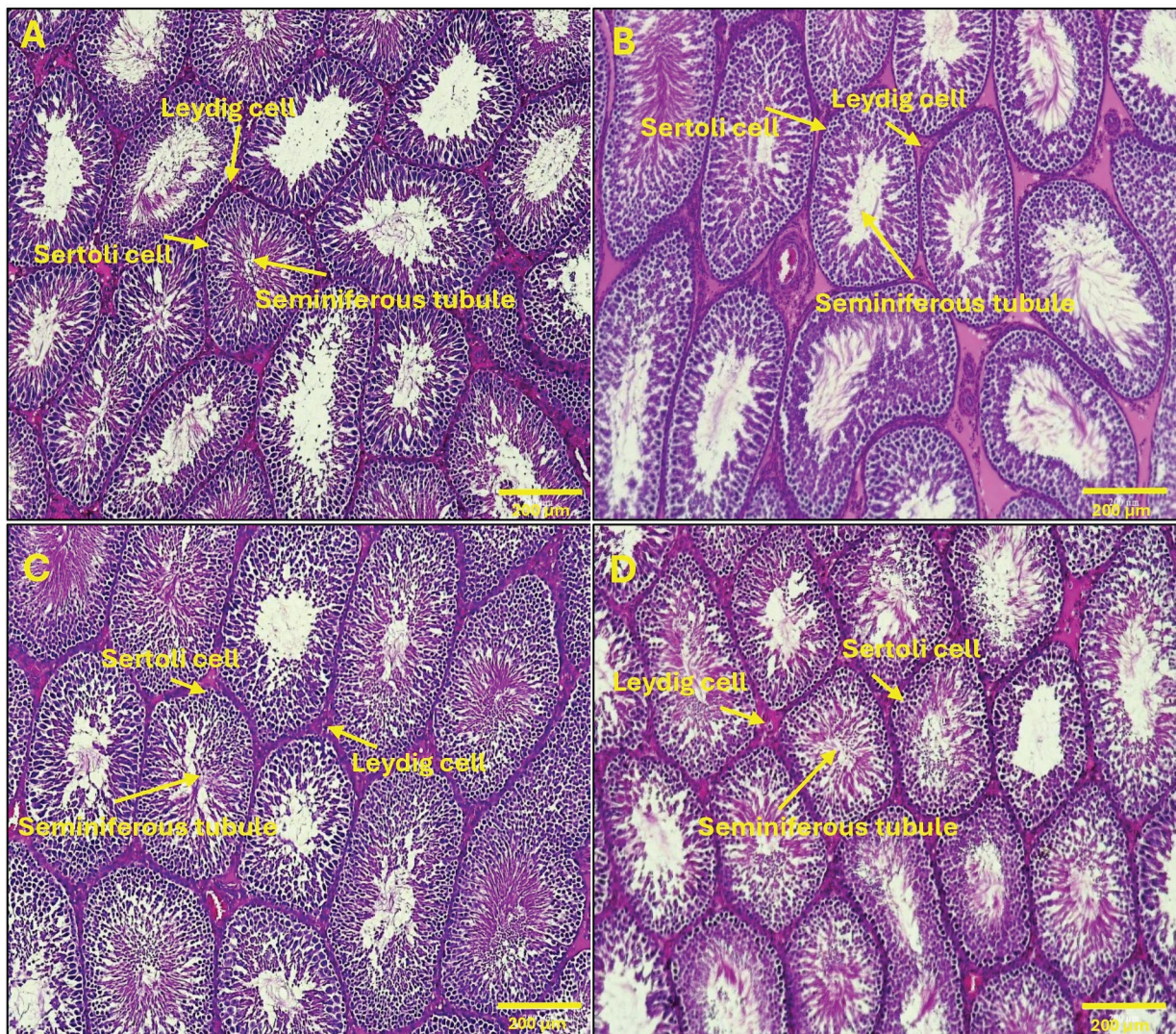


Figure 3. Histopathological images of testicular tissue from diabetic rats, H&E-stained; scale bar = 200 μ m. A: diabetic control group (KN), B: diabetic rats treated with metformin (KP), C: diabetic rats treated with robusta peaberry coffee at 100 mg/kg BW/day (P1), D: diabetic rats treated with robusta peaberry coffee at 200 mg/kg BW/day (P2). The arrows indicate Sertoli cells, Leydig cells, and seminiferous tubules.

Effects of robusta peaberry coffee on serum NO, TNF- α , IL-1 β , and caspase-3

NO levels were highest in the KN group, with a mean of 13.80 ± 0.92 ng/mL, and were significantly higher than those in the KP group, which showed a lower mean of 10.20 ± 0.79 ng/mL ($p < 0.05$). A further decrease was observed in the P1 group, with a mean of 6.04 ± 0.67 ng/mL, which was significantly lower than those in both the KN and KP groups ($p < 0.05$). The P2 group showed the lowest NO level, with a mean of 3.48 ± 0.97 ng/mL, which was significantly lower than those in all other groups ($p < 0.05$). Compared with the KN group, the P2 group showed a reduction of more than 10.32 ng/mL ($p < 0.05$) (Figure 4A).

Figure 4B shows that the TNF- α level was highest in the KN group (79.70 ± 7.58 pg/mL) and was significantly higher than that in the KP group (65.90 ± 7.21 pg/mL) ($p < 0.05$). A greater reduction was observed in the P1 group, with a mean of 53.60 ± 3.01 pg/mL, which was significantly lower than those in the KN and KP groups ($p < 0.05$). The lowest TNF- α level was observed in the P2 group (43.60 ± 2.34 pg/mL), which differed significantly from those of the other groups ($p < 0.05$). Compared with the KN group, the P2 group showed a reduction of more than 36 pg/mL ($p < 0.05$). Greater variability was observed in the KN and KP groups than in the P1 and P2 groups, as indicated by the larger standard deviations.

Figure 4C demonstrates that the IL-1 β level was highest in the KN group, with a mean of 93.55 ± 7.69 pg/mL, and was significantly higher than that in the KP group (65.26 ± 3.52 pg/mL) ($p < 0.05$). The P1 group showed a mean IL-1 β level of 52.70 ± 3.93 pg/mL, which was significantly lower than those in the KN and KP groups ($p < 0.05$). The lowest IL-1 β level was observed in the P2 group, with a mean of 38.02 ± 3.74 pg/mL, which was significantly lower than those in all other groups ($p < 0.05$). Compared with the KN group, the P2 group showed a reduction of more than 55.53 pg/mL ($p < 0.05$).

Caspase-3 activity was highest in the KN group, with a mean of 3.96 ± 0.49 ng/mL, and was significantly higher than that in the KP group (3.14 ± 0.42 ng/mL) ($p < 0.05$). The P1 group showed a mean value of 2.37 ± 0.36 ng/mL, which was significantly lower than those in the KN and KP groups ($p < 0.05$). The lowest caspase-3 activity was observed in the P2 group, with a mean of 1.51 ± 0.28 ng/mL, which was significantly lower than those in all other groups ($p < 0.05$). Compared with the KN group, the P2 group showed a reduction of more than 2.4 ng/mL ($p < 0.05$) (Figure 4D).

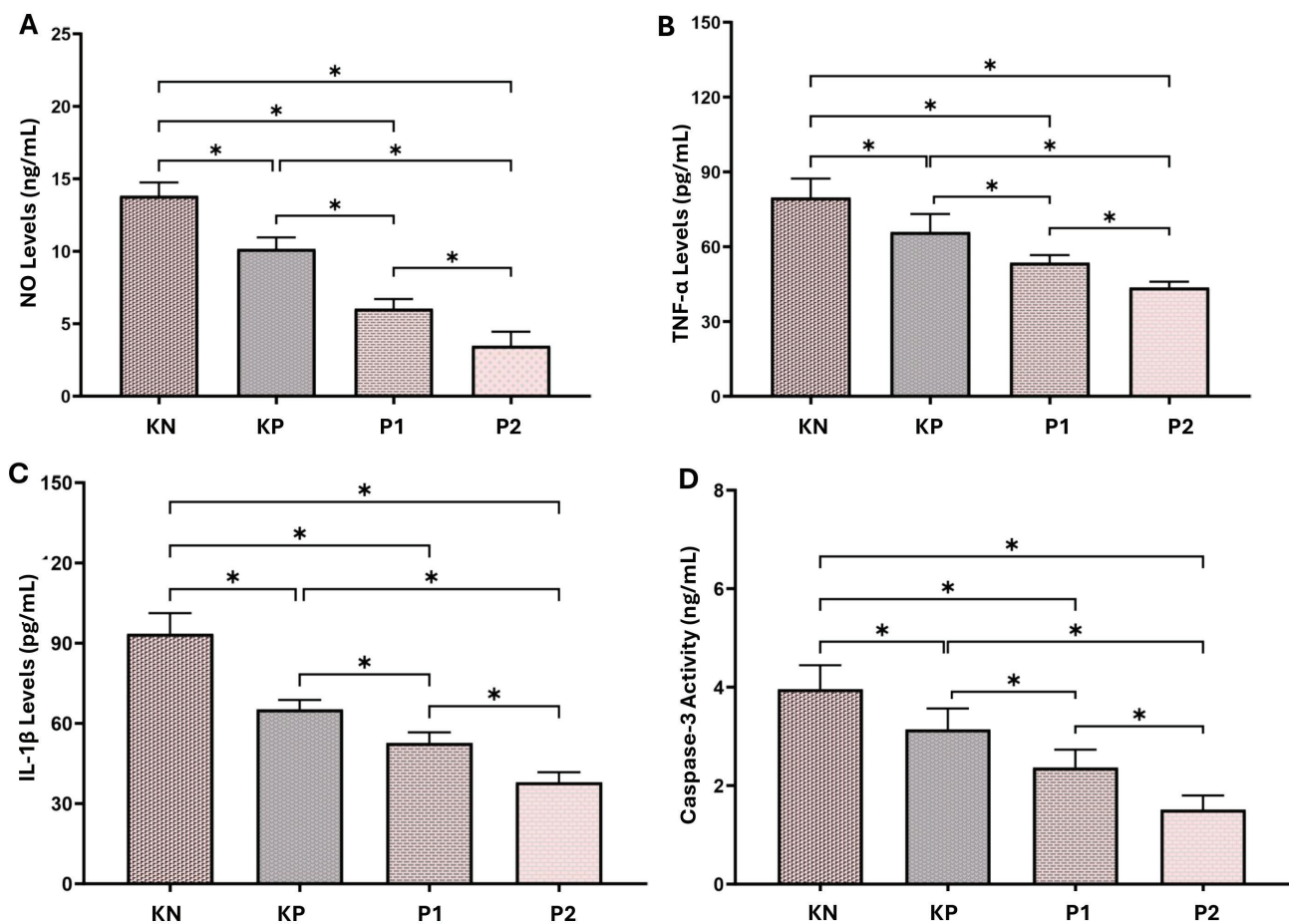


Figure 4. Effects of robusta peaberry coffee. A: serum nitric oxide (NO), B: tumor necrosis factor-alpha (TNF- α), C: interleukin-1 beta (IL-1 β), D: caspase-3, KN: diabetic control, KP: diabetic rats treated with metformin, P1: diabetic rats treated with robusta peaberry coffee at 100 mg/kg BW/day, P2: diabetic rats treated with robusta peaberry coffee at 200 mg/kg BW/day. * $p < 0.05$.

Correlation between NO, TNF- α , IL-1 β , reproductive hormones, caspase-3, Sertoli cells, and Leydig cells

Figure 5 reveals the Pearson correlation heatmap among inflammatory markers, reproductive hormones, the apoptosis marker, and testicular cells. NO and TNF- α showed a strong positive correlation ($r = 0.92$), suggesting that higher NO levels were generally accompanied by higher TNF- α levels in the pooled dataset. However,

this relationship should be interpreted as a statistical association rather than evidence of a direct biological mechanism or causal effect. In contrast, NO and TNF- α showed strong negative correlations with testosterone, LH, FSH, Sertoli cells, and Leydig cells, suggesting inverse statistical associations between inflammatory markers and reproductive parameters.

	NO	TNF- α	Testosterone	LH	FSH	Caspase-3	Sertoli cells	Leydig cells
NO	1.000	0.919	-0.966	-0.955	-0.948	0.910	-0.929	-0.943
TNF- α	0.919	1.000	-0.916	-0.892	-0.910	0.922	-0.897	-0.888
Testosterone	-0.966	-0.916	1.000	0.958	0.972	-0.909	0.946	0.963
LH	-0.955	-0.892	0.958	1.000	0.951	-0.884	0.887	0.929
FSH	-0.948	-0.910	0.972	0.951	1.000	-0.913	0.937	0.941
Caspase-3	0.910	0.922	-0.909	-0.884	-0.913	1.000	-0.849	-0.898
Sertoli cells	-0.929	-0.897	0.946	0.887	0.937	-0.849	1.000	0.913
Leydig cells	-0.943	-0.888	0.963	0.929	0.941	-0.898	0.913	1.000

Figure 5. Pearson correlation heatmap of NO, TNF- α , testosterone, LH, FSH, caspase-3, Sertoli cells, and Leydig cells in diabetic rats. Positive correlations are shown by lighter colors, whereas negative correlations are shown by darker colors.

Caspase-3 showed strong positive correlations with NO ($r=0.91$) and TNF- α ($r=0.92$), but negative correlations with testosterone ($r=-0.91$), LH ($r=-0.88$), FSH ($r=-0.91$), Sertoli cells ($r=-0.85$), and Leydig cells ($r=-0.90$). Conversely, testosterone, LH, and FSH showed positive correlations with Sertoli and Leydig cells, with the strongest associations observed between testosterone and Leydig cells ($r=0.96$), testosterone and FSH ($r=0.97$), and testosterone and LH ($r=0.96$). All correlations were statistically significant ($p<0.001$), indicating strong statistical relationships among the variables included in this study.

Discussion

This research studied how Robusta peaberry coffee influences reproductive hormones and testicular dysfunction in diabetic rats. The findings demonstrated correlations among the variables, supporting the hypothesis that reproductive issues in diabetes stem from oxidative stress that induces inflammation, which activates cell death pathways and hampers steroidogenesis. Diabetes has been reported to lead to an increase in reactive oxygen species (ROS) and an imbalance in antioxidants, thereby exacerbating tissue inflammation, including within the testes.⁸ Subsequently, this cascade disrupts steroidogenesis and triggers apoptosis, resulting in decreased

testosterone levels and impaired spermatogenesis.²⁰

Diabetes mellitus impairs sperm quality and hypothalamic-pituitary function, leading to decreased Leydig cell activity and testosterone production. Increased mitochondrial oxidative stress causes cell death and tissue damage, compromising the blood-testis barrier and leading to lipid peroxidation in germ cell membranes, both of which are crucial for spermatogenesis.²¹ Leydig cells are especially vulnerable, affecting steroidogenic proteins and enzyme expression necessary for testosterone synthesis.¹⁸

Blood glucose levels may be reduced by moderate coffee consumption, and different coffee extract doses can produce different glycemic effects.¹⁷ Robusta coffee has been reported to contain higher CGA than Arabica coffee extract, and aqueous green coffee extracts, particularly Robusta, have shown beneficial effects on pancreatic islet repair in diabetic models.^{16,18,22} In diabetes-associated male infertility, testosterone plays an essential role in spermatogenesis and serves as an important marker of testicular function.^{23,24} In the present study, diabetic control rats showed decreased LH levels, consistent with previous findings that diabetes is associated with reduced reproductive hormone levels.

The observed increases in LH and FSH following robusta peaberry coffee administration may have

several possible explanations. One interpretation is that the reduction in hyperglycemia-associated oxidative stress and inflammation partially restored hypothalamic pituitary gonadal (HPG) axis function, thereby improving pituitary secretion of gonadotropins.^{8,9,28} Alternatively, the elevated LH and FSH concentrations may represent a compensatory endocrine response accompanying the recovery of testicular function rather than a direct stimulatory effect of coffee-derived bioactive compounds on the pituitary gland.²⁷ Improved Leydig cell integrity may enhance testosterone production, while restoration of Sertoli cell function could normalize feedback mechanisms involved in gonadotropin regulation.^{20,27} Because circulating LH and FSH are influenced by multiple physiological factors, including hypothalamic gonadotropin-releasing hormone (GnRH) secretion, pituitary responsiveness, gonadal feedback, metabolic status, and systemic inflammation, the present study cannot determine the primary mechanism responsible for these hormonal changes.^{8,9,27,28}

The strong correlations among NO, TNF- α , and IL-1 β indicate that these inflammatory mediators increase together during activation of pro-inflammatory pathways. Increased NO can amplify oxidative stress, while TNF- α activates NF- κ B and promotes cytokine expression, creating an unfavorable environment for Sertoli and Leydig cells.²⁵ Robusta peaberry coffee treatment reduced these inflammatory mediators, which may be related to its polyphenol and CGA content that can suppress ROS, inflammatory signaling, and cytokine expression in testicular tissue.²⁶

A very strong inverse relationship between testosterone and inflammatory mediators indicates that when the testicular environment is predominantly pro-inflammatory, steroidogenic capacity tends to decline. Physiologically, testosterone is produced by Leydig cells and is highly dependent on mitochondrial integrity, intracellular cholesterol availability, and the expression of cholesterol transport proteins such as StAR and steroidogenic enzymes. Recent evidence suggests that inflammation, particularly through cytokines such as TNF- α , can disrupt androgen production, especially when inflammation occurs alongside oxidative stress.²⁷ This is in line with a meta-analysis that identifies chronic inflammation as an important mediator of fertility disorders in the diabetic population and highlights that targeting inflammation may be a relevant therapeutic strategy.²⁸ Thus, the direction of the correlations in this study confirms that testosterone is not merely decreased, but also serves as a marker of testicular function that is sensitive to inflammatory stress. Interestingly, a recent study on CGA in testicular disorder models also reported that CGA can reduce ROS and pro-inflammatory cytokines in the testes, accompanied by histological repair, which is biologically consistent with recovery of the steroidogenesis pathway.²⁹ If inflammation is suppressed, Leydig cells

are more likely to maintain testosterone synthesis, allowing endocrine signaling to Sertoli cells and seminiferous tissue to recover. Activation of caspase-3 generally represents the execution phase of apoptosis, which can be triggered by mitochondrial damage, oxidative stress, and persistent inflammatory signals. Studies on testicular injury models show that when TNF- α , IL-1 β , and NF- κ B increase, apoptotic indicators, including caspase-3, also tend to rise; conversely, when an intervention suppresses NF- κ B, inflammatory markers and caspase-3 also decrease.³⁰

This study demonstrates that robusta peaberry coffee may increase reproductive hormone levels and preserve Sertoli and Leydig cells in DM rats after 14 days of administration. This supports the findings of Al-Megrin et al., who reported that green coffee possesses exogenous antioxidant properties.⁶ Endogenous antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) can be increased by coffee as an exogenous antioxidant source. Coffee flavonoids act as antioxidants by increasing SOD levels and donating electrons and hydrogen ions to superoxide anions, making them more stable and preventing the oxidation of DNA and lipoproteins. The mineral content of coffee can also inactivate reactive components, while its CGA content scavenges free radicals.³¹

Peaberry coffee contains various health-beneficial compounds, including caffeine, flavonoids, tannins, saponins, trigonelline, carbohydrates, fats, amino acids, organic acids, volatile aromatic compounds, and minerals. Chlorogenic acid is the most abundant acid in coffee, accounting for approximately 8% in coffee beans and 4.5% after roasting.^{32,33} Chemical compounds such as steroids, alkaloids, and flavonoids may act as aphrodisiac components by increasing male sexual activity through vasodilation, nitric oxide production, and elevated testosterone and gonadotropin levels.³⁴ Chlorogenic acid lowers blood glucose levels by inhibiting fatty acid production in both in vivo and in vitro settings, a mechanism similar to that of metformin. Both compounds have antioxidant, antimutagenic, and anticancer properties that affect reactive oxygen species (ROS).¹⁹ The mitigation of oxidative stress in the present study was achieved through the administration of coffee-derived extracts, whose antioxidant properties are attributed to their abundant phenolic and flavonoid contents.³⁵ These bioactive compounds, particularly CGA, are recognized for their ability to enhance insulin sensitivity and improve glucose utilization by peripheral tissues.⁶

Therefore, robusta peaberry coffee may serve as a complementary dietary strategy to modulate glucose metabolism and mitigate metabolic dysfunction associated with diabetes mellitus.¹⁹ Furthermore, bioactive components in coffee, such as CGA and caffeine, exert significant antioxidant potential by scavenging free radicals and inhibiting oxidation

reactions, thereby protecting cellular structures from oxidative damage.

Limitations

This study has several limitations. The relationships among variables were correlational and did not prove direct causality. The sample size was limited, and caspase-3 was measured in serum rather than directly in testicular tissue, so it could not confirm cell-specific apoptosis in Sertoli or Leydig cells. Fertility outcomes, such as sperm quality and reproductive success, were not assessed. In addition, whole robusta peaberry coffee was used, so the specific effects of CGA, caffeine, or other active compounds could not be separated. Detailed phytochemical profiling of each daily extract was also not performed, although preparation consistency was controlled. In addition, the correlation analysis was performed using pooled data from all experimental groups. Therefore, these correlations should be interpreted as exploratory statistical associations rather than evidence of direct mechanistic or causal relationships between variables.

Conclusion

Robusta peaberry coffee reduced inflammatory and apoptosis-related markers and improved reproductive hormone levels, Sertoli cell count, and Leydig cell count in diabetic rats.

These findings indicate that robusta peaberry coffee may support testicular protection under diabetic conditions by modulating inflammation, apoptosis-related responses, and cellular integrity. Further studies are needed to confirm the active compounds, optimal dose, safety profile, and direct fertility outcomes.

Ethical approval

This research has received ethical approval (No. 837/EA/F.XXIII.38/2025) from the Health Ethics Committee of the Polytechnic of Health, Ministry of Health Semarang.

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Conflict of interest

The authors declare no conflicts of interest related to the research, authorship, or publication of this article.

CRedit authorship contribution statement

Mochamad Rizal Maulana: conceptualization, methodology, writing: original draft; **Teguh Budiharjo:** methodology, supervision; **Djoko Priyatno:** investigation, supervision; **Burhannudin:** investigation, discussion of results, resources; **Puji Lestari:** discussion of results, resources; **Sofhia Azqilla Nur:** writing: review and edit; **Yulian Nursaputra Habibina:** writing: review and edit; **Eko Naning Sofyanita:** conceptualization, discussion of

results, writing: review and edit, approval of the final text.

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