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Effect of *Physalis angulata* Active Fraction on The Spermatogenic Cells, mRNA Expression of NFκB and TNF-α in Testicular Diabetic Rat Model

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Abstract

Hyperglycemia in diabetes mellitus (DM) induces oxidative stress, leading to inflammation, reduced spermatogenic cells, and testicular weight, which may affect reproduction and cause infertility. Ciplukan (*Physalis angulata*) contains flavonoid compounds that function as antioxidants to combat free radicals and exhibit protective and therapeutic effects on spermatogenic cells in diabetic rats. Previous studies found that among all fractions of *P. angulata*, fraction I was the best at lowering glucose levels in myoblast cells. This study aims to investigate the protective effect of active fraction I of *P. angulata* on the histology of spermatogenic cells, mRNA expression of NFκB and TNF-α, and testicular weight in a diabetic rat model. This study used a post-test-only control group design with 5 groups: control (C), DM group (diabetic control, DC), DM treated with active fraction I of *P. angulata* at doses of 8.5, 34, and 136 mg/kg BW (PA-1, PA-2, PA-3, respectively). Each group consisted of 5 male Wistar rats (*Rattus norvegicus*). Testicular histology was analyzed with HE staining, mRNA expression was measured using qPCR. To account for variations in body size, the testicular weight was normalized to tibia length, resulting in the testicular weight-to-tibia length ratio. Statistical analysis, including the Shapiro-Wilk normality test and ANOVA, was performed using SPSS. The number of spermatogenic cells, including spermatogonium, primary spermatocytes, and spermatids, in the treatment group given the active fraction of *P. angulata* was significantly higher, whereas the mRNA expression of NFκB and TNF-α were significantly lower than DC group. The ratio of testicle weight per tibia length in PA-1 (34.24±10.95 mg/mm), PA-2 (34.25±2.45 mg/mm) and PA-3 (39.5±2.69 mg/mm) groups were not significantly higher than DC (34.21±2.88 mg/mm) group. Active fraction 1 of *P. angulata* is able to protect the testes in diabetic rats.

Keywords: Diabetes mellitus, Flavonoids, Inflammation, Physalis angulata, Testis

Introduction

Diabetes mellitus is a chronic metabolic disorder that is defined by persistently elevated blood glucose levels or hyperglycemia. This condition is accompanied by a variety of symptoms that endure over an extended period, affecting various aspects of health and well-being. The prevalence, morbidity, and mortality of diabetes mellitus have become serious problems both globally and in Indonesia. Based on data collected from 205 districts and secondary sources such as the Basic Health Research, BPJS Kesehatan, NCD programs, and the Ministry of Health, it is estimated that the prevalence and projected number of deaths due to diabetes in Indonesia will increase from 2020 to 2045 [(1)]. In addition, diabetes-induced metabolic disorders also lead to oxidative stress, which can negatively impact male fertility and reproductive health [(2)] including dysfunction of

the hypothalamic-pituitary-gonadal (HPG) axis, reduced testosterone production and secretion, risk of testicular failure, and abnormalities in spermatogenesis [3].

Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's capacity to neutralize them via its antioxidant defense mechanisms. Reactive oxygen species (ROS), such as free radicals and other highly reactive molecules, are naturally produced as byproducts of normal cellular metabolism. However, oxidative stress occurs when their production surpasses the body's ability to neutralize them with antioxidants. This imbalance triggers a series of harmful effects within the body [4,5]. Overproduction of ROS can damage several cellular members, including DNA, lipids, and proteins, resulting in cellular malfunction and disruptions of regular physiological functions. Such cellular damage may trigger inflammatory responses and impair the functionality of essential intracellular structures, thereby facilitating the initiation and progression of multiple pathological conditions, including diabetes [6].

Oxidative stress in diabetes is caused by the combined effects of several processes, such as the accumulation of by-products from glycolysis, increased activity of the polyol pathway, the formation of advanced glycation end-products (AGEs), activation of protein kinase C (PKC), and stimulation of the hexosamine pathway [7]. Inflammation and oxidative stress are closely linked, as the immune system stimulates the release of pro-inflammatory cytokines and chemokines, which activate macrophages to produce ROS as part of the defense mechanism against pathogens. However, inflammation associated with diabetes causes ongoing production of ROS, which leads to cellular damage and the exhaustion of antioxidant defenses [8]. And then, ROS trigger the production of pro-inflammatory cytokines by activating transcription factors like nuclear factor-kappa B (NFκB). Additionally, the body generates pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) [7]. TNF-α triggers the production of molecules like Nitric Oxide (NO), which can directly or indirectly impact spermatogenesis, damage the sperm membrane, and lower semen quality [9] which can increasing incidence of male reproductive problems, especially infertility.

Physalis angulata L., commonly known as Ciplukan, is a plant from the Solanaceae family that is widely found in tropical regions, especially in Indonesia. It has been traditionally used for treating various health conditions, including diabetes [10]. In the past decade, studies conducted both in vitro and in vivo have shown the medicinal potential of *P. angulata* L., particularly highlighting its anti-inflammatory, antioxidant activity, antifibrotic, and antidiabetic properties [11,12]. A specific active fraction of *P. angulata*, named fraction I, which contains flavonoid compounds [13] can prevent ROS-related processes, thereby mitigating negative effects on the reproductive system [14]. They improve the structure and function of the blood-testis barrier, Leydig and Sertoli cells, as well as spermatogonia, spermatocytes, spermatids, and spermatozoa [15]. Exogenous antioxidants also contribute to the neutralization of ROS, increase the levels of endogenous antioxidants, and enhance the plasma membrane of cells within the sympathetic-adrenal-medullary system and hypothalamic-pituitary-adrenal axis, supporting spermatogenesis [16].

Previous research has not explored the protection effects of fraction I of *P. angulata* in the testes of diabetic rats. Thus, this study aims to investigate whether fraction I can have an impact on histology of spermatogenic cells, expression of NFκB and TNF-α mRNA, and testicular weight.

Materials and Methods

This research has obtained an ethical clearance letter from Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing (FMPHN) Universitas Gadjah Mada (UGM) numbered EC: KE/FK/1703/EC/2023. The research was a quasi-experimental post-test only controlled group design and conducted at FMPHN. Each group consisted of 5 male white rats of the Wistar strain, making a total of 25 rats used. The active fraction was prepared following a method established by the Department of Pharmacology and Therapy. It was obtained from the chloroform extract of *P. angulata* herb using a bioassay-guided approach. The fractionation process was monitored using thin-layer chromatography (TLC), while the bioactivity was evaluated through an in vitro glucose consumption assay.

Kits and chemical

Molecular detection include RNA isolation FAVORGEN Tri RNA Reagent (catalog number: FATRR-001), cDNA synthesis was performed according to the protocol of SMOBIO ExcelRTTM Reverse Transcription Kit II (catalog number: RP1400) purchased from Kairos Jaya Sejahtera. Nuclear Factor kappa B (NFκB) (catalog number 410951695), Tumor Necrosis Factor-alpha (TNF-α) (catalog number 410951751) purchased from Genetica Science Indonesia, and qPCR master mix Bioline SensiFAST SYBR® No-ROX Kit (catalog number BIO-98005) purchased from Genetica Science Indonesia. All chemical and reagents used were hight-quality grade.

Animals and Diabetic Induction

Male Wistar rats aged 8-10 weeks (weighting 200-250 grams) were acclimated for around 7 days and provided with standard feed (RatBio) and drink was provided ad libitum. They were kept in a controlled environment with a temperature of 20-25℃, humidity of 50-60%, and a 12:12 light-dark cycle. They were conditioned for 12 hours without food, then induced with a single dose of Streptozotocin (STZ) 60 mg/kg body weight intraperitoneally [17]. Blood glucose levels were measured 72 hours after the STZ injection. Rats were considered to have DM if their blood glucose levels were >300 mg/dL.

Rats Treated with Active Fraction of P. angulata

The active fraction of *P. angulata* (Fraction I) was obtained from Center for Herbal Medicine, FMPHN UGM. The fraction was dissolved in 0.5% CMC-Na solution in distilled water, and then administered orally using oral gavage in the treatment groups for 60 days at doses of 8.5 (PA-1), 34 (PA-2), and 136 mg/kgBW for (PA-3). Groups Control (C) and diabetic control (DC) received 0.5% CMC-Na in distilled water**.** The dosage based on previous study by Wahyuningsih *et al*. The dose selection in this study was derived from the observed glucose-lowering activity of myoblast cells at a concentration of 100 µg/mL. The dose used in this study was chosen based on how well myoblast cells could lower blood sugar levels at a concentration of 100 µg/mL. This dose was then adjusted for rats, leading to a minimum dose of 8.5 mg/mL [13].

Collection of Testicular Samples

The testes of male Wistar rats of the Wistar strain were collected after a 2-month treatment period using a sacrifice procedure, with an anesthetic cocktail consisting of a mixture of ketamine 50 mg/kg BW, xylazine 2 mg/kg BW, and acepromazine 0.5 mg/kg BW. Afterward, perfusion with 0.9% NaCl was performed through the left ventricle of the heart. Following perfusion, a midline incision was made in the lower abdominal wall to expose the testes. The surrounding fat and connective tissue were carefully removed using fine forceps and iris scissors. The testes were excised at the distal end of the spermatic cord, with care taken to avoid tissue rupture. Dissection was performed under aseptic conditions on a sterile dissecting board.

Subsequently, the left testis was stored in RNA Preservation Solution for NFκB and TNF-α mRNA examination, while the right testis was stored in NBF for histological staining with Hematoxylin and Eosin (HE). Additionally, testicular weight was measured using a digital scale in milligrams (mg). To account for variations in body size, the testicular weight was normalized to tibia length, resulting in the testicular weight-to-tibia length ratio. The tibia collected through a sacrifice procedure, skinned from the hind limb, and soaked in 10% KOH solution for 24 hours. Finally, tibia length was measured in millimeters (mm) using a caliper.

Histological Examination

The testis tissue preserved in Neutral Buffered Formalin (NBF) was then cut longitudinally using microtome with the tissue sections were cut at a thickness of 4-5 micrometers, followed by preparation of paraffin blocks and staining with HE. The testicular histomorphology was analyzed using a light microscope at 400x magnification. The measurement of total count spermatogenic cells, spermatogonium, primary spermatocytes, and spermatids was performed with a software *Image-J* on 25 seminiferous tubules with 5 field of views in each sample. The reading of histological slides is performed by a pathologist.

Quantitative Polymerase Chain Reaction (qPCR)

mRNA expression of NFκB and TNF-α were assessed using Quantitative Polymerase Chain Reaction (qPCR), with β-actin as the housekeeping gene. Quantification of gene expression was performed using 7500 Fast Real-Time PCR System (Applied Biosystem) instrument. Specific primer for NFκB, TNF-α, and housekeeping genes (β-actin) were designed by using sequences in Table 1. In this study, β-actin was used as a reference gene to normalize data. The PCR temperature was set at 95ºC for 2 minutes for polymerase activation (1 cycle), followed by 40 cycles at 95ºC for 5 seconds for the denaturation step and 59ºC for 10 seconds for the annealing step. mRNA expression calculations were performed using this following formula:

The final result of the qPCR analysis is expressed as the fold change in the expression level of the target gene in the experimental sample relative to the reference sample, normalized to the expression of a housekeeping (reference) gene.

- ΔCT (Delta CT): The difference between the cycle threshold (CT) value of the target gene and that of the housekeeping gene.

$\Delta CT = CT_{\text{target gene}} - CT_{\text{housekeeping gene}}$

- ΔΔCT (Delta Delta CT): The difference between the ΔCT of the experimental sample and the ΔCT of the control (reference) sample.

$\Delta\Delta CT = \Delta CT_{\text{experimental}} - \Delta CT_{\text{control}}$

- Relative Expression (R): The relative expression level of the target gene is calculated using the 2^(-ΔΔCT) method.

$R = 2^{-\Delta\Delta CT}$

Table 1. Specification of the primers

Gene	Forward (5'-3')	Reverse (5'-3')	Accession Number
β-actin	GCAGATGTGGATCAGCAAGC	GGTGTAAAACGCAGCT CAGTAA	NM_031144.3
NFκB	CGACAGATGGGCTACACAGA	ATGTGCTGTCTTGTGGAGGA	NM_001276711.2
TNF-α	CGACTCTGACCCCCATTACT	TCGTGTGTTTCTGAGCATCG	NM_012675

Statistical Analysis

Analysis data was performed using SPSS software. ANOVA test was performed to determine the significant mean difference among the groups with *p*-value <0.05, followed by a Post Hoc LSD test.

Results and Discussion

The histological features of the seminiferous tubules observed include spermatogenic cells, consisting of spermatogonium, primary spermatocytes, and spermatids. The morphological results from the H&E staining are presented in Figure 1. The testes of control group (C) at 400X magnification shows well-organized spermatogenic cells at various developmental stages, with spermatozoa filling the seminiferous tubule lumen, indicating normal spermatogenesis. In the diabetic control group (DC), the lumen appears wider with sparse spermatozoa, reflecting impaired spermatogenesis due to diabetes. In the group treated with *P. angulata* active fraction at 8.5 mg/kg (PA-1), there is improvement in the arrangement of spermatogenic cells and denser lumen compared to the diabetic group. At doses of 34 mg/kg (PA-2) and 136 mg/kg (PA-3), the testis histology closely resembles the normal group, with well-organized spermatogenic cells and lumen filled with spermatozoa.

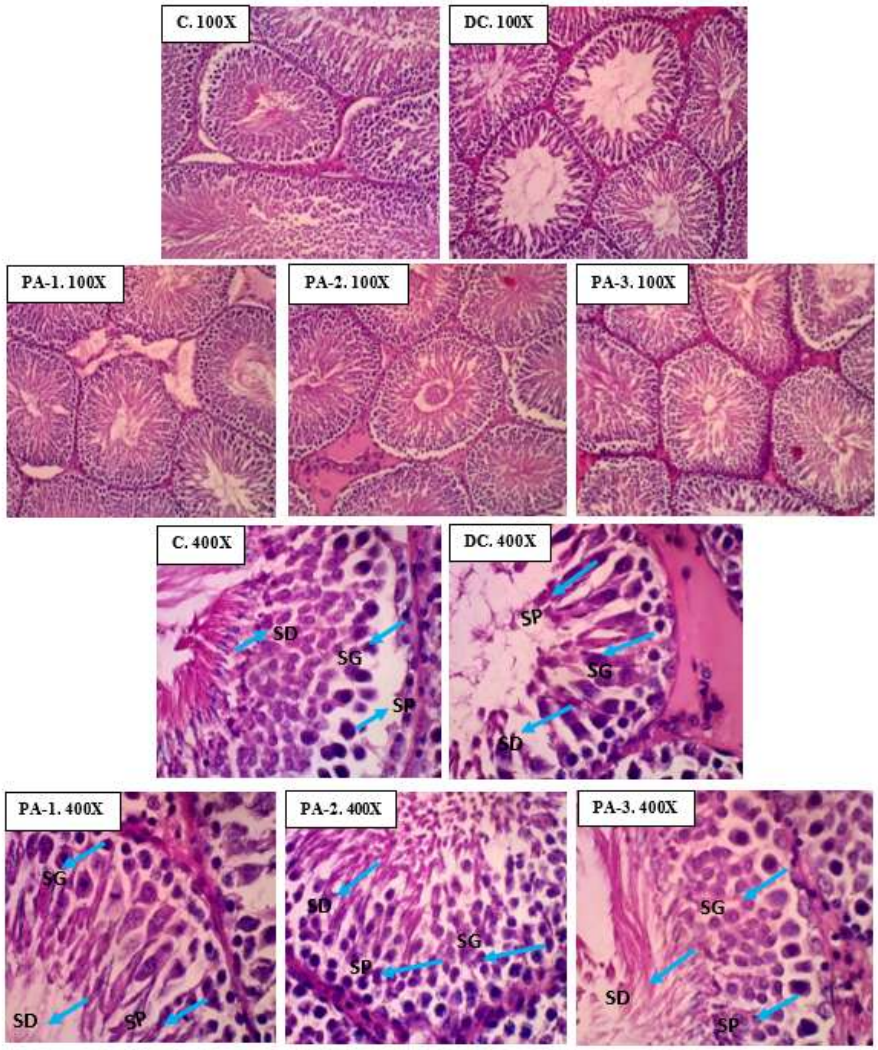

Figure 1. The histological features of the seminiferous tubules of *Rattus norvegicus* treated with *P. angulata*. Control (C), diabetic control (DC), and DM treatment groups doses 8.5; 34; 136 mg/kgBW (PA-1; PA-2; PA-3) were examined using HE staining at 100x and 400x magnification. SG : Spermatogonium SP : Primary spermatocytes .SD : Spermatids

The results of histological examination showed that the number of spermatogenic cells consisting of spermatogonium, primary spermatocytes, and spermatids in treated groups (PA-1, PA-2, and PA-3) were higher compared to the DC group (p<0.05) (Table 2).

Table 2. The number of spermatogenic cells, spermatogonium, primary spermatocytes, and spermatids of *Rattus norvegicus* treated with *P. angulata*

Group	Mean of the number the cells			
	Spermatogonium	Primary Spermatocytes	Spermatid	Spermatogenic
C	17.00 ± 1.00	14.88 ±1.25	42.48 ± 1.06	74.36 ± 1.13
DC	14.36 ± 0.59 *	9.16 ± 1.43 *	15.36 ±0.96 *	38.88 ±1.40 *
PA-1	15.72 ± 1.60	14.96 ± 0.91 #	19.84±0.96 *#	50.52 ± 1.31 *#
PA-2	14.84 ± 0.73 *	13.56 ±0.67 #	29.00 ±1.58 *#	57.40 ±1.14 *#
PA-3	15.96 ± 1.42 #	18.60 ±1.16 *#	30.60 ±1.50 *#	65.16 ± 1.40 *#

Control (C), Diabetic control (DC), and DM treatment groups doses 8.5; 34; 136 mg/kgBW (PA-1; PA-2; PA-3)
* p < 0.05 compared to the control (C)
# p < 0.05 compared to the diabetic control (DC)

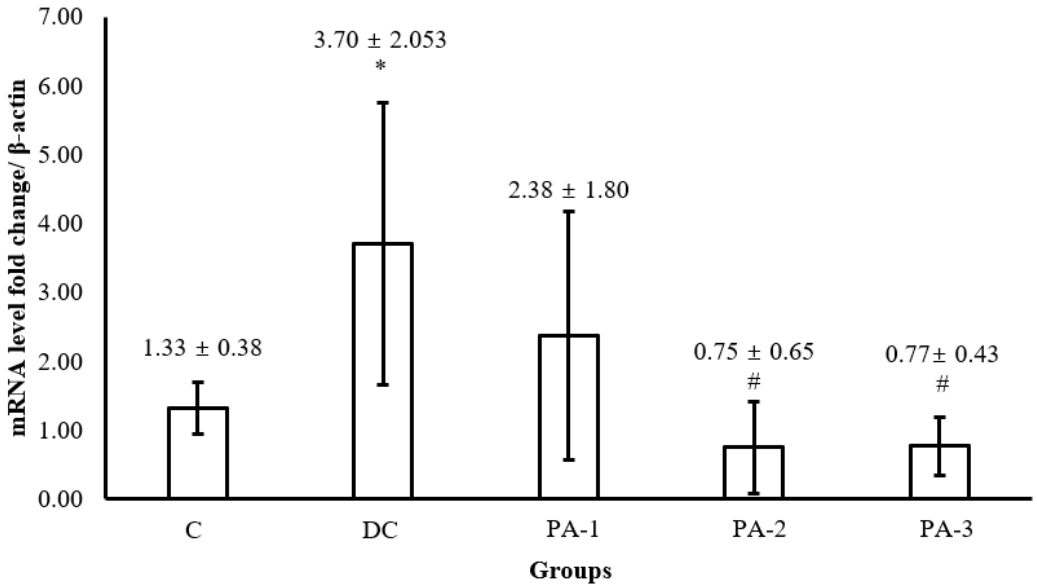

Figure 2. mRNA NFκB expression of *Rattus norvegicus* testes treated with *P. angulata*. Control (C), DM (DC), and DM treatment groups doses 8.5; 34; 136 mg/kgBW (PA-1; PA-2; PA-3)
* p < 0.05 compared to the control (C)
# p < 0.05 compared to the diabetic control (DC)

Figure 2 and 3 showed that a statistically significant difference in both NFκB and TNF-α mRNA expression between the diabetic control (DC) group and the normal control (C) group. The treatment groups (PA-1, PA-2, and PA-3) exhibited lower expression levels compared to the DC group, with PA-1 showing no significant difference, while PA-2 and PA-3 showed significant differences from DC. In both Figures, the PA-1 group had higher expression levels than the control group, though the difference was not statistically significant. Meanwhile, PA-2 and PA-3 had lower values compared to the control group, but the differences were also not statistically significant.

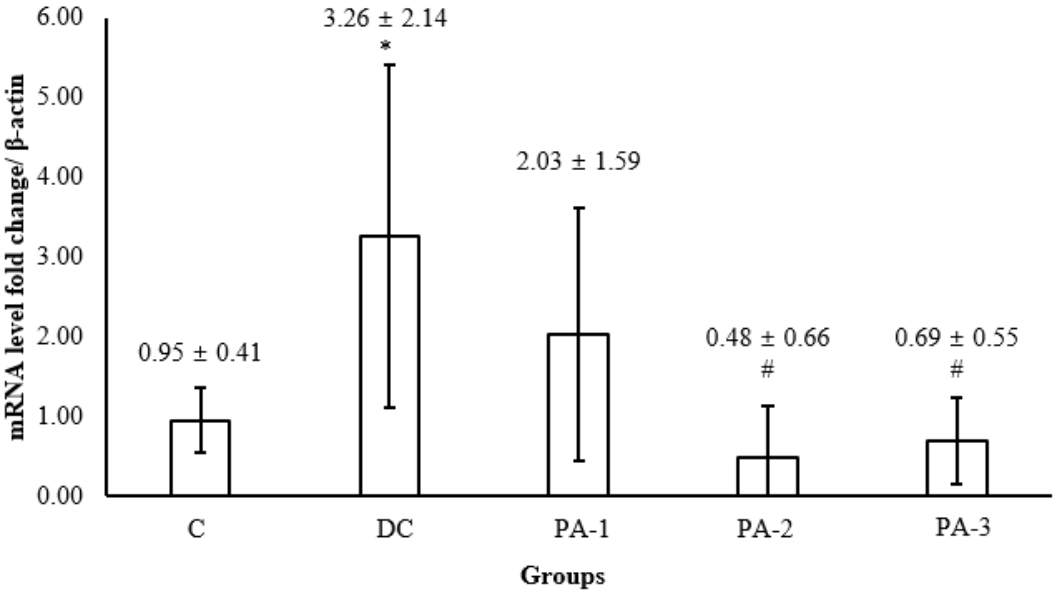

Figure 3. mRNA TNF-α expression of *Rattus norvegicus* testes treated with *P. angulata*. Control (C), DM (DC), and DM treatment groups doses 8.5; 34; 136 mg/kgBW (PA-1; PA-2; PA-3)

* $p < 0.05$ compared to the control (C)

# $p < 0.05$ compared to the diabetic control (DC)

The ratio mean of testicular weight to tibia length in the all treatment groups (PA-1, PA-2 and PA-3) were insignificantly higher than the DC and lower than the C group (Table 3, $p>0.05$). The ratio mean of testicular weight to tibia length in groups PA-1, PA-2 and PA-3 showed that the greater the dose given, the greater the ratio.

Table 3. Ratio of body weight to the length of tibia of *Rattus norvegicus* treated with *P. angulata*.

Group	Ratio mean of testis weight/tibia length ± SD (mg/mm)	*p*- value
C	45.69 ± 1.73	*p*>0.05
DC	34.21 ± 2.88	
PA-1	34.24 ± 2.97	
PA-2	34.25 ± 2.45	
PA-3	39.50 ± 2.69	

Control (C), diabetic control (DC), and DM treatment groups doses 8.5; 34; 136 mg/kgBW (PA-1; PA-2; AP-3)

Diabetes mellitus (DM), characterized by hyperglycemia, can lead to damage in various organs and systems, including dysfunction of the male reproductive system. NFκB, a transcription factor activated by extracellular signals during inflammation, plays a crucial role in inducing pro-inflammatory cytokines such as TNF-α. Its activation can lead to increased TNF-α levels during inflammatory responses. Therefore, inhibiting NFκB may provide a therapeutic strategy for managing inflammatory conditions like diabetes [18]. Diabetes is associated with chronic inflammation, which is linked to increased levels of NFκB and TNF-α [19,20]. This study showed that the active fraction I of *P. angulata* had protective and reparative effects to spermatogenic cells in diabetic rats, as proven by an increase in spermatogenic cell including spermatogonium, primary spermatocytes and spermatids numbers in diabetic rats treated with the active fraction compared to those without treatment. Fraction I of the chloroform extract from *P. angulata* was found to be the most effective in lowering glucose levels among all tested fractions, showing a 26.47% decrease as indicated in earlier studies [21]. This effect is presumably linked to the presence of flavonoid compounds within the fraction [13]. Flavonoids have shown antidiabetic potential, as evidenced by research on kaempferol, a compound found in *Bauhinia forficata*, using HepG2 cell models. The study demonstrated that kaempferol could promote the phosphorylation of AKT in these cells [22].

Secondary spermatocytes are rarely observed due to their brief life span in the process of spermatogenesis (1.1 to 1.7 days). Due to their brief lifespan, secondary spermatocytes are more

challenging to isolate and study compared to other stages in spermatogenesis, such as primary spermatocytes or spermatids. Additionally, secondary spermatocytes are relatively smaller than primary spermatocytes make them more difficult to identify and examine specifically [23]. The expression of mRNA NFκB and TNF-α was also lower in diabetic rats treated with the active fraction of *P. angulata*, indicating a protective and reparative effect on spermatogenic cells in the testes and the result was statistically different from those without treatment.

The diabetic rat group (DC) had fewer spermatogonium than the control group (C), which is consistent with findings that hyperglycemia in diabetes affects spermatogonium by altering mitochondrial structure, reducing their transformation into primary spermatocytes, increasing inactive spermatogonium, and significantly lowering epididymal sperm count. Hyperglycemia raises ROS levels, impairing Leydig cells' testosterone secretion, while Sertoli cells' interaction with germ cells is essential for spermatogenesis [2].

Flavonoids in *Archidendron pauciflorum* were found to increase *Sertoli* and *Leydig* cells by promoting pancreatic B-cell insulin secretion [24]. Similarly, the active fraction of *P. angulata* was shown to enhanced Leydig and Sertoli cell numbers in diabetic rats [25]. Flavonoids also reduce oxidative stress and ROS, improving insulin sensitivity [21]. In the current study, the groups treated with *P. angulata* (PA-1, PA-2, PA-3) had higher spermatogonium counts than the DC group, consistent with previous report showing increased spermatogonium in diabetic rats treated with flavonoids [26].

The diabetic rat group (DC) showed a reduced number of primary spermatocytes compared to the control group (C), consistent with previous findings [27]. Hyperglycemia in diabetes disrupts spermatogenesis in the seminiferous tubules, reducing sperm quality. External factors, including hormonal influences like FSH, affect the meiosis stage of spermatogenic cells, making primary spermatocytes vulnerable to damage and chromosomal abnormalities. Elevated ROS levels in the testes further damage the seminiferous tubules' cell membranes, allowing toxic free radicals to penetrate, a consequence of increased oxidative stress from hyperglycemia.

The mean number of spermatids in the diabetic group (DC) was lower than in the control group (C), consistent with findings attributing this decrease to spermatocyte damage during cell division [28]. Damage to spermatogenic cells in the seminiferous tubules disrupted sperm production. Similarly, diabetes has been reported to disrupt spermatogenesis, reduces spermatogenic cells, increases apoptosis, and lowers sex hormone levels, with mitochondrial and DNA damage contributing to cell death. High ROS levels and oxidative stress were linked to these effects [29].

The expression of NFκB mRNA in the diabetic group (DC) higher compared to the control group (C), in line with findings associating diabetes with elevated NFκB activity [30]. NFκB functions as a transcription factor that regulates immune-related genes, including those for pro-inflammatory molecules, adhesion molecules, and enzymes like cyclooxygenase and nitric oxide synthase. The treatment groups (PA-1, PA-2, PA-3) administered *P. angulata* active fraction showed no significant difference from the control group, indicating a reduction in inflammation and a shift toward a healthier state. Similarly, TNF-α mRNA expression was higher in the DC group, consistent with findings that diabetes-induced inflammation increases pro-inflammatory cytokines (IL-6, IL-8, IL-1, and TNF-α) due to hyperglycemia and oxidative stress from the control group, suggesting that *P. angulata* alleviated inflammation and restored normal physiological conditions [31].

This study also showed a decrease in testis weight, normalized to tibia length, in the DC, PA-1, PA-2, and PA-3 groups compared to C, although not significantly. However, this ratio of testis weight to tibia length in diabetic rats given *P. angulata* (PA-1, PA-2, and PA-3) groups were greater than DC group. Diabetic rats had a lower average testis weight than controls [32]. Testis weight in diabetic rats decreased from day 24 and continued to decline until day 48 [33]. This decrease in testis weight is associated with oxidative stress induced by hyperglycemia, leading to gonadal degeneration, reduced spermatogenic cells, and decreased gonadotropin production [34].

Previous research indicates that potential antioxidant agents, such as flavonoids, can play a role in reducing oxidative stress markers and inflammation caused by hyperglycemia [35]. This suggests that flavonoids could help mitigate the damaging effects of oxidative stress, which is closely associated with conditions like diabetes. It has been hypothesized that *P. angulata*'s flavonoids inhibited apoptosis and enhanced spermatogenesis in diabetic rats, though treated rats had smaller seminiferous tubule diameters than untreated ones [36].

Flavonoid subclasses include anthocyanins, chalcones, flavanones, flavones, flavonols, and isoflavonoids. Flavonoids activate antioxidant pathways by inhibiting lysosomal enzyme and β-glucuronidase secretion, reducing inflammation [37]. Flavonoid compounds are capable of directly neutralizing reactive oxygen species (ROS), thereby lowering oxidative stress inside cells. Moreover, some types of flavonoids can stimulate the Nrf2 signaling pathway, which triggers the activation of antioxidant response elements (AREs)

and promotes the expression of protective genes like heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1). Activation of Nrf2 by flavonoids not only enhances antioxidant defenses but also inhibits pro-inflammatory pathways, such as the NF-κB signaling cascade, thereby reducing inflammation [38].

Flavonol derivatives, such as quercetin and regulins, increase NFκB expression and enhance insulin secretion in response to glucose, while morin effectively lowers inflammatory cytokines like IL-6 and TNF-α. Baicalein, another flavonoid, reduces TNF-α, AGEs, and NFκB activation by regulating the AMPK pathway, which may alleviate insulin resistance and inflammation [39]. In this research demonstrated that the fraction of *P. angulata* can protects testes from damage caused by DM. To developed as a new herbal medicine able to protect male reproductive organs, further researches are needed to determine the safety of the fraction in vivo before finally being tested on humans. Limitation of this study is its reliance on a diabetic rat model, which may not fully mimic the pathophysiology of diabetes and testicular dysfunction in humans. Although the *P. angulata* active fraction showed protective effects on testicular structure, inter-species differences in drug absorption, metabolism, immune responses, and reproductive biology may affect the translation of these findings to humans. Thus, before clinical application, further studies, including pharmacokinetic profiling and human clinical trials, are essential to validate its safety and therapeutic efficacy.

Conclusions

The active fraction of *P. angulata* protected testes of diabetic rats through improving testicular weight, number of spermatogenic cells including spermatogonium, primary spermatocytes, and spermatids, and lowering mRNA expression of NFκB and TNF-α.

Acknowledgments

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Conflicts of Interest

There are no conflicts of interest associated with this study.

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Ethics Statements

The study design was approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing (FMPHN) Universitas Gadjah Mada (UGM) numbered EC: KE/FK/1703/EC/2023.

Author Contribution

The authors confirm contribution to the paper as follows: study and design: DMR, NA; data collection: KDS, MT, FS, SM; analysis and interpretation of results: KDS; draft manuscript preparation: KDS; review and revised the manuscript: RAS. All authors reviewed the results and approved the final version of the manuscript.

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