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Therapeutic Potential of Ethanol Extract from Durian Peel on Testes Microanatomy in Diabetic Male Rats

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Abstract. Diabetes mellitus (DM) is a degenerative disease that can lead to complications such as testicular atrophy. As DM requires lifelong treatment, natural and safe therapeutic alternatives are needed. Durian peel, as a natural material, has potential. This study investigated the effects of ethanol extract of durian peel (EEDP) on testicular histology in diabetic rats. A total of 25 male *Sprague-Dawley* rats, aged 2 months and weight ±200 g, were randomly assigned to five groups: healthy (K0), diabetic (K1), and diabetic+EEDP at doses of 500 (K2), 750 (K3), and 1,000 mg/kgBW (K4), given orally for 28 days. After treatment, the rats were euthanized, and their testes were removed, weighed, and examined using hematoxylin-eosin staining. Observed variables included testis weight, seminiferous tubule diameter, epithelial thickness, and spermatogenesis stages. Statistical analysis ANOVA (α=0,05) showed significant improvements ($p < 0,05$) in the EEDP-treated groups. It is concluded that EEDP at doses of 500-1,000 mg/kgBW improved testicular structure in diabetic rats.. The novelty of this study lies in the utilization of durian peel ethanol extract at varying doses of 500–1,000 mg/kgBW as a natural therapy to improve testicular histological damage caused by diabetes. This study serves as a basis for the development of herbal antidiabetic drugs with protective effects on the reproductive system. The findings are expected to contribute to biomedical science and provide opportunities for utilizing durian peel waste as a value-added material for public health

Keywords: ethanol extract; durian peel; diabetic; antihyperglycemic; testes

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INTRODUCTION

Diabetes mellitus (DM) has become one of the significant global public health problems, including in Indonesia (Baena-Diez et al., 2024). Currently, the number of people with DM worldwide is estimated to have reached 537 million. The global prevalence of DM is projected to rise to 643 million in 2030 and 853 million in 2050. The latest data from the International Diabetes Federation (IDF) indicate that approximately 11.1% of adults aged 20–79 years are living with diabetes (IDF, 2024). In Indonesia, the prevalence of DM is recorded at 11.3% and continues to increase, with projections reaching 21.3 million people with DM by 2030 (Olamoyegun et al., 2024). According to Huang et al. (2024), diabetes mellitus is a degenerative disease that affects the male reproductive system by disrupting the spermatogenesis process in the testes. Recent evidence further suggests that individuals with diabetes have an increased risk of infertility due to impairments in sperm motility, morphology, and vitality, primarily caused by oxidative stress, inflammation, and mitochondrial dysfunction.

The testes are organs of the male reproductive system, with the seminiferous tubules as the main components responsible for sperm and hormone production. Recent findings by Venditti et al. (2024) demonstrated that type 1 diabetes mellitus induces oxidative stress through the nuclear factor erythroid 2–related factor 2 (NRF2) or NOD-like receptor family pyrin domain containing 3 (NLRP3) pathway, damaging somatic and germ cells as well as the integrity of the blood–testis barrier (BTB), which consequently leads to impaired spermatogenesis. Under pathological hyperglycemic conditions, the testes experience oxidative stress that damages the vascular endothelium and decreases the level of nitric oxide (NO). Reduced NO levels result in

microangiopathy, thereby disrupting nutrient distribution to testicular tissue and impairing the stages of spermatogenesis. According to *Frontiers in Endocrinology* (2025), diabetes adversely affects testicular function via pathways involving AGEs accumulation, mitochondrial impairment, and chronic inflammation. Impairments in spermatogenesis affect germ cells and trigger structural and histological alterations in the seminiferous tubules. Furthermore, Zhang et al. (2025) reported that type 1 diabetes significantly disrupts microtubule dynamics by decreasing the expression of several associated proteins, such as MARK4 (microtubule affinity-regulating kinase 4), MAP1A (microtubule-associated protein 1A), and DYNLL1 (dynein light chain LC8-type 1), leading to cytoskeletal disorganization and detrimental effects on germ cell differentiation as well as sperm motility.

Blood sugar control in patients with DM (diabetes mellitus) generally relies on synthetic drugs such as meglitinides, insulin, and metformin, which, when used in the long term, may cause serious side effects. Various plant-based traditional medicines with mechanisms similar to synthetic drugs have been utilized by communities as alternative treatments. Ansari et al. (2023) reported that plant-based diets and phytochemicals play a significant role in managing diabetes by lowering blood glucose levels, improving insulin resistance, and inhibiting pathological mechanisms such as the accumulation of advanced glycation end products (AGEs), mitochondrial dysfunction, and chronic inflammation. The administration of antioxidants under diabetic conditions can suppress oxidative stress and thereby prevent severe damage caused by free radicals (Ansari et al., 2024). One of the plants that can be used as a diabetes therapy is durian. Durian peel extract exhibits promising antioxidant and antidiabetic activities due to its high content of polyphenols and flavonoids. Moreover, durian peel contains antioxidants such as flavonoids, saponins, tannins, alkaloids, and polyphenols, which have potential as therapeutic agents for diabetes (Aziz & Jalil, 2019).

This study used male *Sprague-Dawley* rats induced by alloxan to create diabetes mellitus by administering durian peel ethanol extract at 500, 750, and 1,000 mg/kg doses. These doses are expected to control blood glucose levels and prevent complications of testicular atrophy, so durian peel extract can be developed as a potential antidiabetic and anti-atrophy alternative to treat and prevent complications of diabetes mellitus in Indonesia. The beneficiently of this study is to provide basic information on the potential utilization of natural bioactive compounds from durian peel as an alternative therapy to treat testicular complications due to diabetes, which can support the development of local wisdom-based herbal medicine.

The purpose of this study is to analyze the effect of durian peel ethanol extract on testicular histology, as observed through the variables of testis weight, seminiferous tubule diameter and epithelial thickness, as well as the stages of spermatogenesis in alloxan-induced Sprague-Dawley rats with diabetes mellitus

METHODS

Collection Site

This research was conducted from September 2024 to January 2025. The preparation of the ethanol extract from durian peel was carried out at the Cendekia Nanotech Hutama Laboratory (CNH). The treatment of experimental animals, measurement of blood sugar levels, and observation of testicular preparations were carried out at the Biology Laboratory of Animal Structure and Function, Faculty of Science and Mathematics, Diponegoro University. Testicular preparations are carried out at the Semarang Animal Health Laboratory. This study has obtained ethical clearance from the Health Research Ethics Committee (HREC) of the Faculty of Medicine, Diponegoro University/Dr. Kariadi General Hospital with approval number 100/EC-H/KEPK/FK-UNDIP/X/2024.

Preparation of Durian Peel Extract

The extract was prepared using the mesoderm section of local durian peel through a maceration process. Approximately 1,250 g of durian peel was washed, chopped, and dried in an oven at 50°C for 3-4 days. The dried durian peel was ground into a fine powder and subsequently macerated in 2 L of 70% ethanol for 3 days, with stirring performed once daily for 20 minutes. The resulting filtrate was filtered and concentrated using a rotary evaporator under a pressure of approximately 100 mBar, at a temperature of 40-50°C, and a rotation speed of 50-60 rpm for about 45 minutes, yielding a thick, caramel-like concentrated extract. This procedure is consistent with recent extraction practices for durian peel employing ethanol maceration and solvent evaporation using a rotary evaporator, which have been shown to produce polyphenol-rich fractions with strong antioxidant

and antidiabetic activities (Tran et al., 2024; Noorhashim et al., 2025).

Table 1. Screening Result of Ethanol Extract of Durian Peel

Compound	Result	Description
Flavonoid	+	Positive
Polifenol	+	Positive
Tanin	+	Positive
Saponin	-	Negative
Alkaloid	-	Negative
Terpenoid	+	Positive

Provision of Treatment

This study used *Sprague-Dawley* strain male white rats that were acclimated for 7 days at the Biology Laboratory of Diponegoro University. Rats were randomly placed in 25 containers, thoroughly cleaned with disinfectant, each containing one animal with a cage size of 20×14×24 cm. Individual cages were provided with rice husks, feed bins, and drinking bottles with a steel woven roof. The body weight of white rats is measured before treatment as baseline data.

Baseline blood sugar levels are measured on all rats, except the healthy rat group (K0). Diabetes induction is performed by intraperitoneal injection of alloxan 125 mg/kgBW. After 3 days post-injection, blood sugar levels were measured again using a glucometer to ensure hyperglycemia before treatment. White rats that have blood sugar levels ≥ 140 mg/dL can be declared to have hyperglycemia (Gabriel et al., 2014). White rats in the treatment group that had not experienced hyperglycemia were injected with alloxan again at a dose of 125 mg/kgBW until their blood sugar levels reached ≥ 140 mg/dL.

The rats were divided into five groups, including a healthy control (K0), DM (K1), and three treatment groups (K2-K4). Each group received an ethanol extract of durian peel (EEDP) at varying doses of 500, 750, and 1,000 mg/kgBW for 28 days. The extract treatment was given orally through a cannulated syringe so as not to harm the rats when inserting EEDP into the hull.

Blood Sugar Level Measurement

Blood glucose levels were measured 3 times: at the beginning of the study, after the rats were acclimated for seven days, following alloxan induction, and at the conclusion study after 28 days of treatment. Blood collection began by cleaning the tip of the rat's tail with 70% alcohol, after which the tip was cut approximately 1 mm using sterile scissors. Blood from the caudal vein was applied to a glucose strip until the strip chamber was filled, then left for one minute. Subsequently, the blood glucose value appeared on the glucometer display screen (Hahn et al., 2024).

Microscopic Preparation

Preparation of aortic microanatomy preparations using the paraffin method and hematoxylin-eosin staining. Testicular organs that have been isolated in a 10% BNF fixative solution. The organs that have been fixed are then cut (trimmed) to a 4 mm thickness according to the parts used. The tissue was then put into a tissue cassette and put into a tissue basket to be dehydrated (Kiernan, 2015).

Tissues underwent graded alcohol dehydration at concentrations of 70%, 80%, 90%, and absolute alcohol, with each step performed twice for 30 minutes. The next stage was purification by soaking in silol (2×10 min). Tissue cassettes are removed to continue immersion in liquid paraffin (60℃) to remove air and fill the tissue or cells with paraffin (infiltration).

The infiltrated tissue then enters the process of making tissue blocks using paraffin (embedding). The mold is warmed at 60℃, then the tissue is inserted into each mold. Liquid paraffin is slowly poured into the mold until the entire tissue is submerged and frozen on a cold plate. The paraffin blocks were removed from the molds and stored in the freezer (-20℃) until the paraffin hardened. The hardened tissue in the paraffin block was sectioned with a 3 µm thick rotary microtome and placed in 40℃ warm water until it expanded. The tissue that has expanded, taken with a glass object, and then put into an incubator at 40 ℃ for 24 hours to dry completely, is ready to be stained.

The dried preparations were deparaffinized with xylol (3×5 minutes) and then rehydrated gradually. Rehydration was carried out by immersing the preparations in a graded alcohol series ranging from absolute alcohol to 96% alcohol, 80% alcohol, and 70% alcohol, each for 1 minute, then washed with running water for 1 minute.

Staining of Microscopic Preparations

The tissue staining process uses hematoxylin-eosin. The staining process begins with silanol deparaffinization (3×5 minutes), then rehydration is gradually performed. Rehydration is done by soaking the preparations in a graded alcohol series starting from absolute alcohol, 96% alcohol, 80%

alcohol, 70% alcohol, each for 1 minute, then washed with running water for 1 minute. Preparations were put into hematoxylin dye solution for 5-10 minutes and then washed with running water to remove unbound hematoxylin dye. The next stage is stained with eosin for 1-2 minutes and then washed under running water for 1 minute. The water content in the preparation was withdrawn by dipping the tissue in absolute alcohol as many times as (3×5 dips) and soaking it in silol solution (3×5 mins). The preparations were removed one by one from the silol solution in a wet state, and then one drop of EMT adhesive was given and covered with a cover glass (mounting).

Measurement of Variables

Testis weight, seminiferous tubule diameter, and seminiferous epithelium thickness were variables observed and measured by photomicrograph microscopy.

Research Design

This research design is a complete randomized design (CRD). This experiment was conducted using a completely randomized design (CRD) with 25 male white rats of the Sprague-Dawley strain grouped into five groups. Each treatment group consisted of 5 replicates. This study used a dose of alloxan based on the method used (Kim, 2024). Determination of the use of doses of ethanol extracts from durian peel based on methods carried out (Muhtadi et al., 2015).

Data Analysis

Data obtained, including testicular weight, seminiferous tubule diameter, and seminiferous epithelial thickness, were processed with the statistical software program SPSS version 22.0. Data were analyzed using the Shapiro-Wilk test for checking normality and the Levene test for assessing homogeneity. Data that followed a regular and homogeneous distribution pattern continued with the one-way ANOVA test at the 5% significance level ($\alpha = 0.05$). The significantly different ANOVA results are continued with the Duncan test at the 5% significance level. Non-parametric tests will be conducted if the data is not normally distributed or homogeneous.

RESULTS AND DISCUSSION

Data from the measurement of testicular weight, diameter, and thickness of seminiferous epithelium of diabetes mellitus models after being treated with ethanol extract of durian peel (EEDP) for 28 days had a regular and homogeneous distribution pattern ($P>0.05$). Data were tested using Analysis of Variance (ANOVA) significance at 5% ($\alpha = 0.05$). ANOVA results were significantly different regarding the average testicular weight, diameter of seminiferous tubules, and seminiferous epithelial thickness (Table 3).

Table 2. The results of the analysis of the average testicular weight, diameter, and thickness of the seminiferous tubule epithelium of the testes of Sprague-Dawley rats model of diabetes mellitus after being treated with ethanol extract of durian peel (EEDP) for 28 days.

Variables	Treatment				
	K0 $\bar{x} \pm SD$	K1 $\bar{x} \pm SD$	K2 $\bar{x} \pm SD$	K3 $\bar{x} \pm SD$	K4 $\bar{x} \pm SD$
Testes Weight (g)	1.52 ± 0.05^{c}	0.73 ± 0.09^{a}	1.35 ± 0.17^{b}	1.36 ± 0.06^{b}	1.32 ± 0.10^{b}
Diameter of seminiferous tubules (µm)	315.45 ± 2.20^{c}	173.05 ± 5.31^{a}	249.17 ± 2.79^{b}	245.41 ± 3.68^{b}	244.92 ± 3.17^{b}
Thickness of the seminiferous epithelium (µm)	106.48 ± 2.06^{d}	51.06 ± 0.83^{a}	74.53 ± 3.22^{c}	73.72 ± 1.37^{c}	67.82 ± 1.29^{b}

Description: Data shown as mean ± standard deviation. Numbers followed by different superscripts on the same line indicate significant differences ($P<0.05$); K0 = control not injected with alloxan and only given distilled water; K1 = DM rats; K2 = DM rats + EEDP 500 mg/kgBW; K3 = DM rats + EEDP 750 mg/kgBW; K4 = DM rats + EEDP 1.000 mg/kgBW (K4).

Testicular Weight

Testicular weights in the EEDP-treated group were higher than those in the diabetic group. Duncan test results showed that the average testicular weight between K0 and K1 was significantly different (Table 2). The average testicular weights between treatments K2, K3, and K4 were not significantly different, but significantly different from K0 and K1. Testicular weights in groups K2, K3, and K4 were higher than in K1, although they had not reached normal weights as in K0. Based on the statement of Setchell et al. (1994) and Sriparitiwi (2019), seminiferous tubules make up 80-85% of the main constituents of white rat testes. Interstitial tissue components consisting of Leydig cells, blood vessels, macrophages, and connective tissue filling the space between seminiferous tubules make up 10-12% of the testicular weight. Tunica albuginea, as a layer of fibrous connective tissue wrapping the testis, accounts for about ±5% of the total weight of the testis. The testicular rete and efferent ducts contribute 1-3% to the testicular weight.

Testicular weight can be maintained when bioactive compounds improve the structure of the testicular seminiferous tubules. Hassanpour & Doroudi (2023) stated that the improvement of testicular structure through EEDP treatment is thought to be related to the effects of flavonoid and polyphenol compounds, which can reduce the production of free radicals. These compounds possess hydroxyl groups that donate hydrogen atoms to abolish free radicals, a process known as scavenging (Speisky et al., 2022), chelate transition metal ions such as iron that trigger ROS formation (Rudrapal et al., 2022), and inhibit the activity of ROS-producing enzymes like NADPH oxidase or xanthine oxidase (Zahra et al., 2024). In addition, flavonoids and polyphenols can also activate cellular antioxidant pathways through Nrf2 (Nuclear factor erythroid 2–related factor 2), which enhances endogenous antioxidant enzymes and produces active metabolites with higher antioxidant potential. Nrf2 is a transcription factor that plays an important role in regulating the expression of antioxidant genes and detoxifying enzymes, thereby being strongly associated with cellular defense mechanisms against oxidative stress (Rudrapal et al., 2024). Through these mechanisms, damage to the organic materials constituting cells or tissues, including testicular structure, can be prevented. The research results by Ostovan et al. (2022) stated that the flavonoid antioxidant content of Rydinga persica extract at a dose of 600 mg/kg can improve weight shrinkage in diabetic rat testes. The seminiferous tubules in the testes did not appear to experience thickening of the basal lamina and spermatogenic apoptosis, hyperplasia of Leydig cells, and edema of the interstitial space that occurred were lighter than those in the DM group.

Seminiferous Tubule Diameter

The data in Table 2 show that the average diameter of seminiferous tubules between K0 and K1 is significantly different. In contrast, the average diameter of seminiferous tubules between treatments K2-K4 is not significantly different. The three treatments with EEDP doses of 500, 750, and 1.000 mg/kgBW produced an average diameter of seminiferous tubule significantly different from K0 and K1.

The average diameter of testicular seminiferous tubules was significantly different between K0 and K1, showing that alloxan induction triggered diabetic conditions that significantly affected structural changes in testicular seminiferous tubules through the mechanism of ROS formation and inhibition of the glucokinase enzyme. According to Adelati et al. (2016), structural damage leads to a reduction in the diameter of seminiferous tubules, which is caused by impaired mitotic activity in spermatogenic cells and ultimately inhibits spermatogenesis.

Data from this study showed an improvement in the diameter of testicular seminiferous tubules in diabetic animals after treatment. Although there was an increase in seminiferous tubule diameter, the treatment effect was not strong enough to produce significant differences among groups K2, K3, and K4. This lack of significance indicates that the observed variation in seminiferous tubule diameter among the three treatment groups was likely due to the relatively similar antihyperglycemic activity of the administered doses. These findings are consistent with the report of Toprak et al. (2023), who demonstrated that antioxidant therapy in diabetic rats improved the narrowed seminiferous tubule diameter, although differences among moderate doses were not always statistically significant. Similar results were also reported by Liu et al. (2024), who found that antioxidant administration in diabetic rats ameliorated testicular structural damage, including improvements in seminiferous tubule diameter, although the variations among doses within the effective range did not show significant differences.

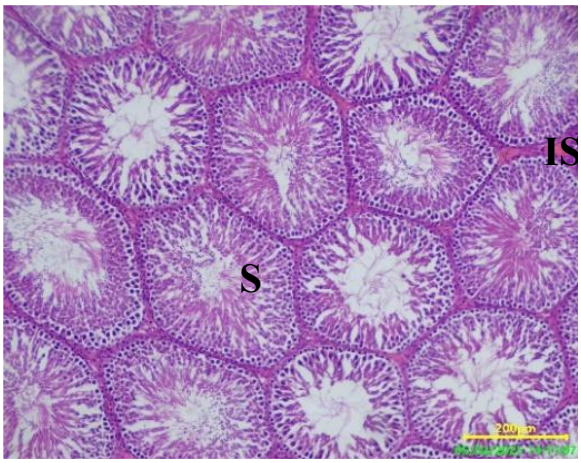

Figure 1. Histology of a cross-section of the seminiferous tubule of a K0 white rat (H&E, 100×). Seminiferous tubule (ST); interstitial space (IS).

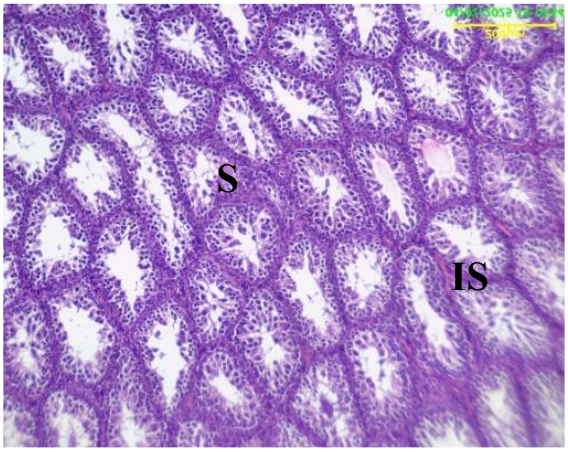

Figure 2. Histology of a cross-section of the seminiferous tubule of a K1 white rat (H&E, 100×). Seminiferous tubule (ST); interstitial space (IS).

Figure 3. Histology of a cross-section of the seminiferous tubule of a K2 white rat (H&E, 100×). Seminiferous tubule (ST); interstitial space (IS).

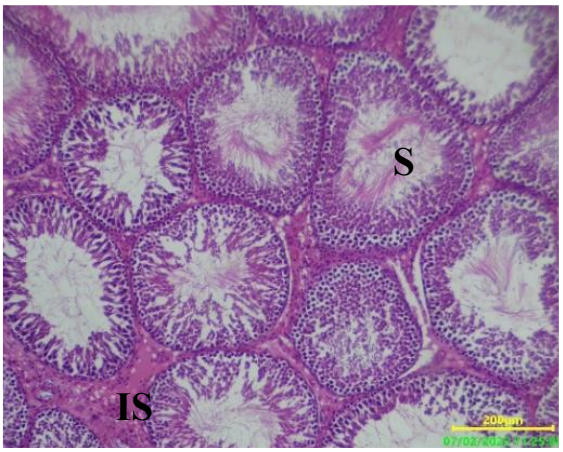

Figure 4. Histology of a cross-section of the seminiferous tubule of a K3 white rat (H&E, 100×). Seminiferous tubule (ST); interstitial space (IS).

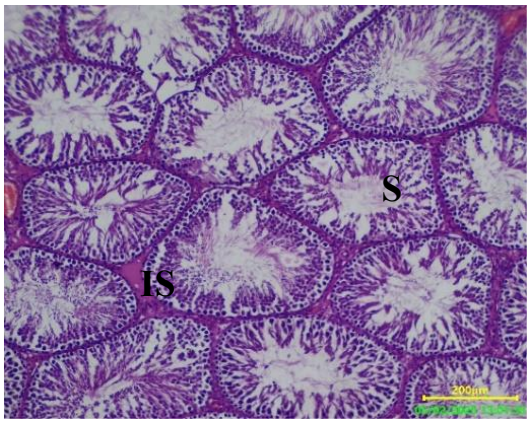

Figure 5. Histology of a cross-section of the seminiferous tubule of a K4 white rat (H&E, 100×). Seminiferous tubule (ST); interstitial space (IS).

The shape of the seminiferous tubules in K0 is round and broad, while in K1 it tends to be oval and narrow. The condition of the diameter of the seminiferous tubules K2, K3, K4 tends to be round and wider than K1, indicating the protective effect of polyphenol and flavonoid compounds in the ethanol extract of durian peel (EEDP). The study results of Mohlala et al. (2023) showed that flavonoid content, especially quercetin, works by binding directly to the active site of α-amylase, thus blocking the substrate (starch) from binding. Quercetin also disrupts the conformation of the α-glucosidase enzyme, so it cannot bind the substrate optimally.

According to Mahfur (2019), the improvement of testicular structure by EEDP treatment is thought to be related to the effects of flavonoid and polyphenol compounds that can reduce the production of free radicals, so that

damage to organic materials that make up cells or tissues can be prevented. Feng et al. (2018) reported that flavonoids contained in durian skin have activity in counteracting 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, increasing nitric oxide (NO) production, and playing a role in the capture of superoxide anion radicals (O_2^-). Meanwhile, research by Liu et al. (2020) revealed that durian peel extract can significantly inhibit the formation of reactive oxygen species (ROS) triggered by exposure to hydrogen peroxide (H_2O_2).

Thickness of Seminiferous Tubule Epithelium

Ethanol extract of durian peel (EEDP) significantly affects the variable thickness of seminiferous tubule epithelium. The variable in diabetic test animals after EEDP treatment was higher compared to diabetic test animals without treatment. Duncan test results showed that the average thickness of seminiferous tubule epithelium between K0 and K1 was significantly different; between treatments, K2, K3, and K4 were significantly different from K0 and K1. The following result is that the average between treatments K2 and K3 is not significantly different, but between K2, K3, and K4 is significantly different (Table 3).

The group of rats given alloxan had thinner seminiferous tubule epithelium compared to normal rats without alloxan. Rat group treated with alloxan experiences hyperglycemia, which triggers oxidative stress. The oxidative stress causes a decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which disrupts the production and maturation of germ cells, and then has an impact on decreasing epithelial thickness, as evidenced by K1, which is significantly different from K0.

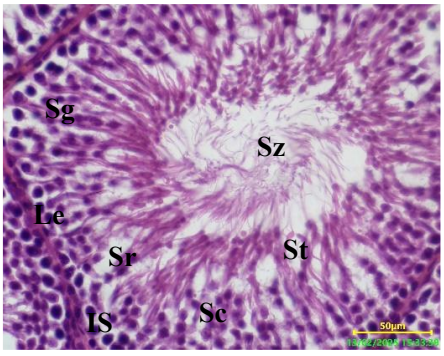

Figure 6. Histology of a cross-section of the seminiferous tubule of a K0 white rat (H&E, 400×). Spermatogonia (Sg); spermatocytes (Sc); spermatids (St); spermatozoa (Sz); Sertoli cells (Sr); Leydig cells (Le); interstitial space (IS).

Based on the histological characteristics shown in Figure 6, spermatogenesis in the K0 group occurs normally. This condition is indicated by several histological features, including spermatogonia (Sg) clearly arranged along the basal membrane of the seminiferous tubules, spermatocytes (Sc) appearing in normal number and morphology within the adluminal compartment, and spermatids (St) observed progressing toward the lumen of the tubules. Spermatozoa (Sz) are present abundantly within the lumen, indicating optimal maturation. Sertoli cells (Sr) are visible, supporting the structure and development of spermatogonia and spermatocytes, while Leydig cells (Le) appear morphologically normal and are located within the interstitial space (IS) between the seminiferous tubules. Overall, the seminiferous tubules in the control group exhibit intact structural organization, absence of cellular degeneration, and normal spermatogenesis.

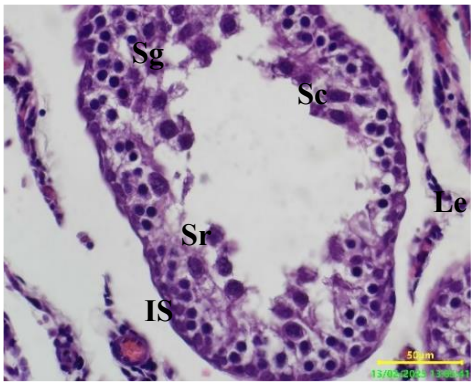

Figure 7. Histology of a cross-section of the seminiferous tubule of a K1 white rat (H&E, 400×). Spermatogonia (Sg); spermatocytes (Sc); spermatids (St); spermatozoa (Sz); Sertoli cells (Sr); Leydig cells (Le); interstitial space (IS).

Figure 7 shows that the rats in the K1 treatment group, namely the diabetic rats, experienced degeneration of the seminiferous tubules as a result of hyperglycemia. This degeneration is indicated by several histological characteristics observed in the tissue preparations, including the adluminal compartment and lumen appearing empty, without spermatids and spermatozoa. Spermatogonia (Sg) are still present along the basal membrane, but in fewer numbers compared to the K0 and other treatment groups (K2, K3, K4). Spermatocytes (Sc) appear in reduced numbers and are loosely arranged. The epithelium of the seminiferous tubules appears thinner, and the interstitial space (IS) is widened.

The number and morphology of Leydig cells (Le) and Sertoli cells (Sr) also appear altered, with a noticeable decrease in both cell type populations. The diabetic condition in the K1 group induces oxidative stress, leading to cellular apoptosis or necrosis, and subsequently disrupts the spermatogenesis process.

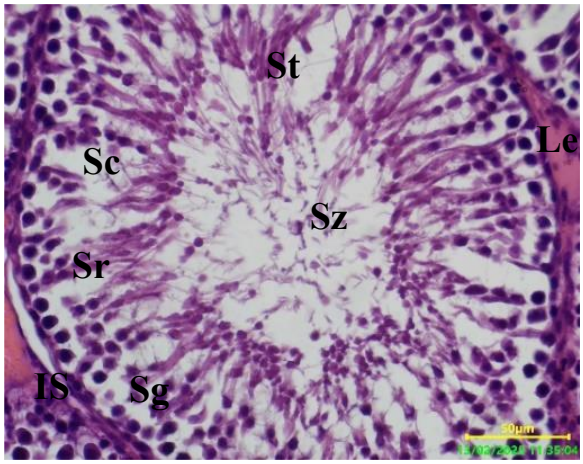

Figure 8. Histology of a cross-section of the seminiferous tubule of a K2 white rat (H&E, 400×). Spermatogonia (Sg); spermatocytes (Sc); spermatids (St); spermatozoa (Sz); Sertoli cells (Sr); Leydig cells (Le); interstitial space (IS).

Figure 8 shows a recovery of testicular structure in diabetic rats following treatment with EEDP. The histological structure of the seminiferous tubules demonstrates tissue repair, indicated by several features, including spermatogonia (Sg) arranged more densely along the basal membrane, and an increased number of spermatocytes (Sc) and spermatids (St) compared to the K1 group. Spermatozoa (Sz) are clearly visible within the lumen, although still fewer than in the K0 group. Sertoli cells (Sr) appear to regain their structural function in supporting spermatogonia and spermatids. The interstitial space (IS) becomes more compact and improved, with Leydig cells (Le) appearing normal in both structure and distribution. The durian peel extract begins to restore spermatogenesis through its antioxidant activity, which reduces damage to germinal cells and other epithelial cells within the seminiferous tubules.

Figure 9 shows a more pronounced recovery of testicular structure from diabetic conditions following EEDP treatment. The histological structure of the seminiferous tubules is characterized by a normal and noticeably thicker epithelial layer. Spermatogonia (Sg), spermatocytes (Sc), spermatids (St), and spermatozoa (Sz) appear more completely represented, present in optimal numbers, and are arranged in a more orderly manner. The number of spermatozoa within the lumen is higher compared to the K2 group. The interstitial space (IS), as well as Sertoli cells (Sr) and Leydig cells (Le), appear more abundant and structurally supportive of spermatogonia and spermatocytes. Among the treatment groups, the diabetic rats in the K3 group exhibit the most optimal histological structure of the seminiferous tubules, closely resembling the normal condition observed in the K0 group.

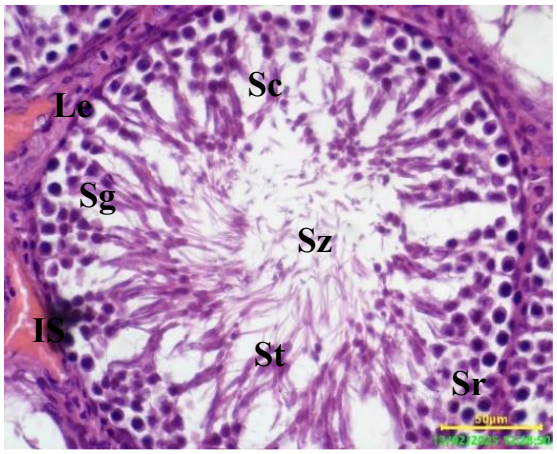

Figure 9. Histology of a cross-section of the seminiferous tubule of a K3 white rat (H&E, 400×). Spermatogonia (Sg); spermatocytes (Sc); spermatids (St); spermatozoa (Sz); Sertoli cells (Sr); Leydig cells (Le); interstitial space (IS).

Figure 10 shows that diabetic rats treated with EEDP exhibit improvements in the structure of the seminiferous tubules, although the recovery is slightly reduced compared to the K3 group. The histological characteristics observed indicate that spermatogenesis proceeds well, although the epithelial thickness appears to be decreased. Spermatogonia (Sg), spermatocytes (Sc), and spermatids (St) are present in complete developmental layers, supported by Sertoli cells (Sr). The number of spermatozoa (Sz) within the lumen is lower compared to that in the K3 group. The interstitial space (IS) appears normal, with Leydig cells (Le) displaying relatively similar morphology and quantity to those in K3. Overall, the EEDP dosage administered in the K4 group provides structural improvement to the seminiferous tubules in diabetic rats, though the degree of recovery is not as optimal as that observed in the K3 treatment group.

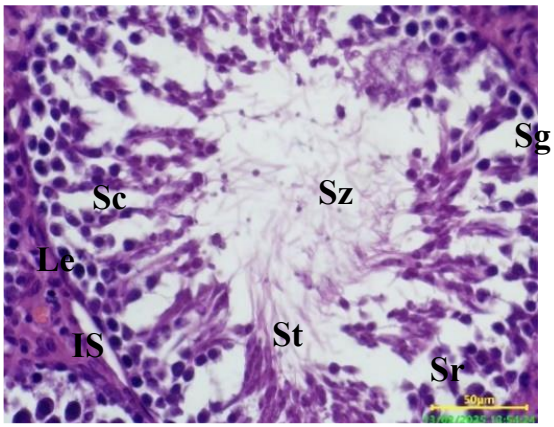

Figure 10. Histology of a cross-section of the seminiferous tubule of a K4 white rat (H&E, 400×). Spermatogonia (Sg); spermatocytes (Sc); spermatids (St); spermatozoa (Sz); Sertoli cells (Sr); Leydig cells (Le); interstitial space (IS).

The accumulation of ROS triggered by alloxan causes a decrease in reproductive hormones such as LH (luteinizing hormone) and FSH (follicle-stimulating hormone) which disrupts the production and maturation of germ cells, then has an impact on decreasing epithelial thickness (Wijayanti & Lestari, 2018) as evidenced by K1 which is significantly different from K0 as observed in this study's result.

The average testicular seminiferous tubule epithelium thickness between K2, K3, and K4 significantly differed from K0 and K1. The results of this study indicate that the administration of EEDP to alloxan-induced diabetic rats can repair spermatogenic cells in the seminiferous tubules, which is characterized by the increased thickness of the seminiferous tubule epithelium. The decrease in blood glucose levels caused by flavonoids due to EEDP treatment can reduce oxidative stress. According to Liu et al. (2019), flavonoids are antioxidant compounds that function to bind metal ions, such as Cu2+ and Fe2+. Copper and iron ions in cells can bind H_2O_2 and form ROS through enzymatic pathways. Metal ions bound by flavonoids can reduce oxidative damage to cells and prevent the death of spermatogenic cells in testicular seminiferous tubules, so the thickness of the seminiferous tubules does not decrease. Flavonoids are also known to increase TGF-β expression during the early proliferation phase, which supports fibroblast activation, extracellular matrix deposition, and tissue formation (Alfatinnisa et al., 2024).

The results of this study's phytochemical screening of durian skin reported that they also found it to contain flavonoids, tannins, and terpenoids. According to Zhan et al. (2021), tannins are known to have the ability to increase signaling pathways that lead to the expression of genes encoding endogenous antioxidant enzymes, including (SOD) superoxide dismutase, catalase, and (GPx) glutathione peroxidase. These three antioxidant enzymes play a role in detoxifying reactive oxygen species. The investigation carried out by Budiastuti et al. (2020) stated that terpenoids contribute electrons to prevent oxidative damage to lipids, proteins, and testicular cells.

The average data on the epithelium thickness of the seminiferous tubule in the K2 and K3 treatment groups are significantly different from K4, which is indicated by the thicker epithelium of K2 and K3. The average epithelial thickness in K4 with a 1.000 mg/kgBW dose was 67.82 µm, lower than in the K2 and K3 treatments. EEDP given at a dose of 1.000 mg/kgBW has a less than optimal effect on the repair of spermatogenic cells, presumably because high doses of antioxidants can turn into prooxidants. The results of research by Monageng et al. (2023) support this study, which states that high doses of antioxidants needed for tissue repair in organs can undergo bioconversion into prooxidants and trigger the production of ROS.

Martemucci et al. (2023) carried out an investigation that indicated antioxidant supplementation should be adjusted to physiological conditions to avoid prooxidant effects because antioxidants have dualistic effects, which can be beneficial and harmful. Administration of low to moderate doses of polyphenols and flavonoids can neutralize free radicals. However, at high doses, they can potentially undergo auto-oxidation, thus producing new free radicals. Yücel et al. (2020) stated that antioxidants such as polyphenols and flavonoids can bind to metal ions in the body, such as Fe^{2+} and Cu^{+} , which trigger the Fenton reaction. The Fenton reaction results in hydroxyl radicals, which have reactive properties and damage lipids and proteins in cells. Oxidative damage caused by hydroxyl radicals can activate the apoptotic pathway, especially in the pancreas, kidney, and testicular seminiferous tubules.

This study demonstrates that ethanol extract of durian peel (EEDP) at doses of 500–1,000 mg/kg body weight can improve testicular structure and stages of spermatogenesis in alloxan-induced diabetic rats. The bioactive compounds found in EEDP, such as flavonoids,

polyphenols, and terpenoids, play a key role in reducing oxidative stress, which is a major cause of testicular damage. The novelty of this research lies in the development of a treatment material in the form of durian peel ethanol extract (DPEE), which shows effects on the testicular histology of alloxan-induced diabetic Sprague-Dawley rats, particularly on testis weight, seminiferous tubule diameter and epithelial thickness, as well as spermatogenesis stages. Another novelty of this study is the use of varying doses (500, 750, and 1,000 mg/kgBW), which have not been previously tested, thus allowing the determination of the optimal dose as a candidate for natural therapy. An additional novelty aspect is the utilization of durian peel, commonly regarded as waste, as a potential bioactive source with antidiabetic and protective effects on male reproductive organs.

The benefits of this research include, first, providing an alternative natural therapy to reduce diabetes complications, particularly reproductive organ damage (testicular atrophy and spermatogenesis disorders). Second, providing information on the optimal dose of durian peel ethanol extract that is effective in improving the testicular histology of diabetic rats. Third, adding a comparative reference for similar studies investigating medicinal plants as antihyperglycemic and antioxidant agents. The contribution of this research to science is to expand biomedical studies on the relationship between herbal antidiabetics and reproductive function, serve as a foundation for further research in pharmacology, histology, and reproduction related to the utilization of phytochemicals from plant waste, and provide a reference for the development of herbal antidiabetic drugs with protective effects on the male reproductive organ. The contribution of this research to society is to encourage the utilization of durian peel, which is usually considered waste, into a value-added product as a raw material for herbal medicine; to provide insights into safer alternative therapies compared to long-term synthetic drugs that have side effects on the kidneys and other organs; and to potentially support male reproductive health programs for diabetic patients

CONCLUSIONS

Ethanol extract of durian peel (EEDP) provides testicular protection in diabetic rats by increasing testicular weight, diameter, and epithelial thickness of seminiferous tubules, and stages of spermatogenesis. EEDP has potential as a natural therapy in improving spermatogenesis dysfunction and preventing diabetes-induced atrophy. Future research needs to conduct toxicity tests of EEDP and studies on the optimal dose of EEDP to ensure its more effective and efficient use. Potential strategies in the treatment of reproductive disorders due to diabetes include using nanoparticles to increase the bioavailability of bioactive compounds and combining them with other therapies for more optimal results. Future studies are suggested to analyze hormonal profiles and molecular mechanisms of durian peel ethanol extract, as well as to identify its active compounds and validate efficacy through broader preclinical trials. Explain suggestions for future research that have not been examined in this study.

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AUTHOR CONTRIBUTION STATEMENT

The authors contributed significantly to the work reported in this manuscript. The CES (Cinta Etna Syakera) was responsible for data collection and analysis, as well as conducting the literature review. The SI (Sri Isdadiyanto) conceived and designed the study, verified the data analysis, and supported data visualization. The SS (Sunarno Sunarno) provided substantial input during the writing and revision process of the manuscript. The LOIJ (La Ode Irman Jaya) performed verification, correction, and improvement of the manuscript in English. All authors have approved the final manuscript and accept responsibility for all aspects of the work.

INFORMED CONSENT STATEMENT

All participants provided informed consent, as stipulated in the Ethical Clearance (EC) approved by the Health Research Ethics

Committee (HREC) of the Faculty of Medicine, Diponegoro University/Dr. Kariadi General Hospital, Semarang, Central Java Province, Indonesia, with approval number 100/EC-H/KEPK/FK-UNDIP/X/2024. Prior to participation, each subject received a detailed explanation regarding the purpose, procedures, potential risks, and benefits of the study. All participants voluntarily agreed to participate and signed a written informed consent form.

CONFLICT OF INTEREST STATEMENT

The authors certify that they have no competing interests that could have influenced the work reported in this manuscript.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors certify that no artificial intelligence (AI) technologies were employed in the preparation, analysis, or writing of this manuscript. All research activities, including data collection, interpretation, and manuscript preparation, were performed exclusively by the authors without AI assistance.

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