THE ETHANOL EXTRACT OF JENGKOL (ARCHIDENDRON PAUCIFLORUM) FRUIT PEEL CAN REPAIR THE HISTOLOGICAL STRUCTURE OF THE TESTES IN MALE WISTAR DIABETIC RAT MODELS

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ABSTRACT

Diabetes Mellitus is a major health problem that adversely affects a patient's life quality worldwide due to its severe complications It has been recognized that chronic hyperglycemia with oxidative stress is a major cause of male infertility. The fruit peel of Jengkol (Archidendron Pauciflorum) is known to contain several antioxidant compounds, such as flavonoids, tannins, saponin, and polyphenols. This study aims to determine the effectiveness of antidiabetic ethanol extract of jengkol fruit peel (EEJFP) in repairing the damage testicular structure in streptozotocin-induced diabetic rats. This research used an experimental method in a laboratory with Completely Random Design (CRD) using 5 treatments and 5 replications. Treatment was administered for 54 consecutive days, comprising a negative control (NC), positive control (PC), comparison (glibenclamide dose 10 mg/kg BW), T1, and T2 (EEJFP dose 385 and 770 mg/kg BW). Diabetic induction was performed with streptozotocin dose of 65 mg/kg BW in male Wistar rats except for the NC group. The parameters observed included morphological aspect of testis (weight, length, width, and volume of testis) and histological structure of testis (seminiferous tubule diameter, lumen diameter, spermatogenic epithelial thickness, and membrane basal thickness). The obtained data were analyzed by ANOVA test and followed by Duncan's test. The results showed that the morphological parameters and histological parameters in the T2 group were not significantly different from the NC group. Based on the study's results, it can be concluded that the EEJFP dose of 770 mg/kg BW is an effective dose in repairing the testicular structure in streptozotocin-induced diabetic male Wistar rats.

Keyword: *Antidiabetic, Antioxidant, Diabetes Mellitus, Testicular structure*

1. INTRODUCTION

Diabetes mellitus (DM) is a degenerative disease prevalent in the modern era. According to the World Health Organization [1], the number of DM patients reached 415 million adults in 2015, marking a fourfold increase since the 1980s. It is projected to rise further to 642 million adults by 2040. Indonesia ranks seventh globally in the prevalence of DM sufferers, with an estimated 10 million adults affected. As highlighted by Ding et al. [2], DM is characterized by hyperglycemia and hypoinsulinemia, affecting neurological, endocrinological, and reproductive functions.

The conditions of DM can be induced through pancreatectomy and the use of chemical compounds known as diabetogenic agents. Commonly used chemical substances as inductors of DM, such as streptozotocin (STZ), have been discussed by Suharmiati [3]. STZ can impact the characteristics of blood insulin and glucose concentration in pancreatic β cells, leading to hyperglycemia and hypoinsulin conditions [4]. Hyperglycemic conditions can cause oxidative stress, resulting in damage to cell mitochondria and dysfunction in various organs. Oxidative stress occurs when the balance between antioxidants and Reactive Oxygen Species (ROS) is disrupted [5].

This imbalance can elevate the number of ROS, causing damage to cell membranes and resulting in lipid peroxidation or malondialdehyde (MDA). If left unchecked, this process can lead to damage in the cell membrane system and eventual cell death [6]. Vignera et al. [7] reported that oxidative stress contributes to infertility in 35% of individuals with diabetes mellitus.

One of the male reproductive organs sensitive to increased ROS is the testes. The accumulation of ROS in the testes can lead to damage in the structure and function of its functional cells [5]. Specifically, hyperglycemic conditions have adverse effects on sperm concentration and motility due to alterations in energy production and free radicals [2]. DM can result in an increase in the thickness of the basement membrane in the seminiferous tubules and a decrease in the total size of the seminiferous tubules due to a reduction in sperm production caused by cell degeneration [8]. According to Khaneshi et al [8], DM can also lead to a reduction in the number of Sertoli cells and germ cells, reducing the rate of spermatogenesis, ultimately resulting in a decrease in testicular size, diameter, length, and weight. Testicular size is influenced by the number of Sertoli cells, the process of spermatogenesis, and is closely related to germ cells [9]

The treatment for diabetes mellitus (DM) can involve insulin therapy or the use of synthetic oral drugs to control blood sugar levels. One commonly used synthetic oral drug from the sulfonylurea class is glibenclamide. This medication functions by stimulating the release of insulin in the β-pancreatic cells that are still functioning properly due to containing sulfonylurea. Glibenclamide has dominant side effects, namely hypoglycemia, weakness, pallor, sweating, and palpitations [10]. Therefore, an alternative in the form of traditional medicine, which has a minimal impact on the body, is sought.

The development of plants for use in traditional medicine has been long known in Indonesia. One such plant used to treat diabetes mellitus is the jengkol (*Archidendron. pauciflorum*) fruit peel. According to Malini et al. [11], the people of Karangwangi Village consume boiled water made from dried jengkol fruit peel to treat diabetes. Jengkol fruit peel also contains alkaloid compounds, flavonoids, tannins, saponins, and polyphenols, which have been tested to reduce blood sugar levels in alloxan-induced rats [12]. Flavonoid compounds, a subgroup of polyphenols, possess the ability to inhibit lipid peroxidation, engage in metal chelation-oxidation-reduction activities, and hinder processes associated with ROS [13]. Being antioxidants, flavonoid compounds can also protect cells from damage caused by free radicals [14]. The ethanol extract of jengkol fruit peel (EEJPF) exhibits polyphenol levels of 28.82%, flavonoids of 0.23%, and tannins of 3.83% [15]. Malini et al. [16] stated that the EEJPF 770 mg/kg BW was the most effective dose in reducing blood glucose levels (65.4%) and increasing plasma insulin levels (230%) in rats streptozotocin-induced diabetic rats.

Building upon these prior findings, we envisioned that EEJPF might possess the potential to ameliorate structural damage in the testes. Therefore, this study aimed to investigate the effectiveness of EEJPF against structural damage in the testes, both morphologically and histologically, in streptozotocin-induced diabetic rats.

2. METHODS

2.1 Instruments

The instruments used in the study included a sterile dissection kit, a rotary evaporator (Eyela N1100SWD), a rotary microtome (Microm HM310), a heating plate (Thermatic EDS-89), a light microscope (Olympus CX-21), an oven (Cole Palmer), and a staining jar.

2.2 Materials

Twenty-five male Wistar rats (180-200 g), provided by the Biosystem Laboratory in the Department of Biology, were utilized. These rats were housed under standard environmental conditions, with room temperatures ranging from 25 to 32˚C and a 12-hour dark-light cycle, as described by Adelakun et al. [16]. They were granted free access to drinking water and a standard pellet diet (CP-551, PT. Charoen Pokphand). The rats underwent a one-week acclimation period in holding facilities before the commencement of treatments, following the protocol outlined by Sefidgar et al. [18]. The fruit peel of *A. Pauciflorum* was sourced from Payakumbuh, West Sumatra. Taxonomic identification of the samples was conducted in the Taxonomy laboratory within the Biology Department at the Faculty of Mathematics and Sciences, University of Padjadjaran.

2.3 Experimental design

The experimental using completely randomized design with six treatments and four replications each. Animals were randomly assigned to control and treated groups, as described below.

1. Negative Control (NC): Non-STZ + CMC solution 0.5%
2. Positive Control (PC): STZ + CMC solution 0.5%
3. Treatment 1 (T1): STZ + EEJFP dose 385 mg/kg BW
4. Treatment 2 (T2): STZ + EEJFP dose 770 mg/kg BW
5. Comparison (C): STZ + Glibenclamide dose 10 mg/kg BW

2.4 Preparation of Etanol Extract from Jengkol Fruit Peel

The jengkol fruit peels were air-dried until a constant weight was achieved and then blended into a coarse powder. The dried powder was soaked and macerated in 96% ethanol (ratio 1:6) for 72 hours, with the macerate collected every 24 hours. Subsequently, the macerate was evaporated using a rotary evaporator at a temperature of 40-50°C and freeze-dried to obtain a paste extract [19].

2.5 Induction of Diabetes and Administration of EEJFP

Prior to the induction of diabetes using STZ, the animals underwent an overnight fast. Their baseline fasting glucose levels were determined using a glucometer by collecting blood via tail cut. Diabetes was induced by intravenous injection of a freshly prepared STZ solution at a dose of 65 mg/kg BW in a 10 mM citrate buffer solution (pH 4.5) for five groups, while the negative control rats were injected with the vehicle alone. Subsequently, the animals underwent another overnight fast for 72 hours after the administration of STZ. Blood was collected via tail cut to determine their fasting glucose levels. Animals with glucose levels exceeding 250 mg/dL were categorized as diabetic rats and utilized for further experimentation.

Both normal non-diabetic control and diabetic control rats were administered a 0.5% carboxymethyl cellulose (CMC) solution, while EEJFP or glibenclamide was orally administered to diabetic rats using an intragastric tube once a day for 54 days. The chosen treatment duration aligns with the duration of one spermatogenesis cycle [20].

2.6. Preparation for the observation of morphological and histological aspects of the testicles.

On the 55th day, following an overnight fast, the animals were weighed and then euthanized through cervical dislocation, and their testicular organs were isolated. The organs were rinsed with 0.9% NaCl to eliminate blood residue and dried using filter paper. The observed testis morphology included testicular weight, testicular size in terms of length and width, and testicular volume.

Histologic preparations of the testicular organs were made by fixing the isolated testicular organs in Bouin solution for 24 hours. The testicular organs were then transversely cut, washed in 70% alcohol for 24 hours, dehydrated in a series of alcohol and 100% alcohol-based clarification (xylol). Subsequently, the testicular organs were infiltrated in xylol:paraffin and embedded in paraffin using an oven set at 60-70°C. The organs were then cut using a microtome with a thickness of 5 microns at a temperature below 24°C. Staining was performed using Hematoxylin-Eosin (HE). The staining process involved deparaffinization, rehydration in a series of alcohols, immersion in Hematoxylin solution for 25 minutes, rinsing with flowing tap water, immersion in Eosin solution for 10 seconds, dehydration in a series of alcohols, purification in a series of xylol solutions, drying at room temperature, and covering with a glass slide. The histological sections of the stained testicular tissue were then observed for histological structures using a light microscope.

Observation of the testicular structure involved measuring the diameter of the seminiferous tubules, the thickness of the germinal tubule epithelial layer, the thickness of the basal membrane, and the diameter of the seminiferous tubule lumen. Measurements were conducted using a digital microscope with a magnification of 100x-400x and analyzed with the computer program ImageJ.

3. Result

The results of the analysis of variance (ANOVA) for morphological parameters, including weight, length, width, and volume of the testes, indicate that the F counts are greater than the F table values. This suggests that the treatment significantly influences the weight, width, length, and volume of the testes in male Wistar rats, prompting further examination with the Duncan test.The average testicular weight, length, width, and volume in rats with the STZ-induced diabetes model and those treated with EEJFP are presented in Table 1.

Table 1 Average weight, length, width and volume of the testes of male Wistar rats after treatment

Treatment	Morphological Parameters			
	Organ Weight ± SD (g)	Organ Length ± SD (cm)	Organ Width ± SD (cm)	Organ Volume ± SD (cm^2)
Negative Control (NC)	1.62 ± 0.09 [c]	2.21 ± 0.14 [c]	1.25 ± 0.09 [b]	1.82 ± 0.38 [c]
Positive Control (PC)	1.17 ± 0.20 [a]	1.99 ± 0.08 [a]	1.08 ± 0.06 [a]	1.22 ± 0.11 [a]
Treatment 1 (T1)	1.47 ± 0.05 [b,c]	2.14 ± 0.04 [b,c]	1.20 ± 0.07 [b]	1.64 ± 0.18 [b,c]
Treatment 2 (T2)	1.61 ± 0.19 [c]	2.18 ± 0.08 [b,c]	1.23 ± 0.10 [b]	1.73 ± 0.24 [b,c]
Comparison (C)	1.30 ± 0.25 [a,b]	2.06 ± 0.07 [a,b]	1.15 ± 0.04 [a,b]	1.42 ± 0.09 [a,b]

Inf: The data are presented as mean ± SD. Data analysis employed an ANOVA test with Duncan's test at a 95% confidence level ($\alpha = 0.05$). The presence of the same letter in one column indicates no significant difference.

Histological observations of seminiferous tubules involved measuring tubular diameter, lumen diameter, epithelial thickness, and basal membrane thickness in 30 testicular histological sections. The mean values for seminiferous tubule diameter, lumen diameter, spermatogenic epithelial thickness, and basement membrane thickness in rats are presented in Table 2. The ANOVA test results for these parameters (seminiferous tubule diameter, lumen diameter, spermatogenic epithelial thickness, and basement membrane thickness) indicate that the F count values are greater than the F table value. This implies that there is a significant treatment effect on male rats. Subsequently, the Duncan test was conducted to identify the treatment groups that are significantly different from each other.

Table 1. The average diameter of seminiferous tubule, lumen diameter, spermatogenic epithelial thickness, and basement membrane thickness in the testicular seminiferous tubules of Wistar male rats after treatment.

Treatment	Histological Parameters (µm)			
	Seminiferous Tubule Diameter ± SD	Lumen Diameter ± SD	Spermatogenic Epital Thickness ± SD	Basal Membrane Thickness ± SD
Negative Control (NC)	89.14 ± 4.88 [c]	43.87 ± 5.28 [a]	43.39 ± 2.63 [d]	0.96 ± 0.21 [a]
Positive Control (PC)	77.33 ± 2.54 [a]	52.89 ± 6.55 [b]	23.77 ± 2.10 [a]	1.41 ± 0.15 [b]
Treatment 1 (T1)	85.69 ± 1.18 [b,c]	45.06 ± 1.45 [a]	39.97 ± 0.50 [c]	1.00 ± 0.25 [a]
Treatment 2 (T2)	87.80 ± 4.31 [c]	44.21 ± 3.84 [a]	42.41 ± 2.56 [c,d]	0.99 ± 0.16 [a]
Comparison (C)	82.54 ± 1.21[b]	46.52 ± 1.07 [a]	34.15 ± 1.01 [b]	1.01 ± 0.22 [a]

Inf: The data are presented as mean ± SD. Data analysis employed an ANOVA test with Duncan's test at a 95% confidence level ($\alpha = 0.05$). The presence of the same letter in one column indicates no significant difference.

4. Discussion

Table 1 illustrates that the intravenous induction of STZ at a dose of 60 mg/kg BW caused the positive control rats (PC) to exhibit the lowest testicular weight, length, width, and volume, significantly differing from negative control rats (NC) that were not induced by STZ. Streptozotocin (STZ) is a diabetogenic agent capable of causing damage to β-pancreatic cells [4]. According to Pathak et al. [21], STZ is cytotoxic specifically to β-pancreatic cells, resulting in decreased function of these cells to secrete the hormone insulin, leading to DM. The results of this study align with the research of Abbasi et al. [22], indicating a decrease in testicular weight, length, width, and volume in DM rats due to oxidative stress on tissues and organs. DM conditions can also lead to a reduction of Sertoli cells and spermatogenic cells, as mentioned by Slegtenhorst-Eegdeman et al. [19]. Testis size is influenced by the number of Sertoli cells, the process of spermatogenesis, and is closely related to germ cells. Reproductive organ dysfunction in diabetics has been reported to be associated with ROS, causing oxidative stress [23]. This is thought to contribute to a decrease in testicular weight, length, width, and volume in the diabetic PC group rats. Glibenclamide 10 mg/kg BW in the comparison treatment group (T1 and T2) demonstrated that testicular weight, length, width, and volume were significantly different from the NC group but not significantly different from PC based on the Duncan test (Table 1). Male rats given oral medication with glibenclamide, a sulfonylurea

group according to Confederat et al. [24], may experience side effects of toxicity to cells, resulting in cell necrosis. The cell damage caused by Glibenclamide is thought to be the reason for its inability to increase the weight, length, width, and volume of the testicles in diabetic rats. Therefore, Glibenclamide at a dose of 10 mg/kg BW is considered ineffective in repairing the damage caused by DM in STZ-induced testes of rats.

Table 1 demonstrates that all EEJFP treatments (T1, T2) significantly enhance testicular weight, length, width, and volume compared to the positive control (PC). The testicular parameters, including weight, length, width, and volume at T1 with EEJFP administered at a dosage of 385 mg/kg and at T2 with EEJFP at a dose of 770 mg/kg BW, did not exhibit significant differences when compared to the negative control (NC).

Jengkol fruit skin ethanol extract (EEJFP) comprises various bioactive compounds, such as flavonoids, alkaloids, tannins, and saponins. The findings of Heim et al. [13] research indicate that flavonoid compounds belong to the polyphenol group, exhibiting the capability to inhibit lipid peroxidation, engage in metal chelation-oxidation-reduction activities, and participate in processes associated with ROS. Specifically, flavonoids can target biological elements relevant to type 2 diabetes mellitus, including α-glycosidase and DPP-4, as reported by Sarian et al. [25]. Kim et al. [26] further note that compounds within the polyphenol group can increase glucose uptake and enhance the regulation of glucose transporters in skeletal muscle, ultimately leading to a reduction in blood glucose levels in diabetic rats. Other bioactive compounds found in EEJFP include tannins, as indicated by Kumari and Jain [27]. Tannins, according to their research, can enhance glucose absorption and inhibit adipogenesis, suggesting their potential to lower blood sugar levels in individuals with DM. Additionally, tannins exhibit antioxidant activity and contribute to hypoglycemic effects by promoting glycogenesis. Alkaloid compounds are also identified in EEJFP, with findings from Malini et al. [16} demonstrating that alkaloids in the EEJFP can reduce blood glucose levels and elevate insulin levels in mice induced with STZ. The activity of saponin compounds, as highlighted by Barky et al. [28], involves regulating blood glucose levels and preventing diabetes complications through their antioxidant properties. Saponins, when associated with dyslipidemia, aid in reducing the risk of atherosclerosis and cardiovascular diseases in individuals with diabetes.

The bioactive compounds present in EEJFP, such as flavonoids, tannins, saponins, and alkaloids, are believed to possess antioxidant potential. This aligns with the findings of Onyeso et al. [28] on Watermelon (*Citrullus Lanatus*) seed extract, where high phenolic and flavonoid compounds, along with alkaloids, tannins, and saponins, demonstrated hypoglycemic effects and antioxidant potential. Antioxidants play a crucial role in repairing organ damage by reducing the abundance of free radicals associated with DM. Caro-Ordieres et [30] suggest that normalizing free radical levels can aid in tissue and organ repair. In individuals with DM, the condition generates an abundance of ROS. Excessive ROS within cells can lead to reduced sperm motility, sperm morphological abnormalities [31], decreased penetration capacity of spermatozoa with oocytes, and diminished fertility [32]. The results indicate that administering EEJFP, containing flavonoids, tannins, saponins, and alkaloids, can restore testicular size. This aligns with the research conducted by Khanesi et al. [8], where diabetic rats experienced a significant reduction in testicular weight, testis diameter, length, and volume compared to the group treated with sesame seeds *(S. Indicum L*), known for its antioxidants and anti-inflammatory properties. A study by Zhou et al. [33] also demonstrated that STZ-induced mice given *Lycium Chinensis*, which contains antioxidant compounds, experienced an increase in testicular weight compared to the diabetic rat group.

The histological structure of the seminiferous tubules of the testes after 54 days of treatment, observed microscopically in each group, is depicted in Figure 1. Figure 1A illustrates the histological cross-section of the seminiferous tubule in the rat testis under negative control (NC) treatment. In the figure, it is evident that the seminiferous tubule comprises the basement membrane and the spermatogenic epithelium. According to Heffner and Danny [34], the testes consist of a collection of seminiferous tubules, each equipped with basal burns serving as the site of progenitor cells in the form of spermatogonia cells for spermatozoa production. Each tubule is composed of epithelial cells containing developing spermatogenic cells, referred to as the seminiferous epithelium or germinal epithelium. Germ cells within the seminiferous tubules consist of layers of spermatogenic epithelial cells sequentially arranged from the outermost to the innermost layers, namely young mass cells (spermatogonia or germ cells), primary spermatocytes, secondary spermatocytes, and mature cells (spermatids). Spermatozoa, as the end result of the maturation process of spermatids, are observed in the lumen of the seminiferous tubules [35].

Figure 1B depicts a histological cross-section image of the seminiferous tubule of the rat testis from the positive control (PC) treatment group, induced by STZ. In this figure, it is noticeable that the size of the seminiferous tubules is smaller compared to the negative control (NC) group. The intravenous induction of STZ in PC mice leads to conditions associated with diabetes mellitus (DM). According to Khaneshi et al. [8], DM conditions can result in a reduction in the overall size of the seminiferous tubules and an increase in the thickness of the basement membrane of the seminiferous tubules. This reduction is attributed to a decrease in sperm production

caused by cell degeneration. Cell degeneration or cell death occurs due to damage to the cell membrane, leading to lipid peroxidation or malondialdehyde (MDA) formation resulting from oxidative stress caused by DM [6].

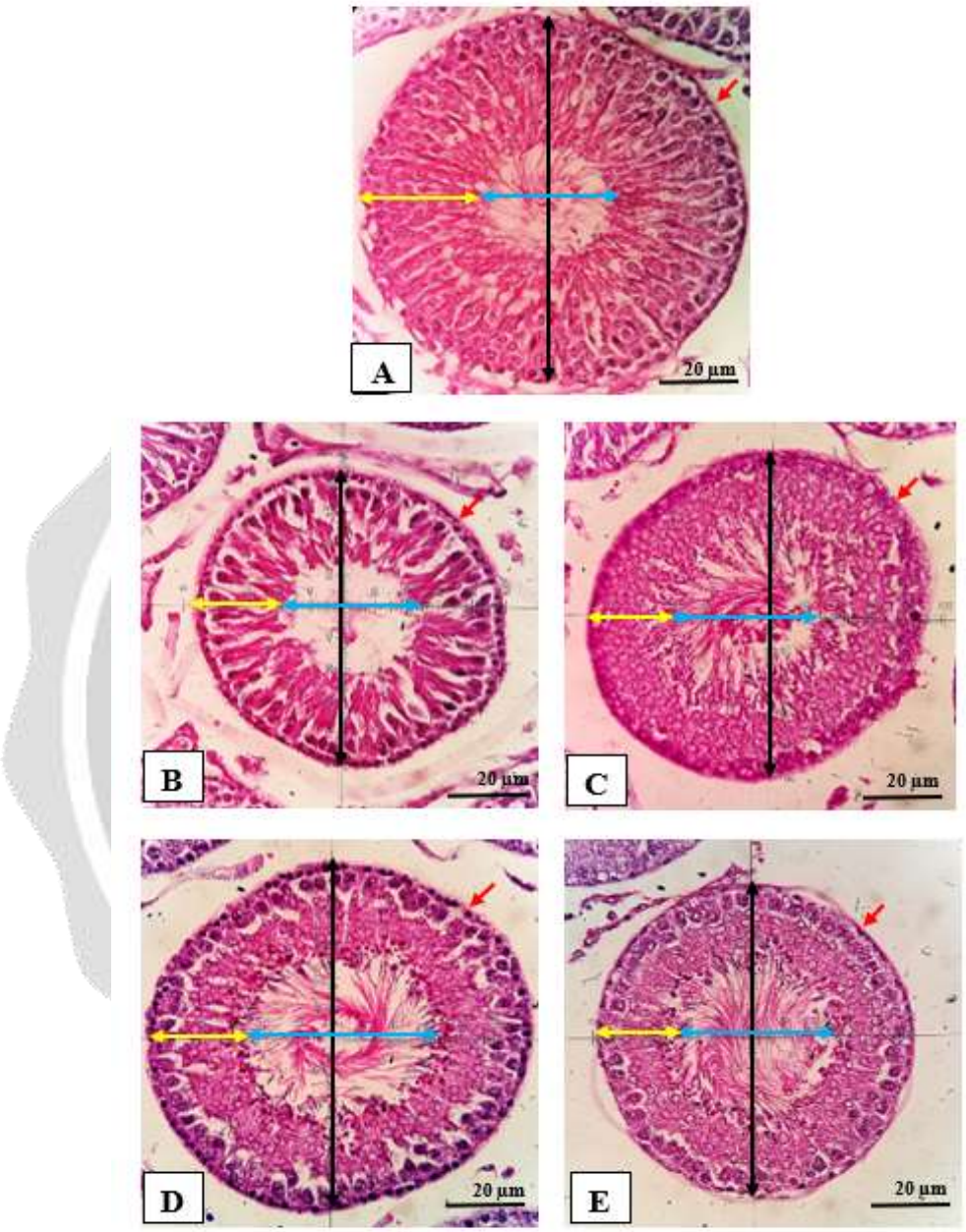

Figure 1. Transverse Incision of Rat Testis Organ with H&E staining and 400x magnification
Information: (A) NC (non-STZ, CMC 0.05%); (B) PC (STZ 60 mg/kg BW+CMC 0.05%); (C) T1 (STZ 60 mg/kg+CMC 0.05% + EEJFP 385 mg/kg BW); (D) T2 (STZ 60 mg/kg BW+CMC 0.05%+EEJFP 770 mg/kg BW); (E) Pb (STZ 60 mg/kg+CMC 0.05%+Glibenclamide 10 mg/kg BW). Blackline; Seminiferous tubule diameter, blue line; lumen diameter, yellow line; thick spermatogenic epitels, red arrows; basement membrane thickness

Figure 1C and 1D depict histological cross-sections of the seminiferous tubules in the testicles of rats treated with EEJFP. The figures reveal structures and sizes that are not significantly different from the negative control (NC) group. This similarity is attributed to the presence of antioxidant compounds in EEJFP, including flavonoids, tannins, saponins, and alkaloids. Antioxidants play a crucial role in repairing organ damage by reducing the number of free radicals caused by DM. According to Caro-Ordieres et al. [30], lowering the number of free radicals to normal levels can facilitate the repair of damage in tissues and organs.

Figure 1 E displays a histological cross-section of the seminiferous tubules in the testicles of rats treated with Glibenclamide at a dose of 10 mg/kg BW in the comparison group (Pb). The figure indicates that the size of the testicular seminiferous tubules in these mice is smaller than that of the NC group but not significantly different from the positive control (PC) group. This is because the administration of Glibenclamide can induce toxic side effects on cells, leading to cell necrosis [24]. As noted by Slegtenhorst-Eegdeman et al. [9], the death of germ cells, Sertoli cells, and Leydig cells in the seminiferous tubules of the testes results in a decrease in testicular size.

The results of the analysis of histological testicular preparations for rats (Table 2) revealed significant differences in the positive control group (PC) rats induced with intravenous STZ at a dose of 60 mg/kg BW. Parameters such as seminiferous tubule diameter, lumen diameter, spermatic epithelial thickness, and basal membrane thickness differed significantly compared to negative control mice (NC) that were not induced with STZ. Streptozotocin (STZ) is commonly used as a diabetogenic agent to induce diabetes models in studies, leading to DNA damage. STZ induces damage to pancreatic β cells, resulting in alterations in blood insulin and glucose concentrations. This is attributed to the presence of glucose molecules in STZ, which bind to glucose receptors on the plasma membrane, blocking glucose-induced insulin secretion [36]. As stated by Erwin et al. [37], STZ causes DNA damage in pancreatic β cells, preceded by the disruption of adenosine triphosphate (ATP) ordering in the mitochondria due to free radical formation in the cells. Consequently, there is an increase in the xanthine oxidase enzyme, leading to the inhibition of the Krebs Cycle. Intravenous induction of STZ in male rats can induce conditions resembling diabetes mellitus in animal research models.

Overcoming hyperglycemia conditions in DM leads to an increase in ROS, and the activation of excess ROS in tissues induces oxidative stress. This oxidative stress interferes with cellular respiration, causing damage to the mitochondrial membrane and impairing its function [38]. According to Semenkovich et al. [39], this situation triggers a transition in mitochondrial permeability, reflecting the function of the mitochondrial membrane and membrane leakage. This leads to membrane depolarization and activation of apoptotic factors, initiating the apoptotic process. Apoptotic cell death contributes to organ atrophy and low levels of sexual hormones, such as testosterone, luteinizing hormone, and follicle-stimulating hormone in male mice [40]. Additionally, there is a decrease in seminiferous tubule size and a reduction in the spermatogenic cell series [41]. This disruption affects the spermatogenesis process, resulting in a decrease in seminiferous tubule diameter, an increase in lumen diameter, a decrease in spermatogenic epithelial thickness, and an increase in basement membrane thickness.

The administration of Glibenclamide at a dose of 10 mg/kg BW in the comparison treatment group (Pb) demonstrated lower values for tubular diameter, lumen diameter, spermatogenic epithelial thickness, and basement membrane thickness, significantly different from the negative control (NC) group, but not significantly different from the positive control (PC) group (Table 2). The use of Glibenclamide at this dosage induces hypoglycemic effects in rats [10]. Research by Adaramoye et al.[42} also indicated that Glibenclamide administration can lead to decreased sperm quality and testicular dysfunction, accompanied by an increase in lipid peroxidation and a decrease in the activity of antioxidant enzymes. This condition disrupts the spermatogenesis process, resulting in a decrease in the size of the seminiferous tubules in the testes.

Table 2 illustrates that all treatments with EEJFP lead to a significant increase in seminiferous tubule diameter, a decrease in lumen diameter, an increase in spermatogenic epithelium thickness, and a reduction in basement membrane thickness compared to the PC. The measurements of seminiferous tubule diameter, lumen diameter, spermatogenic epithelium thickness, and basement membrane thickness at T1 are not significantly different from the NC. This consistency is believed to be attributed to the presence of bioactive compounds acting as antioxidants in the jengkol fruit peel, including flavonoids, alkaloids, tannins, and saponins. Caro-Ordieres et al. [30] suggest that flavonoids exhibit an anti-diabetic effect, primarily by locally modulating the inflammatory, oxidative, and lipotoxic microenvironment. This modulation provides protection to tissues and organs directly affected by DM and its consequences. Alkaloid compounds in EEJFP, as indicated by research conducted by Malini et al. [16], have the potential to lower blood glucose levels in mice induced with STZ and also increase insulin levels. The absence of insulin in the blood circulation of individuals with DM can lead to glucose being transported from the blood circulation to problematic cells, prompting cells to seek alternative energy sources through fat, protein, and muscle sugar systems. This metabolic process can generate by-products such as free radicals [43]. According to Darmawan

[44], an increase in the number of free radicals can induce apoptosis in sperm cells by damaging the mitochondrial membrane.

Other compounds found in EEJFP include tannins. According to Kumari and Jain [27], tannins are potential antioxidants capable of ameliorating the pathological oxidative state of DM by reducing free radicals and activating antioxidant enzymes. Tannins can enhance glucose uptake through insulin signaling pathways, such as Phosphoinositide 3-kinase (PI3K), p38 Mitogen-Activated Protein Kinase (MAPK), and translocation activation of GLUT-4. The EEJFP also contains saponin compounds. Kumar et al. [45] reported that saponins can lower blood glucose and increase insulin levels in blood plasma by reducing gluconeogenesis through the inhibition of the two main enzymes, glucose-6-phosphate and fructose-1.6-bisphosphatase. Saponins also increase glucose oxidation by activating glucose-6-phosphate. Additionally, saponins inhibit molecular absorption and cause disruption in the glucose transporter system, leading to glucose absorption [46].

The bioactive compounds in EEJFP, such as flavonoids, tannins, saponins, and alkaloids, are believed to hold potential as antioxidants. Research by Malini et al. {19} demonstrated that the EEJFP, with an antioxidant activity value of 66.82%, was capable of repairing damaged β-pancreatic cells and normalizing the levels of ROS in individuals with DM. This aligns with the findings of Onyeso et al. [29] regarding Watermelon (*Citrullus lanatus*) seed extract, which revealed that phenolic compounds, flavonoids, alkaloids, tannins, and saponins have hypoglycemic effects and antioxidant potential. Antioxidants are thought to mitigate the production of free radicals resulting from DM conditions. Kim et al.'s [26] research highlighted an effective approach to reducing elevated glucose levels and neutralizing the amount of ROS by inhibiting α-glycosidase through the use of inhibitors like routine, quercetin, and isoquercetin found in flavonoids. The α-glycosidase inhibitors prevent glucose uptake, thereby reducing postprandial blood glucose levels and neutralizing the ROS count.

The results revealed that the administration of EEJFP treatment led to an increase in seminiferous tubule diameter, a decrease in lumen diameter, thickening of the germinal epithelium, and thinning of the basement membrane compared to rats in the positive control (PC) group. This effect is attributed to the presence of antioxidants in EEJFP. These findings align with the research results of Ghanbari et al. [23], demonstrating that administering Royal Jelly to mice as an antidiabetic and antioxidant significantly reduced the thickness of the tunica albuginea and significantly increased the diameter of seminiferous tubules compared to STZ-induced diabetic mice. Khaneshi et al. [8] also reported that antioxidants and anti-inflammatory properties found in sesame seeds (*Sesamum Indicum* L) given to STZ-induced diabetic rats increased seminiferous tubule diameter, thickened germinal epithelial cells, normalized interstitial spaces between tubules, and decreased basement membrane thickness, with spermatogenesis cells and Leydig cells appearing normal.

5. Conclusion

The test animals administered with EEJFP, when examined morphologically, showed an increase in testicular weight, length, width, and volume, and these parameters were not significantly different from those of the negative control group (NC). Similarly, in terms of histological structure, EEJFP resulted in an increase in seminiferous tubule diameter, a decrease in lumen diameter, thickening of the spermatogenic epithelium, and depletion of the basement membrane, with no significant differences from the negative control group (NC). This is attributed to the presence of bioactive compounds in EEJFP, such as flavonoids, tannins, saponins, and alkaloids, believed to possess antioxidant potential. The results of the statistical analysis indicated that the effective dose for repairing rat testicular structures was EEJFP at a dose of 770 mg/kg BW, compared to EEJFP at a dose of 385 mg/kg BW. Therefore, it can be concluded that administering a dose of 770 mg/kg BW of EEJFP is effective in repairing the testicular structure in Wistar male rats induced with STZ.

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