

Fruit Extract of Black Pepper (*Piper Nigrum* L.) Ameliorates Male Reproductive Dysfunction in Alloxan-Induced Diabetic Rats

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Purpose: Diabetes mellitus is a chronic hyperglycemic condition leading to metabolic problems that cause organ damage and result in serious complications. Long-term complications of diabetes can cause serious health problems, such as sexual and reproductive dysfunction in men and women. Black pepper (*Piper nigrum* L.) is a medicinal plant proven to increase testosterone hormone levels, sexual function (libido), and spermatogenesis parameters in male rats. In this study, the effect of black pepper fruit extract on sexual function and reproductive function (fertility) in alloxan-induced diabetic male rats was evaluated.

Materials and Methods: A total of 30 male Sprague Dawley rats (*Rattus norvegicus*), aged 2.5 to 3 months and weighing 100-150 g, were divided into five groups of 6 individuals each. Group I consisted of rats given only standard feed. Group II consisted of alloxan-induced hyperglycemic rats given standard feed. Groups III and IV were alloxan-induced hyperglycemic rats given black pepper extract at 122.5 and 245 mg/kg BW, respectively, for 8 days. Group V consisted of alloxan-induced hyperglycemic rats given sildenafil therapy or zinc plus ascorbic acid. After 8 days of treatment, the erectile function and libido of the rats were assessed, followed by an evaluation of spermatozoa and testicular histology. Data were analyzed using ANOVA with significance set at $p < 0.05$.

Results: Alloxan-induced diabetic rats showed significant impairment in erectile function, libido, sperm quality, and testicular histology ($p < 0.001$ vs. control). Administration of black pepper extract at a dose of 122.5 mg/kg BW increased total penile reflexes (9.33 ± 1.03 vs. 6.00 ± 1.26 in the diabetes group, $p = 0.002$) and improved libido, with a decrease in courtship latency (5.50 ± 0.55 s vs. 21.00 ± 9.47 s, $p = 0.013$) and an increase in mounting frequency (18.05 ± 5.99 vs. 7.17 ± 1.83 , $p = 0.009$). The extract dose of 245 mg/kg BW increased sperm concentration (158.16 ± 29.80 vs. $12.6 \pm 1.3 \times 10^6$, $p < 0.001$), progressive sperm motility ($65.0 \pm 35\%$ vs. $27.0 \pm 30\%$, $p = 0.006$), and normal sperm morphology ($82.9 \pm 5.7\%$ vs. $35.0 \pm 10.8\%$, $p < 0.001$). The number of Leydig cells increased significantly in the 122.5 mg/kg BW extract group (59.33 ± 4.08 vs. 30.50 ± 3.86 ; $p < 0.001$) compared to the diabetes group. There was no significant difference in spermatocyte count (640 ± 86.5 vs. 491 ± 37.0 ; $p = 0.119$).

Conclusion: *Piper nigrum* fruit extract ameliorates sexual dysfunction and reproductive impairment in alloxan-induced diabetic rats, particularly at 122.5 mg/kg BW, with significant improvements in erectile function, libido, sperm quality, and testicular histology. These findings suggest its potential as a natural therapeutic agent for diabetes-related male reproductive dysfunction.

Keywords: diabetes mellitus; hyperglycemia; black pepper; *piper nigrum*; erectile function; libido; spermatozoa count; spermatocyte count

INTRODUCTION

Diabetes mellitus (DM) is one of the most common chronic diseases in the world, characterized by carbohydrate metabolism disorders. There will be approximately 589 million people aged 20 to 79 years suffering from diabetes mellitus in 2024. Diabetes mellitus can be caused by disorders in insulin production, cellular resistance to insulin, or both. This chronic hyperglycemic condition can interact with other metabolic

problems in people with diabetes mellitus, causing organ damage and resulting in serious complications.^(1,2) These complications include microvascular conditions such as retinopathy, nephropathy, and neuropathy, as well as macrovascular conditions causing coronary artery, peripheral artery, and cerebrovascular diseases.⁽³⁾ Long-term complications of diabetes can cause serious health problems, one of which is sexual dysfunction in men and women. In men, sexual dysfunction due to diabetes manifests as erectile dysfunction, with a prev-

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Table 1. Erectile Function Parameters of Studied Rats

Group	QF	LF	ER	TPR	TPR p-value#
I (only fed)	3.67 ± 0.82 ^a	1.33 ± 0.52 ^a	4.67 ± 0.82 ^a	9.67 ± 1.03 ^a	vs II = <0.001 ; vs III = 0.246 ; vs IV = 1.000 ; vs V = 0.135
II (alloxan)	1.83 ± 0.75 ^a	0.33 ± 0.52 ^a	3.83 ± 0.75 ^a	6.00 ± 1.26 ^a	vs I = <0.001 ; vs III = 0.02 ; vs IV = <0.001 ; vs V = 0.038
III (extract 122.5 mg/kg BW)	3.33 ± 1.03 ^a	1.67 ± 0.52 ^a	4.33 ± 0.52 ^a	9.33 ± 1.03 ^a	vs I = 0.246 ; vs II = 0.02 ; vs IV = 0.747 ; vs V = 1.000
IV (extract 245 mg/kg BW)	2.83 ± 0.75 ^a	0.67 ± 0.82 ^a	3.33 ± 0.82 ^a	6.83 ± 0.98 ^a	vs I = 1.000 ; vs II = <0.001 ; vs III = 0.747 ; vs V = 0.436
V (sildenafil citrate)	2.17 ± 0.75 ^a	1.33 ± 0.52 ^a	4.67 ± 0.52 ^a	8.17 ± 1.17 ^a	vs I = 0.135 ; vs II = 0.038 ; vs III = 1.000 ; vs IV = 0.436

QF = Quick Flip, LF = Long Flip, ER = Erection, TPR = Total Penile Reflexes, a = Presented in mean and Standard Deviation (SD), # = LSD Post Hoc test

alence 3.5 times higher than in men without diabetes.⁽⁴⁾ Erectile dysfunction in men with diabetes mellitus is also associated with decreased fertility due to hypothalamic-pituitary-gonadal axis dysfunction, as well as spermatogenesis and maturation disorders.⁽⁵⁾ Erectile dysfunction and reproductive disorders in men are related to testosterone levels. Many studies have shown that testosterone deficiency is common in men with diabetes, including both type 1 and type 2 diabetes. In people with type 2 diabetes, there is a decrease in free testosterone of up to 57%, while in people with type 1 diabetes, the decrease in free testosterone reaches 20.3%.^(6,7) This further demonstrates that the impact of DM can interfere with erectile function and male reproductive function.

Apart from testosterone serving as a risk factor for diabetes, low testosterone levels are known to cause decreased sexual function and fertility in men. Low sexual function is characterized by a loss of erection, decreased sexual desire, and a decreased frequency of sexual intercourse.⁽⁸⁾ Research on animal models has shown that testosterone is also a determining factor in male fertility because it affects spermatogenesis. The critical processes in spermatogenesis that are influenced by testosterone include maintaining the blood-testis barrier, supporting the meiosis process, facilitating the adhesion of spermatids to Sertoli cells, and promoting the release of sperm.⁽⁹⁾

Currently, there are many types of drugs commonly used to treat DM, including metformin, sulfonylureas, meglitinides, thiazolidinediones, and DPP-4 inhibitors. Unfortunately, all of these drugs have side effects. Metformin, for example, causes side effects such as nausea, vomiting, abdominal bloating, diarrhea, heartburn, headache, agitation, dizziness, tiredness, chills, abdominal cramps or pain, loss of appetite, asthenia, and myalgia.⁽¹⁰⁾ Therefore, the search for DM drugs derived from plants continues to grow. One of the medicinal plants that has the potential to possess anti-diabetic properties is black pepper (*Piper nigrum* L.). Tests on alloxan-induced diabetic rats showed that black pepper fruit extract was effective in lowering blood sugar levels and

was also effective in lowering cholesterol levels.^(11,12) Apart from having potential as an anti-diabetic agent, black pepper fruit extract has also been proven to increase testosterone hormone levels, sexual function (libido), and spermatogenesis parameters in male rats. Mating tests on male rats given black pepper fruit extract showed a significant increase in libido, indicated by a shorter courtship latency.⁽¹³⁾ The fertility parameters of male rats given black pepper fruit extract also increased, indicated by an increase in epididymal sperm concentration, spermatocyte counts, spermatid counts, and the weight of epididymal tubules.⁽¹⁴⁾ Although black pepper has shown antidiabetic and reproductive benefits, its effect on DM-related erectile dysfunction is not well established. Specifically, its influence on erectile function, libido parameters, spermatozoa parameters, and testicular histology parameters has not been fully evaluated. To our knowledge, this is the first study to demonstrate the potential of black pepper (*Piper nigrum* L.) fruit extract to improve sexual function, as depicted by libido parameters, spermatozoa parameters, and testicular histology parameters, in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Study Design

This study was an experimental, randomized controlled laboratory investigation to assess the effects of black pepper (*Piper nigrum* L.) fruit extract on erectile function, libido parameters, spermatozoa parameters, and testicular histology parameters in alloxan-induced diabetic rats. Rats were randomly assigned into control, diabetic, extract-treated, and positive control groups to allow for a direct comparison of treatment outcomes. Data were collected and analyzed using IBM SPSS Statistics version 31 software (New York, USA). The normality of the data was assessed with the Shapiro-Wilk test. Differences between groups were evaluated using One-Way Analysis of Variance (ANOVA), followed by a Least Significant Difference (LSD) post hoc test to identify specific intergroup differences. A *p*-value of < 0.05 was considered statistically significant.

Table 2. Libido Parameters of Courtship Latency (CL) and Mount Latency (ML) of Studied Rats

Group	CL (s)	CL p-value^	ML (s)	ML p-value#
I (only fed)	5.17 ± 0.41a	vs II = 0.003 ; vs III = 0.060 ; vs IV = 0.241 ; vs V = 0.005	8.50 ± 3.51a	vs II = <0.001 ; vs III = 0.033 ; vs IV = 0.006 ; vs V = <0.001
II (alloxan)	21.00 ± 9.47a	vs I = 0.003 ; vs III = 0.013 ; vs IV = 0.003 ; vs V = 0.003	37.17 ± 6.31avs	vs I = <0.001 ; vs III = 0.009 ; vs IV = <0.001 ; vs V = 0.075
III (extract 122.5 mg/kg BW)	5.50 ± 0.55a	vs I = 0.06 ; vs II = 0.013 ; vs IV = 0.204 ; vs V = 1.000	19.00 ± 10.81a	vs I = 0.033 ; vs II = 0.009 ; vs IV = 0.464 ; vs V = 0.006
IV (extract 245 mg/kg BW)	7.00 ± 1.10a	vs I = 0.241 ; vs II = 0.003 ; vs III = 0.204 ; vs V = 0.019	27.67 ± 6.53avs	vs I = 0.006 ; vs II = <0.001 ; vs III = 0.464 ; vs V = 0.026
V (sildenafil citrate)	9.00 ± 4.82a	vs I = 0.005 ; vs II = 0.003 ; vs III = 1.000 ; vs IV = 0.019	16.00 ± 8.27avs	vs I = <0.001 ; vs II = 0.075 ; vs III = 0.007 ; vs IV = 0.026

CL = Courtship Latency, ML = Mount Latency, a = Presented in mean and Standard Deviation (SD), ^ = Mann-Whitney test, # = LSD Post Hoc test, (s) = time in seconds

Table 3. Mount Frequency (MF) of Studied Rats

Group	MF (n)	MF p-value#
I (only fed)	17.00 ± 3.74 ^a vs II = <0.001 ;	vs III = 0.351 ; vs IV = 0.649 ; vs V = 0.157
II (alloxan)	7.17 ± 1.83 ^a vs I = <0.001 ;	vs III = 0.002 ; vs IV = <0.001 ; vs V = 0.006
III (extract 122.5 mg/kg BW)	18.05 ± 5.99 ^a vs I = 0.351 ;	vs II = 0.002 ; vs IV = 0.17 ; vs V = 0.615
IV (extract 245 mg/kg BW)	13.33 ± 3.45 ^a vs I = 0.649 ;	vs II = <0.001 ; vs III = 0.17 ; vs IV = 0.066
V (sildenafil citrate)	14.83 ± 5.74 ^a vs I = 0.157 ;	vs II = 0.006 ; vs III = 0.615 ; vs IV = 0.066

MF = Mount Frequency, ^a= Presented in mean and Standard Deviation (SD), # = LSD Post Hoc test, (n) = number of mountings in 30 minutes

Plant Preparation

Fresh black pepper fruit was collected from a farmer in Ngarip Village, Ulubelu District, Tanggamus Regency, Lampung Province. The fresh fruit was washed and rinsed until clean, and then dried. After drying, the fruit was ground into powder using a blender. Extraction was performed by soaking the black pepper powder in 95% ethanol at room temperature. The supernatant was collected every 24 hours for three days and filtered to remove unwanted components. The filtrate was concentrated using a rotary evaporator at a temperature of 40°C and a pressure of 60 mbar. The extract was stored in a refrigerator as stock until used.

Animal Preparation

This study utilized 30 male Sprague Dawley rats (*Rattus norvegicus*), aged 2.5-3 months and weighing 100-150 grams, obtained from the animal house at IPB University, Bogor, Indonesia. All procedures were conducted in accordance with and approved by the guidelines of the Health Research Ethics Commission of the Faculty of Medicine, Lampung University (No.3468/UN26.18/PP.05.02.00/2024). The rats were acclimated for a week at a temperature of 25°C with stable room humidity and lighting, and were given standard feed ad libitum.

The studied rats were divided into five groups of 6 individuals each. Group I consisted of rats that were given only standard feed. Group II consisted of alloxan-induced hyperglycemic rats given standard feed. Groups III and IV were alloxan-induced hyperglycemic rats given black pepper extract at 122.5 and 245 mg/kg BW, respectively, for 8 days. Group V consisted of alloxan-induced hyperglycemic rats given sildenafil at 1 mg/kg BW or zinc plus ascorbic acid at 1 mg/kg BW.

Alloxan induction in the studied rats was performed intraperitoneally using normal saline solvent at a dose of 150 mg/kg BW. To prevent alloxan-induced rats from suffering from severe hypoglycemia, the rats were given a 20% glucose solution (5-10 ml) orally after 6 hours. Furthermore, after being maintained for 24 hours, the rats were given an additional 5% glucose solution to prevent hypoglycemia. Rats were categorized as having DM if they experienced glycosuria and hyperglycemia with blood glucose levels of 200 to 300 mg/dL.⁽¹⁵⁾

Phosphodiesterase-5 inhibitor (PDE-5i) therapy was administered one hour before the observation of erectile function and libido assessment. The therapy utilized Sildenafil Citrate, a drug from the PDE-5i class, at a dose of 1 mg/kg BW as a positive control for the recovery of erectile dysfunction caused by DM.⁽¹⁶⁾ Sildenafil treatment was performed following Gurbuz et al. to decrease advanced glycation end products, malondialdehyde (MDA), and inducible nitric oxide synthase (iNOS), and to preserve neuronal nitric oxide synthase (nNOS) and cyclic guanosine monophosphate (cGMP) contents in penile tissue.⁽¹⁷⁾

Erectile Function Assessment

The erectile function of the rats was assessed on the ninth day, one hour before the mating test was performed. The animals were placed in a supine position with partial restraint. The preputial sheath was pushed behind the glans penis and held in this manner for a period of 15 minutes to elicit the penile reflex. Total Penile Reflexes (TPR) for each animal were calculated as the sum of Erection (E), Long Flips (LF), and Quick Flips (QF). Mathematically, it can be expressed using the following formula: $TPR = E + QF + LF$.^(18,19)

Libido Assessment

To determine the libido level of the studied rats, a mating test was conducted on the ninth day. The mating test was conducted by placing male and female rats in a cage with a partition in the middle. The cage was placed in a room with low lighting. Observation and analysis of the mating behavior of the rats were conducted through video recording. Three mating behavior parameters were assessed: courtship latency, mounting latency, and mounting frequency. Courtship latency is the time from the partition being opened until the male sniffs the female's genitals or other body parts. Mounting latency is the time from the partition being opened until the male mounts the female's back. Mounting frequency is the number of times the male mounts the female's back during the 30 minutes (1800 seconds) of the mating test.

Sperm Analysis and Testis Histology Examination

After erectile function and mating behavior were assessed, sperm and histological analyses of the studied

Table 4. Sperm Analysis Parameters of Sperm Concentration and Sperm Progressive Motility of Studied Rats

Group	SC (10 ⁶)	SC p-value#	SPM (%)	SPM p-value#
I (only fed)	75.81 ± 52.9 ^a	vs II = 0.002 ; vs III = 0.004 ; vs IV = 0.483 ; vs V = <0.001	57.0 ± 33.0 ^a	vs II = 0.024 ; vs III = 0.07 ; vs IV = 0.121 ; vs V = 0.556
II (alloxan)	12.6 ± 1.3 ^a	vs I = 0.002 ; vs III = 0.877 ; vs IV = 0.01 ; vs V = <0.001	27.0 ± 30.0 ^a	vs I = 0.024 ; vs III = 0.697 ; vs IV = 0.436 ; vs V = 0.006
III (extract 122.5 mg/kg BW)	62.95 ± 29.4 ^a	vs I = 0.483 ; vs II = 0.01 ; vs V = 0.019 ; vs IV = <0.001	36.6 ± 23.0 ^a	vs I = 0.121 ; vs II = 0.436 ; vs V = 0.721 ; vs IV = 0.037
IV (extract 245 mg/kg BW)	158.16 ± 29.8 ^a	vs I = <0.001 ; vs II = <0.001 ; vs V = <0.001 ; vs III = <0.001	65.0 ± 35.0 ^a	vs I = 0.556 ; vs II = 0.006 ; vs V = 0.021 ; vs III = 0.037
V (Zinc plus ascorbic acid)	19.2 ± 6.7 ^a	vs I = 0.004 ; vs II = 0.877 ; vs III = 0.019 ; vs IV = <0.001	31.8 ± 23.0 ^a	vs I = 0.07 ; vs II = 0.697 ; vs III = 0.721 ; vs IV = 0.021

SC = Sperm Concentration, SPM = Sperm Progressive Motility, ^a= Presented in mean and Standard Deviation (SD), # = LSD Post Hoc test

Table 5. Sperm Analysis Parameters of Normal Sperm Morphology of Studied Rats

roup	SNM (%)	SNM <i>p</i> -value#
I (only fed)	75.0 ± 13.7 ^a	vs II = <0.001 ; vs III = <0.001 ; vs IV = 0.119 ; vs V = 0.273
II (alloxan)	35.0 ± 10.8 ^a	vs I = <0.001 ; vs III = 0.04 ; vs IV = <0.001 ; vs V = <0.001
III (extract 122.5 mg/kg BW)	62.0 ± 14.7 ^a	vs I = 0.119 ; vs II = <0.001 ; vs V = 0.017 ; vs IV = 0.011
IV (extract 245 mg/kg BW)	82.9 ± 5.7 ^a	vs I = 0.273 ; vs II = <0.001 ; vs V = <0.001 ; vs III = 0.011
V (Zinc plus ascorbic acid)	40.9 ± 7.8 ^a	vs I = <0.001 ; vs II = 0.04 ; vs III = 0.017 ; vs IV = <0.001

SNM = Sperm Normal Morphology, ^a= Presented in mean and Standard Deviation (SD), # = LSD Post Hoc test

rats were performed. The sperm analysis and testis histology examination were conducted in the Anatomy Pathology Laboratory of the Faculty of Medicine, Lampung University. The rats were terminated via an injection of ketamine at a dose of 100 mg/kg BW and cervical dislocation. A total of 30 right testes (testicular weight between 800 mg and 1000 mg) and cauda epididymides were removed using a dissecting kit. The cauda epididymides were pinched to squeeze the spermatozoa into a petri dish. Subsequently, a suspension of spermatozoa was formed after being mixed with 0.9% NaCl, as mentioned in previous studies.⁽²⁰⁾ Spermatozoa were counted using a Neubauer hemocytometer under a light microscope (400x) and expressed as millions/ml. Sperm motility was evaluated by counting motile and immotile sperm. Sperm morphology was assessed from a smear of the epididymal filtrate prepared on a clean glass slide with 1% eosin staining. After the object dried, observation was performed under a Richter Optica HS-3B-3 light microscope (China) at 400x magnification, and abnormalities of either the head or tail were noted. Testicular histology examination was performed using Hematoxylin & Eosin (HE) staining, and spermatocyte were counted in 10 random seminiferous tubule cross-sections per testis. Leydig cells were counted in 10 non-overlapping interstitial fields with a thickness of 4-5 µm; counts were normalized to area using National Institutes of Health ImageJ software version 1.8.0 (New York, USA) (calibrated to µm²) at 400x magnification.^(20,21)

RESULTS

Erectile Function

The erectile function parameters of alloxan-induced rats treated with black pepper fruit extract are presented in Table 1. Group I, consisting of normal rats with normal feed, showed the highest average values for the quick flip and erection parameters. As the reference group in this study, Group I also had the highest average TPR values compared to the other groups. Among the alloxan-induced rats, Group III (treated with black pepper extract at a dose of 122.5 mg/kg BW) demonstrated av-

erage quick flip, long flip, and erection values that were comparable to those of Group I. The average TPR value in Group III was also similar to that of Group I. However, Group IV, which received a higher dose of black pepper extract (245 mg/kg BW), showed relatively lower average values for quick flip, long flip, and erection compared to Group III, with TPR values more comparable to those of Group II. As shown in Table 1, Group III had relatively higher average TPR values compared to Group V. The treatment effect was observed only in the TPR parameter, whereas the differences in ER, QF, and LF parameters were not statistically significant. Diabetic rats given black pepper fruit extract at a dose of 122.5 mg/kg BW (Group III) showed statistically significant differences in TPR values compared to alloxan-induced rats with normal feed (Group II).

Libido

The effect of administering black pepper extract on the libido of rats using the parameters of courtship latency, mounting latency, and mounting frequency can be seen in Table 2 and Table 3. Courtship Latency (CL) and Mount Latency (ML) showed significant differences among groups. The alloxan group (II) had the longest CL (21.00 ± 9.47, *p* = 0.003 vs. Group I) and ML (37.17 ± 6.31, *p* < 0.001 vs. Group I). In contrast, the extract 122.5 mg/kg BW group (III) demonstrated a CL of 5.50 ± 0.55 and an ML of 19.00 ± 10.81, both significantly different from Group II (*p* = 0.013 and *p* = 0.009, respectively) and closer to Group I. The extract 245 mg/kg BW group (IV) also showed shorter CL (7.00 ± 1.10) and ML (27.67 ± 6.53) compared with Group II (*p* = 0.003 and *p* < 0.001, respectively). The sildenafil group (V) exhibited a CL of 9.00 ± 4.82 and an ML of 16.00 ± 8.27, with significant differences from Group II (*p* = 0.003 and *p* = 0.075, respectively). Mount Frequency (MF) was lowest in Group II (7.17 ± 1.83), which was significantly different from Group I (17.00 ± 3.74, *p* < 0.001). Group III (18.05 ± 5.99) was not significantly different from Group I (*p* = 0.351), while Group IV (13.33 ± 3.45) and Group V (14.83 ± 5.74) showed higher MF compared with Group II (*p* < 0.001 and *p* = 0.006, respectively).

Table 6. Testicular Histology Parameters.

Group	LC	LC <i>p</i> -value	SgC	SgC <i>p</i> -value
I (only fed)	60.83 ± 5.1 ^a	vs II = <0.001 ; vs III = <0.001 ; vs IV = 0.581 ; vs V = 0.001	511 ± 73.4a	vs II = 0.593 ; vs III = <0.001 ; vs IV = 0.294 ; vs V = 0.013
II (alloxan)	30.50 ± 3.8 ^a	vs I = <0.001 ; vs III = 0.035 ; vs IV = <0.001 ; vs V = 0.001	491 ± 37.0a	vs I = 0.593 ; vs III = <0.001 ; vs IV = 0.119 ; vs V = 0.042
III (extract 122.5 mg/kg BW)	59.33 ± 4.0 ^a	vs I = <0.001 ; vs II = 0.035 ; vs IV = <0.001 ; vs V = <0.001	640 ± 86.5a	vs I = 0.294 ; vs II = 0.119 ; vs V = <0.001 ; vs IV = 0.001
IV (extract 245 mg/kg BW)	50.67 ± 2.7 ^a	vs I = 0.581 ; vs II = <0.001 ; vs III = <0.001 ; vs IV = 0.003	414 ± 32.5a	vs I = 0.013 ; vs II = 0.042 ; vs V = 0.014 ; vs III = 0.001
V (Zinc plus ascorbic acid)	34.8 ± 7.5 ^a	vs I = 0.001 ; vs II = <0.001 ; vs III = <0.001 ; vs IV = 0.003	319 ± 64.5a	vs I = <0.001 ; vs II = <0.001 ; vs III = <0.001 ; vs IV = 0.014

LC = Leydig Cell Count, SgC = Spermatogonium Cell Count, ^a = Presented in mean and Standard Deviation (SD), # = LSD Post Hoc test

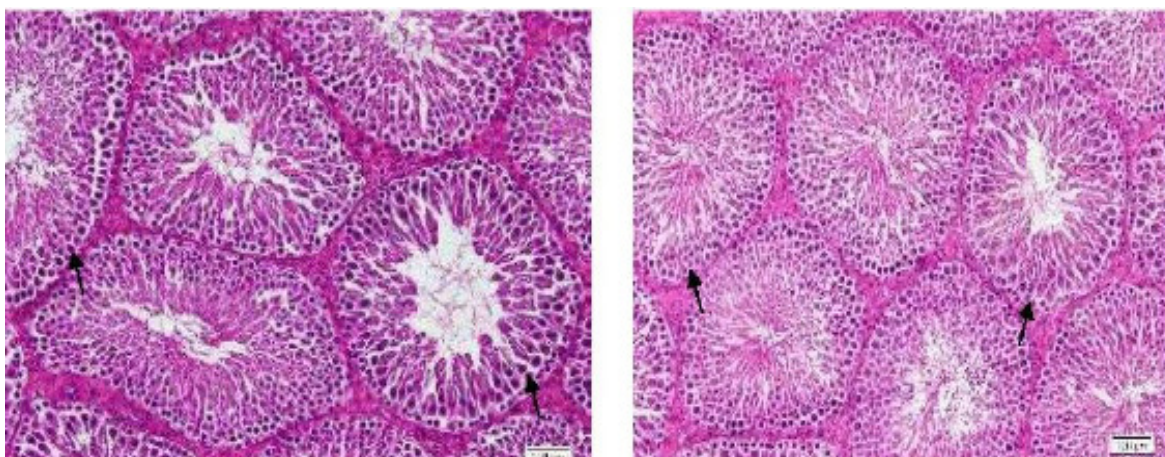


Figure 1. Higher amounts of spermatocyte were observed in Group III with black pepper extract at 122.5 mg/kg BW (A) as compared to Group II without black pepper extract (B).

Sperm Analysis

The results of the assessment of spermatozoa parameters of alloxan-induced hyperglycemic rats given black pepper fruit extract are presented in Table 4 and Table 5. Diabetic rats given black pepper extract at a dose of 245 mg/kg BW (Group IV) showed a significant increase in sperm concentration and motility. Normal sperm morphology in diabetic rats given black pepper extract at a dose of 245 mg/kg BW also significantly increased compared to rats given other treatments.

The diabetic control group (II) had reduced sperm concentration ($12.6 \pm 1.3 \times 10^6$), low progressive motility ($27.0 \pm 30\%$), and lower normal morphology ($35.0 \pm 10.8\%$). Black pepper extract at 245 mg/kg BW (Group IV) significantly enhanced sperm concentration ($158.16 \pm 29.80 \times 10^6$, $p = 0.001$ vs. Group II) and progressive motility ($65.0 \pm 35\%$, $p = 0.006$ vs. Group II), while increasing normal sperm morphology ($82.9 \pm 5.7\%$, $p < 0.001$ vs. Group II). At the 122.5 mg/kg BW dose (Group III), improvements in concentration ($62.95 \pm 29.40 \times 10^6$) and progressive motility ($36.6 \pm 23\%$) were modest and not significant, while normal sperm morphology decreased ($62.0 \pm 14.7\%$).

Testicular Histology Analysis

Table 6 presents the examination results of the testicular histology of alloxan-induced diabetic rats treated with black pepper extract. The data in the table show that black pepper extract at a dose of 122.5 mg/kg BW significantly restored the number of Leydig cells and the spermatocyte count. The diabetic control group (II) exhibited the lowest Leydig cell count (30.50 ± 3.86) and reduced spermatocyte count (491 ± 37.0). Administration of black pepper extract at 122.5 mg/kg BW (Group III) significantly restored Leydig cell numbers (59.33 ± 4.08 , $p = 0.035$ vs. Group II) and spermatocyte count (640 ± 86.5 , $p = 0.119$ vs. Group II), which were comparable to normal controls. The higher extract dose (Group IV: 50.67 ± 2.73 ; 414 ± 32.5) improved Leydig cells but significantly reduced spermatogonia compared to Group III. Group V did not significantly restore Leydig cells (34.8 ± 7.55) and spermatocyte (319 ± 64.59), remaining close to the diabetic controls.

DISCUSSION

The present study demonstrated that the administration of *Piper nigrum* fruit extract ameliorated reproductive parameters in alloxan-induced diabetic male rats, as evidenced by increased sperm concentration, motility, serum testosterone levels, and improved morphology of seminiferous tubules. These findings are consistent with previous reports showing that black pepper extract can enhance fertility potential and improve reproductive outcomes in diabetic or hyperglycemic animal models, most likely through the attenuation of oxidative stress and the restoration of metabolic status.^(20,21)

The decline in erectile function in alloxan-induced diabetic rats may be related to insulin resistance. Insulin resistance is known to be a cause of erectile dysfunction and hypogonadism.⁽²²⁾ Persistent hyperglycemia triggers Endoplasmic Reticulum Stress (ERS) in the Leydig cells of the testis, causing increased expression of ERS-related factors, resulting in Leydig cell apoptosis and disrupting testosterone production, which leads to decreased erectile function.^(23,24) Insulin deficiency in diabetes mellitus also causes excessive secretion of arginase II in the corpus cavernosum so that Nitric Oxide (NO) cannot function properly, resulting in inadequate blood flow to the penis. This can trigger erectile dysfunction.^(25,26)

It can be seen that the administration of black pepper fruit extract significantly improved the decreased libido due to hyperglycemia caused by alloxan induction. Based on the values of courtship latency, mounting latency, and mounting frequency, it was revealed that the best dose of black pepper extract to restore the libido of hyperglycemic male rats was a dose of 122.5 mg/kg BW. The therapeutic effect of sildenafil citrate also significantly improved the libido of the rats.

The study revealed that alloxan induction (Group II) significantly decreased libido parameters in male rats. According to various reports, this occurred because hyperglycemic conditions caused severe gonadal dysfunction, reproductive dysfunction, and diminished body and reproductive organ weight.⁽²⁷⁾

Male rats with alloxan-induced hyperglycemia treated with black pepper extract were found to have improved sexual function. This recovery is very likely due to the influence of piperine in the black pepper fruit. Piper-

ine is an active ingredient typically found in Piperaceae plants. Piperine extracted from the fruit of the *Piper retrofractum* L. plant, for example, is known to have an aphrodisiac effect on the libido of male rats.⁽²⁸⁾

The restoration of sexual function by black pepper fruit extract is related to the effect of piperine, which can inhibit the action of testosterone 5 α -reductase so that testosterone levels remain high. In addition, black pepper fruit extract is also known to contain fatty acids, such as lauric acid, myristic acid, and palmitic acid, which affect androgen secretion and metabolism. Black pepper fruit extract is also known to contain zinc, which promotes increased sex hormones, including testosterone in serum.^(13,14) Testosterone is a hormone that has been proven to increase sexual desire or libido in hypogonadal men.⁽²⁹⁾

This study shows that alloxan induction in male rats (Group II) causes a significant decrease in spermatozoa concentration and Leydig cell count compared to normal rats (Group I). This may be related to endocrine imbalance and impaired glucose transport and metabolism in diabetic rats. Endocrine imbalance—dysfunction of the HPG axis—occurs in diabetic patients and can cause infertility. Hyperglycemia exposure causes decreased sensitivity of the pituitary to gonadotropin-releasing hormone (GnRH) stimulation, resulting in abnormal secretion of FSH and LH. FSH plays a role in stimulating testicular Sertoli cells to promote sperm maturation in the seminiferous tubules, while LH plays a role in stimulating Leydig cells to increase serum testosterone levels. This decreased sensitivity of the pituitary to GnRH causes disorders in the male reproductive system.⁽²⁷⁾

This research showed that the administration of black pepper fruit extract at a dose of 245 mg/kg to alloxan-induced diabetic rats significantly increased the number, motility, and normal morphology of spermatozoa. On the other hand, the administration of black pepper extract at a concentration of 122.5 mg/kg significantly increased the number of Leydig cells and the number of spermatogonia. This result is not surprising because the study reported by Chinta et al. showed that the administration of piperine to rats causes hormonal imbalance by altering the serum levels of follicle-stimulating hormone, luteinizing hormone, sex hormone-binding globulin, and both serum and testicular testosterone.⁽³⁰⁾

Other research results showing piperine effects that are in line with the findings of this study were reported by Chen et al. Piperine increased serum testosterone, Leydig cell number, cell size, multiple steroidogenic pathway proteins, 3 β -hydroxysteroid dehydrogenase 1, 17 α -hydroxylase/20-lyase, and steroidogenic factor 1 expression levels. Piperine stimulates pubertal Leydig cell development by increasing the number of Leydig cells and promoting their maturation. However, because piperine reduces FSH, it inhibits spermatogenesis.⁽³¹⁾ Another researcher (Ere et al.) reported that the administration of black pepper fruit extract to rabbits increased testicular morphometric parameters, but caused damage to the seminiferous tubules and decreased spermatogenesis.⁽³²⁾

CONCLUSIONS

The administration of black pepper fruit extract to male alloxan-induced diabetic rats significantly increased erectile function, libido, spermatozoa count, Leydig

cell count, and spermatocyte count. Therefore, it can be concluded that black pepper extract (*Piper nigrum* L.) has potential as an ingredient to overcome sexual dysfunction and reproductive dysfunction in males.

SUMMARY

This study shows that black pepper extract improves sexual and reproductive functions, such as erection, libido, and sperm quality, in diabetic male rats. It offers a potential natural treatment for diabetes-related male reproductive issues.

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None declared.

CONFLICT OF INTEREST

There is no conflict of interest.

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