TRANSPUBLIKA
Precise. Resilience. Felicitous.

Pharmacology, Medical Reports, Orthopedic, and Illness Details
https://ojs.transpublika.com/index.php/COMORBID
Online ISSN 2809-9745

https://doi.org/10.55047/comorbid.v4i4.1935

Original Article

Aphrodisiac Effect of Ethanol Extract of Tapak Liman *(Elephantopus scaber* L.*)* Leaves on Testosterone Levels and Testicular Histology in Rats

Yuneka Saristiana[1*], Novyananda Salmasfattah[2], Fendy Prasetyawan[3], Lisa Savitri[4]

[1-3]Pharmacist Professional Education Study Program, Faculty of Health Sciences, Universitas Kadiri, Indonesia
[4]Medical Laboratory Technology, Faculty of Health Sciences, Universitas Kadiri, Indonesia
Email: [1)] yunekasaristiana@gmail.com, [2)] novynandasalmasfattah@gmail.com, [3)] Fendy.pra@gmail.com, [4)] lisasavitri@unik-kediri.ac.id

Received : 25 August - 2025
Accepted : 05 October - 2025
Published online : 10 October - 2025

Abstract

The demand for aphrodisiac use is substantial, however, many marketed herbal products contain synthetic drugs, underscoring the urgent need for safer natural therapeutic alternatives. This study aimed to evaluate the aphrodisiac potential of ethanol extract of *Elephantopus scaber* (Tapak Liman) leaves on serum testosterone levels and testicular histology in male Wistar rats. The extract was obtained through maceration with 70% ethanol and subjected to phytochemical screening, which revealed the presence of flavonoids, tannins/phenolics, and triterpenoids. Healthy male Wistar rats were divided into five groups: normal control, negative control, positive control (sildenafil), and two treatment groups receiving 100 mg/kg BW and 200 mg/kg BW of the extract for 21 days. Serum testosterone levels were measured using ELISA, while histological examination focused on the diameter of seminiferous tubules. The results demonstrated that the 200 mg/kg BW dose significantly increased testosterone levels (209.41 ± 0.62 ng/dL) compared with the negative control (120.32 ± 1.55 ng/dL) and restored the diameter of seminiferous tubules (220.56 ± 4.98 µm) close to normal conditions (225.30 ± 5.12 µm). The 100 mg/kg BW dose produced a moderate effect, while the positive control also showed notable improvement. The observed increase in testosterone and recovery of seminiferous tubule diameter indicate that the extract enhances spermatogenic activity and testicular function. In conclusion, the ethanol extract of *E. scaber* leaves at 200 mg/kg BW demonstrated significant aphrodisiac activity through hormonal stimulation and improvement of testicular histology, suggesting its potential as a natural agent to support male reproductive health.

Keywords: Aphrodisiac, *Elephantopus Scaber*, Reproductive Health, Seminiferous Tubule, Testosterone.

1. Introduction

Aphrodisiacs are defined as substances or agents that stimulate or enhance sexual desire, libido, and reproductive function. According to Katzung in Basic and Clinical Pharmacology, aphrodisiacs act through central nervous system stimulation or by increasing sex hormones such as testosterone (Vanderah, 2024). These agents may be derived from synthetic chemicals or natural sources such as medicinal plants. Natural aphrodisiacs are believed to cause fewer side effects compared to synthetic ones (Brunetti et al., 2020). Their pharmacodynamic effects include improved blood circulation, neurotransmitter release, and endocrine hormone elevation. Some aphrodisiacs also act through antioxidant mechanisms

Copyright: © 2025 by the authors.
This is an open access article distributed under the terms and conditions of the CC BY 4.0.

that reduce oxidative stress in reproductive organs. Therefore, aphrodisiacs are considered important topics in the development of safer therapies for sexual dysfunction.

Aphrodisiacs play a crucial role in maintaining reproductive health, especially among men with reduced libido and sexual dysfunction. Sexual dysfunction negatively affects quality of life, self-confidence, and interpersonal relationships. This condition is often linked to declining testosterone levels due to aging or unhealthy lifestyles. The use of aphrodisiacs aims to normalize sexual function, improve sperm production, and enhance vitality. In addition, aphrodisiacs may provide further benefits such as mood enhancement, stress reduction, and improved physical performance. In holistic medicine, aphrodisiacs are regarded as part of energy restoration and hormonal balance therapy (Elterman et al., 2021).

The demand for aphrodisiacs continues to rise in line with increasing awareness of the importance of sexual quality of life. Modern lifestyles, work-related stress, lack of exercise, and poor diet contribute to growing cases of sexual dysfunction. Many men are seeking safe and effective natural solutions. This demand has driven the popularity of herbal-based aphrodisiacs in the market. Herbal products are perceived as more natural, affordable, and less likely to cause side effects. Yet, such popularity must be balanced with education and scientific evidence. Consumers must be protected from unstandardized and unsafe products. Hence, research on validated herbal aphrodisiacs is essential to meet this market demand.

In contrast, many so-called herbal aphrodisiacs illegally contain pharmaceutical chemicals (also known as *Bahan Kimia Obat* or BKO). These substances are often illegally added to herbal products to enhance their pharmacological effects. Substances such as sildenafil, tadalafil, and nitrate derivatives are often added to create rapid effects without proper dosage control or legal authorization. Long-term consumption of these substances may lead to serious adverse effects such as hypotension, cardiovascular disorders, and reproductive organ dysfunction. The Indonesian FDA (BPOM) has reported multiple poisoning cases caused by adulterated herbal products. This highlights gaps in regulation and public awareness regarding the risks of illegal aphrodisiacs. Therefore, the development of natural, safe, and evidence-based alternatives is urgently needed (BPOM RI, 2021).

Various chemical compounds such as flavonoids, steroids, triterpenoids, and sesquiterpene lactones have been reported to possess activities related to the enhancement of sexual function. Several studies have demonstrated that flavonoids and isoflavonoids can promote testicular steroidogenesis (increasing testosterone production), protect Leydig cells from oxidative stress, and improve reproductive parameters or sexual behavior in certain animal models (Martin & Touaibia, 2020). Other studies on steroidal saponins and related compounds have often been associated with elevated testosterone levels and enhanced sexual behavior (Zhu et al., 2017). Triterpenoids and triterpene saponins have also been identified in several species to exhibit sexual function-enhancing activities, with isolation studies reporting their involvement in reproductive activity and improvements in stamina or aphrodisiac effects (Enema et al., 2018).

These findings strengthen the hypothesis that the presence of such bioactive compounds in plants may contribute to aphrodisiac activity. Tapak Liman (*Elephantopus scaber L.*) is a traditional medicinal plant that has been empirically used in the community for various therapeutic purposes, including the improvement of stamina and vitality. Phytochemical analyses have revealed that this plant contains flavonoids, steroids, triterpenoids, and sesquiterpene lactones. Therefore, Tapak Liman holds potential as a herbal candidate with aphrodisiac properties through the synergistic actions of its secondary metabolites. However, to date, no specific studies have been conducted to evaluate the aphrodisiac potential of Tapak

Liman originating especially in East Java region. Hence, further research is required to scientifically validate its ethnomedicinal claims.

Recent pharmacological studies show that Tapak Liman contains a number of key bioactive compounds, particularly sesquiterpene lactones (deoxyelephantopin and its derivatives), as well as flavonoids and phytosterols that have been identified by modern phytochemical studies and shown to have biological activities such as antioxidant, anti-inflammatory, and cytotoxic effects (Djati et al., 2024). In pharmaceutical contexts, it exhibits antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial activities. Its flavonoid and phenolic contents play a key role in protecting cells from oxidative damage, including testicular cells (Martin & Touaibia, 2020). Reports also suggest that Tapak Liman may modulate hormonal systems (Djati et al., 2021). Its steroid compounds are believed to naturally elevate testosterone levels. Nevertheless, scientific evidence supporting its aphrodisiac effects remains limited. In vivo studies are necessary to strengthen its pharmacological basis. This creates opportunities for further research into Tapak Liman as a safe and effective aphrodisiac.

This study employs an in vivo experimental method using ethanolic extracts of Tapak Liman leaves in male Wistar rats. Test animals are divided into negative control, positive control (sildenafil), and two treatment groups with extract doses. The main parameters include serum testosterone levels measured by ELISA and histological structures of testes using HE staining. Statistical analyses such as ANOVA and Tukey's test are applied to validate the results. Microscopic documentation is also conducted to assess spermatogenic cell count and seminiferous tubule diameter. These methods are expected to provide strong evidence for the aphrodisiac efficacy of Tapak Liman extract.

Research on Tapak Liman is expected to create new opportunities for the development of high-quality herbal aphrodisiac products grounded in local wisdom. The findings may provide a strong scientific foundation for the formulation of safe and effective phytopharmaceuticals. Furthermore, the results have the potential to be utilized as teaching materials, contribute to scientific publications, and support future product patent applications. By focusing on testosterone enhancement and improvements in testicular histological structure, this study aims to strengthen the empirical evidence supporting the traditional use of Tapak Liman. Additionally, it encourages the sustainable utilization of Indonesia's rich biodiversity and the preservation of ethnomedicinal knowledge. In the long term, this research is expected to contribute to the development of locally based phytopharmaceuticals and facilitate the integration of traditional medicine into modern healthcare. Through a rigorous scientific approach, it supports the downstreaming of local resources into tangible solutions for male reproductive health. Therefore, this study aims to investigate the aphrodisiac potential of the ethanolic extract of Elephantopus scaber L. leaves by evaluating its effects on serum testosterone levels and testicular histology in male Wistar rats.

2. Literature Review

Aphrodisiacs are substances that enhance libido, reproductive function, and sperm quality through psychogenic, peripheral, and endocrine mechanisms. Testosterone, the principal hormone of the male reproductive system, plays a crucial role in regulating libido, spermatogenesis, and reproductive organ health. Thus, its decline is often associated with hypogonadism, infertility, and sexual dysfunction. Histological assessment, particularly the

diameter of seminiferous tubules, also serves as an important indicator of spermatogenic activity, with changes reflecting either stimulation or impairment of reproductive function.

Several medicinal plants, including *Eurycoma longifolia*, *Tribulus terrestris*, and *Panax ginseng*, have been reported to exert aphrodisiac effects through testosterone enhancement and germ cell protection. *Elephantopus scaber L.* (Tapak Liman), a widely distributed Indonesian herbal plant, contains flavonoids, triterpenoids, and sesquiterpene lactones with antioxidant properties that may support reproductive function, although scientific evidence remains limited. Therefore, this study aims to evaluate the effects of ethanol extract of Tapak Liman leaves on testosterone levels and seminiferous tubule diameter in male Wistar rats.

2.1. Aphrodisiacs and Their Mechanisms

An aphrodisiac is defined as a substance capable of enhancing sexual desire, libido, and reproductive function. Theoretically, aphrodisiacs act through three main mechanisms: (1) stimulation of the central nervous system (psychogenic), (2) increased blood flow to the reproductive organs (peripheral), and (3) hormonal modulation, particularly through the elevation of testosterone levels (Al-Madhagi & Tarabishi, 2024; Nouioura et al., 2024; Ulloa del Carpio et al., 2024). Classical pharmacology literature states that testosterone plays a pivotal role in regulating libido, spermatogenesis, and the development of male reproductive organs (Hall, 2016; Li et al., 2024). Thus, the endocrine mechanism serves as an important theoretical foundation in the development of aphrodisiacs.

2.2. Reproductive System and the Role of Testosterone

The theory of male reproduction highlights the essential role of the testes as the organ responsible for sperm and testosterone production. Leydig cells produce testosterone in response to luteinizing hormone (LH), while Sertoli cells support spermatogenesis through androgen-binding protein (Hall, 2016). Adequate testosterone levels maintain the integrity of spermatogenesis, libido, and reproductive organ health. A decline in testosterone levels (hypogonadism) results in infertility, loss of muscle mass, and erectile dysfunction (Bhasin et al., 2018).

2.3. Assessment of Seminiferous Tubule Diameter under Normal Conditions

The seminiferous tubules are the site of spermatogenesis and represent a critical parameter in evaluating male reproductive health. Under optimal physiological conditions, the diameter of seminiferous tubules in adult Wistar rats ranges between 180–280 µm, with lumens fully occupied by spermatogenic cells at various developmental stages (Abarikwu et al., 2020). An optimal diameter reflects healthy spermatogenic activity, active germ cell regeneration, and continuous sperm production. A reduction in tubule diameter generally indicates impaired spermatogenesis caused by oxidative stress, toxic exposure, or hormonal deficiency, while an increase in diameter may suggest spermatogenic stimulation due to elevated testosterone levels (Sitasiwi et al., 2023). Thus, measuring the diameter of seminiferous tubules serves as a relevant histological indicator in assessing the aphrodisiac effects of a substance.

2.4. Testosterone Levels

Testosterone is the primary androgenic hormone produced by Leydig cells, playing a crucial role in spermatogenesis, libido, and the maintenance of male sexual function. In healthy adult men, normal testosterone levels range from 300–1000 ng/dL (Bhasin et al., 2018). Meanwhile, in male Wistar rats, normal testosterone levels are reported to range from 10–15 nmol/L (≈288–433 ng/dL), with a median value of approximately 13.1 nmol/L (Dalla

et al, 2023). These values indicate a healthy endocrine condition to support optimal spermatogenesis. Decreased testosterone levels are often associated with hypogonadism, chronic stress, or aging, whereas increased levels within the physiological range reflect favorable hormonal stimulation due to pharmacological interventions or administration of herbal aphrodisiacs.

2.5. Previous Research

Several studies have demonstrated that medicinal plants possess aphrodisiac potential. *Eurycoma longifolia* (tongkat ali), *Tribulus terrestris*, *Panax ginseng*, and *Mucuna pruriens* have been reported to enhance libido and sperm quality through hormonal and antioxidant mechanisms (Ara et al., 2023; Choowong-in et al., 2022; Lee et al., 2020; Leisegang et al., 2022). Several medicinal plants have been shown to possess aphrodisiac properties through hormonal, vascular, and neurochemical mechanisms. *Eurycoma longifolia* enhances steroidogenesis and testosterone production through its quassinoid content, while *Tribulus terrestris* contains steroidal saponins such as protodioscin that modulate androgen levels and improve libido. *Panax ginseng* is rich in ginsenosides that stimulate nitric oxide pathways, enhance penile vasodilation, and exert antioxidant effects, whereas *Mucuna pruriens* contains L-DOPA, which enhances central dopaminergic activity and improves sexual behavior. In addition to these hormonal and neurotransmitter-mediated effects, many of these plants also contain flavonoids and phenolic compounds that protect testicular tissue from oxidative stress and support spermatogenesis. Tapak liman contains sesquiterpene lactones, flavonoids, terpenoids, and saponins compound classes associated with antioxidant and steroidogenic effects (Jamal & Jose, 2017; Martin & Touaibia, 2020). The similarity in phytochemical profiles between *E. scaber* and established aphrodisiac plants provides a strong rationale for investigating its potential aphrodisiac activity via hormonal and histological assessments.

3. Methods

This study is in vivo laboratory experimental research using a completely randomized design (CRD) with male Wistar rats as experimental animals. This design was chosen to minimize bias and ensure the reliability of the results. The study was conducted at the Laboratory of the Center for Food and Nutrition Studies, while histological preparation was carried out at the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada. The histological slides were examined at the Laboratory of Anatomical Pathology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. The research was conducted from June to August 2025.

3.1. Materials and Instruments

The materials used in this study included *Elephantopus scaber* L. (Tapak Liman) leaves which contain 70% ethanol, physiological saline 0.9%, carboxymethyl cellulose sodium (CMC-Na 1%), sildenafil citrate (Novell), testosterone ELISA kit, 10% buffered formalin solution, and standard phytochemical reagents such as Dragendorff, Mayer, $FeCl_3$, concentrated HCl, Mg powder, and concentrated H_2SO_4. The instruments employed were a rotary evaporator, microtome, light microscope, ELISA reader, and standard surgical instruments for animal dissection, along with an analytical balance, animal cages, and standard laboratory glassware.

3.2. Plant Extraction

Leaves of *E. scaber* were dried, powdered, and extracted using the maceration method with 70% ethanol for seven days with daily stirring. The filtrate was filtered and concentrated

using a rotary evaporator to obtain a viscous extract, which was then stored in an airtight container at 4 °C until further use.

3.3. Phytochemical Screening

The extract was subjected to phytochemical screening to identify the presence of secondary metabolites following standard procedures (Harborne, 1998). Alkaloids were detected using Dragendorff's and Mayer's reagents, while flavonoids were confirmed through the Mg–HCl reaction. The presence of tannins was evaluated using the $FeCl_3$ test, and saponins were identified by the froth test. Steroids and terpenoids were examined using the Liebermann–Burchard reaction.

3.4. Experimental Animals and Treatments

The experimental animals were healthy male Wistar rats, 8–10 weeks old, weighing 250–300 g. Animals were acclimatized for 7 days under standard laboratory conditions with free access to food and water. Rats were randomly divided into five groups (n = 7 each):

1) Normal control (physiological saline).
2) Negative control (CMC-Na 1%).
3) Positive control (Sildenafil 50 mg/kg BW).
4) Treatment group I (Tapak Liman extract 100 mg/kg BW).
5) Treatment group II (Tapak Liman extract 200 mg/kg BW).

All treatments were administered orally using an oral gavage (sonde) once daily for 21 consecutive days.

3.5. STZ Induction

Streptozotocin (STZ) induction in experimental animals has been shown to significantly reduce testosterone levels through multifactorial mechanisms. STZ selectively destroys pancreatic β-cells, leading to hyperglycemia resembling type 1 diabetes mellitus, which subsequently enhances oxidative stress and systemic inflammation. These metabolic disturbances directly impair Leydig cell function in the testes, the primary site of testosterone production. Previous studies have reported that STZ-induced diabetes downregulates steroidogenic enzyme expression, disrupts gonadotropin (LH and FSH) stimulation, and diminishes the steroidogenic capacity of Leydig cells (Martin & Touaibia, 2020; Nna et al., 2019). Moreover, oxidative damage and lipid peroxidation in testicular tissue further aggravate the decline in testosterone levels. Collectively, STZ not only induces metabolic disorders but also contributes to male reproductive dysfunction by suppressing the hypothalamic–pituitary–gonadal axis. STZ induction was administered after the experimental animals had undergone acclimatization, specifically on day 7. The successful induction of STZ is typically observed on the third day following administration.

3.6. Measurement of Serum Testosterone Levels

On day 22, rats were ethically sacrificed, and blood samples were collected from the orbital vein or directly from the heart. Blood was allowed to clot and centrifuged to obtain serum. Testosterone levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) according to the manufacturer's protocol.

3.7. Histological Examination of the Testicular

Testicular were excised, fixed in 10% buffered formalin for at least 24 hours, processed, sectioned into 4–5 µm thickness using a microtome, and stained with Hematoxylin–Eosin (HE). Observations were carried out under a light microscope to evaluate Diameter of

seminiferous tubules. Five random fields of view per sample were analyzed, and the mean values were calculated for comparison between groups.

4. Results and Discussion

4.1. Results of Phytochemical Identification of 70% Ethanol Extract of *Elephantopus scaber* Leaves (Tapak Liman)

The phytochemical screening revealed that the 70% ethanol extract of *Elephantopus scaber* leaves (Tapak Liman) contained flavonoids, tannins/phenols, and triterpenoids, whereas alkaloids, steroids, and saponins were not detected. The results of phytochemical testing of Tapak Liman herbs are shown in Table 1 below:

Table 1. Phytochemical Screening Results of 70% Ethanol Extract of *Elephantopus scaber* Leaves

No	Phytochemical Group	Screening Result
1	Alkaloids	–
2	Flavonoids	+
3	Tannins/Phenols	+
4	Steroids	–
5	Triterpenoids	+
6	Saponins	–

The results of the phytochemical identification are consistent with previous findings indicating that tapak liman is a significant source of flavonoids and other phenolic compounds. Yuliani et al. (2019) reported that leaf extracts of tapak liman possess high levels of total phenolics and flavonoids, particularly in plants growing at mid-altitude habitats. Similarly, Martin & Touaibia (2020) observed that the ethanol extract of tapak liman contained quantifiable amounts of phenolics and flavonoids.

The phytochemical screening of the 70% ethanol extract of Tapak Liman revealed the presence of flavonoids, tannins/phenolics, and triterpenoids. The presence of these three groups of bioactive compounds may explain the biological activities of the extract related to male reproductive health.

Flavonoids are secondary metabolites known for their strong antioxidant activity through mechanisms such as free radical scavenging and lipid peroxidation inhibition. This antioxidant activity is crucial in protecting testicular cells from oxidative stress, which is known to impair spermatogenesis and reduce testosterone levels (Aitken & Krausz, 2001). In addition, flavonoids have also been reported to play a role in hormonal modulation by enhancing the activity of Leydig cells, the primary producers of testosterone in the testes (Yakubu et al., 2005). Thus, the presence of flavonoids in the Tapak Liman extract makes a significant contribution to its aphrodisiac potential.

In addition to flavonoids, phenolic compounds in the form of tannins were also detected in the extract. Tannins act as protective agents with antioxidant and anti-inflammatory properties, helping maintain the integrity of germ cells in the seminiferous tubules. This protection is important to ensure optimal spermatogenesis and to improve the histological structure of the testes affected by oxidative stress. Therefore, tannins further support the extract's potential in preserving reproductive function.

Meanwhile, the presence of triterpenoids indicates that the extract may also influence the endocrine system. Triterpenoids are known to stimulate the secretion of luteinizing hormone (LH) from the anterior pituitary, which subsequently stimulates Leydig cells to

increase testosterone production. The rise in testosterone levels is closely associated with enhanced libido, erectile function, and spermatogenesis quality. Therefore, triterpenoids represent another important component contributing to the aphrodisiac activity of Tapak Liman extract.

The results also demonstrated that alkaloids and saponins were not detected in the extract. Although both classes of compounds are recognized for certain biological activities, their presence at high concentrations can be toxic. Some alkaloids are known to affect the central nervous system and cause neurological side effects, while high concentrations of saponins can induce hemolysis of red blood cells (Hussain et al., 2018). Therefore, the absence of these compounds in the extract may indicate a better safety profile for therapeutic use.

Overall, the combination of flavonoids, tannins/phenolics, and triterpenoids found in the extract supports its potential as a natural aphrodisiac. The underlying activity is most likely derived from the synergistic effects of antioxidant mechanisms and hormonal stimulation, which collectively contribute to increasing testosterone levels and improving both the function and structure of reproductive tissues.

4.2. Measurement of Testosterone Hormone Levels

After 21 days of treatment, the rats were ethically sacrificed, and blood samples were collected through the orbital vein or cardiac puncture. The collected blood was analyzed for testosterone levels. The measurement of testosterone hormone concentration was carried out using the Enzyme-Linked Immunosorbent Assay (ELISA) method. The results of testosterone measurements were compared among groups to determine whether the extract influenced the hormonal system. An increase in testosterone levels serves as one of the main indicators of the aphrodisiac activity of the tested material. The average testosterone levels are shown in table 2.

Table 2. The Average Testosterone Levels

Group	Mean (ng/dl) ± SD
Normal control	269.09 ± 1.87
Negative control	120.32 ± 1.55
Positive control	199.40 ± 1.97
Tapak Liman extract 100 mg/kg BW	135.52 ± 1.52
Tapak Liman extract 200 mg/kg BW	209.41 ± 0.62

The results of this study showed that the normal control group had the highest testosterone level (269.09± 1.87 ng/dL), whereas the negative control group exhibited the lowest level (120.32 ± 1.55 ng/dL). Administration of the 200 mg test dose significantly increased testosterone levels (209.41 ± 0.62 ng/dL), approaching those of the normal control, while the 100 mg dose produced only a moderate increase (235.52 ± 1.52 ng/dL). The positive control group also displayed relatively high levels (199.40 ± 1.97 ng/dL). Further analysis using the Tukey HSD test revealed highly significant differences ($p < 0.001$) in almost all group comparisons, indicating that the treatment had a pronounced effect on testosterone levels, with the 200 mg dose being more effective than the 100 mg dose.

Physiologically, testosterone plays a crucial role in regulating spermatogenesis through the activation of Sertoli cells, which support the development of germ cells into mature spermatozoa. A decrease in testosterone levels has been shown to cause seminiferous tubule atrophy and a reduction in spermatogenic activity (Grande et al., 2022). The diameter of the seminiferous tubules is strongly influenced by testosterone concentration, where hormonal deficiency results in lumen shrinkage due to impaired proliferation and differentiation of germ

cells (Souza et al., 2017). In this study, the negative control group with reduced testosterone levels likely experienced a decrease in seminiferous tubule diameter. Conversely, the group treated with the 200 mg dose, which demonstrated testosterone levels close to normal, was likely to exhibit recovery of seminiferous tubule diameter toward physiological conditions. These findings are consistent with the report of Rahmah et al. (2021), which indicated that increasing testosterone through pharmacological or herbal interventions could enhance seminiferous tubule diameter in hypogonadal rat models.

In conclusion, this study emphasizes that increasing testosterone levels through administration of the 200 mg dose not only restored levels close to physiological conditions but also had a positive impact on maintaining seminiferous tubule diameter.

4.3. Calculation of the Average Diameter of Seminiferous Tubules in Testicular Histology

The histology of the testes serves as an important parameter for assessing both physiological and pathological conditions that affect reproductive function, particularly through the evaluation of seminiferous tubule structures. Morphological alterations such as reduced tubule diameter, disorganization of the germinal epithelium, decreased numbers of spermatogenic cells, and degeneration of Sertoli and Leydig cells indicate impaired spermatogenesis as a consequence of oxidative stress, apoptosis, or hormonal deficiencies. One of the most commonly used agents to model such conditions is streptozotocin (STZ), a diabetogenic compound that selectively destroys pancreatic β-cells, leading to persistent hyperglycemia. Chronic hyperglycemia induced by STZ enhances the production of reactive oxygen species (ROS) and reduces endogenous antioxidant activity, ultimately resulting in testicular tissue damage. Therefore, the use of STZ in experimental research provides significant value in evaluating the impact of hyperglycemia on the histological structure of seminiferous tubules.

The reduction in seminiferous tubule diameter occurs as a result of oxidative stress, germ cell apoptosis, decreased testosterone production due to Leydig cell damage, Sertoli cell dysfunction, and impaired testicular vascularization. Collectively, these factors contribute to thinning of the germinal epithelium and degeneration of spermatogenesis, which manifests as shrinkage of tubule diameter. Based on histopathological findings, the seminiferous tubule diameter in STZ-induced diabetic experimental animals tends to decrease compared with normal control animals. Under physiological conditions, seminiferous tubule diameters generally range from 220–300 µm, whereas in diabetic groups, shrinkage can reach 160–200 µm or even smaller, depending on the degree of tissue damage. This reduction in diameter is closely associated with the loss of germinal cell layers, including spermatogonia, spermatocytes, and spermatids, leading to a decreased number of spermatogenic cells. Furthermore, degeneration of the germinal epithelium results in thinning of the tubule wall, while the lumen appears enlarged due to the diminished presence of spermatogenic cells. These histopathological features clearly indicate impaired spermatogenesis, reflecting the detrimental effects of hyperglycemia and oxidative stress induced by STZ on male reproductive function. Representative histological images of normal seminiferous tubule diameters are shown in Figure 1-5.

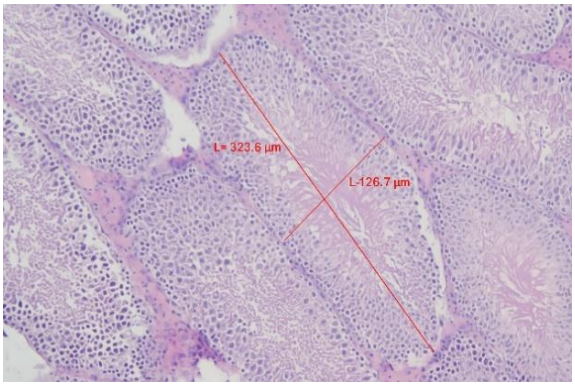


Figure 1. Histology of seminiferous tubules in the normal control group showing an intact spermatogenic layer with a wide tubular diameter

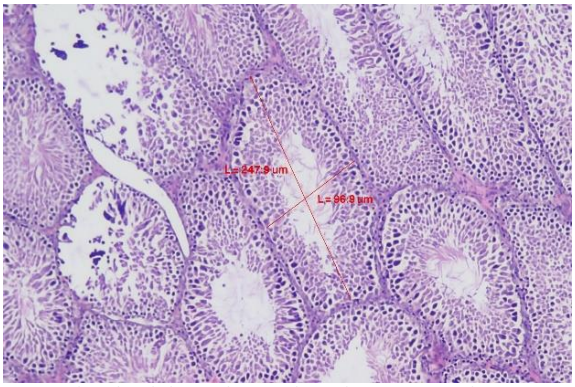


Figure 2. Seminiferous tubule histology in the negative control group exhibiting tubular shrinkage and disrupted spermatogenic layers

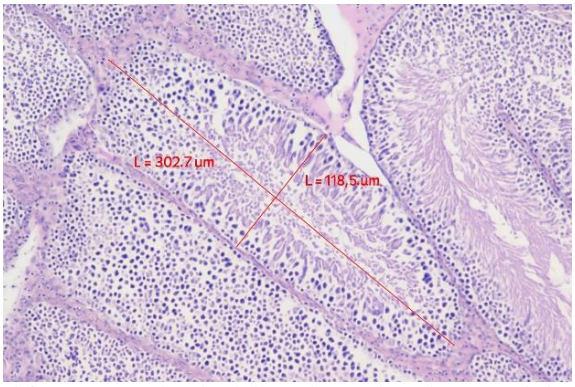


Figure 3. Seminiferous tubule histology in the positive control group

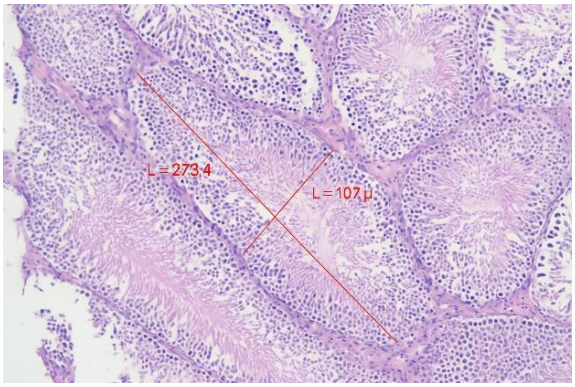


Figure 4. Seminiferous tubule histology in the treatment group receiving *Elephantopus scaber* extract at a dose of 100 mg/kg BW

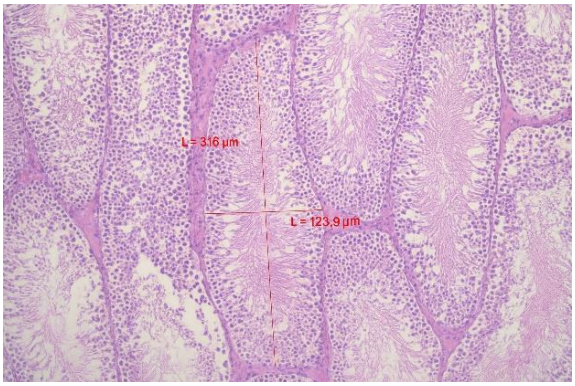


Figure 5. Seminiferous tubule histology in the treatment group receiving *Elephantopus scaber* extract at a dose of 200 mg/kg BW, showing structural recovery approaching normal and resembling the diameter observed in the positive control group

Further, the average diameter of seminiferous tubules in each treatment group is presented in Table 3.

Table 3. Average diameter of seminiferous tubules (µm)

Group	Mean (µm) ± SD
Normal control	225,30 ± 5.12
Negative control	172,45 ± 4.87
Positive control	210,67 ± 6.03
Tapak Liman extract 100 mg/kg BW	190,23 ± 5.15
Tapak Liman extract 200 mg/kg BW	220,56 ± 4.98

Based on the average diameter of the seminiferous tubules presented in Table 3, there is a tendency that experimental groups with higher testosterone levels exhibit larger seminiferous tubule diameters. In the normal control group, the mean testosterone level was within the physiological range and corresponded to relatively larger tubule diameters compared with the negative control group. Conversely, groups with reduced testosterone levels demonstrated a narrowing of tubule diameter, indicating impaired spermatogenesis due to diminished hormonal stimulation. This finding is consistent with the critical role of testosterone as the principal hormone supporting Sertoli cell function and maintaining the structural integrity of the seminiferous tubules. Therefore, the observed positive relationship between testosterone levels and tubule diameter suggests that a decline in testosterone directly impacts the histological quality of the testes.

Testosterone is the primary hormone regulating spermatogenesis in the testes. One of the key histological indicators of testicular health is the diameter of the seminiferous tubules. Larger tubule diameters generally reflect active spermatogenesis, whereas narrowed diameters may indicate impaired testicular function. The data in Table 3 demonstrate a positive correlation between testosterone levels and seminiferous tubule diameter, meaning that higher testosterone concentrations are associated with larger tubule diameters. This finding is consistent with the role of testosterone in stimulating germ cell proliferation and supporting the maintenance of tubule structure. Increased tubule diameters are typically accompanied by thicker layers of spermatogenic cells, reflecting enhanced spermatogenic activity. Conversely, reduced testosterone levels may lead to tubule atrophy (narrowing of diameter) as a result of diminished hormonal support for Sertoli cells and germ cells.

5. Conclusion

The ethanol extract of *Elephantopus scaber* (Tapak Liman) leaves demonstrated aphrodisiac activity as evidenced by its ability to increase serum testosterone levels and improve testicular histology in male Wistar rats. Administration of the extract at a dose of 200 mg/kg BW significantly elevated testosterone concentrations close to normal physiological levels and restored the diameter of seminiferous tubules. Phytochemical screening confirmed the presence of flavonoids, tannins/phenolics, and triterpenoids, which may contribute synergistically to these effects. These findings suggest that *E. scaber* extract at an optimal dose has potential as a natural agent to support male reproductive health.

Testosterone is a key factor in maintaining male reproductive function; however, other hormones such as follicle-stimulating hormone (FSH) and prolactin also play important roles in regulating spermatogenesis and influencing seminiferous tubule structure. Future studies should consider measuring these additional hormonal parameters to provide a more comprehensive understanding of the extract's effects on the reproductive endocrine system and testicular function.

6. Acknowledgement

The authors would like to express their sincere gratitude to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for the financial support provided through the Saintek Research Program, under DIPA No. SP DIPA-139.04.1.693320/2025. The authors also extend their appreciation to the institution's leadership, colleagues, and all parties who have contributed valuable input, facilities, and encouragement throughout the implementation of this research. Without their support, this work would not have been successfully completed.

7. References

Abarikwu, S. O., Simple, G., & Onuoha, C. S. (2020). Morphometric Evaluation of the Seminiferous Tubules and the Antioxidant Protective Effects of Gallic Acid and Quercetin in the Testis and Liver of Butyl Phthalate Treated Rats. *Indian Journal of Clinical Biochemistry*, *35*(1), 20–31. https://doi.org/10.1007/s12291-018-0788-0

Aitken, R., & Krausz, C. (2001). Oxidative stress, DNA damage and the Y chromosome. *Reproduction*, *122*(4), 497–506. https://doi.org/10.1530/rep.0.1220497

Al-Madhagi, H., & Tarabishi, A. A. (2024). Nutritional aphrodisiacs: Biochemistry and Pharmacology. *Current Research in Food Science*, *9*, 100783. https://doi.org/10.1016/j.crfs.2024.100783

Ara, A., Vishvkarma, R., Mehta, P., & Rajender, S. (2023). The Profertility and Aphrodisiac Activities of Tribulus terrestris L.: Evidence from Meta-Analyses. *Andrologia*, *2023*(1). https://doi.org/10.1155/2023/7118431

Bhasin, S., Brito, J. P., Cunningham, G. R., Hayes, F. J., Hodis, H. N., Matsumoto, A. M., Snyder, P. J., Swerdloff, R. S., Wu, F. C., & Yialamas, M. A. (2018). Testosterone Therapy in Men With Hypogonadism: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, *103*(5), 1715–1744. https://doi.org/10.1210/jc.2018-00229

BPOM RI. (2021). *Public Warning Obat Tradisional, Suplemen Kesehatan, dan Kosmetika Mengandung Bahan Kimia Obat/Bahan Dilarang Tahun 2021*. BPOM RI-Pusat Data dan Informasi Obat dan Makanan. https://www.pom.go.id/siaran-pers/siaran-pers-public-warning-obat-tradisional-suplemen-kesehatan-dan-kosmetika-mengandung-

bahan-kimia-obat-bahan-dilarang-tahun-2021

Brunetti, P., Lo Faro, A. F., Tini, A., Busardò, F. P., & Carlier, J. (2020). Pharmacology of Herbal Sexual Enhancers: A Review of Psychiatric and Neurological Adverse Effects. *Pharmaceuticals*, *13*(10), 309. https://doi.org/10.3390/ph13100309

Choowong-in, P., Sattayasai, J., Boonchoong, P., Poodendaen, C., Wu, A. T., Tangsrisakda, N., Sawatpanich, T., Arun, S., Uabundit, N., & Iamsaard, S. (2022). Protective effects of Thai Mucuna pruriens (L.) DC. var. pruriens seeds on sexual behaviors and essential reproductive markers in chronic unpredictable mild stress mice. *Journal of Traditional and Complementary Medicine*, *12*(4), 402–413. https://doi.org/10.1016/j.jtcme.2021.12.001

Djati, M. S., Christina, Y. I., Dwijayanti, D. R., & Rahayu, S. (2024). Synergistic modulation of proinflammatory mediators and cytokines in lipopolysaccharide-activated RAW 264.7 macrophages: The therapeutic potential of Elephantopus scaber and Sauropus androgynus ethanol extract. *Veterinary World*, *17*(3), 728–734. https://doi.org/10.14202/vetworld.2024.728-734

Djati, M. S., Christina, Y. I., & Rifa'i, M. (2021). The combination of Elephantopus scaber and Sauropus androgynus promotes erythroid lineages and modulates follicle-stimulating hormone and luteinizing hormone levels in pregnant mice infected with Escherichia coli. *Veterinary World*, 1398–1404. https://doi.org/10.14202/vetworld.2021.1398-1404

Elterman, D. S., Bhattacharyya, S. K., Mafilios, M., Woodward, E., Nitschelm, K., & Burnett, A. L. (2021). The Quality of Life and Economic Burden of Erectile Dysfunction. *Research and Reports in Urology*, *13*, 79–86. https://doi.org/10.2147/RRU.S283097

Enema, O., Umoh, U., Umoh, R., Ekpo, E., Adesina, S., & Eseyi, O. (2018). Chemistry and Pharmacology of Aphrodisiac Plants: A Review. *Journal of Chemical and Pharmaceutical Research*, *10*(7). https://www.jocpr.com/articles/chemistry-and-pharmacology-of-aphrodisiac-plants-a-review.pdf

Grande, G., Barrachina, F., Soler-Ventura, A., Jodar, M., Mancini, F., Marana, R., Chiloiro, S., Pontecorvi, A., Oliva, R., & Milardi, D. (2022). The Role of Testosterone in Spermatogenesis: Lessons From Proteome Profiling of Human Spermatozoa in Testosterone Deficiency. *Frontiers in Endocrinology*, *13*. https://doi.org/10.3389/fendo.2022.852661

Hall, J. E. (2016). *Guyton and Hall Textbook of Medical Physiology* (13th ed.). Elsevier.

Harborne, A. J. (1998). *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*. Springer Netherlands.

Hussain, G., Rasul, A., Anwar, H., Aziz, N., Razzaq, A., Wei, W., Ali, M., Li, J., & Li, X. (2018). Role of Plant Derived Alkaloids and Their Mechanism in Neurodegenerative Disorders. *International Journal of Biological Sciences*, *14*(3), 341–357. https://doi.org/10.7150/ijbs.23247

Jamal, R. K., & Jose, V. (2017). Determination of phytochemicals by GC-MS in methanol extract of Elephantopus scaber L. *Journal of Pharmacognosy and Phytochemistry*, *6*(6). https://www.phytojournal.com/archives/2017.v6.i6.2165/determination-of-phytochemicals-by-gc-ms-in-methanol-extract-of-elephantopus-scaber-l

Lee, H. W., Kil, K.-J., & Lee, M. S. (2020). Ginseng for Improving Semen Quality Parameters: A Systematic Review. *The World Journal of Men's Health*, *38*(3), 377. https://doi.org/10.5534/wjmh.190125

Leisegang, K., Finelli, R., Sikka, S., & Panner Selvam, M. (2022). Eurycoma longifolia (Jack) Improves Serum Total Testosterone in Men: A Systematic Review and Meta-Analysis of Clinical Trials. *Medicina*, *58*(8), 1047. https://doi.org/10.3390/medicina58081047

Li, L., Lin, W., Wang, Z., Huang, R., Xia, H., Li, Z., Deng, J., Ye, T., Huang, Y., & Yang, Y. (2024). Hormone Regulation in Testicular Development and Function. *International Journal of Molecular Sciences*, *25*(11), 5805. https://doi.org/10.3390/ijms25115805

Martin, L. J., & Touaibia, M. (2020). Improvement of Testicular Steroidogenesis Using

Flavonoids and Isoflavonoids for Prevention of Late-Onset Male Hypogonadism. *Antioxidants*, *9*(3), 237. https://doi.org/10.3390/antiox9030237

Nna, V. U., Bakar, A. B. A., Ahmad, A., & Mohamed, M. (2019). Down-regulation of steroidogenesis-related genes and its accompanying fertility decline in streptozotocin-induced diabetic male rats: ameliorative effect of metformin. *Andrology*, *7*(1), 110–123. https://doi.org/10.1111/andr.12567

Nouioura, G., Lyoussi, B., & Derwich, E. (2024). Rekindling desire: Unveiling the Aphrodisiac potential of Apiaceae Elixirs. *Phytomedicine Plus*, *4*(2), 100530. https://doi.org/10.1016/j.phyplu.2024.100530

Rahmah, N., Wurlina, W., Madyawati, S., Utomo, B., Hernawati, T., & Safitri, E. (2021). Kaliandra honey improves testosterone levels, diameter and thickness of tubulus seminiferous epithelial of white rat (Rattus norvegicus) through stimulation of HSP70 due to malnutrition. *Open Veterinary Journal*, *11*(3), 401. https://doi.org/10.5455/OVJ.2021.v11.i3.11

Sitasiwi, A. J., Mardiati, S. M., & Melati, A. K. (2023). Struktur Histologi Testis Tikus Putih (Rattus Norvegicus L.) Setelah Pemberian Sediaan Nanokitosan Ekstrak Etanol Daun Mimba (Azadirachta Indica A.Juss). *Buletin Anatomi Dan Fisiologi*, *8*(2), 122–129. https://doi.org/10.14710/baf.8.2.2023.122-129

Souza, A. P. B. de, Schorr-Lenz, Â. M., Lucca, F., & Bustamante, I. C. (2017). The epididymis and its role on sperm quality and male fertility. *Animal Reproduction*, *14*(Suppl. 1), 1234–1244. https://doi.org/10.21451/1984-3143-AR955

Ulloa del Carpio, N., Alvarado-Corella, D., Quiñones-Laveriano, D. M., Araya-Sibaja, A., Vega-Baudrit, J., Monagas-Juan, M., Navarro-Hoyos, M., & Villar-López, M. (2024). Exploring the chemical and pharmacological variability of Lepidium meyenii: a comprehensive review of the effects of maca. *Frontiers in Pharmacology*, *15*. https://doi.org/10.3389/fphar.2024.1360422

Vanderah, T. W. (2024). *Katzung's Basic & Clinical Pharmacology*. McGraw-Hill.

Yakubu, M. T., Akanji, M. A., & Oladiji, A. T. (2005). Aphrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. *Asian Journal of Andrology*, *7*(4), 399–404. https://doi.org/10.1111/j.1745-7262.2005.00052.x

Yuliani, Rachmadiarti, F., Dewi, S. K., Asri, M. T., & Soegianto, A. (2019). Total phenolic and flavonoid contents of elephantopus scaber and ageratum conyzoides (Asteraceae) leaves extracts from various altitude habitats. *Ecology, Environment and Conservation*. https://www.scopus.com/pages/publications/85071093288

Zhu, W., Du, Y., Meng, H., Dong, Y., & Li, L. (2017). A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of Tribulus terrestris. *Chemistry Central Journal*, *11*(1), 60. https://doi.org/10.1186/s13065-017-0289-x