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Original Research Article

Ethanol extract of *Imperata cylindrica* Roots Potential as an Aphrodisiac in Male Wistar Rats

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ABSTRACT

The roots of *Imperata cylindrica* are used by many ethnic groups in Indonesia as an aphrodisiac. This study determined the aphrodisiac properties of the ethanol extract of *I. cylindrica* roots in male Wistar rats. The experimental animal was 25 male and 15 female Wistar rats aged 4 – 5 months. The male rate had at least two previous copulation experiences. The ethanol extract of *I. cylindrica* was administered orally to the treatment group for 28 days at 100, 200, and 400 mg/kg. The group given sildenafil citrate (5 mg/kg BW) and distilled water served as positive and negative controls, respectively. On day 29, the serum testosterone level and sexual behavior parameters (mounting and intromission latency, mounting and intromission frequency, ejaculation latency, and postejaculatory mounting interval) were observed and assessed. Administration of the ethanol extract of *I. cylindrica* roots for 28 days resulted in decreased mounting and intromission latency and increased mounting and intromission frequency at all doses. There was an increase in ejaculation latency at 100 and 200 mg/kg BW doses and a decrease in the postejaculatory mounting interval at 100 and 200 mg/kg BW doses. A 100 mg/kg BW dose approached the giving sildenafil effect (5 mg/kg BW) as a positive control. Serum testosterone levels increased at all doses of the extract. The ethanol extract of *I. cylindrica* roots has the potential to act as an aphrodisiac, with the possible mechanism of action being an increase in testosterone levels.

***Keywords*:** aphrodisiac, root, *Imperata cylindrica*, ethanol extract, sexual behavior, herbal plants, testosterone

Introduction

Male sexual dysfunction refers to disturbances in the response to the sexual cycle, which encompasses drive/desire, arousal, and orgasm. Its prevalence is found to exceed 50% among men aged 40–70 years and tends to increase with age.[1] Erectile dysfunction disorders are found in 10%–20%, sexual drive disorders in ~8%–18%, and premature ejaculation disorders in ~30% of individuals.[2] In China, the reported prevalence of sexual dysfunction among married men aged 30–60 years with one or more symptoms was 15%. Additionally, the prevalence of decreased sex drive was 11.1%, erectile dysfunction was 4.3%, and premature ejaculation was 4.7%.[3] At present, there is no reported prevalence of sexual dysfunction in Indonesia, except for erectile dysfunction, which has been reported at 35.6% (22.3% mild, 13.7% mild-moderate, 3.1% moderate, and 0.8% severe). The highest prevalence was observed among individuals aged ≥60 years, with a rate of 88.0%.[4]

Sexual dysfunction itself can have implications for infertility, well-being, interpersonal functioning, relationships with partners, and overall quality of life.[5] Psychologically, the impact of sexual dysfunction in men is evident in the form of depression and anxiety. In a study involving 76 men with erectile dysfunction, with spinal cord injury, the incidences of depression and anxiety were reported at 16.47% and 58.7%, respectively. Furthermore, in another study with 64 men experiencing erectile dysfunction and impaired sexual drive, without spinal cord injury, the depression and anxiety rates were 30.0% and 48.75%.[6]

The primary cause of male sexual dysfunction is a decline in testosterone levels within the blood circulation. Testosterone levels naturally decrease as men age, with a gradual decline of 0.4%–2% per year, starting at ~30 years of age. Among men aged 70 and above, ~35% have lower testosterone levels compared with younger men, and 13% of these individuals have hypogonadism. Late-onset hypogonadism (LOH) is the term used to describe male sexual dysfunction in people over 65 with low testosterone levels.[7] Alongside diminished testosterone levels, sexual dysfunction in men can also be attributed to various factors, including diabetes mellitus, cancer, stroke, hypertension, depression, anxiety, and penile trauma.[1]

Testosterone hormone replacement therapy remains the primary treatment choice for sexual dysfunction associated with LOH. However, excessive testosterone supplementation has been linked to breast cancer, prostate cancer, polycythemia, erythrocytosis, and severe obstructive sleep apnea.[8] The high cost of this therapy poses a barrier for patients with limited incomes.[9] In cases of erectile dysfunction, drugs from the phosphodiesterase-inhibitor class, such as sildenafil, vardenafil, and tadalafil, are commonly used for treatment.[10] Nonetheless, the efficacy of conventional medications is limited, and there are contraindications in certain disease states and side effects, including headaches, dizziness, facial flushing, nasal congestion, dyspepsia, and visual changes such as photophobia, cyanopsia, and hazy vision, which can cause discomfort to users.[11]

If sexual dysfunction is linked to the physiological processes of the sexual response cycle, dietary factors are just as essential as the widespread use of medicines to treat it. Sexual dysfunction can be reversed through modest lifestyle modifications and hormonal management. Implementing lifestyle changes, such as regular exercise and adopting a nutritious diet for weight loss, are key approaches to addressing sexual dysfunction.[8,12] Providing specific nutrition can

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contribute to an improved quality of life and enhanced sexual health. Herbal medicines, with aphrodisiac properties, can aid in this process.[13] The utilization of herbal plants as aphrodisiacs for the treatment of sexual disorders is rising.[14] Herbal plants with aphrodisiac properties are easily accessible and have minimal to no side effects.[15]
In Indonesia, besides ginger (*Zingiber officinale Roscoe*), areca nut (*Areca catechu*), long jack (*Eurycoma longifolia Jack*), and pepper (*Piper nigrum*), cogon grass roots (*Imperata cylindrica*) are widely used as an aphrodisiac by various ethnic groups. Specifically, 19 ethnic groups in Indonesia, namely Basap, Gayo, Pubian, Postejaculatory mounting intervalPeminggir, Sunda Priangan, Osing, Bawean, Madura, Baduy, Suaid, Dayak Tomum, Pitap, Dayak Bentian, Kutai, Dayak Bahau, To Manui'i, Tialo, Jawan, and Helong, utilize cogon grass roots as an aphrodisiac herb.[16-17] However, there have been no reported studies regarding the aphrodisiac properties of *I. cylindrica* roots. This study evaluated the aphrodisiac properties of *I. cylindrica* roots in male Wistar rats.

Materials and Methods

Collection and authentication of plant materials
I. cylindrica roots were collected from rice fields in the Subak Mundeh area of Nyambu Village, Kediri-Tabanan District, Bali (8°34'43.7"S 115°08'40.4"E) in July 2022. The plants grew in dry soil and were characterized by their mature stage and brownish-yellow leaves. The roots obtained were identified and authenticated by the Directorate of Scientific Correction Management at the "Eka Karya" Botanical Gardens in Bedugul, Bali (National Research and Innovation Agency) (number of sample 1617-97127-1).

Extraction
The extraction was done at the Integrated Services Laboratory of the Faculty of Agricultural Technology, Universitas Udayana. The maceration method was used to prepare the extract using 96% ethanol (PT. Brataco, Indonesia). Approximately 4 kg of previously washed and air-dried *I. cylindrica* roots were cut into small pieces (1–1.5 cm) and dried in a dehydrator at 40°C–50°C for 24 h. They were blended until a smooth consistency was achieved and passed through a 40-mesh sieve. The powdered roots were then subjected to maceration with 96% ethanol in a ratio of 1:10 (sample: solvent). The maceration process involved stirring the mixture for 15 min at 2-h intervals, for a total maceration time of 48 h. Then, the sample was filtered using ordinary filter paper until the extract no longer dripped. The filtrate was evaporated using an evaporator until no solvent was dripping from the apparatus. The evaporation process was carried out at 40°C, with 100 rpm flask rotation speed and 100 Mbar vacuum pressure. The total amount of extract obtained after the evaporation process was 42 g.

ELISA kit and reagents
Ethinyl estradiol (Ovalumon) and progesterone (Potahormon) were obtained from Wonderindo, Indonesia. Sildenafil citrate was obtained from Viagra 100 mg, manufactured by Pfizer in Indonesia. The ELISA Kit reagent used to measure serum testosterone levels was from BT Lab (Code: EA0023Ra), provided by CV Gamma Scientific Biolab in Malang, Indonesia.

Grouping of experimental animals and administration of extracts
This research protocol was approved by the Ethics Commission Unit of the Faculty of Medicine, Udayana University, with approval number 2416/UN14.2.2.VII.14/LT/2022. A total of 25 male Wistar rats, aged 4–5 months, weighing 200–250 g, and 15 female Wistar rats, aged 4–5 months, weighing 150–200 g, were used in this study. The male rats had at least two previous copulation experiences. The experimental animals were obtained from the Department of Histology, Faculty of Medicine, Universitas Udayana.
The animals were housed in cages measuring 46 × 38 × 24 cm, which were maintained in a clean condition. They were provided with access to water and CP551 pellet food. The room was properly ventilated, and the temperature was maintained at 25°C ± 2°C. The light cycle followed a 12-h natural light period and a 12-h dark period. Experimental animals were closely monitored and weighed daily throughout the treatment period. Male Wistar rats were randomly divided into five groups, including three treatment and two control groups. Each group consisted of five rats. The treatment groups received different doses of the ethanol extract of *I. cylindrica* roots: 100, 200, and 400 mg/kg BW. The positive control group received sildenafil citrate at 5 mg/kg BW, while the negative control group was administered 2 ml distilled water. Since the ethanol extract of *I. cylindrica* roots and sildenafil are not soluble in water, Tween 20 was added to each treatment solution. The distilled water solution given to the negative control group also contained Tween 20. The extracts and drugs were administered orally for 28 days using an orogastric tube.

Rat sexual behavior trial procedure
Before the research was conducted, all experimental animals were acclimatized for one week. To assess the sexual behavior activity of male rats, the preparations and procedures followed the method described by Singh et al.[18] Before the treatment, male rats were trained by exposing them to sexually receptive females once a day for four consecutive days. The female rats needed to be artificially induced into the estrus phase before mating with the male rats because female rats only accept mating when they are in the estrus phase. It was achieved by administering ethinyl estradiol at 100 µg dose per rat (Ovalumon, 0.01 ml) subcutaneously 48 h before pairing and a subcutaneous injection of 1 mg progesterone per rat (Potahormon, 0.01 ml) 4 h before pairing. The acceptance of female rats was confirmed by exposing them to male rats that were not part of the research sample. The most receptive females were then selected and paired with male rats in a one-to-one ratio. The experiments were conducted during the dark phase, approximately from 8:00 p.m. to 4:00 a.m. local time on day 28 after treatment. Before mating, the male and female rats were placed in a glass cage (46 cm × 38 cm × 24 cm) for a 30-minute adaptation period. Thirty minutes after the male rats were given the extract, they were placed in the glass box. Ten minutes later, the female rats in heat were introduced into the cage. The mating process was observed immediately after the female rats were placed in the male rats' cage. The observation lasted for 30 min and was recorded using the Xiaomi Home Security Camera 360º CCTV camera with its infrared feature activated. The parameters evaluated were mounting latency: the time from when the male rat was paired with the female rat until the male rat first mounted the female rat (measured in seconds); intromission latency: the time from when the male rat was paired with the female rat until the male rat first performed intromission (measured in seconds); ejaculatory latency: the time from the first intromission to ejaculation (measured in seconds); postejaculatory mounting interval: the time from the first ejaculation to the next mounting (measured in seconds); mounting frequency: the number of mountings during the 30-min observation period; intromission frequency: the number of intromissions during the 30-min observation period. After the mating process occurred, the cage needed to be cleaned regularly to eliminate the urine odor, as this can significantly affect the subsequent mating process of the rats to be tested.

Blood collection and serum preparation
Blood samples were collected on day 29. Before blood collection, the rats were anesthetized with 10% ketamine at a dose of 50 mg/kg BW and 2% xylazine at a dose of 20 mg/kg BW intramuscularly in their thigh muscles. Blood sampling was collected at the orbital plexus, located at the inner corner of the eye. The blood was then transferred to a sterile tube and centrifuged at 1,200 g for 5 min at room temperature. The serum was collected and stored at −20°C.

Examination of serum testosterone levels
The serum testosterone examination procedure was carried out according to the protocol in the reagent kit from BT Lab (Code: EA0023Ra). Sample, standard, and biotinylated antigen was incubated for 60 minutes at 37°C, aspirated, and washed for 5 times. Then, avidin-HRP was added and incubated for 60 minutes at 37°C. The same procedure was conducted after adding substrate solution A and B. The OD value was analyzed within 10 minutes at 450 nm after adding stop solution.[19]

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Data analysis

The data are presented as mean ± SEM and analyzed using SPSS 25 and GraphPad Prims nine. Tests for different means for the five groups used one-way ANOVA + post hoc Tukey HSD for normally distributed data with the same variance and one-way ANOVA Welch + post hoc Dunnett T3 if the variant is different. The data is said to be significantly different if the p-value is <0.05.

Results and Discussion

Effects of I. cylindrica root ethanol extract on sexual behavior

Assessing rat sexual behavior, including mounting (frequency and latency), provides insights into sexual drive and motivation. An increase in mounting frequency and a decrease in mounting latency indicate heightened sexual drive (motivation). The parameters related to intromission (frequency and latency) reflect the rat's erectile ability, while the ejaculation parameter signifies sexual satisfaction. These mounting, intromission, and ejaculation parameters serve as indicators to measure libido, sexual potency, and sexual vigor.[20]

The results revealed that the mounting latency in all treatment groups (100, 200, and 400 mg/kg BW) and the positive control group (sildenafil, 5 mg/kg BW) were significantly decreased compared with that of the negative control group (aquades) ($^{a}p < 0.05$). Moreover, the 400 mg/kg BW dose showed an enhancement and significant difference compared with the 200 mg/kg BW dose ($^{*}p < 0.05$) and the positive control ($p < 0.01$) (Figure 1A). The mounting latency assessment results were closely aligned with those of intromission latency. Figure 1B demonstrates that the intromission latency in all treatment and positive control groups decreased significantly compared with that of the negative control group ($^{a}p < 0.05$). In the treatment groups, intromission latency exhibited an enhancement and significant difference between the 200 and 400 mg/kg BW doses ($^{*}p < 0.05$). The mean intromission latency of the 400 mg/kg BW treatment group also significantly differed from that of the positive control group ($^{**}p < 0.01$). Mounting latency and intromission latency did not significantly differ between the 100 and 200 mg/kg BW treatment groups and the positive control group ($^{b}p > 0.05$). The 200 mg/kg BW group closely resembled the positive control group in terms of mounting latency and intromission latency parameters.

The ejaculatory latency parameter was increased in the treatment group. Statistical analysis demonstrated a significant difference in the average ejaculatory latency time between the 100 and 200 mg/kg BW treatment groups compared with that of the negative control group ($^{*}p < 0.05$), as depicted in Figure 2. Furthermore, there was no significant difference in the average ejaculatory latency between the positive control group and the 100 and 200 mg/kg BW treatment groups ($^{b}p > 0.05$). This suggests that the effect of *I. Cylindrica* root extract at doses of 100 and 200 mg/kg BW had a comparable ejaculatory latency time to sildenafil (5 mg/kg).

The postejaculatory mounting interval parameters were found to be decreased in the treatment groups. Figure 3 presents the results of the Dunnett T3 post hoc test, indicating a significant difference in the average postejaculatory mounting interval time between the negative control group and the treatment groups administered with cogon grass root extract at doses of 100 and 200 mg/kg BW ($p < 0.05$).

Statistical analysis revealed no significant difference in the postejaculatory mounting interval between the positive control and the treatment groups (100 and 200 mg/kg BW, $^{a}p > 0.05$). This indicates that the administration of *I. cylindrica* root extract at doses of 100 and 200 mg/kg BW resulted in a reduction in postejaculatory mounting interval comparable to that of the positive control group.

Furthermore, the mounting frequency and intromission frequency in the treatment groups increased. As shown in Figure 4, the mounting frequency of the negative control group notably differed from that of the treatment groups at all extract doses ($^{a}p < 0.05$). Additionally, there was no significant difference in the average mounting frequency between the positive control group and the 100 mg/kg BW treatment group ($^{b}p > 0.05$). This indicates that the 100 mg/kg BW dose exhibited a mounting frequency that was nearly comparable to that of the positive control group.

The post hoc test using Tukey HSD revealed a significant difference in the mean intromission frequency between the negative control group and all treatment groups ($^{a}p < 0.05$). Furthermore, the Tukey HSD post hoc test results indicated no significant difference in the mean intromission frequency between the positive control group and the 100 mg/kg BW treatment group ($^{b}p > 0.05$). These findings suggest that a dose of 100 mg/kg BW has a similar effect on intromission frequency when compared with 5 mg/kg BW sildenafil.

The observed decrease in mounting latency, intromission latency, and postejaculatory mounting interval, along with the increased mounting frequency, suggests heightened arousal and enhanced sexual motivation in the male rats. Additionally, the increased intromission frequency indicates improved erectile function. Therefore, similar results were reported by Ramalan et al.,[21] who studied the aphrodisiac effect of methanol extract from *Borassus aethiopum Mart.* in Wistar rats.

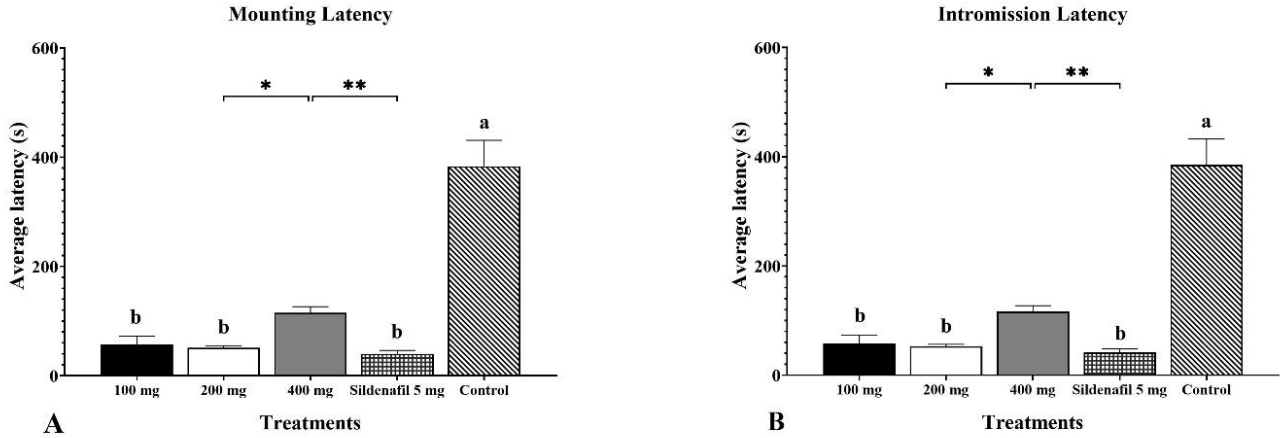

Figure 1: The effect of administering ethanol extract of cogon grass (*Imperata cylindrica*) root on mounting latency [A] and Intromission latency [B]. The graphs represent the results of the Dunnett T3 post hoc test. Graphs labeled with the letter *a* indicate a significant difference compared with graphs not labeled with the letter *a* ($p < 0.05$). The graph labeled with the letter *b* did not show a significant difference from the group labeled with the letter *b* ($p > 0.05$). The data presented are expressed as mean ± SEM; n = 5; $^{*}p < 0.05$; $^{**}p < 0.01$.

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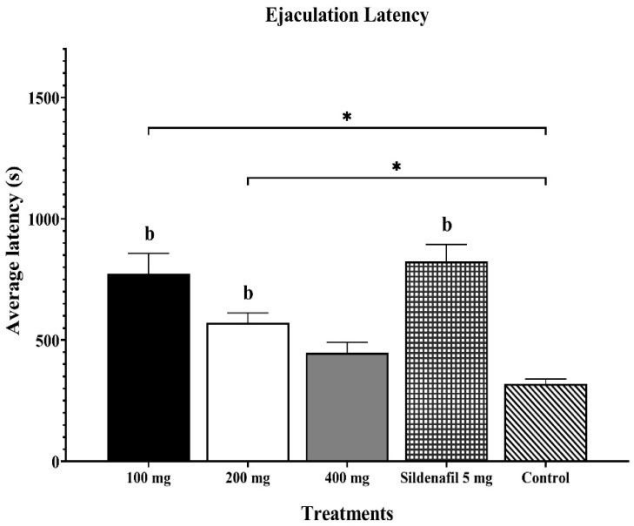

Figure 2: The effect of administering ethanol extract of *Imperata cylindrica* roots on ejaculation latency. Graphs labeled with the letter *b* indicate no significant difference. The data presented represent the mean ± SEM; n = 5; *p < 0.05.

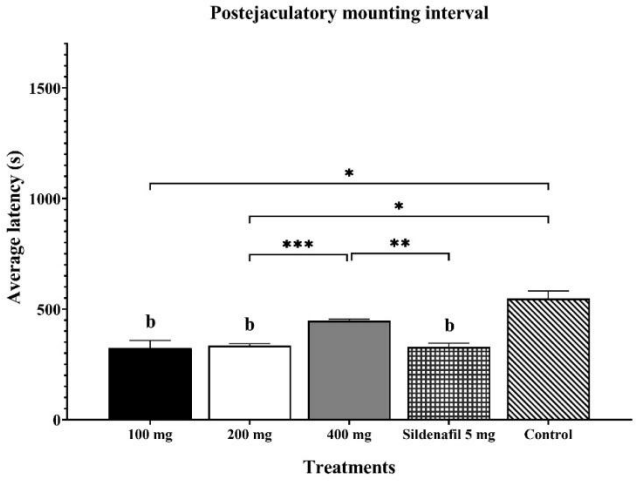

Figure 3. Effect of giving cogon grassroots ethanol extract (*Imperata cylindrica*) on the postejaculatory mounting interval. The graph above is a Dunnett T3 post hoc result. Graphs labeled with the letter *b* are not significantly different. The data presented are the mean ± SEM; n = 5; *p < 0.05; **p < 0.01; *** p < 0.001.

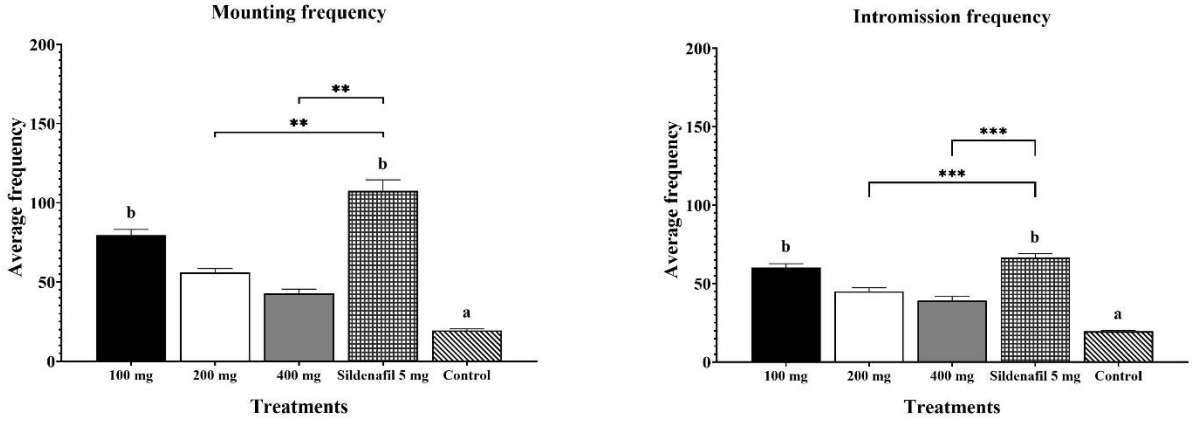

Figure 4: The effect of administering *Imperata cylindrica* root ethanol extract on mounting frequency and intromission frequency. The graph representing mounting frequency is the result of the Dunnett T3 post hoc test, while the graph illustrating intromission frequency is based on the Tukey HSD post hoc test. Graphs marked with the letter *a* indicate a significant difference compared with graphs without the label *a* ($p < 0.05$). Graphs labeled with the letter *b* did not show a significant difference from the group labeled with the same letter ($p > 0.05$). The data presented in the graphs represent the mean ± SEM; n = 5; **p < 0.01; ***p < 0.001.

The results showed that the mounting frequency, intromission frequency, and ejaculation frequencies were increased on days 7 and 28, along with an increased of extract doses. Mounting latency and intromission latency were decreased compared with negative control.[21] These findings are also consistent with other previous studies by Fouche et al.,[22] Kenmogne et al.,[23] and Tang et al.[24] The prolonged ejaculation latency observed in this study indicates an enhancement of rat sexual function due to the aphrodisiac effects of *I. cylindrica* root extract, as reported by Singh et al.,[25] Fouche et al.,[22] and Ramalan et al.[21] Notably, increasing the extract dose to 400 mg/kg BW did not further increase ejaculatory latency or decrease postejaculatory mounting interval, which is in agreement with the findings of Erhabor and Idu.[26]

Effects of I. cylindrica root ethanol extract on serum testosterone levels

Testosterone plays a crucial role in sexual function, as documented in numerous studies. It is responsible for regulating sexual motivation, sex drive, and sexual performance. In men, testosterone is vital for maintaining erectile function.[27] Testosterone exerts its influence on sexual function and response through mechanisms in the brain or periphery[28] and is primarily produced by the Leydig cells in the testes.[29]

Serum testosterone levels were observed to increase in all treatment groups compared with the control groups. Figure 5 shows the statistical test results indicating a significant difference in the mean serum testosterone levels between the negative control group and all treatment groups ($^{****}p < 0.0001$).

The serum testosterone level decreased slightly with the increased of the dosses. The highest levels were observed at a dose of 100 mg/kg BW, whereas the lowest levels were observed at 400 mg/kg BW. Nwokike et al. also reported an increase in serum testosterone levels following the administration of *I. cylindrica* root extract, although their results showed higher testosterone levels with higher extract doses.[30] The precise mechanism through which cogon grass (*I. cylindrica*) roots elevate serum testosterone levels remains unclear and warrants further investigation. However, the Tukey HSD post hoc analysis revealed no significant difference in the mean serum

testosterone levels among the treatment groups. This indicates that all doses of *I cylindrica* root extract administered for 28 days have the potential to increase serum testosterone levels.

Compounds in plants that have aphrodisiac effects generally contain saponins, flavonoids, and alkaloids. Saponin compounds affect androgenic and gonadotrophic activity. Flavonoids have androgenic effects, while alkaloid compounds play a role in increasing cholesterol synthesis in the testes, which is necessary for the synthesis of the hormone testosterone, increasing levels of nitric oxide, and relaxing the smooth muscle of the corpus cavernosum.[31] Jayalakshmi et al .[32] and Khaerunnisa et al.[33] reported the presence of flavonoids as a secondary metabolite found in reed root extract using water or alcohol solvents. Dhianawaty et al.[34] reported that from 96 mg of alcoholic extract of the roots of the reeds, the flavonoid content was found to be 1.17%. Based on the results of the studies above, it can be linked that reed roots can act as an aphrodisiac because the extract contains flavonoids, which have an androgenic effect (increases the synthesis of the hormone testosterone). The exact mechanism still requires further research.

This study has certain limitations, particularly concerning the measurement of serum testosterone levels and the assessment of rat sexual behavior. It is recommended that both serum testosterone levels and rat sexual behavior be measured weekly to provide a more comprehensive understanding of the effects of *I. cylindrica* root extract. Additionally, the study did not include measurements of luteinizing hormone and follicle-stimulating hormone levels, which are important hormonal markers in the regulation of sexual function. Future studies could consider including these hormone measurements to gain further insights into the mechanisms underlying the observed effects.

Conclusion

Overall, this study provides evidence that the ethanol extract of *I. cylindrica* roots have potential as an aphrodisiac, aligning with their traditional use in Indonesian ethnic groups as a traditional medicine. Particularly, a dose of 100 mg/kg BW of the extract demonstrated promising results in terms of its aphrodisiac effects. The observed increase in serum testosterone levels suggests that this may be one of the mechanisms through which the extract exerts its aphrodisiac properties. Considering its androgenic effects, further research on cogon grass root extract could be explored for potential applications in hypogonadism and male infertility cases.

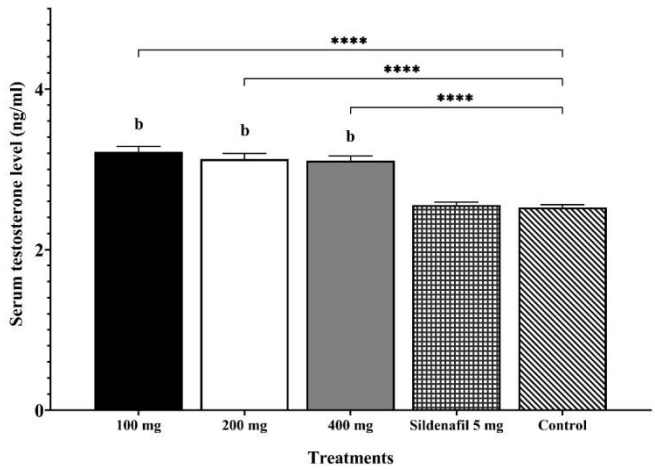

Figure 5: The effect of administering ethanol extract of *Imperata cylindrica* roots on serum testosterone levels, analyzed with Tukey HSD post hoc analysis. Graphs labeled with the letter *b* indicate no significant difference. The unlabeled graphs also show no significant difference ($p > 0.05$). The data presented represents the mean ± SEM; n = 5; ****$p < 0.0001$.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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