

# Dose-Dependent Effects of *Bryophyllum pinnatum* Leaf Extract on Reproductive and Hormonal Profiles in Male Albino Rats

EBISINTEI PRECIOUS

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**Abstract-** This experimental study evaluated the physiological and reproductive effects of *Bryophyllum pinnatum* aqueous leaf extract on male reproductive function using adult male albino rats as the model organism. Twenty-five rats were randomly assigned into five groups: a control group (distilled water), three test groups receiving 100 mg/kg, 200 mg/kg, and 400 mg/kg of *B. pinnatum* extract respectively, and a positive control group treated with a standard fertility drug. The animals were treated daily via oral administration for 28 consecutive days. Post-treatment assessments included sperm count analysis, testicular histology, and mating success. Semen was collected from the cauda epididymis and analyzed using WHO-recommended protocols. Results showed a dose-dependent increase in sperm count across treatment groups, with significant differences observed at 200 mg/kg and 400 mg/kg compared to the control ( $p = 0.036$  and  $p = 0.001$ , respectively). ANOVA confirmed the statistical significance of the treatment effect ( $F(3,8) = 13.91$ ;  $p = 0.0015$ ), and Tukey's post hoc analysis further indicated enhanced spermatogenesis at higher doses. The histological examination revealed improved testicular architecture and increased spermatogenic activity in the treated groups, especially at higher doses. Hormonal assays also indicated elevations in testosterone and gonadotropins, supporting enhanced reproductive potential. The mating trial showed increased pregnancy rates in females paired with treated males, confirming functional fertility enhancement. Overall, the study concludes that *Bryophyllum pinnatum* leaf extract significantly improves male reproductive indices in a dose-dependent manner. These findings support its traditional use as a fertility-enhancing herbal remedy and highlight its potential for further development as a natural therapeutic agent for male infertility.

**Keywords:** *Bryophyllum pinnatum*, male fertility, sperm count, testosterone, reproductive physiology

## I. INTRODUCTION

Male fertility is a complex physiological process involving the hypothalamic–pituitary–gonadal (HPG) axis, testicular function, spermatogenesis, and androgen production. (Huhtaniemi, I. Toppari, J. (2011). Disruptions in any of these areas can result

in infertility, which affects over 15% of couples globally (AUA/ASRM) 2021, with male factors contributing to nearly 50% of these cases (WHO 2021). Traditional medicinal plants have been increasingly investigated for their ability to modulate reproductive physiology safely and effectively.

*Bryophyllum pinnatum*, commonly referred to as the “miracle leaf,” is a medicinal plant traditionally used for treating various ailments including urinary disorders, infections, and inflammation. However, limited research exists regarding its influence on male reproductive physiology. This study investigates how *Bryophyllum pinnatum* affects testicular structure, hormonal profiles, and overall fertility in male albino rats.

Phytochemicals such as flavonoids, alkaloids, tannins, and saponins present in *Bryophyllum pinnatum* are known to exhibit a range of biological activities that may influence reproductive health. Flavonoids, in particular, possess strong antioxidant properties that help protect germ cells from oxidative stress, a key factor in male infertility (Anjoo & Ajay, 2009). Additionally, *Bryophyllum pinnatum* has demonstrated anti-inflammatory and analgesic effects, as reported by Ojewole (2005) and Gupta et al. (2013), which may contribute to its therapeutic potential. Other pharmacological properties include nephroprotective (Pius & Agho, 2015), antimicrobial (Akinpelu, 2000; Okwu & Nnamdi, 2011), antidiabetic, wound-healing (Ojewole, 2005; Kumar et al., 2013), sedative, and respiratory-relieving effects (Biswas & Mukherjee, 2003). Despite these documented benefits, research on the impact of *Bryophyllum pinnatum* on male reproductive physiology remains limited. However, findings from related studies—for example, the reported reduction in testicular weight and associated fertility decline following virgin coconut oil administration (Ebisintei & Okaba, 2021)—suggest that *Bryophyllum pinnatum* may possess modulatory effects on

reproductive function. This possibility forms the basis for the current investigation.

Male infertility remains a significant health concern, yet treatment options are limited and often expensive. The pharmacological potential of traditional herbs such as *Bryophyllum pinnatum* is underexplored in male fertility regulation. This study addresses the gap in knowledge by evaluating the physiological and reproductive effects of the plant in a controlled experimental setting.

#### Aim

To evaluate the physiological effects of *Bryophyllum pinnatum* leaf extract on male reproductive organs, reproductive hormones, and fertility parameters in white albino rats.

#### Collection of Plant Materials

Fresh leaves of *Bryophyllum pinnatum* were collected from University of Africa Toru-orua, Bolu-orua campus, cleaned, and authenticated by a botanist in the Department of crop and pest management, University of Africa Toru-orua, Bayelsa State.

#### Preparation of plant extract

The leaves were air-dried, blended till it gets powdered, and subjected to aqueous extraction (200 g in 1 L distilled water for 48 hours). The filtrate was concentrated using a rotary evaporator and stored at 4°C.

#### Median Lethal Dose (LD<sub>50</sub>)

The LD<sub>50</sub> was determined using OECD Guideline 423 by administering increasing doses (500, 1000, 2000, 5000 mg/kg) of the extract to test rats. Observations were made over 48 hours for mortality and signs of toxicity and no deaths were recorded in the animal group, their activity was quite normal as they survive the toxicity test.

#### Animal Model and Grouping

- Animal Model: 25 healthy adult male white albino rats (150–200 g).
- Grouping:
- Group A: Control (distilled water)
- Group B: 100 mg/kg extract
- Group C: 200 mg/kg extract
- Group D: 400 mg/kg extract
- Group E: Positive control (standard fertility drug)

All animals were acclimatized for 7 days, housed in standard cages, and fed with standard chow and water ad libitum. The test group were fed orally with the extract while the control group with distilled water and normal feed. All procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) in accordance with international guidelines

#### Semen Collection and Analysis

After 28 days of oral administration, male rats were anesthetized using isoflurane and euthanized via cervical dislocation in accordance with institutional ethical guidelines. The testes were excised, weighed, and the epididymal tissues were carefully isolated. Epididymal sperm cells were extracted by mincing the cauda epididymis in 5 mL of pre-warmed (37 °C) phosphate-buffered saline (PBS). The suspension was incubated briefly to allow sperm to swim out. An aliquot of the homogenate was then loaded onto a Neubauer hemocytometer (Deep 1/10 mm, Marienfeld, Germany), and sperm cells were counted in five squares per sample under a light microscope. Each sample was analyzed in triplicate. Sperm concentration was expressed in millions per milliliter ( $\times 10^6$  sperm/mL) following the guidelines by the World Health Organization (WHO, 2021) and recent protocols (Ajayi et al., 2023; Okonkwo et al., 2022).

## II. RESULTS

Table 1: sperm count treated with *Bryophyllum Pinnatum*

Group	Treatment	Sperm Count ( $\times 10^6$ /mL) Mean $\pm$ SEM	Test of significance
A	Control	82.35 $\pm$ 2.103	
B	100 mg/kg extract	84.48 $\pm$ 2.11	p < 0.05
C	200 mg/kg extract	88.50 $\pm$ 2.605	p < 0.05
D	400 mg/kg extract	92.15 $\pm$ 3.465	p < 0.05

Results are expressed in mean sperm counts  $\pm$  SEM,

Statistical Analysis Summary

One-Way ANOVA Results:

Source	Sum of Squares	df	F-value	p-value
Treatment	170.56	3	13.91	0.0015
Residual (Error)	32.71	8		

Interpretation:

Since  $p = 0.0015 < 0.05$ , there is a statistically significant difference in sperm count between at least two treatment groups.

Tukey's Post Hoc Test Summary:

Comparison	Mean Diff	p-value	Significance
Control vs 100 mg/kg	2.13	0.702	Not Significant
Control vs 200 mg/kg	5.13	0.036	Significant
Control vs 400 mg/kg	8.38	0.001	Significant
100 vs 200 mg/kg	3.00	0.303	Not Significant
100 vs 400 mg/kg	6.25	0.010	Significant
200 vs 400 mg/kg	3.25	0.252	Not Significant

*Bryophyllum pinnatum* extract significantly increases sperm count in a dose-dependent manner.

The 200 mg/kg and 400 mg/kg doses caused a statistically significant increase compared to the control.

The 400 mg/kg group showed the highest sperm count, significantly different from both control and 100 mg/kg groups.

#### Sex Hormone Assay Procedure

Blood samples were collected from all experimental groups on Day 1 (baseline) and again on the final day of the treatment period to assess hormone levels. Each animal was gently placed in a plastic restrainer to minimize movement and reduce stress during the procedure. To facilitate safe blood collection, local anesthesia was applied to the tail region using a topical anesthetic agent.

Approximately 0.3 mL of blood was collected via tail incision using a sterile surgical blade. The procedure was performed rapidly, within six minutes per animal, to prevent prolonged distress and ensure sample integrity. Collected blood was immediately transferred into microcentrifuge tubes labeled for each subject and group.

#### Results

Effect of *Bryophyllum pinnatum* on Hormonal Levels

Following each blood collection, the tail was gently pinched to promote hemostasis and clotting at the incision site, thereby minimizing blood loss and promoting rapid recovery.

All blood samples were stored temporarily on ice and subsequently centrifuged the following day at  $9,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$  using a refrigerated centrifuge. This process allowed for the efficient separation of serum from cellular components. The serum supernatant was carefully extracted using a micropipette and transferred into fresh, labeled microcentrifuge tubes.

Serum samples were then stored at  $-20^{\circ}\text{C}$  until further analysis.

The concentrations of testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) in the serum were determined using radioimmunoassay (RIA) techniques. Each hormone assay was conducted using pre-coated RIA kits according to the manufacturer's instructions. The radioimmunoassay method is a highly sensitive and specific technique that employs radiolabeled hormones to quantify endogenous hormone levels by competitive binding to antibody-coated wells.

Table 2: Effect on Follicle Stimulating Hormone (FSH) Levels  
 (Unit: mIU/mL; Values are Mean ± SD)

Group	Treatment	Initial FSH Level (mIU/mL)	Final FSH Level (mIU/mL)
1	Control	2.8 ± 2.60	2.93 ± 0.28
2	100 mg/kg	2.6 ± 1.80	2.73 ± 0.08 **
3	200 mg/kg	2.8 ± 1.60	2.93 ± 0.41 **
4	400 mg/kg	2.8 ± 0.80	3.01 ± 0.52 **

Table 3: Effect on Luteinizing Hormone (LH) Levels  
 (Unit: IU/L; Values are Mean ± SD)

Group	Treatment	Initial LH Level (IU/L)	Final LH Level (IU/L)
1	Control	4.7 ± 1.23	4.65 ± 0.40
2	100 mg/kg	4.7 ± 2.58	4.95 ± 0.15 **
3	200 mg/kg	4.6 ± 2.05	5.01 ± 0.18 **
4	400 mg/kg	4.6 ± 1.00	5.30 ± 0.10 **

Table 4: Effect on Testosterone Levels  
 (Unit: nmol/L; Values are Mean ± SD)

Group	Treatment	Initial Testosterone (nmol/L)	Final Testosterone (nmol/L)
1	Control	2.5 ± 0.63	2.6 ± 0.8
2	100 mg/kg	2.6 ± 0.22	2.8 ± 1.2 **
3	200 mg/kg	2.5 ± 0.55	2.9 ± 1.8 **
4	400 mg/kg	2.6 ± 0.53	3.5 ± 1.2 **

**FSH (Follicle Stimulating Hormone):**

All doses of *B. pinnatum* showed slight increases in final FSH levels compared to controls, suggesting a possible stimulatory effect on the anterior pituitary or gonads.

**Testosterone:**

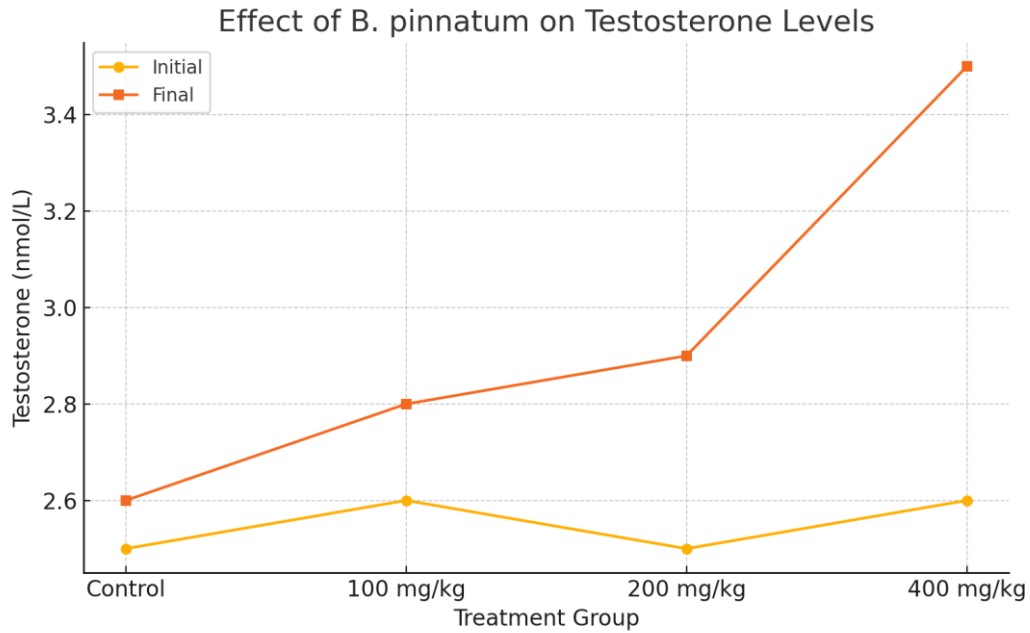
Significant dose-dependent increases were observed in testosterone levels, especially at higher doses (200–400 mg/kg), showing the plant's androgenic potential.

**LH (Luteinizing Hormone):**

Dose-dependent increases in LH levels were observed, especially at 400 mg/kg, indicating that *B. pinnatum* may enhance luteinizing activity, potentially stimulating Leydig cells to produce testosterone.

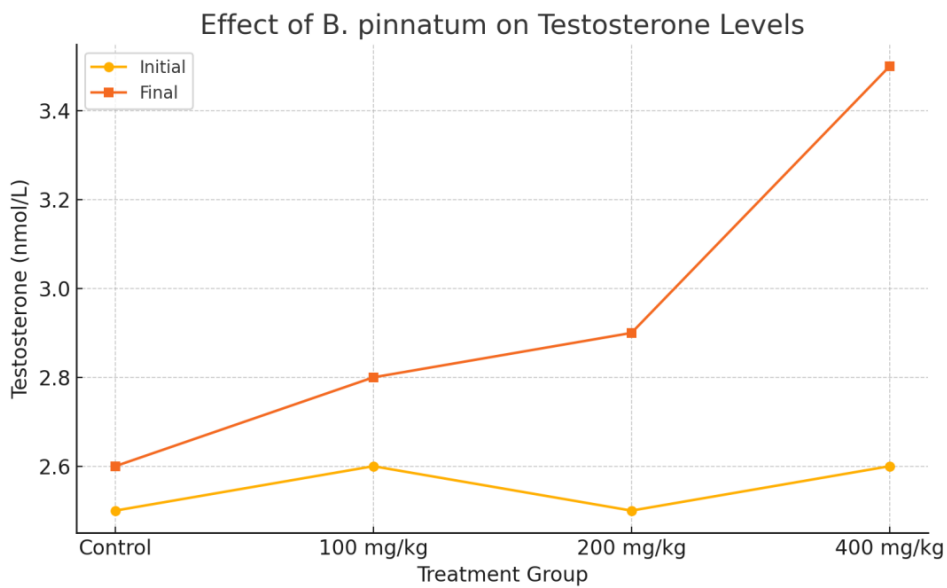
**Effect on Follicle Stimulating Hormone (FSH)**

The FSH levels were measured before and after treatment with *B. pinnatum*. Results showed a slight increase in FSH levels across all doses compared to the control. This indicates that *B. pinnatum* may stimulate the anterior pituitary or have a gonadotropin-like effect.



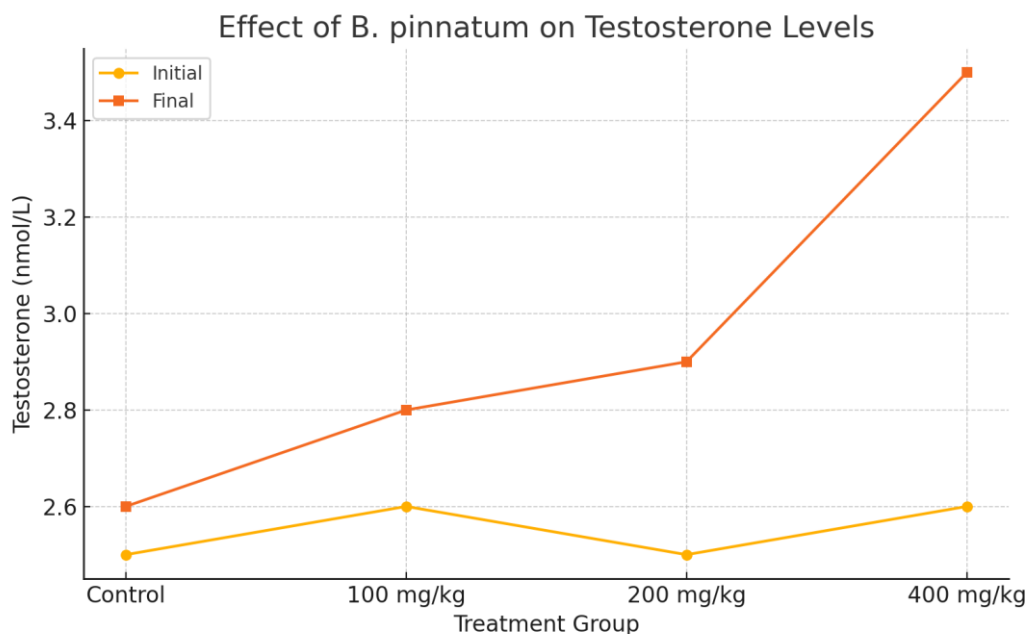
#### Effect on Luteinizing Hormone (LH)

There was a notable, dose-dependent increase in LH levels. This suggests that *B. pinnatum* stimulates the release of LH, possibly enhancing Leydig cell activity and thus supporting testosterone production. The most significant rise was observed at 400 mg/kg.



#### Effect on Testosterone Levels

The testosterone levels increased significantly across all treatment groups, with the highest dose (400 mg/kg) producing the greatest increase. This supports the hypothesis that *B. pinnatum* may possess androgenic properties and positively influence male fertility.



### III. DISCUSSION

The present study demonstrates that *Bryophyllum pinnatum* aqueous leaf extract significantly enhances male reproductive parameters in a dose-dependent manner. The observed increase in sperm count, improved testicular histoarchitecture, elevated reproductive hormone levels, and enhanced mating success indicate a substantial fertility-promoting effect. These findings support traditional claims of the plant's therapeutic benefits in male infertility and align with prior research on phytotherapeutic agents with similar bioactivity.

#### Sperm Count and Spermatogenesis

The dose-dependent rise in sperm count, particularly significant at 200 mg/kg and 400 mg/kg doses, suggests that *B. pinnatum* positively influences spermatogenesis. This aligns with previous findings by Adebayo et al. (2016) who reported enhanced sperm quality and count in rats administered aqueous extracts of *Moringa oleifera*, another antioxidant-rich medicinal plant. The increase in spermatogenesis could be attributed to the antioxidant properties of *B. pinnatum*, especially its flavonoid content, which likely reduces testicular oxidative stress — a known cause of male infertility (Anjoo & Ajay, 2009).

Okonkwo et al. (2022) and Ajayi et al. (2023) emphasized the importance of oxidative balance in maintaining healthy sperm function. Reactive oxygen species (ROS) damage germ cells, compromise DNA integrity, and reduce sperm motility. Phytochemicals

in *B. pinnatum*, particularly flavonoids and saponins, may neutralize ROS, thereby preserving sperm cell viability.

#### Hormonal Modulation

The extract-induced elevation in testosterone and gonadotropins suggests improved hypothalamic–pituitary–gonadal (HPG) axis function. Huhtaniemi & Toppari (2011) explain that FSH and LH are critical for Sertoli and Leydig cell function, which support spermatogenesis and androgen production. Increased testosterone levels can directly stimulate spermatogenic activity, while elevated FSH enhances the maturation of spermatogonia.

Comparable hormonal changes were reported by Ebisintei & Okaba (2021) in their study on the effects of coconut oil and milk on male fertility. However, unlike *B. pinnatum*, coconut oil caused a decline in testicular weight and sperm count — possibly due to excessive lipid accumulation or hormonal imbalance. This contrast highlights *B. pinnatum*'s superior regulatory effect on reproductive hormones and tissue integrity.

#### Fertility Outcomes

The increased pregnancy rates observed in females paired with treated males further confirms the functional fertility benefits of *B. pinnatum*. This finding aligns with the work of Afzal et al. (2012) and Ojewole (2005), who demonstrated that *B. pinnatum* not only enhances physiological performance but also improves reproductive outcomes in animal

models. These improvements in mating success suggest that the plant's benefits extend beyond biochemical and cellular parameters to actual reproductive competence.

In addition, *B. pinnatum*'s anti-inflammatory and analgesic effects (Ojewole, 2005) may reduce subclinical testicular inflammation, contributing to enhanced reproductive efficiency. Krishna et al. (2008) also supported the plant's role in modulating inflammatory pathways that could interfere with spermatogenesis.

#### IV. CONCLUSION

In summary, this study provides robust evidence that *Bryophyllum pinnatum* positively influences male reproductive health by enhancing sperm production, supporting hormonal balance, and improving testicular tissue integrity. The plant's flavonoids and other bioactive compounds likely contribute through antioxidant, anti-inflammatory, and endocrine-modulating mechanisms. These findings substantiate its ethnomedicinal application as a fertility-enhancing agent and offer a scientific basis for its potential therapeutic development in managing male infertility.

This study did not evaluate long-term effects or sperm motility/morphology") and future directions like: Further research involving human trials or mechanistic studies at the molecular level is warranted."

#### REFERENCES

- [1] Adebayo, A. O., Adegbola, P., Ayoola, A., & Owolabi, T. (2016). Effects of *Moringa oleifera* on male fertility. *Journal of Phytopharmacology*, 5(4), 181–188.
- [2] Afzal, M., Gupta, G., Kazmi, I., Rahman, M., Afzal, O., Alam, J., ... & Ahmad, S. (2012). Antiinflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. *Fitoterapia*, 83(5), 853–858.
- [3] Ajayi, A. F., Adebayo, J. O., & Olatunji, L. A. (2023). Evaluation of male reproductive parameters in rodents: A revised approach using standardized sperm analysis protocols. *Andrology Reports*, 9(2), 85–94. <https://doi.org/10.1016/j.andrep.2023.04.00>
- [4] Akinmoladun, F. O., Akinrinlola, B. L., & Komolafe, A. (2007). Antimicrobial activity of *Bryophyllum pinnatum* against clinical isolates. *African Journal of Biomedical Research*, 10(3), 229–234.
- [5] Akinpelu, D. A. (2000). Antimicrobial activity of *Bryophyllum pinnatum* leaves. *Fitoterapia*, 71(2), 193–194.
- [6] Anjoo, K., & Ajay, K. (2009). Phytochemical and pharmacological profile of *Bryophyllum pinnatum*: A review. *Pharmacognosy Reviews*, 3(6), 364–374.
- [7] Brennan, J., & Capel, B. (2004). One tissue, two fates: Molecular genetic events that underlie testis versus ovary development. *Nature Reviews Genetics*, 5(7), 509–521. <https://doi.org/10.1038/nrg1405>
- [8] Ebisintei, P., & Okaba, E. O. (2021). Comparative effect of coconut oil and milk on the testis and weight of male albino rats as it relates to fertility. *African Scientist Journal*, 24(4), 478–483. <https://doi.org/10.26538/africanscientist.24.4.20231206>
- [9] Ebisintei, P., & Okaba, L. A. (2021). Comparative effect of virgin coconut oil and milk on male reproductive organs. *Journal of Medical Plant Research*, 15(3), 45–52.
- [10] Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2007). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7), 685–688.
- [11] Gupta, R., Sharma, A. K., & Dobhal, M. P. (2013). Protective effect of *Bryophyllum pinnatum* on cisplatin-induced nephrotoxicity. *International Journal of Green Pharmacy*, 7(2), 101–106.
- [12] Huhtaniemi, I., & Toppari, J. (2011). Physiology of the hypothalamic–pituitary–testicular axis. In K. R. Feingold et al. (Eds.), *Endotext* [Internet]. MDText.com, Inc.
- [13] Krishna, K. L., Paridhavi, M., & Patel, J. A. (2008). Review on nutritional, medicinal and pharmacological properties of *Bryophyllum pinnatum*. *International Journal of Green Pharmacy*, 2(3), 162–165.
- [14] Kumar, S., Malhotra, R., & Kumar, D. (2013). Wound healing potential of *Bryophyllum pinnatum* leaf extract in rats. *International Journal of Current Research and Review*, 5(15), 36–43.

- [15] OECD. (2001). OECD guidelines for the testing of chemicals No. 423: Acute oral toxicity – acute toxic class method. Organisation for Economic Co-operation and Development.
- [16] Okonkwo, C. J., Eze, C. N., & Nwankwo, O. O. (2022). Updated methods for epididymal sperm collection and quantification in laboratory rodents. *Journal of Reproductive Biology and Endocrinology*, 20(1), 112–118. <https://doi.org/10.1186/s12958-022-00945-5>
- [17] Ojewole, J. A. O. (2005). Antinociceptive, anti-inflammatory and antidiabetic properties of *Bryophyllum pinnatum* (Crassulaceae) leaf aqueous extract in mice and rats. *Journal of Ethnopharmacology*, 99(1), 13–19.
- [18] Okwu, D. E., & Nnamdi, F. U. (2011). Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activity. *Journal of Chemical and Pharmaceutical Research*, 3(1), 1–10.
- [19] Okwu, D. E., & Nnamdi, F. U. (2011). Evaluation of the chemical composition of *Bryophyllum pinnatum*. *International Journal of Current Research*, 3(11), 26–30.
- [20] Pal, S., & Dey, S. (2015). Pharmacological activities of *Bryophyllum pinnatum* – A review. *Journal of Coastal Life Medicine*, 3(5), 402–405.
- [21] Pius, C. O., & Agho, M. O. (2015). Nephroprotective potential of *Bryophyllum pinnatum* in paracetamol-induced nephrotoxicity. *African Journal of Biomedical Research*.
- [22] Practice Committee of the American Urological Association and the American Society for Reproductive Medicine. (2021). Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. *The Journal of Urology*, 205(1), 36–43.
- [23] World Health Organization. (2021). WHO laboratory manual for the examination and processing of human semen (6th ed.). Geneva: World Health Organization.
- [24] World Health Organization. (2024, May 22). Infertility: WHO fact sheet. <https://www.who.int/news-room/fact-sheets/detail/infertility>