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The Antiproliferative and Spermicide Effects of D-Limonene on Gonadal Function in Male Albino Rats: A Comprehensive Study on Spermatogenesis and Gonadal Morphometry

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Abstract

This study investigates the effects of D-Limonene on reproductive health parameters in albino rats, focusing on sperm quality and gonadal morphology. The study involved administering D-Limonene at various doses to assess their impacts on sperm parameters, including motility, viability, and abnormalities, as well as gonadal morphometry. The D-Limonene was applied at doses of 50, 100, and 150 mg/kg BW for 60 days, followed by histopathological analysis of testicular tissues. Results indicated that D-Limonene treatment revealed a dose-dependent decline in sperm quality parameters. Specifically, total motility, progressive motility, and viability decreased, while abnormality rates increased with higher D-Limonene doses. Notably, the highest dose (150 mg/kg BW) led to the most significant reductions in sperm motility and viability. Additionally, the presence of necrotic cells and structural changes in testicular tissues were observed, supporting the adverse impact of D-Limonene on reproductive function. The findings suggest that D-Limonene exhibits potential as an effective spermicide, highlighting its utility in population control applications. The study underscores the need for further research to fully understand the mechanisms behind these effects and their implications for reproductive health.

Keywords

Reproductive health, D-Limonene, Spermicidal activity, Control population, Leptospirosis.

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Introduction

Leptospirosis is a zoonotic disease of global significance, primarily transmitted through the urine of infected animals, particularly rodents, which serve as major reservoirs. The control of rodent populations is crucial not only for managing the spread of leptospirosis but also for mitigating other public health threats posed by these animals (Pal and Hadush, 2017). Traditional rodent control methods, such as chemical rodenticides, often raise concerns regarding environmental toxicity and the potential development of resistance (McGee *et al*., 2020). As a result, there is a growing interest in alternative strategies that target the reproductive capacity of rodents as a means of population control. Natural compounds with spermicidal and antiproliferative properties have emerged as promising candidates for such interventions (Nascimento *et al*., 2022). D-Limonene, a bioactive compound found in citrus peels, has shown significant potential due to its diverse biological activities, including antimicrobial, anti-inflammatory, and cytotoxic effects (Lin *et al*., 2024). However, its application in reproductive health, particularly in the context of controlling rodent populations, remains underexplored. The ability of D-Limonene to inhibit spermatogenesis and reduce sperm viability could offer a novel approach to population control in rodent species, thereby contributing to the prevention of leptospirosis and other rodent-borne diseases.

Despite ongoing efforts to control rodent populations, zoonotic diseases such as leptospirosis remain a significant public health concern (Karpagam and Ganesh, 2020). Leptospirosis is caused by *Leptospira* bacteria, transmitted primarily through the urine of infected animals, with rodents serving as the principal reservoirs (Soo *et al*., 2020). Current rodent control strategies rely heavily on chemical rodenticides. While these agents are effective in reducing rodent numbers, they raise serious environmental concerns, including toxicity to non-target species and the potential for soil and water contamination (Bradley and Lockaby, 2023). Moreover, the continuous use of chemical rodenticides can lead to the development of resistance in rodent populations, exacerbating the problem over time (Imrat *et al*., 2013). Furthermore, existing control methods inadequately address the reproductive capacity of rodents, which is a critical factor in their population resilience. Rodents possess a high reproductive rate, allowing their populations to quickly rebound following control interventions (Allan *et al*., 2015). This underscores the urgent need for alternative, more sustainable approaches that specifically target rodent reproductive functions (Blasdell *et al*., 2019). D-Limonene, a bioactive compound found in citrus peels, has shown promise as an antiproliferative and spermicidal agent. Preliminary studies indicate that D-Limonene can inhibit spermatogenesis and reduce sperm viability, potentially limiting rodent reproductive capacity (Salehi *et al*., 2019). However, the efficacy and mechanisms of D-Limonene in the context of rodent population control have not been thoroughly investigated (Tahir *et al*., 2020). This study aims to fill this gap by evaluating the impact of D-Limonene on gonadal function and sperm quality in male rats and assessing its potential as an environmentally friendly rodent population control agent (Nagpal and Abraham, 2017).

This study explores the spermicidal and antiproliferative effects of D-Limonene on

gonadal function in male rats, with the goal of assessing its potential as a population control agent. By investigating its impact on spermatogenesis, gonadal morphometry, and sperm quality, this research seeks to determine the viability of D-Limonene as a natural method for reducing the reproductive capacity of rodents, thereby contributing to more sustainable approaches to disease vector management. The current literature on rodent population control primarily focuses on chemical rodenticides, with limited exploration of biological alternatives that target reproduction (Massei *et al*., 2024). While D-Limonene has been recognized for its broad-spectrum biological activities, its application in controlling rodent fertility remains largely unexplored. This study fills a critical gap by providing comprehensive data on the effects of D-Limonene on male reproductive parameters, positioning it as a potential tool for integrated pest management strategies that prioritize environmental safety and efficacy. The objectives of this research are to evaluate the spermicidal and antiproliferative effects of D-Limonene on male reproductive parameters and assess its potential as a natural rodent population control agent.

This research introduces a novel approach to rodent population control by harnessing the spermicidal and antiproliferative properties of D-Limonene. Unlike traditional methods, which often involve toxic chemicals, this study explores a natural compound with the potential to disrupt the reproductive cycle of rodents, thereby offering a sustainable and targeted solution for managing vector populations (Witmer, 2018). The findings of this study could pave the way for new, environmentally friendly strategies in the control of leptospirosis and other rodent-borne diseases, underscoring the broader implications for public health and pest management.

Materials and Methods

Study Design

This study employed an experimental design to evaluate the antiproliferative and spermicidal effects of D-Limonene on male rats. The experiment was structured as a controlled laboratory study, with subjects randomly assigned to different treatment groups. The study included a control group receiving no treatment and three experimental groups receiving varying doses of D-Limonene. The doses were selected based on preliminary toxicity studies to ensure safety while allowing for effective analysis of the compound's biological effects. The study duration was set at 8 weeks, sufficient to assess the impact of D-Limonene on gonadal function and sperm parameters.

Population and Sample

The study population consisted of adult male Wistar rats (*Rattus norvegicus*), aged 8–10 weeks, and weighing between 200–250 g. This age range was chosen to ensure sexual maturity, facilitating the evaluation of reproductive parameters. A total of 40 rats were used, with 10 rats assigned to each of the four groups (control, low dose, medium dose, high dose). The sample size was calculated based on power analysis to ensure adequate statistical power for detecting differences between groups.

Research Procedure

The rats were acclimatized for one week before the commencement of the experiment. During this period, they were housed in standard laboratory conditions, with a 12-hour light/dark cycle, controlled temperature (24°C),

and free access to food and water. D-Limonene was administered orally using a gavage, daily, for the duration of the study. The control group received an equivalent volume of the vehicle solution.

The study involved a controlled administration of D-Limonene to male rats over a period of 60 days. The rats were divided into four groups based on the concentration of D-Limonene administered. The control group received 0 mg/kg BW (body weight) of D-Limonene, while the treatment groups were administered D-Limonene at concentrations of 50 mg/kg BW (T1), 100 mg/kg BW (T2), and 150 mg/kg BW (T3), respectively. D-Limonene was prepared and administered orally once daily. The dosing solution was prepared fresh each day to ensure stability and effectiveness. Rats in the control group received an equivalent volume of the vehicle solution (without D-Limonene), ensuring consistency in administration across all groups.

At the end of the treatment period, the rats were euthanized using CO_2 inhalation, followed by cervical dislocation. The testes were immediately removed, cleaned of surrounding tissues, and weighed using a precision balance. Testicular morphometry was performed to measure the length and width of the testes using a digital caliper. These measurements were used to assess any changes in testicular size as a result of the treatment.

Histopathological Preparation and Analysis

The removed testes were fixed in 10% buffered formalin for 24 hours, then dehydrated in a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were cut using a microtome and stained with hematoxylin and eosin (H&E) for histopathological examination.

Microscopic examination was performed to evaluate the histological architecture of the testes, with specific attention to the seminiferous tubules. The number of spermatogenic cells, including spermatogonia, spermatocytes, and spermatids, was counted per cross-section of the seminiferous tubules. The presence and condition of Leydig cells, which are responsible for testosterone production, and Sertoli cells, which support and nourish developing sperm cells, were also assessed. The counts were averaged across multiple sections to provide a comprehensive assessment of the testicular response to D-Limonene treatment. Necrotic cells were identified by their characteristic morphological features, including cell shrinkage, loss of membrane integrity, and nuclear fragmentation. The extent of necrosis was quantified by counting necrotic cells per field in multiple sections of the testes.

Sperm Quality Assessment

Sperm quality was evaluated from samples collected from the cauda epididymis. The sperm parameters assessed included sperm count, motility, viability, and morphological abnormalities. Sperm motility was analyzed using computer-assisted sperm analysis (CASA), while viability was assessed using eosin-nigrosin staining. Morphological abnormalities were recorded by examining sperm smears under a microscope.

Data Analysis

Data were analyzed using SPSS software (version 25.0). Descriptive statistics were used to summarize the data, including means and standard deviations. The normality of data distribution was assessed using the Shapiro-Wilk test. Differences between groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test

for pairwise comparisons. Statistical significance was set at $p < 0.05$. The results were presented as mean ± standard deviation, and graphical representations were used to illustrate key findings.

Results and Discussion

The data presented in Table 1 highlight the impact of different doses of D-Limonene on the body weight of rats over a 60-day treatment period. The control group, which received no D-Limonene, exhibited a healthy weight gain of 40 ± 0.11 g with no observed alopecia. In contrast, rats treated with D-Limonene at 50 mg/kg BW (T1) showed a reduced weight gain of 33 ± 0.45 g and mild alopecia, indicating the onset of side effects. As the dose increased to 100 mg/kg BW (T2), weight gain further decreased to 20 ± 0.56 g, and the severity of alopecia became moderate. This suggests a dose-dependent effect of D-Limonene on body weight and the development of alopecia.

The highest dose of 150 mg/kg BW (T3) resulted in the lowest weight gain of 15 ± 0.33 g and moderate alopecia. This significant reduction in weight gain, combined with severe alopecia, reflects the detrimental effects of the highest D-Limonene dose on the rats' health. The observed trends indicate that higher doses of D-Limonene lead to increased severity of adverse effects, including reduced body weight gain and more pronounced alopecia. These findings underscore the need for careful consideration of dosing to balance therapeutic effects with potential side effects.

Table 1. Body Weight Progression of Rats Over 60 Days of Treatment

Group	Mean ± SD			
	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Alopecia Severity
Control (0 mg/kg BW)	180 ± 1.34^{a}	220 ± 1.22^{a}	40 ± 0.11^{a}	None
T1 (50 mg/kg BW)	182 ± 0.65^{ab}	215 ± 0.09^{ab}	33 ± 0.45^{a}	Mild
T2 (100 mg/kg BW)	185 ± 1.33^{b}	205 ± 2.54^{bc}	20 ± 0.56^{b}	Moderate
T3 (150 mg/kg BW)	180 ± 1.22^{a}	195 ± 2.11^{c}	15 ± 0.33^{c}	Moderate

Note: Different superscript or note significance indicate significant differences $p<0.05$

The data in Table 2 present the gonadal morphometry results of rats treated with different doses of D-Limonene for 60 days. The measurements include the weight, length, and width of the left and right testes, with significant findings observed across different treatment groups. The control group, receiving no D-Limonene, had the highest testicular measurements: left testis weight of 1.30 ± 0.07 g, right testis weight of 1.32 ± 0.08 g, and average lengths and widths of 19.0 ± 0.7 mm and 10.5 ± 0.4 mm, respectively, showing normal gonadal development. In contrast, rats treated with 50 mg/kg BW D-Limonene (T1) exhibited a decrease in testicular measurements, with left testis weight of 1.25 ± 0.06 g, right testis weight of 1.26 ± 0.06 g, and average lengths and widths of 18.5 ± 0.6 mm and 10.2 ± 0.3 mm. This

reduction was more pronounced in the 100 mg/kg BW group (T2), where the left and right testis weights further decreased to 1.15 ± 0.05 g and 1.16 ± 0.05 g, respectively, and the average lengths and widths were 17.8 ± 0.5 mm and 9.8 ± 0.3 mm. The most significant reductions were observed in the 150 mg/kg BW group (T3), with left and right testis weights of 1.00 ± 0.04 g and 1.02 ± 0.05 g, respectively, and average lengths and widths of 16.5 ± 0.4 mm and 9.0 ± 0.2 mm.

Statistical analysis revealed significant differences between the treatment groups and the control group, with the 150 mg/kg BW treatment showing the most pronounced reductions in all parameters ($p < 0.01$). These findings indicate a dose-dependent effect of D-Limonene on testicular morphometry, with higher doses leading to more severe reductions in testicular size. The observed changes in gonadal morphometry underscore the impact of D-Limonene on testicular health and development, suggesting potential adverse effects at higher doses.

Table 2. Effects of D-Limonene Treatment on Testis Morphometry in Male Rats

Parameter	Mean ± SD			
	Control (0 mg/kg BW)	T1 (50 mg/kg BW)	T2 (100 mg/kg BW)	T3 (150 mg/kg BW)
Left Testis Weight (g)	1.30 ± 0.07^{a}	1.25 ± 0.06^{ab}	1.15 ± 0.05^{bc}	1.00 ± 0.04^{c} **(p<0.01)**
Right Testis Weight (g)	1.32 ± 0.08^{a}	1.26 ± 0.06^{ab}	1.16 ± 0.05^{bc}	1.02 ± 0.05^{c} **(p<0.01)**
Left Testis Length (mm)	19.0 ± 0.7^{a}	18.5 ± 0.6^{ab}	17.8 ± 0.5^{bc}	16.5 ± 0.4^{c} **(p<0.01)**
Right Testis Length (mm)	19.2 ± 0.8^{a}	18.6 ± 0.6^{ab}	17.9 ± 0.5^{bc}	16.6 ± 0.5^{c} **(p<0.01)**
Left Testis Width (mm)	10.5 ± 0.4^{a}	10.2 ± 0.3^{ab}	9.8 ± 0.3^{b}	9.0 ± 0.2^{c} **(p<0.01)**
Right Testis Width (mm)	10.6 ± 0.4^{a}	10.3 ± 0.3^{ab}	9.9 ± 0.3^{b}	9.2 ± 0.2^{c} **(p<0.01)**

Note: Different superscript or note significance indicate significant differences p<0.05

The histopathological examination of the testes from the control and D-Limonene-treated groups revealed significant alterations in the testicular architecture, particularly in the seminiferous tubules, across the different treatment doses. In the control group, the seminiferous tubules displayed normal histological features with well-organized spermatogenic cells, including spermatogonia, spermatocytes, and spermatids, alongside healthy Leydig and Sertoli cells. Few to no necrotic cells were observed.

In contrast, the testes of rats treated with D-Limonene showed dose-dependent disruptions in the testicular structure. The seminiferous tubules in the T1 group (50 mg/kg BW) exhibited slight disorganization of spermatogenic cells with a minor increase in necrotic cells. The T2 group (100 mg/kg BW) displayed moderate disorganization and a noticeable decrease in the number of

spermatogenic cells, with a significant increase in necrosis. The T3 group (150 mg/kg BW) showed severe damage to the seminiferous tubules, with a marked reduction in spermatogenic cells and widespread necrosis. Leydig and Sertoli cells were also significantly affected, with observable degeneration and a reduction in their numbers, particularly at the highest dose.

Table 3. Histopathological Findings in Testicular Tissue Following D-Limonene Treatment

Group	Mean ± SD				
	Spermatogenic	Leydig Cells/Section	Sertoli Cells/Section	Necrotic Cells/Field	Significance
Control (0 mg/kg BW)	85 ± 5	15 ± 2	12 ± 1	1 ± 0	-
T1 (50 mg/kg BW)	75 ± 6	13 ± 2	10 ± 2	5 ± 1	**p < 0.05**
T2 (100 mg/kg BW)	60 ± 7	10 ± 3	8 ± 2	15 ± 3	**p < 0.01**
T3(150 mg/kg BW)	40 ± 8	6 ± 2	5 ± 1	30 ± 5	**p < 0.001**

The data in Table 4 illustrate the impact of D-Limonene treatment on sperm quality parameters in albino rats. In the control group, which did not receive any D-Limonene, sperm quality metrics were optimal: total motility was 87.3 ± 1.2%, progressive motility was 63.5 ± 1.5%, viability was 92.1 ± 1.0%, and abnormality was relatively low at 10.2 ± 1.1%. These values indicate healthy sperm function and minimal abnormalities.

Treatment with D-Limonene resulted in a dose-dependent decline in sperm quality. For the 50 mg/kg BW group (T1), total motility decreased to 81.7 ± 1.5%, progressive motility to 54.2 ± 1.8%, and viability to 87.5 ± 1.2%, with an increase in abnormality to 16.3 ± 1.2%. The 100 mg/kg BW group (T2) showed further deterioration with total motility at 73.4 ± 1.8%, progressive motility at 43.7 ± 2.0%, viability at 83.2 ± 1.5%, and abnormality rising to 22.8 ± 1.4%. The most severe effects were observed in the 150 mg/kg BW group (T3), where total motility dropped to 65.9 ± 2.0%, progressive motility to 32.5 ± 2.5%, viability to 76.4 ± 1.8%, and abnormality increased to 30.1 ± 1.6%. These results clearly indicate that higher doses of D-Limonene have a detrimental effect on sperm quality, underscoring potential risks to reproductive health.

Table 4. Sperm Quality Assessment in Albino Rats Treated with D-Limonene

Group	Mean ± SD			
	Total Motility (%)	Progressive Motility (%)	Viability (%)	Abnormality (%)
Control (0 mg/kg BW)	87.3 ± 1.2^{a}	63.5 ± 1.5^{a}	92.1 ± 1.0^{a}	10.2 ± 1.1^{a}
T1 (50 mg/kg BW)	81.7 ± 1.5^{b}	54.2 ± 1.8^{b}	87.5 ± 1.2^{ab}	16.3 ± 1.2^{b}
T2 (100 mg/kg BW)	73.4 ± 1.8^{bc}	43.7 ± 2.0^{c}	83.2 ± 1.5^{b}	22.8 ± 1.4^{c}
T3 (150 mg/kg BW)	65.9 ± 2.0^{d}	32.5 ± 2.5^{d}	76.4 ± 1.8^{c}	30.1 ± 1.6^{d}

Note: Different superscript or note significance indicate significant differences $p<0.05$

The results of this study provide robust evidence of D-Limonene's antiproliferative effects on male reproductive tissues. The significant reductions in testicular weight, length, and width observed in all treatment groups indicate a substantial impact on testicular development (Hussein *et al*., 2020). These morphometric changes are indicative of impaired spermatogenesis, as evidenced by the decreased number of spermatogenic cells within the seminiferous tubules (Cordeiro *et al*., 2018). Histological examination revealed a notable reduction in spermatogonia, spermatocytes, and spermatids, suggesting that D-Limonene disrupts the normal progression of spermatogenesis. This disruption is likely due to the compound's cytotoxic effects, which interfere with cell division and differentiation processes. The observed decrease in testicular size and spermatogenic cell populations aligns with existing literature on the antiproliferative effects of various bioactive compounds, which often exert their effects through mechanisms such as oxidative stress induction or disruption of cellular signaling pathways. In this study, D-Limonene's ability to inhibit cell proliferation and induce cell death within the seminiferous tubules underscores its potential as an effective reproductive toxicant. The broad impact on both germinal and supporting cells further emphasizes the compound's potential utility in controlling rodent reproductive capacity, highlighting its relevance for pest management and environmental control strategies (Hamoud, 2019).

The disruption of spermatogenesis by D-Limonene is evident from the detailed histopathological analysis, which revealed a significant reduction in key spermatogenic cell types within the seminiferous tubules. The observed decline in spermatogonia, spermatocytes, and spermatids suggests that D-Limonene adversely affects the entire process of sperm cell development. Spermatogenesis, a complex and tightly regulated process, relies on the precise interaction between germ cells and supportive Sertoli cells (Ni *et al*., 2019). The reduction in the number of these germ cells implies that D-Limonene interferes with the differentiation and proliferation of spermatogonia, which are the progenitor cells for all subsequent stages of sperm development. Moreover, the concurrent decrease in Sertoli cells, which are crucial for nurturing and supporting the developing sperm cells, indicates that D-Limonene may also impair the supportive environment necessary for spermatogenesis. This disruption

can be attributed to the compound's potential to induce oxidative stress or alter hormonal signaling pathways that regulate testicular function. The marked decrease in Leydig cells, responsible for testosterone production, further compounds the disruption by affecting hormonal support essential for spermatogenic processes (Oduwole *et al*., 2021). Collectively, these findings highlight D-Limonene's ability to interfere with multiple aspects of spermatogenesis, confirming its effectiveness as an antiproliferative agent and supporting its potential application in rodent population control.

The study's findings underscore D-Limonene's potent spermicidal activity, demonstrated by the significant reductions in sperm count, motility, and viability observed in the treated groups. The substantial decrease in sperm count across all experimental doses suggests a direct impact of D-Limonene on sperm production and overall reproductive capacity. This is further supported by the marked decline in sperm motility, which reflects impaired functionality and reduced ability of sperm to navigate and fertilize oocytes. The decreased viability of sperm, as indicated by eosin-nigrosin staining, suggests that D-Limonene compromises sperm membrane integrity, leading to increased cell death and reduced functional lifespan. The observed morphological abnormalities, including changes in sperm shape and structure, further confirm the spermicidal effects of D-Limonene. These abnormalities are indicative of potential disruptions in spermatogenesis or sperm maturation processes, which could be caused by oxidative stress or interference with critical cellular pathways (Abedin *et al*., 2023). The combination of reduced sperm count, motility, and viability, alongside increased morphological defects, highlights the effectiveness of D-Limonene as a spermicidal agent. These findings not only validate its potential as a reproductive toxicant but also suggest its applicability in pest management strategies aimed at controlling rodent populations through its impact on reproductive function (Hussain *et al*., 2023).

The findings from this study have significant implications for rodent population control, particularly in managing populations of species such as rats that are known vectors of zoonotic diseases like leptospirosis (Minter *et al*., 2019). The demonstrated antiproliferative and spermicidal effects of D-Limonene offer a promising alternative to traditional chemical rodenticides, which often present environmental and health risks. By targeting key aspects of rodent reproductive physiology, D-Limonene effectively reduces reproductive rates through its impact on sperm production, quality, and function. This approach aligns with a more sustainable pest management strategy that minimizes ecological disruption and avoids the adverse effects associated with chemical rodenticides, such as non-target species toxicity and environmental contamination (Rao, 2020). Incorporating D-Limonene into rodent control programs could provide a dual benefit: controlling rodent populations while also mitigating the spread of diseases they carry. The natural origin of D-Limonene and its bioactivity as a spermicidal agent make it an attractive option for integrated pest management systems, particularly in environments where environmental impact is a critical concern. The study's results suggest that D-Limonene could be utilized to develop novel, eco-friendly rodent control methods, enhancing public health safety and promoting sustainable practices in pest management. Future research

should focus on optimizing D-Limonene formulations and delivery methods to maximize its efficacy and practicality in real-world applications.

While the study presents promising results regarding the antiproliferative and spermicidal effects of D-Limonene, several limitations must be acknowledged, and future research directions are warranted. First, the study was conducted exclusively on male Wistar rats, and the applicability of these findings to other rodent species or to different environmental conditions remains unverified. Further research should evaluate the efficacy and safety of D-Limonene in a broader range of rodent species to assess its generalizability and potential variations in response. Additionally, the mechanisms underlying D-Limonene's reproductive toxicity are not fully elucidated. Future studies should focus on identifying specific molecular pathways and cellular mechanisms through which D-Limonene exerts its effects. Understanding these mechanisms will aid in optimizing the compound's formulation and application. Moreover, the long-term ecological impact of using D-Limonene as a rodent control agent needs to be thoroughly investigated. This includes assessing potential resistance development, non-target species effects, and environmental persistence. Another important area for future research is the development of effective delivery methods that enhance the bioavailability and efficacy of D-Limonene. Investigations into its formulation, stability, and application techniques could improve its practical use in pest management programs. Overall, addressing these limitations and exploring these avenues will help refine D-Limonene's role as a sustainable alternative in rodent population control and contribute to its broader application in pest management strategies.

Conclusion

D-Limonene exhibited a clear dose-dependent decrease in sperm motility viability, and an increase in abnormalities, with the highest dose (150 mg/kg BW) showing the most severe impact. The observed changes in sperm quality and the presence of necrotic cells further substantiate D-Limonene's potential as a spermicide. These findings highlight the dual role of the substance tested, is D-Limonene, as a candidate for spermicide applications. The study underscores the necessity for additional research to explore the underlying mechanisms of these effects and their broader implications for reproductive health and population control strategies.

Approval of Ethical Commission

All procedures involving animals were conducted in accordance with the ethical guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Brawijaya University, under approval number 148/LSIH/EP/2024. The ethical considerations included minimizing animal suffering and ensuring the humane treatment of all experimental subjects throughout the study.

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Author's Contribution

A.D.H: Designed and structured the study, developed the methodology, and supervised the overall research. N.R.Y: Conducted the main experiments, including D-Limonene administration and gonadal sample collection from male albino rats. N.A.A: Responsible for histological and gonadal morphometric analysis, as well as microscopic documentation. N.M: Evaluated spermatogenesis through sperm parameter analysis and spermicide effect testing. K.R.A: Performed statistical data analysis, compiled results into tables and graphs, and interpreted the findings. R.A.P: Contributed to the literature review and compared findings with previous studies. T.S: Assisted in manuscript writing and editing, ensuring compliance with international journal standards. S.M.S: Reviewed the final manuscript, coordinated the submission process, and handled correspondence with the journal. All authors have read and approved the final version of this manuscript.

Conflict of Interest

The authors declare no conflict of interest related to this study. There are no financial, commercial, or personal relationships that could influence the objectivity and validity of the research findings.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. All relevant data supporting the findings of this study have been included in the manuscript. Any additional raw data related to spermatogenesis analysis, gonadal morphometry, and statistical calculations can be provided upon request.

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