OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Dispensing Opticianry**

Exploring the Effects of Cucumis Sativus Aqueous Leaf Extract on Reproductive Health in Adult Male Wistar Rats

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DOI: https://doi.org/10.36348/sjmps.2026.v12i03.007 | **Received:** 16.01.2025 | **Accepted:** 21.02.2025 | **Published:** 31.03.2026

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Abstract

This study investigates the effects of *Cucumis sativus* (cucumber) leaf extract on hormonal levels, semen quality, and sperm cell morphology in male Wistar rats. Thirty rats were randomized into three groups: control, 400 mg/kg, and 800 mg/kg extract-treated groups, and administered for 28 days. Hormonal assays revealed significant dose-dependent reductions in follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels in treated groups ($p < 0.05$). Semen quality analysis showed a significant decline in semen color, consistency, pH, sperm motility, and live proportion, alongside increased abnormal sperm morphology and non-motile spermatozoa, especially in the 800 mg/kg group. Furthermore, differential sperm abnormalities, including head deformities, twisted tails, and bent mid-pieces, were significantly elevated in treated groups, indicating structural and functional damage to sperm cells. The results suggest that *Cucumis sativus* leaf extract, at higher doses, impairs male reproductive hormones, semen quality, and sperm morphology, potentially due to oxidative stress or phytochemical-induced toxicity. This aligns with prior studies on phytotoxic effects of plant extracts, where flavonoids, alkaloids, and other bioactive compounds were implicated in reproductive toxicity. However, the exact mechanism underlying these effects remains unclear and warrants further exploration. This study provides a foundation for understanding the reproductive toxicity of *Cucumis sativus* leaf extract, emphasizing the need for cautious use of herbal preparations containing this plant.

Keywords: *Cucumis sativus*, reproductive toxicity, Wistar rats, sperm morphology, hormones, semen quality, phytochemicals, oxidative stress.

1.0. INTRODUCTION

The reproductive health of males has become a significant area of concern globally due to increasing cases of infertility and subfertility. Male infertility, which accounts for nearly 40% of infertility cases worldwide, is often linked to hormonal imbalances and poor sperm parameters such as low sperm count, motility, and morphology (Kumar & Singh, 2015; Sharma *et al*., 2021). Environmental factors, lifestyle changes, and exposure to toxins have all been implicated in the decline of male reproductive health (Kumar *et al*., 2014).

Natural products and plant-based remedies have historically played a pivotal role in addressing health challenges, including fertility-related issues. *Cucumis sativus*, commonly known as cucumber, is a widely consumed fruit belonging to the Cucurbitaceae family. Known for its antioxidant and anti-inflammatory properties, the leaves of *Cucumis sativus* contain phytochemicals such as flavonoids, phenols, and alkaloids, which have demonstrated potential therapeutic benefits (Khan *et al*., 2022). Several studies have highlighted the role of antioxidants in protecting spermatozoa from oxidative stress, which is a leading cause of male infertility (Gharagozloo and Aitken, 2011; Adewoyin *et al*., 2017; De Luca *et al*., 2021). Phytochemicals in *Cucumis sativus* leaf extract may influence hormone profiles, such as testosterone and follicle-stimulating hormone (FSH), and improve sperm parameters by mitigating oxidative damage and enhancing testicular function (Azu, 2009).

Male infertility has become a significant public health issue, particularly in developing countries where access to advanced fertility treatments is limited.

Conventional treatments for male infertility often come with side effects, high costs, and limited accessibility (Schlegel *et al*., 2021). Furthermore, the etiology of male infertility is multifactorial, making its management complex. Although natural remedies are increasingly being explored for their potential to enhance fertility, there is a scarcity of evidence-based studies focusing on the effects of *Cucumis sativus* leaf extract on male reproductive health. Understanding the influence of this extract on hormonal regulation and sperm quality could provide an affordable and accessible alternative for managing male infertility.

Exploring plant-based remedies for improving reproductive health is not only cost-effective but also aligns with the global shift toward sustainable and natural healthcare solutions (Kandwal *et al*., 2024). *Cucumis sativus* is an easily accessible plant, widely cultivated in various parts of the world, making its use in fertility treatments economically viable. Moreover, investigating the effects of *Cucumis sativus* leaf extract on hormone profiles and sperm parameters can pave the way for developing novel therapeutic interventions. By elucidating its mechanisms of action, this study will provide valuable insights into the potential of *Cucumis sativus* as a complementary or alternative treatment for male infertility.

2.0. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant Materials

Fresh fruits of *Cucumis sativus* were purchased from a local market in Umuerim Nekede, Owerri West L.G.A., Imo State, Nigeria. The plant material was identified and authenticated by a botanist, Dr. C.N. Duru, in the Department of Environmental Biology, Federal Polytechnic Nekede.

2.1.2 Animals

Adult male Wistar rats weighing between 150-200 g were obtained from a reputable breeder in Ihiagwa, Owerri West L.G.A., Imo State. The rats were housed in well-ventilated stainless steel wire cages under standard laboratory conditions, with a 12-hour light/dark cycle, temperature of 22 ± 2°C, and relative humidity of 55 ± 5%. They were fed standard rat pellets and provided with clean water ad libitum. A two-week acclimatization period was allowed before the commencement of the experiment.

2.1.3 Chemicals and Reagents

All chemicals and reagents used were of analytical grade and obtained from standard suppliers.

2.1.4 Instruments/Equipment

Laboratory instruments and equipment, including a top-loading balance, pH meter, microscope, and glass slides, were provided by the Department of Pharmaceutical Technology, Federal Polytechnic Nekede, Owerri.

2.2 Methods

2.2.1 Preparation of Plant Material and Extraction

Fresh leaves of *Cucumis sativus* were collected, washed, and air-dried at room temperature. The dried leaves were ground into a fine powder using an electric grinder. The powder was subjected to extraction using methanol using a maceration method. The resulting extract was concentrated using a rotary evaporator and stored at 4°C for further use.

2.2.2 Animal Grouping and Treatment

Male Wistar rats were randomly divided into three groups of Ten (10) animals each, as follows:

Group 1 (Normal Control): Received distilled water only.

Group 2 (Low Dose): Administered *Cucumis sativus* Leaf extract at 400 mg/kg body weight.

Group 3 (High Dose): Administered *Cucumis sativus* Leaf extract at 800 mg/kg body weight.

The treatments were administered orally once daily for 28 consecutive days.

2.3. Biochemical Assay

2.3.1. Hormonal Analysis:

Blood samples were collected via retro-orbital puncture on Day 29 for serum analysis. Hormonal levels (testosterone, FSH, luteinizing hormone) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Shariati *et al*., 2008).

2.3.2. Sperm Analysis:

The cauda epididymis was dissected, and spermatozoa were collected. Sperm count, motility, viability, and morphology were assessed using standard procedures (Jalili *et al*., 2018).

3.0. RESULTS

3.1. Effect of Cucumis sativus leaf extract on hormones of Wistar rats

Table 1: Significant changes in LH, FSH, and Testosterone of *Cucumis sativus-treated* Wistar rats

Treatments	FSH (miu/ml)	LH (miu/ml)	Testosterone (mg/L)
Control	7.88±0.41^{b}	4.93±0.27^{c}	12.83±0.85^{b}
400 mg/kg leaf extract	5.08±0.25^{a}	4.04±0.18^{b}	9.86±0.48^{a}
800 mg/kg leaf extract	4.45±0.38^{a}	3.39±0.44^{a}	9.75±1.49^{a}

Values are expressed as mean ± standard deviation (n = 4), and values with unlike superscripts are significantly ($p<0.05$) different from paired mean within the column

The results indicate that *Cucumis sativus* leaf extract reduces FSH, LH, and testosterone levels in a dose-dependent manner. The control group consistently exhibited the highest values for all parameters, while increasing doses of the extract (400 mg/kg and 800 mg/kg) led to a significant reduction. However, the reductions between the 400 mg/kg and 800 mg/kg groups were not significant for FSH and testosterone, suggesting a potential plateau effect at higher doses.

3.2. Effect of Cucumis sativus leaf extract on Sperm quality of Wistar rats

Table 2: Significant changes in semen quality of *Cucumis sativus-treated* Wistar rats

Treatments	Semen colour	Semen consistency	Semen pH	Sperm progressive motility (%)	Sperm cells with aberrant movement (%)
Control	2.00±0.00^{c}	3.00±0.00^{b}	7.19±0.03^{b}	74.55±3.71^{b}	14.78±1.60^{c}
400 mg/kg leaf extract	1.50±0.58^{b}	2.75±0.00^{b}	7.13±0.04^{b}	71.01±1.05^{b}	15.46±0.54^{c}
800 mg/kg leaf extract	1.00±0.00^{a}	1.50±0.00^{a}	7.07±0.07^{a}	55.34±2.34^{a}	20.84±0.71^{d}

Values are expressed as mean ± standard deviation (n = 4), and values with unlike superscripts are significantly ($p<0.05$) different from paired mean within the column

The analyzed data shows the effects of *Cucumis sativus* leaf extract on semen parameters, with significant differences observed across the treatments ($p < 0.05$). Semen color and consistency showed a dose-dependent decline, with the control group (2.00 ± 0.00c and 3.00 ± 0.00b, respectively) exhibiting the highest values, while the 800 mg/kg group (1.00 ± 0.00a and 1.50 ± 0.00a) had the lowest, indicating significant differences among all groups. Semen pH also decreased significantly, with the control group (7.19 ± 0.03b) being higher than both the 400 mg/kg (7.13 ± 0.04b) and 800 mg/kg (7.07 ± 0.07a) groups, where the latter had the lowest pH. Sperm progressive motility reduced significantly from the control (74.55 ± 3.71b) to the 800 mg/kg group (55.34 ± 2.34a), while sperm cells with aberrant movement increased significantly, from 14.78 ± 1.60c in the control to 20.84 ± 0.71d in the 800 mg/kg group. These findings indicate that higher doses of *Cucumis sativus* leaf extract negatively affect semen quality and sperm motility, likely due to its impact on spermatogenesis or sperm physiology.

Table 2: Significant changes in semen quality of *Cucumis sativus-treated* Wistar rats

Treatments	Non motile spermatozoa (%)	Live proportion (%)	Sperm count (x10^{6}/CEP)	Sperm with normal morphology (%)	Abnormal spermatozoa (%)
Control	13.25±0.90^{c}	80.12±1.23^{b}	115.93±4.20^{b}	92.06±0.93^{b}	7.44±0.46^{b}
400 mg/kg leaf extract	14.06±0.82^{c}	82.11±1.18^{b}	112.47±3.29^{b}	90.66±0.64^{b}	9.34±0.64^{c}
800 mg/kg leaf extract	23.95±1.45^{d}	68.36±2.21^{a}	88.14±1.15^{a}	82.69±1.68^{a}	16.31±1.32^{d}

Values are expressed as mean ± standard deviation (n = 4), and values with unlike superscripts are significantly ($p<0.05$) different from paired mean within the column

The analyzed data demonstrates the impact of *Cucumis sativus* leaf extract on sperm quality parameters, with significant differences across treatments ($p < 0.05$). Non-motile spermatozoa significantly increased from the control group (13.25 ± 0.90c) to the 800 mg/kg group (23.95 ± 1.45d), indicating a dose-dependent reduction in sperm motility. The live proportion of sperm decreased significantly, with the 800 mg/kg group (68.36 ± 2.21a) having lower values compared to the control (80.12 ± 1.23b) and 400 mg/kg group (82.11 ± 1.18b), showing significant impairment in sperm viability at higher doses. Sperm count also decreased significantly from the control (115.93 ± 4.20b) to the 800 mg/kg group (88.14 ± 1.15a), suggesting reduced spermatogenesis or sperm production with increasing extract concentration. Sperm with normal morphology significantly declined from the control (92.06 ± 0.93b) to the 800 mg/kg group (82.69 ± 1.68a), while abnormal spermatozoa significantly increased, from 7.44 ± 0.46b in the control to 16.31 ± 1.32d in the 800 mg/kg group. These results indicate that higher doses of the leaf extract adversely affect sperm motility, viability, morphology, and overall quality, reflecting potential reproductive toxicity.

3.3. Effect of Cucumis sativus leaf extract on Differential sperm cell abnormalities of Wistar rats

Table 3: Significant changes in differential sperm cell abnormalities of *Cucumis sativus-treated* Wistar rats

Treatments	Total Head Abnormality (%)	Sperm cell with twisted tail (%)	Bent mid-piece abnormality (%)	Total cytoplasmic droplets (%)	Entangled/twisted spermatozoa (%)
Control	1.05±0.08^{b}	1.01±0.02^{b}	1.01±0.10^{a,b}	3.44±0.49^{b}	1.13±0.04^{b}
400 mg/kg leaf extract	1.16±0.02^{b}	0.88±0.06^{a,b}	1.85±0.47^{b}	3.99±0.06^{c}	2.01±0.05^{c}
800 mg/kg leaf extract	1.66±0.04^{c}	1.25±0.03^{c}	4.29±0.38^{c}	6.75±0.06^{d}	3.03±0.07^{d}

Values are expressed as mean ± standard deviation (n = 4), and values with unlike superscripts are significantly ($p<0.05$) different from paired mean within the column

The analyzed data highlights the differential sperm cell abnormalities induced by *Cucumis sativus* leaf extract**,** showing significant differences across treatments ($p < 0.05$). Total head abnormalities increased significantly from the control group (1.05 ± 0.08b) to the 800 mg/kg group (1.66 ± 0.04c), indicating dose-dependent damage to sperm head morphology. Sperm cells with twisted tails were significantly higher in the 800 mg/kg group (1.25 ± 0.03c) compared to the control (1.01 ± 0.02b) and 400 mg/kg group (0.88 ± 0.06a,b), with the latter showing no significant difference from the control. Bent mid-piece abnormalities increased significantly, from 1.01 ± 0.10a,b in the control to 4.29 ± 0.38c in the 800 mg/kg group, showing marked structural damage at higher doses. Total cytoplasmic droplets were significantly higher in the 800 mg/kg group (6.75 ± 0.06d) compared to the control (3.44 ± 0.49b) and 400 mg/kg group (3.99 ± 0.06c). Similarly, entangled or twisted spermatozoa increased significantly, from 1.13 ± 0.04b in the control to 3.03 ± 0.07d in the 800 mg/kg group. These results suggest that the extract induces significant structural abnormalities in sperm cells in a dose-dependent manner, with the highest dose (800 mg/kg) causing the most pronounced damage.

4.0. DISCUSSION

The effects of *Cucumis sativus* (cucumber) leaf extract on reproductive parameters in male Wistar rats, as observed in this study, highlight potential dose-dependent reproductive toxicity. The findings are consistent with earlier studies reporting the impact of phytochemical-rich plant extracts on reproductive health, potentially mediated through oxidative stress, hormonal disruption, or direct cellular damage to reproductive tissues.

4.1. Effect on Hormonal Levels

The reduction in follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels with increasing doses of the extract suggests endocrine disruption. Testosterone, primarily regulated by LH and FSH, is critical for spermatogenesis and maintaining secondary sexual characteristics in males (Oduwole *et al*., 2021). A decline in these hormones could reflect impaired hypothalamic-pituitary-gonadal (HPG) axis function (Meethal and Atwood, 2005). Recent studies have investigated the effects of various plant extracts on reproductive hormones in rats. Aqueous extract of *Cucumis sativus* (cucumber) increased serum testosterone and luteinizing hormone (LH) levels in male rats (Obembe *et al*., 2023). However, in female rats, high doses of cucumber extract showed potentially harmful effects on ovarian histomorphology (Okafor *et al*., 2023). Celery leaf extract at 200 mg/kg significantly decreased LH levels in male rats, but had no significant effect on testosterone or follicle-stimulating hormone (FSH) levels (Kooti *et al.*, 2015). In diabetic male rats, Nigella sativa seed powder treatment increased testosterone levels significantly compared to untreated diabetic rats, but its effect on LH levels was not significant (Aithal *et al*., 2015). These studies demonstrate that plant extracts can influence reproductive hormone levels, but their effects may vary depending on the plant species, dosage, and the animal's physiological state.

4.2. Effect on Semen Quality

The study revealed significant alterations in semen parameters with increasing doses of the extract, including reductions in sperm progressive motility, semen pH, and the proportion of live spermatozoa, alongside increased non-motile sperm and sperm cells with aberrant movement. These observations align with studies demonstrating the potential of certain plant phytochemicals to induce oxidative stress, leading to lipid peroxidation and membrane damage in sperm cells (Adewoyin *et al*., 2017). Changes in semen pH could indicate altered seminal plasma composition, potentially impairing sperm motility and fertilization capacity.

The significant decline in sperm count and normal morphology with higher doses of *Cucumis sativus* extract also reveals its potential to disrupt spermatogenesis. Research has suggested that flavonoids and alkaloids present in plants can exert toxic effects on the testes, reducing the efficiency of Sertoli cells, which are essential for nurturing developing sperm (Gao *et al.*, 2015; Yan *et al*., 2021). The observed increase in abnormal spermatozoa, including twisted tails and bent mid-pieces, could result from disrupted microtubule formation or mitochondrial dysfunction, as these structures are critical for motility and energy production in sperm cells (Fawzy *et al*., 2020).

4.3. Differential Sperm Cell Abnormalities

The significant increase in total head abnormalities, cytoplasmic droplets, and entangled or twisted spermatozoa with higher doses of the extract further confirms its deleterious effects. Cytoplasmic droplets, typically shed during sperm maturation, are indicative of immaturity or impaired epididymal function when present in excess (Cooper, 2005; Kumar, 2023). The increased incidence of twisted tails and bent mid-pieces may also reflect oxidative damage to the axonemal structure or dysfunction in accessory structures like the epididymis (Siddique *et al.*, 2011; Kumar, 2023).

The toxicological effects observed may be attributed to bioactive compounds in *Cucumis sativus* leaves, such as flavonoids, alkaloids, and tannins, known for their antioxidant properties but capable of exerting pro-oxidant effects at higher concentrations (Rajashekar *et al*., 2023). Excessive accumulation of reactive oxygen species (ROS) can overwhelm cellular antioxidant defenses, leading to damage in testicular and epididymal tissues (Dutta *et al*., 2021). Furthermore, oxidative stress has been implicated in DNA damage, lipid peroxidation, and apoptosis in germ cells, which aligns with the observed increase in abnormal sperm morphology and reduction in sperm count (Aitken *et al.*, 2012; Jannatifar *et al*., 2019).

4.4. Directions for Future Research

The results of this study have implications for the potential use of *Cucumis sativus* leaf extract in therapeutic contexts. While lower doses may retain antioxidant benefits, higher doses could pose risks to reproductive health. Future studies should focus on isolating specific bioactive compounds responsible for these effects, investigating the molecular mechanisms underlying their action, and evaluating potential protective strategies, such as co-administration with antioxidants like Vitamin E or selenium. Additionally, histopathological analysis of testicular tissues would provide further insights into the extent of cellular damage caused by the extract.

5.0. CONCLUSION

The findings demonstrate that *Cucumis sativus* leaf extract exerts dose-dependent adverse effects on male reproductive parameters, including hormonal disruption, reduced semen quality, and increased sperm cell abnormalities. These effects reveal the importance of cautious dosing in the use of herbal remedies and highlight the need for further research to delineate their safety profiles.

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