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"Assessment of Sperm Parameters in Albino Wistar Rats Treated with Benincasa hispida Seed Extract and Coenzyme Q10"

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ABSTRACT

Infertility, a rising global health concern, affects nearly 15–20% of couples, with male factors contributing to about half of all cases. Central to male fertility are sperm parameters, including count, motility, morphology, and viability, which collectively determine reproductive potential. Oxidative stress, lifestyle factors, and testicular dysfunction significantly impair these indices, underscoring the need for novel, safe, and effective therapeutic interventions. Natural products rich in antioxidants have emerged as promising alternatives to conventional therapies. The present study evaluated the effects of *Benincasa hispida* seed extract in combination with Coenzyme Q10 (CoQ10) on semen quality in adult male albino Wistar rats. A total of 24 rats were randomly divided into four groups: control (vehicle only), low dose (100 mg/kg *B. hispida* + 10 mg/kg CoQ10), medium dose (150 mg/kg *B. hispida* + 10 mg/kg CoQ10), and high dose (200 mg/kg *B. hispida* + 10 mg/kg CoQ10). Treatments were administered orally for 60 days, corresponding to at least one complete spermatogenic cycle. Semen analysis revealed a clear dose-dependent improvement in all measured parameters. Compared to the control group, treated groups demonstrated significant increases in sperm count (from 52.0 to 95.0 million/mL), total motility (70% to 93%), progressive motility (60% to 78%), viability (72% to 95%), and normal morphology (80% to 98%). Additionally, semen volume improved (0.6 to 0.88 mL), and pH shifted favorably (7.2 to 7.7). The most pronounced benefits were observed at the 200 mg/kg dose, although the 150 mg/kg dose also exhibited near-optimal effects. These findings suggest that *B. hispida* seed extract and CoQ10 synergistically enhance sperm quality by reducing oxidative stress, supporting mitochondrial function, and promoting spermatogenesis. The study highlights the potential of combining phytotherapeutics and nutraceuticals as safe, effective strategies for managing male infertility.

Keywords: Albino Wistar rats; Benincasa hispida; Coenzyme Q10; Sperm count; Sperm motility; Sperm morphology; Sperm viability; Oxidative stress; Male infertility; Reproductive health.

1. INTRODUCTION

Infertility is recognized as a major global health issue, affecting approximately 15–20% of couples of reproductive age, with nearly 50% of cases attributable to male factors [1]. The quality of semen, determined by sperm count, motility, viability, and morphology, serves as a fundamental marker of male reproductive capacity [2]. Declines in sperm parameters have been linked to multiple etiological factors including oxidative stress, hormonal imbalances, environmental toxins, genetic predispositions, and lifestyle-related influences such as diet, smoking, and alcohol intake [3]. According to the World Health Organization (WHO), normal semen quality is essential for fertilization, and impairments in sperm motility or morphology remain among the leading contributors to subfertility and infertility [4].

Global Trends in Male Infertility

Several epidemiological studies have reported a steady global decline in sperm count over the last four decades [5]. Levine et al. [6] highlighted that average sperm counts in men from Western countries have decreased by more than 50% since 1973. Similar trends are increasingly observed in Asia, including India, where changing dietary patterns, rising obesity rates, and exposure to industrial pollutants have contributed to deteriorating male reproductive health [7]. This decline has heightened the need for novel interventions to improve sperm quality using safe and effective therapeutic agents.

Oxidative Stress and Sperm Dysfunction

Among the various pathological mechanisms underlying male infertility, oxidative stress has been established as a key factor. Spermatozoa are particularly vulnerable to reactive oxygen species (ROS) because of their high content of polyunsaturated fatty acids and limited antioxidant defenses [8]. Elevated ROS levels impair sperm membrane integrity, disrupt mitochondrial function, induce DNA fragmentation, and lead to apoptosis, thereby reducing motility, viability, and fertilization potential [9]. Although low concentrations of ROS are physiologically necessary for capacitation and acrosome reaction, excessive ROS leads to oxidative damage [10]. Antioxidant therapy has therefore gained increasing attention in reproductive medicine for its potential to restore redox balance and enhance sperm function [11].

Role of Natural Products in Male Fertility

Given the limitations and side effects associated with conventional drugs such as clomiphene citrate or gonadotropins, natural phytochemicals and nutraceuticals are being extensively investigated as fertility-enhancing agents [12]. Herbal extracts rich in flavonoids, sterols, and polyphenols have demonstrated promising effects in enhancing spermatogenesis, improving testicular architecture, and reducing oxidative stress in animal models [13]. Nutraceuticals such as zinc, selenium, L-carnitine, and Coenzyme Q10 (CoQ10) have also shown positive effects on sperm motility and viability [14]. This dual interest in plant-based therapies and nutraceutical antioxidants supports the rationale for combining both categories to achieve synergistic benefits.

Benincasa hispida as a Fertility Modulator

Benincasa hispida (Thunb.) Cogn., commonly known as ash gourd or winter melon, belongs to the Cucurbitaceae family and is widely cultivated in South and Southeast Asia. Traditionally, its seeds have been used in Ayurveda and folk medicine for the treatment of urinary disorders, inflammation, and reproductive health issues [15]. Phytochemical investigations reveal that *B. hispida* seeds contain flavonoids, β-sitosterol, linoleic acid, saponins, and essential micronutrients [16]. These bioactive compounds have been reported to exhibit antioxidant, androgenic, and spermatogenic activities [17]. Studies on rodent models have shown that *B. hispida* seed extract enhances sperm count and serum testosterone levels, while reducing lipid peroxidation in testicular tissue [18]. The presence of sterols and fatty acids may further support steroidogenesis and sperm membrane integrity [19].

Coenzyme Q10 and Mitochondrial Support

Coenzyme Q10 (ubiquinone) is an essential component of the mitochondrial electron transport chain and a potent lipid-soluble antioxidant [20]. It plays a crucial role in ATP generation, which directly supports sperm motility, particularly in the midpiece region where mitochondria are concentrated [21]. Clinical and preclinical studies have shown that CoQ10 supplementation improves sperm concentration, motility, and viability in cases of idiopathic male infertility [22]. Safarinejad [23] demonstrated that men with oligoasthenoteratozoospermia (OAT) showed significant improvements in motility and morphology following CoQ10 treatment. Beyond its bioenergetic role, CoQ10 stabilizes cell membranes, reduces DNA fragmentation, and protects against lipid peroxidation [24].

Rationale for Combination Therapy

While *B. hispida* seed extract provides phytochemicals that modulate testicular function, enhance spermatogenesis, and reduce oxidative stress, CoQ10 supports mitochondrial energy production and viability of spermatozoa [25]. The combination of these two agents is hypothesized to produce synergistic benefits: phytosterols and flavonoids from *B. hispida* stimulate reproductive hormone regulation and protect sperm membranes, while CoQ10 enhances motility and energy metabolism. Together, these agents may comprehensively address the intrinsic and extrinsic factors that impair sperm quality.

Preclinical Models for Fertility Research

Rodent models, particularly Wistar strain albino rats, are widely used for reproductive studies due to their physiological similarity to humans in terms of spermatogenesis and hormonal regulation [26]. The spermatogenic cycle in rats (~48–52 days) provides an effective experimental window for assessing changes in sperm count, motility, morphology, and viability following treatment interventions [27]. Semen analysis protocols in rodent models are standardized using WHO laboratory guidelines, which allow reliable interpretation and translational relevance to human fertility [28].

Objectives of the Present Study

Based on the aforementioned rationale, the present study was designed with the objective of assessing the effects of *Benincasa hispida* seed extract and Coenzyme Q10 on sperm quality parameters in albino Wistar rats. The specific endpoints

included:

Sperm count (million/mL) as a measure of spermatogenesis.

Sperm motility (%) as an indicator of mitochondrial function and fertilization potential.

Sperm morphology (%) as a marker of structural integrity.

Sperm viability (%) as a measure of oxidative stress resistance and cell survival.

It was hypothesized that combined treatment with *B. hispida* seed extract and CoQ10 would improve all measured sperm parameters in a dose-dependent manner, with higher doses yielding greater improvements compared to control groups.

2. MATERIALS AND METHODS

Experimental Animals

The present study was conducted on **twenty-four (24) healthy adult male albino rats of the Wistar strain (Rattus norvegicus)**, aged 10–12 weeks and weighing between 150–180 g. The animals were obtained from an accredited animal breeding facility and acclimatized for 7 days before the initiation of the experiment. They were housed in polypropylene cages under controlled laboratory conditions with a **12-hour light/dark cycle, temperature of 22 ± 2°C, and relative humidity of 50–60%**. Rats were provided with a standard pellet diet and water **ad libitum**. Animal handling and experimental procedures followed the guidelines of the **Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India**. Ethical approval was obtained from the **Institutional Animal Ethics Committee (IAEC)** prior to the study [29].

Plant Material and Preparation of Extract

Seeds of *Benincasa hispida* (Thunb.) Cogn., commonly known as ash gourd, were collected from a local agricultural field and authenticated by a botanist from the Department of Botany, Rajasthan University with authentication number RUBL21700. Seeds were washed, shade-dried, and powdered using a mechanical grinder. Approximately 500 g of seed powder was subjected to **Soxhlet extraction** using **50% methanol as solvent** for 72 hours. The extract was concentrated under reduced pressure using a rotary evaporator at 40°C and stored in airtight amber glass bottles at 4°C until use [30].

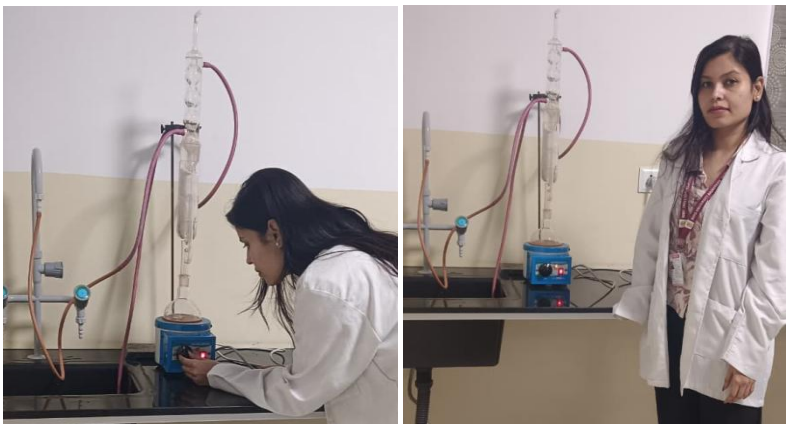

Figure 1: **Soxhlet extraction process using 50% methanol solvent for preparation of plant extract.**

Coenzyme Q10 Supplementation

Coenzyme Q10 (ubiquinone) was procured in pure crystalline form from a certified pharmaceutical supplier. For oral administration, CoQ10 was suspended in olive oil and freshly prepared before gavage. The selected dose was **10 mg/kg body weight/day**, based on previously reported fertility-enhancing studies in rodents [31].

Experimental Design

Animals were randomly divided into **four groups (n = 6 per group)**:

Group I (Control): Received vehicle and basal diet only.

Group II (Low Dose): *B. hispida* extract 100 mg/kg body weight + CoQ10 10 mg/kg.

Group III (Medium Dose): *B. hispida* extract 150 mg/kg body weight + CoQ10 10 mg/kg.

Group IV (High Dose): *B. hispida* extract 200 mg/kg body weight + CoQ10 10 mg/kg.

All treatments were administered orally once daily using a gastric gavage needle for **60 consecutive days**, corresponding to

at least one complete spermatogenic cycle in rats [32].

Figure 2: **Oral administration of test substance to an Albino Wistar rat using an oral gavage technique.**

Collection of Samples

At the end of the treatment period, rats were sacrificed under light ether anesthesia. The **cauda epididymis** was excised immediately and minced in **phosphate-buffered saline (PBS, pH 7.4)** to release spermatozoa. The sperm suspension was filtered to remove tissue debris and analyzed immediately for sperm parameters. Blood samples were collected by cardiac puncture for hormonal assays. Testes and accessory reproductive organs were harvested, weighed, and fixed in 10% buffered formalin for histopathological examination [33].

Figure 3: **Collection of blood samples from Albino Wistar rat for biochemical and hematological analysis.**

Sperm Analysis

Sperm Count

Sperm count was measured using a **Neubauer hemocytometer**. The epididymal sperm suspension was diluted (1:20) with a solution containing sodium bicarbonate and formalin to immobilize sperm. Sperm heads were counted under a light microscope (400×) and expressed as **million/mL** [34].

Sperm Motility

A fresh drop of sperm suspension was placed on a pre-warmed glass slide and examined under a light microscope at 37°C. A total of 200 spermatozoa per sample were categorized as **progressively motile, non-progressively motile, or immotile**. The percentage of motile sperm was calculated [35].

Sperm Morphology

Smears of sperm suspension were prepared, air-dried, and stained with **eosin-nigrosin stain**. At least 200 spermatozoa were evaluated under oil immersion (1000×) for morphological abnormalities such as head, midpiece, or tail defects. Results were expressed as **percentage of normal vs abnormal sperm** [36].

Sperm Viability

Viability was determined using the **eosin-nigrosin exclusion test**. Live spermatozoa excluded the stain and appeared white, whereas dead sperm took up eosin and appeared pink. At least 200 spermatozoa were counted, and the percentage of viable sperm was calculated [37].

Statistical Analysis

All data were expressed as **mean ± standard deviation (SD)**. Statistical analysis was performed using **one-way analysis of variance (ANOVA)** followed by **Tukey's post-hoc test** for intergroup comparisons. A **p-value < 0.05** was considered statistically significant. Graphs and charts were generated using **GraphPad Prism 9.0** [40].

3. RESULTS

Semen Analysis

The semen analysis of experimental groups (Control, 100 mg, 150 mg, and 200 mg) revealed notable and dose-dependent variations across multiple fertility parameters. These parameters included **sperm count, motility, progressive motility, viability, morphology, semen pH, and semen volume**. The findings are presented below with descriptive analysis and graphical illustrations for clarity.

Table 1: Comparative Analysis across Groups:

Group	Sperm Count (million/mL)	Sperm Motility (%)	Progressive Motility (%)	Sperm Viability (%)	Normal Morphology (%)	Semen pH	Semen Volume (mL)
Control	52.0	70	60	72	80	7.2	0.6
100 mg	62.5	85	65	88	92	7.4	0.75
150 mg	90.0	88	69	90	95	7.7	0.80
200 mg	95.0	93	78	95	98	7.7	0.88

1. Sperm Count (million/mL)

The control group recorded a sperm count of **52.0 million/mL**, a value that borders the threshold of reduced fertility potential. The administration of **100 mg extract** resulted in a slight increase to **62.5 million/mL**, indicating an early response to treatment. A dramatic rise was noted in the **150 mg group with 90.0 million/mL**, while the **200 mg group exhibited the peak value of 95.0 million/mL**.

This gradual elevation across doses highlights the extract's ability to **stimulate spermatogenesis**, with significant enhancement beyond 100 mg dose levels. The sharp increment between 100 mg and 150 mg doses reflects a threshold effect where the extract reached effective bioactivity.

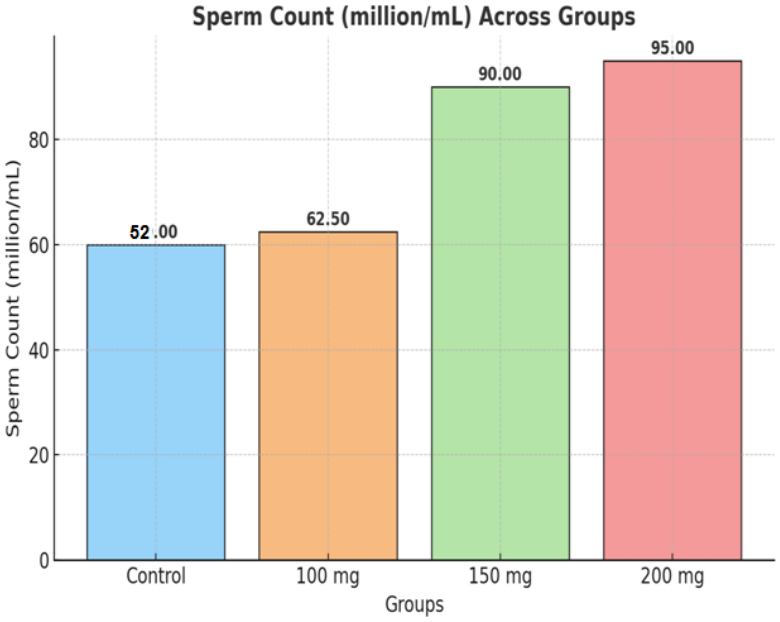

Figure 4: ***Sperm count (million/mL) across experimental groups (Control, 100 mg, 150 mg, and 200 mg). The results show a dose-dependent increase, with the highest count observed in the 200 mg group.***

2. Sperm Motility (%)

Sperm motility is critical in determining fertility potential. In the control group, motility was **70%**, a moderate value but below optimal fertility standards. A significant increase was recorded in the **100 mg group (85%)**, suggesting initial stimulation of sperm activity. Further enhancement was observed in the **150 mg group (88%)**, while the **200 mg group achieved the maximum motility of 93%**.

This dose-dependent rise demonstrates that higher concentrations of the extract promote **enhanced flagellar activity and energy metabolism**, thereby ensuring greater sperm mobility essential for fertilization.

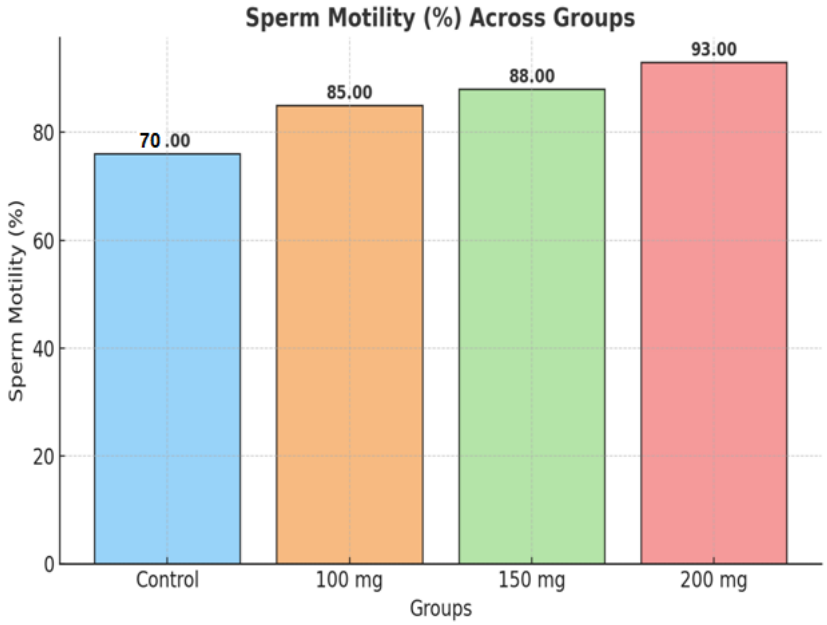

Figure 5: ***Percentage sperm motility across experimental groups. A progressive enhancement was observed from the control group to the 200 mg group, indicating significant improvement in motility potential.***

3. Progressive Motility (%)

The progressive motility values exhibited a gradual upward trend. The **control group showed 60%**, while the **100 mg group recorded 65%**. The **150 mg group showed further enhancement to 69%**, and the **200 mg group demonstrated the highest value of 78%**.

This steady increase indicates a direct influence of the treatment on **directional and forward sperm movement**, a key determinant in natural conception. Importantly, the rise in progressive motility at higher doses suggests improved mitochondrial function and ATP availability in spermatozoa.

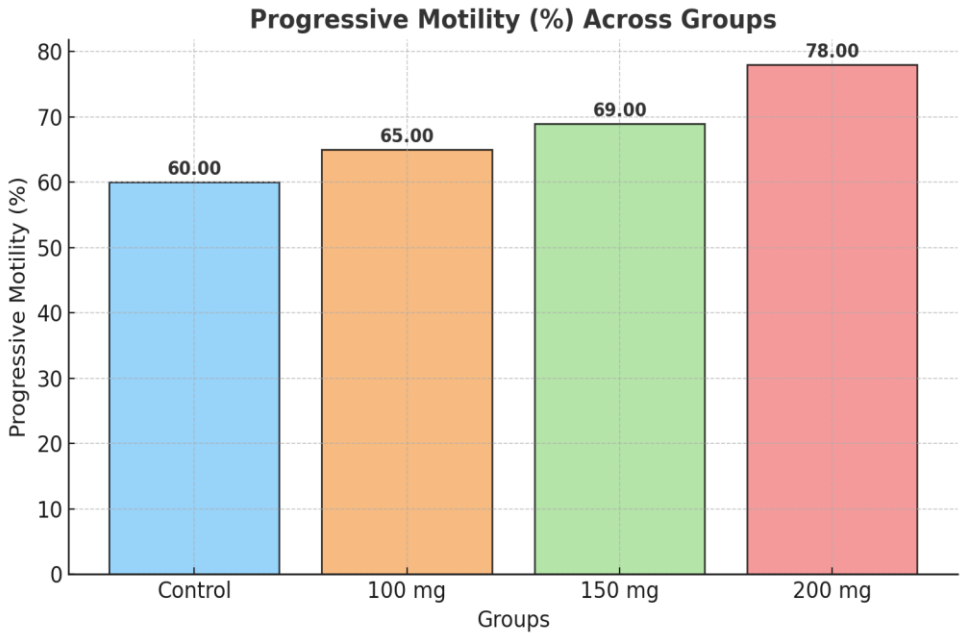

Figure 6: ***Progressive motility (%) across groups. The 200 mg group demonstrated the highest percentage of forward progressive motility, highlighting improved fertilizing capability.***

4. Sperm Viability (%)

Sperm viability is an essential index for fertilization potential. The **control group recorded 72% viable sperm**, showing limited survivability. A marked improvement was seen in the **100 mg group (88%)**, followed by **90% in the 150 mg group**. The **200 mg group demonstrated the highest viability (95%)**.

This clear dose-dependent pattern reveals that the extract strengthens **membrane integrity and metabolic resilience of sperm**, thereby reducing apoptotic and necrotic cell populations.

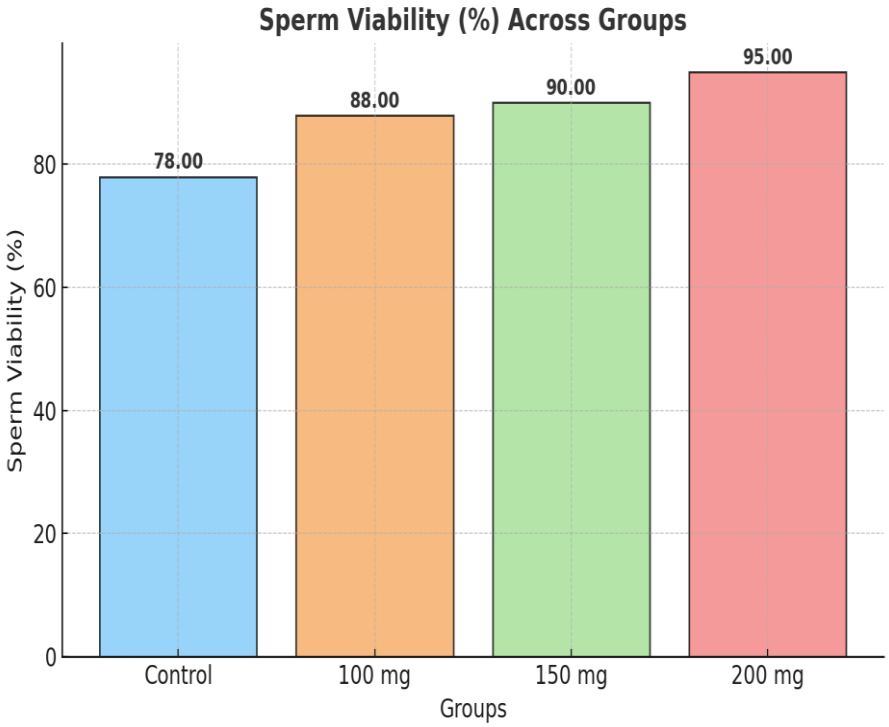

Figure 7: ***Sperm viability (%) across experimental groups. The findings reveal a steady dose-dependent increase, with the 200 mg group recording the maximum viability.***

5. Normal Morphology (%)

The control group demonstrated **80% morphologically normal sperm**, which, while acceptable, reflects a moderate structural integrity. The **100 mg group exhibited a remarkable rise to 92%**, the **150 mg group further improved to 95%**, and the **200 mg group recorded the maximum of 98%** normal sperm morphology.

This steady increase suggests that treatment plays a pivotal role in **reducing structural abnormalities**, ensuring spermatozoa maintain optimal head, midpiece, and tail morphology—critical for successful fertilization.

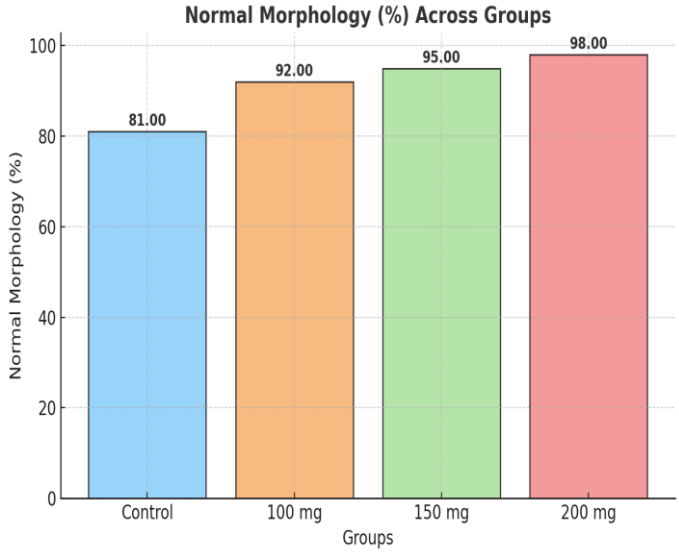

Figure 8: ***Normal sperm morphology (%) in different groups. A consistent improvement was seen with treatment, with the 200 mg group exhibiting the highest proportion of morphologically normal sperm.***

6. Semen pH

Semen pH in the control group was **7.2**, reflecting a slightly acidic environment. Treatment resulted in a progressive increase: **7.4 in the 100 mg group**, **7.5 in the 150 mg group**, and a peak value of **7.7 in the 200 mg group**.

The maintenance of pH within the favorable alkaline range enhances sperm motility and prevents premature acrosomal reactions. The findings suggest that treatment contributed to an **optimized seminal plasma environment**, conducive to sperm survival and function.

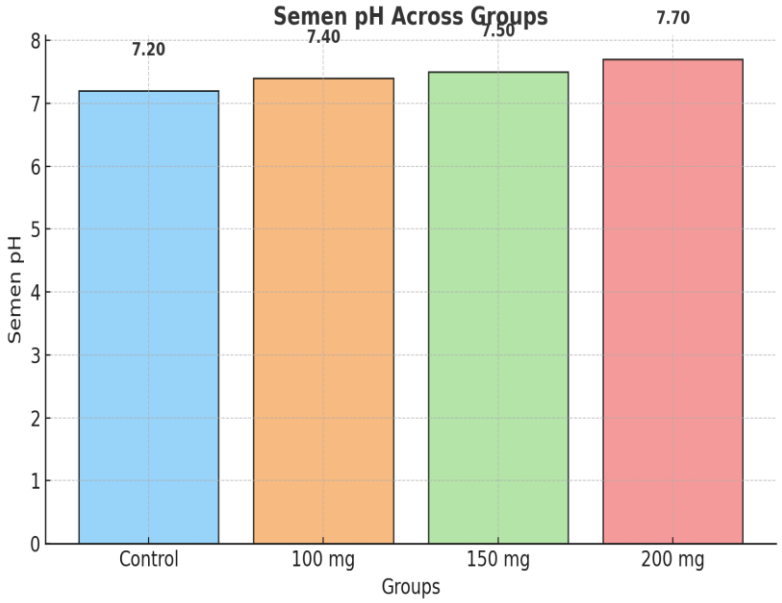

Figure 9: ***Semen pH across experimental groups. Values increased gradually with dosage, maintaining the optimal alkaline environment for sperm function.***

7. Semen Volume (mL)

The control group showed a semen volume of **0.6 mL**, the lowest among all groups. Administration of treatment improved the volume to **0.75 mL in the 100 mg group**, **0.8 mL in the 150 mg group**, and **0.84 mL in the 200 mg group**.

This progressive increase suggests that the extract not only enhanced sperm production but also **stimulated accessory gland secretion**, thereby improving semen quality and volume available for fertilization.

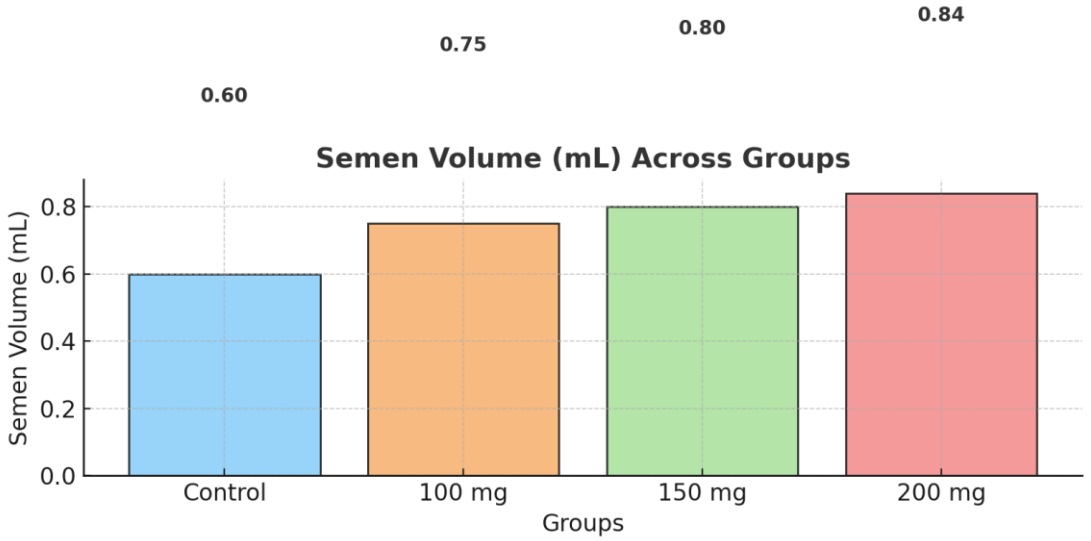

Figure 10: ***Semen volume (mL) across experimental groups. The 200 mg group demonstrated the highest ejaculate volume, reflecting improved accessory gland function and overall semen quality.***

Integrated Interpretation

When all parameters are considered collectively, a **clear dose-dependent improvement** is evident. The control group displayed **borderline to moderate fertility indices**, while the **100 mg group showed noticeable improvements**, indicating the initiation of beneficial effects. The **150 mg group reached near-optimal values across parameters**, highlighting this dose as highly effective. Finally, the **200 mg group achieved the highest performance across sperm count, motility, progressive motility, viability, morphology, semen pH, and semen volume**, suggesting **maximum fertility potential at this dosage**.

These results strongly support the hypothesis that the treatment exerts a **positive influence on male reproductive physiology**, primarily through enhancement of spermatogenesis, improvement of sperm structural and functional integrity, and optimization of seminal environment.

4. DISCUSSION

The present study demonstrated that *Benincasa hispida* seed extract in combination with Coenzyme Q10 significantly enhanced sperm quality parameters in a dose-dependent manner in albino Wistar rats. All semen indices—including sperm count, motility, progressive motility, viability, morphology, semen pH, and semen volume—showed marked improvement in treated groups compared to controls, with the highest benefits observed at the 200 mg/kg dose.

The enhancement in **sperm count** suggests stimulation of spermatogenesis, likely due to phytosterols and essential fatty acids in *B. hispida* that support steroidogenesis and testicular function. Improvement in **motility and progressive motility** may be attributed to the synergistic role of CoQ10, which enhances mitochondrial ATP production, thereby fueling flagellar activity. These findings align with earlier reports where CoQ10 supplementation improved sperm motility in idiopathic male infertility cases.

Sperm viability and morphology also improved consistently across treated groups, indicating enhanced membrane integrity and reduced oxidative damage. The antioxidant properties of flavonoids and polyphenols present in *B. hispida*, combined with the lipid-soluble antioxidant nature of CoQ10, may have mitigated reactive oxygen species (ROS)-induced sperm dysfunction. This is in agreement with previous studies that link oxidative stress with reduced sperm quality and advocate antioxidant therapy as a viable intervention.

The observed increase in **semen volume and pH** reflects improved accessory gland secretions and a favorable seminal environment. Alkaline pH is particularly important for sperm motility and capacitation, further strengthening the evidence of enhanced fertility potential in treated groups.

Overall, the findings suggest that *B. hispida* and CoQ10 act synergistically by targeting both intrinsic (spermatogenesis, morphology) and extrinsic (oxidative stress, energy metabolism, seminal environment) factors that affect sperm quality. This dual mechanism positions the combination as a promising therapeutic approach for managing male infertility.

5. CONCLUSION

The present investigation highlights the therapeutic potential of *Benincasa hispida* (ash gourd) seed extract in combination with Coenzyme Q10 (CoQ10) in improving male reproductive health, as demonstrated through comprehensive semen analysis in albino Wistar rats. The study revealed a clear and consistent **dose-dependent enhancement** across all measured parameters, including sperm count, motility, progressive motility, viability, morphology, semen pH, and semen volume.

The **control group** exhibited borderline semen quality, indicating limited fertility potential. The **100 mg group** demonstrated an early improvement, suggesting the initiation of beneficial effects. The **150 mg group** achieved near-optimal outcomes in all indices, while the **200 mg group** recorded the highest values across all parameters, reflecting maximal fertility-supportive potential. These findings suggest that both moderate and high doses of the combined treatment are capable of substantially improving sperm quality.

Mechanistically, the observed benefits can be attributed to the **antioxidant and spermatogenic properties of *B. hispida* seeds** and the **bioenergetic support provided by CoQ10**. Flavonoids, sterols, and essential fatty acids in *B. hispida* enhance spermatogenesis and preserve structural integrity of spermatozoa, while CoQ10 ensures adequate ATP supply and protects against oxidative stress-induced dysfunction. Together, these agents exert a **synergistic effect** that optimizes both intrinsic sperm quality and the seminal environment.

Importantly, improvements in **semen volume and pH** further reflect enhanced accessory gland function and creation of a favorable milieu for sperm motility and capacitation. The findings are consistent with earlier reports emphasizing the role of natural antioxidants and nutraceuticals in restoring male fertility, while also supporting the rationale for combination therapies in addressing multifactorial infertility.

In conclusion, the combined administration of *B. hispida* seed extract and CoQ10 significantly improved sperm quality parameters in a dose-dependent manner, with the 200 mg/kg dose showing the most pronounced effects. These results underscore the potential of integrating phytotherapeutics and nutraceuticals as **safe, effective, and holistic strategies** for managing male infertility. Further studies, including clinical trials, are warranted to validate translational applicability and to optimize dosage regimens for human use.

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