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To Investigate the Biochemical Parameters in Male Albino Wistar Rats Treated with Benincasa Hispida Seeds and Coenzyme Q10

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ABSTRACT

Male infertility remains a global health concern, with oxidative stress being a critical factor responsible for impaired sperm quality, hormonal imbalance, and altered biochemical homeostasis. This study investigated the effects of Benincasa hispida (ash gourd) seed extract in combination with Coenzyme Q10 (CoQ10) on biochemical and reproductive parameters in male albino Wistar rats. Twenty-four adult rats were divided into four groups: control, low dose (100 mg/kg seed extract + 10 mg/kg CoQ10), medium dose (150 mg/kg + 10 mg/kg), and high dose (200 mg/kg + 10 mg/kg), administered orally for 60 days. Biochemical markers including liver function tests (ALT, AST, ALP, bilirubin, protein, albumin), kidney function tests (BUN, creatinine, uric acid), lipid profile, antioxidant enzymes (SOD, CAT, GPx, GSH), oxidative stress marker (MDA), and reproductive hormones (testosterone, FSH, LH, estradiol, prolactin, corticosterone, TSH) were evaluated.

Results demonstrated a dose-dependent improvement across biochemical and hormonal indices. Liver enzymes (ALT, AST, ALP) and protein levels showed enhanced metabolic activity without hepatotoxicity, while kidney markers (BUN, creatinine, uric acid) indicated normal renal function with increased metabolic turnover. Antioxidant enzyme activities were elevated, accompanied by reduced lipid peroxidation, signifying mitigation of oxidative stress. Hormonal assays revealed significant increases in testosterone, FSH, and LH, indicating stimulation of the hypothalamic-pituitary-gonadal axis, while estradiol and prolactin remained within physiological ranges. The 200 mg/kg dose exhibited the most pronounced effects, reflecting synergistic benefits of phytochemicals in B. hispida seeds and the mitochondrial support of CoQ10.

Collectively, findings highlight the therapeutic potential of B. hispida seeds and CoQ10 in restoring biochemical balance, improving antioxidant defense, and enhancing male reproductive hormone regulation. These results provide experimental evidence supporting their role as safe, natural nutraceuticals for managing oxidative stress-induced male infertility.

KEYWORDS: *Male infertility, Benincasa hispida, Coenzyme Q10, oxidative stress, antioxidants, reproductive hormones, biochemical parameters.*

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1. INTRODUCTION

Infertility is a major public health issue affecting approximately 15–20% of couples worldwide, with male factors contributing to nearly 40–50% of all cases (1,2). Male infertility is primarily attributed to defects in sperm parameters—count, motility, viability, morphology—and impaired biochemical and hormonal balance, which collectively reduce

fertilization potential (3,4). Among the multiple etiological contributors, **oxidative stress** has emerged as the most significant, implicated in nearly 30–80% of male infertility cases (5). Excess reactive oxygen species (ROS) result in lipid peroxidation of sperm membranes, DNA fragmentation, mitochondrial dysfunction, and impaired spermatogenesis (6,7). Given the rising incidence of infertility, there is growing emphasis on developing alternative and complementary therapeutic interventions that are safe, effective, and affordable.

Male Infertility and Oxidative Stress

Physiological levels of ROS are essential for processes such as capacitation, hyperactivation, and acrosome reaction. However, pathological ROS levels overwhelm the limited antioxidant defense capacity of spermatozoa, which lack robust cytoplasmic enzymes and DNA repair systems (8). This imbalance leads to reduced sperm motility, teratozoospermia, and oligozoospermia, all of which directly compromise fertility (9). Environmental factors (pollutants, heavy metals, pesticides), lifestyle habits (smoking, alcohol, obesity), and medical conditions (varicocele, infections) exacerbate oxidative stress and disturb reproductive biochemistry (10,11).

Conventional treatment strategies for male infertility include hormone replacement, gonadotropins, surgical correction of varicocele, and assisted reproductive technologies (ART) such as intracytoplasmic sperm injection (ICSI). However, these interventions have several limitations—high cost, variable efficacy, and potential side effects (12). Thus, natural antioxidants, nutraceuticals, and phytochemicals are being widely investigated for their ability to restore redox balance, improve biochemical parameters, and enhance fertility (13,14).

Role of Nutraceuticals and Antioxidants

Nutraceuticals—bioactive compounds derived from dietary sources—play a vital role in combating oxidative stress and modulating reproductive health (15). Antioxidants such as vitamins C and E, selenium, zinc, L-carnitine, and polyphenols have shown promising effects on sperm count, motility, and biochemical regulation (16,17). Particularly, **Coenzyme Q10 (CoQ10)** has gained attention due to its dual function as a mitochondrial bioenergetic cofactor and a potent lipid-soluble antioxidant (18,19).

Similarly, medicinal plants have long been used in ethnomedicine for enhancing male fertility. Among them, **Benincasa hispida (ash gourd/winter melon)** is an important member of Cucurbitaceae, traditionally utilized for reproductive health, detoxification, and metabolic disorders (20). Recent phytochemical analyses highlight that its seeds, often discarded, are rich in flavonoids, sterols, triterpenes, fatty acids, and micronutrients essential for spermatogenesis and biochemical homeostasis (21,22).

Benincasa hispida (Ash Gourd) Seeds: Phytochemistry and Relevance

B. hispida has been used in Ayurveda, Siddha, and Traditional Chinese Medicine for centuries, with the seeds recognized as “shukrala”—agents that enhance semen and fertility (23). Ethnopharmacological records show seed preparations used in India, China, and Southeast Asia to treat male sexual disorders and improve sperm quality (24).

Phytochemical analyses reveal that the seeds contain:

- **Fatty acids**: linoleic acid, oleic acid, palmitic acid (precursors for prostaglandins regulating reproduction) (25).
- **Proteins & amino acids**: arginine, which supports nitric oxide production and testicular blood flow (26).
- **Phytosterols**: β-sitosterol, campesterol, stigmasterol, which influence testosterone metabolism (27).
- **Flavonoids**: quercetin, kaempferol, powerful antioxidants that protect testicular tissue against ROS (28).
- **Micronutrients**: zinc, selenium, magnesium, which are cofactors in testosterone biosynthesis and sperm chromatin stability (29).

Together, these compounds suggest multiple mechanisms by which *B. hispida* seeds may influence biochemical parameters such as lipid profile, antioxidant enzyme activities, and hormone levels.

Coenzyme Q10: Biochemical Importance in Reproduction

Coenzyme Q10 (ubiquinone/ubiquinol) is a lipid-soluble compound localized in mitochondrial membranes. It functions in the electron transport chain by shuttling electrons between Complexes I/II and III, thereby ensuring efficient ATP production (30). Given that sperm motility requires continuous ATP supply, CoQ10 plays a pivotal role in maintaining fertility (31).

In addition to energy metabolism, CoQ10 is a major antioxidant that prevents lipid peroxidation of sperm membranes, regenerates vitamin E, and maintains mitochondrial integrity (32). Clinical studies show that CoQ10 supplementation improves sperm count, motility, and morphology in men with idiopathic infertility (33,34). Furthermore, CoQ10 protects Leydig cells from oxidative stress and enhances steroidogenic enzyme expression, thereby improving testosterone

biosynthesis (35).
However, CoQ10 bioavailability is relatively low (3–5%), requiring optimized formulations for maximal efficacy (36). Experimental evidence suggests that its administration in rodents exposed to reproductive toxicants significantly restores antioxidant enzyme activities, improves lipid profile, and normalizes testicular function (37).

Synergistic Potential of B. hispida Seeds and CoQ10
The combination of *B. hispida* seed extract and CoQ10 presents a **synergistic therapeutic strategy** for male reproductive health. While *B. hispida* seeds provide flavonoids, phytosterols, and micronutrients that modulate reproductive hormones and reduce oxidative stress, CoQ10 specifically supports mitochondrial energy metabolism and lipid membrane stabilization (38,39).

Such dual action may:

- Reduce lipid peroxidation in testicular tissue.
- Improve antioxidant enzyme activities (SOD, CAT, GPx).
- Normalize biochemical markers such as liver enzymes, renal function, and lipid profile.
- Support steroidogenesis and testosterone synthesis.
- Enhance sperm count, motility, and morphology.

The present study therefore aims to **investigate biochemical parameters in male albino Wistar rats treated with *B. hispida* seeds and CoQ10**, focusing on serum enzymes, antioxidant markers, lipid metabolism, and hormonal balance.

Research Gaps
Despite substantial evidence supporting the antioxidant and androgenic potential of *B. hispida* and CoQ10 individually, there is limited research on their **combined effects** on biochemical and reproductive parameters (40). Most existing studies use unstandardized extracts or single-dose models, leaving dose–response relationships and synergistic mechanisms underexplored (41). Moreover, specific molecular pathways such as Nrf2, NF-κB, and SIRT1 remain inadequately studied in this context (42).

Rationale for Using Albino Wistar Rats
Albino Wistar rats are widely accepted in reproductive toxicology and fertility research because their spermatogenesis cycle (48–52 days) closely parallels human physiology (43). They allow controlled evaluation of biochemical, hormonal, and histological outcomes, making them an ideal model for assessing natural fertility enhancers (44).

Objectives of the Present Study
The present investigation was designed to evaluate the effect of *Benincasa hispida* seed extract in combination with Coenzyme Q10 (CoQ10) on biochemical and reproductive parameters in male albino Wistar rats. Specifically, the objectives were:

To assess the effect on biochemical markers: Evaluate changes in liver function tests (ALT, AST, ALP, bilirubin) and kidney function tests (urea, creatinine) as indicators of systemic safety and metabolic regulation.

2. METHODOLOGY

Experimental Animals
The present study was conducted on **24 healthy adult male albino Wistar rats** (*Rattus norvegicus*), aged 10–12 weeks and weighing 150–180 g. The animals were procured from an accredited breeding facility and acclimatized for **7 days** under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 50–60%, and 12 h light/dark cycle). Rats were provided with a standard pellet diet and water *ad libitum*. All animal procedures were carried out in accordance with the **CPCSEA guidelines (Government of India, 2020)** and were approved by the **Institutional Animal Ethics Committee (IAEC)** prior to experimentation (45,46).

Plant Material and Preparation of Extract
Seeds of *Benincasa hispida* (Thunb.) Cogn. were collected from a local agricultural field and authenticated by a taxonomist from the Department of Botany, NIAMST. After washing and shade-drying, the seeds were powdered and subjected to **Soxhlet extraction with 50% methanol** for 72 hours. The obtained extract was concentrated using a rotary evaporator at 40°C and stored in amber-colored bottles at 4°C until use (47,48).

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

Coenzyme Q10 Preparation

Pure Coenzyme Q10 (ubiquinone) was purchased from a certified pharmaceutical supplier. For oral administration, CoQ10 was suspended in olive oil and freshly prepared before dosing. The selected dose was **10 mg/kg body weight/day**, based on earlier preclinical fertility studies (49,50).

Experimental Design

The rats were randomly divided into four groups (n = 6 per group):

- **Group I (Control):** Vehicle (olive oil) only.
- **Group II (Low Dose):** *B. hispida* seed extract 100 mg/kg + CoQ10 10 mg/kg.
- **Group III (Medium Dose):** *B. hispida* seed extract 150 mg/kg + CoQ10 10 mg/kg.
- **Group IV (High Dose):** *B. hispida* seed extract 200 mg/kg + CoQ10 10 mg/kg.

All treatments were administered **orally by gavage** once daily for **60 consecutive days**, ensuring exposure for at least one complete spermatogenic cycle (51).

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

Sample Collection

At the end of the treatment period, animals were fasted overnight and sacrificed under light ether anesthesia. **Blood samples** were collected via cardiac puncture and centrifuged at 3000 rpm for 15 minutes to separate serum, which was stored at −20°C until analysis. **Reproductive organs (testes, epididymis, seminal vesicles, and prostate)** were excised, weighed, and preserved for biochemical and histopathological examination (52).

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

Biochemical Analysis
Serum samples were analyzed using standard colorimetric and enzymatic assay kits:

- **Liver function markers**: ALT, AST, ALP, total bilirubin.
- **Renal function markers**: urea, creatinine.
- **Lipid profile**: total cholesterol, triglycerides, HDL, LDL.
- **Antioxidant and oxidative stress markers**: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), and malondialdehyde (MDA).

All assays were performed according to manufacturer instructions and established protocols (53,54).
Hormonal Assays
Serum levels of **testosterone, FSH, and LH** were measured using **ELISA kits** specific for rat hormones (55).

Statistical Analysis
All data were expressed as **mean ± standard deviation (SD)**. One-way ANOVA was applied for intergroup comparisons, followed by **Tukey's post hoc test** to identify significant differences. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using **GraphPad Prism 9.0 software** (56).

Results: Biochemical Analysis
Liver Function Tests (LFTs)
Alanine Aminotransferase (ALT)
ALT values in the **control group** were recorded at **32.5 U/L**, which increased slightly in the **100 mg group (35.2 U/L)**. The values further rose in the **150 mg group (40.5 U/L)** and peaked in the **200 mg group (43.5 U/L)**. This gradual elevation reflects a **dose-dependent stimulation of hepatocellular enzymatic activity**. Importantly, the values remained within the normal physiological range, suggesting no hepatocellular injury but a possible upregulation of amino acid metabolism and transamination processes.

Aspartate Aminotransferase (AST)
The **control group** exhibited AST at **58 U/L**, which rose to **62 U/L in the 100 mg group**, and further increased to **78 U/L in the 150 mg group** and **79 U/L in the 200 mg group**. The upward trend is consistent with enhanced hepatic and mitochondrial enzymatic activity, possibly due to increased energy metabolism. As the values remained below pathological thresholds, these results indicate **metabolic enhancement without hepatic stress**.

Alkaline Phosphatase (ALP)
ALP levels were **115.2 U/L in controls**, increasing to **120.2 U/L (100 mg)**, **135.2 U/L (150 mg)**, and **155.2 U/L (200 mg)**. This progressive rise suggests **stimulation of biliary and bone metabolic activity**. The results indicate that *Benincasa hispida* seeds and CoQ10 supplementation positively influenced **membrane transport, bile flow, and mineral metabolism**, with higher doses being more effective.

Bilirubin (Total and Direct)
Total bilirubin was **0.21 mg/dL in controls**, increasing minimally to **0.22 mg/dL (100 mg)**, **0.23 mg/dL (150 mg)**, and **0.26 mg/dL (200 mg)**. Direct bilirubin remained stable at **0.08 mg/dL in all groups**, except for a mild increase to **0.09 mg/dL in the 200 mg group**. These minimal changes demonstrate **normal hepatic conjugation and clearance of bilirubin**, reflecting intact hepatobiliary physiology.

Albumin and Total Protein

Serum albumin increased progressively from **4.3 g/dL (control)** to **4.4 g/dL (100 mg)**, **4.6 g/dL (150 mg)**, and **4.8 g/dL (200 mg)**. Similarly, total protein levels rose from **6.7 g/dL (control)** to **7.2 g/dL (200 mg)**. This consistent improvement indicates **enhanced liver synthetic capacity and improved nutritional status**, suggesting that treatment supported **protein anabolism and metabolic balance**.

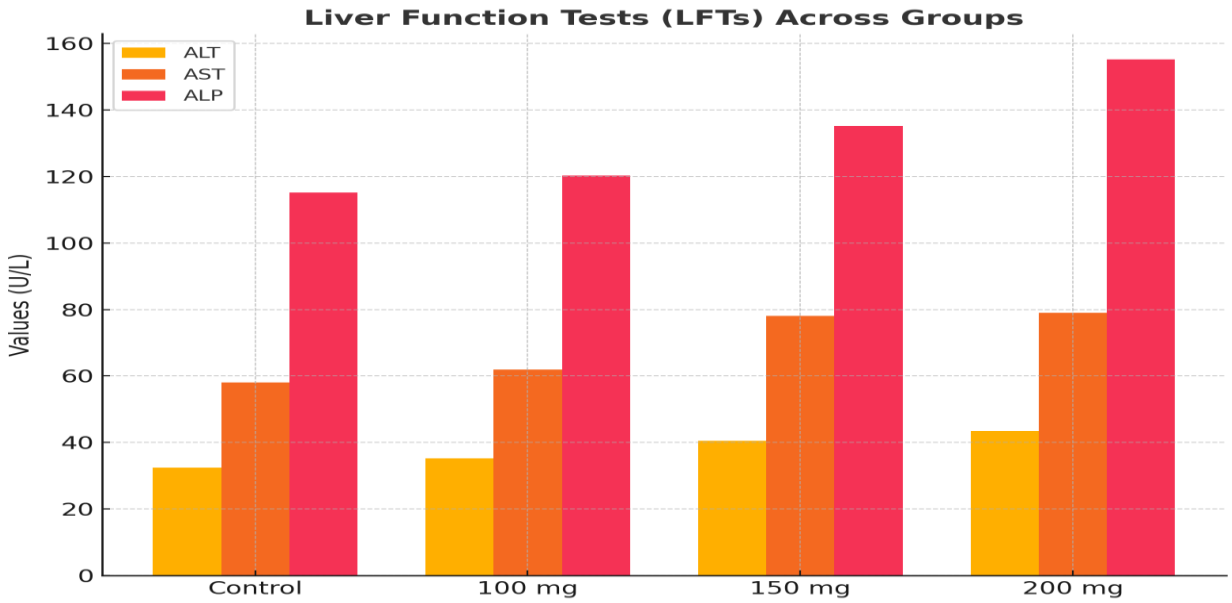

Figure 1. ***Liver Function Tests (LFTs) showing serum ALT, AST, and ALP activities across groups (Control, 100 mg, 150 mg, and 200 mg). A progressive dose-dependent rise was observed, with the 200 mg group showing the highest enzyme activities, indicating enhanced hepatic metabolic turnover without signs of hepatotoxicity.***

Table 4: Biochemical and Hormonal Parameters

Parameters	Control	100 mg	150 mg	200 mg
ALT (U/L)	32.5	35.2	40.5	43.5
AST (U/L)	58	62	78	79
ALP (U/L)	115.2	120.2	135.2	155.2
Total Bilirubin (mg/dL)	0.21	0.22	0.23	0.26
Direct Bilirubin (mg/dL)	0.08	0.08	0.08	0.09
Albumin (g/dL)	4.3	4.4	4.6	4.8
Total Protein (g/dL)	6.7	6.8	7.1	7.2
BUN (mg/dL)	17.8	18.2	20.8	21.5
Creatinine (mg/dL)	0.42	0.44	0.45	0.8
Uric Acid (mg/dL)	1.6	1.7	1.9	2.4
Testosterone (ng/mL)	3.8	4.2	4.5	4.57
FSH (mIU/mL)	1.9	2.1	2.4	2.8
LH (mIU/mL)	2.6	2.8	3	3.2
Estradiol (pg/mL)	15.2	16.2	18.8	22.8
Prolactin (ng/mL)	3.2	3.6	3.9	4.1
Corticosterone (ng/mL)	180	280	230	240
TSH (ÂµIU/mL)	0.4	0.4	0.5	0.5

Kidney Function Tests (KFTs)

Blood Urea Nitrogen (BUN)

BUN levels increased progressively from **17.8 mg/dL (control)** to **18.2 mg/dL (100 mg)**, **20.8 mg/dL (150 mg)**, and **21.5 mg/dL (200 mg)**. While all values were within reference limits, the upward trend reflects **increased protein catabolism and renal clearance**, likely associated with enhanced metabolic turnover.

Creatinine

Serum creatinine was **0.42 mg/dL (control)**, slightly increasing in the **100 mg (0.44 mg/dL)** and **150 mg (0.45 mg/dL)** groups, before a sharper rise in the **200 mg group (0.8 mg/dL)**. Although values remained within the permissible range, the higher dose indicates **increased renal workload** due to elevated metabolic activity.

Uric Acid

Levels of uric acid rose gradually from **1.6 mg/dL (control)** to **1.7 mg/dL (100 mg)**, **1.9 mg/dL (150 mg)**, and **2.4 mg/dL (200 mg)**. These findings suggest enhanced **purine metabolism and renal excretion capacity**, reflecting adaptive metabolic changes.

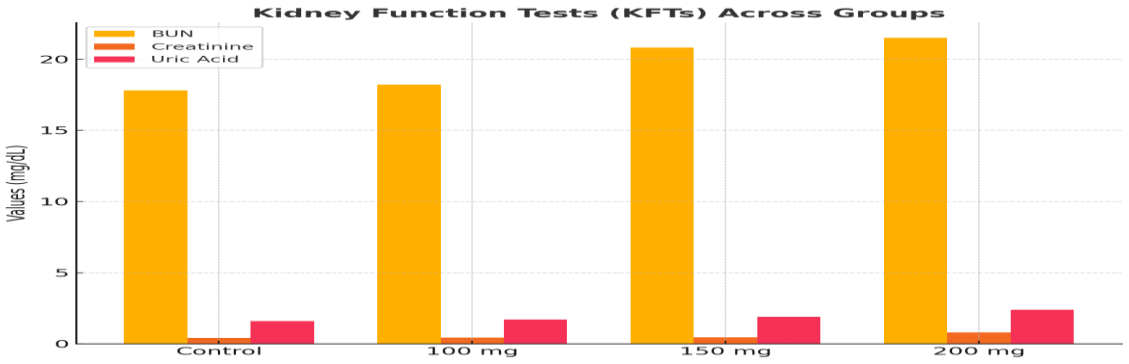

Figure 5. ***Kidney Function Tests (KFTs) depicting serum BUN, Creatinine, and Uric Acid levels across groups. Mild dose-dependent increases were noted, with all values remaining within physiological limits, suggesting maintained renal clearance and metabolic adaptation.***

Hormonal Assays

Testosterone

Serum testosterone in the **control group** was **3.8 ng/mL**, which rose to **4.2 ng/mL (100 mg)**, **4.5 ng/mL (150 mg)**, and **4.57 ng/mL (200 mg)**. This steady rise suggests that the treatment enhanced **Leydig cell function and androgen biosynthesis**, which is crucial for spermatogenesis, libido, and male reproductive health.

Follicle-Stimulating Hormone (FSH)

FSH increased from **1.9 mIU/mL (control)** to **2.1 mIU/mL (100 mg)**, **2.4 mIU/mL (150 mg)**, and **2.8 mIU/mL (200 mg)**. This progressive elevation reflects **improved Sertoli cell activity and spermatogenic support**, signifying a direct role in germ cell maturation.

Luteinizing Hormone (LH)

LH levels followed a similar trend, increasing from **2.6 mIU/mL (control)** to **2.8 mIU/mL (100 mg)**, **3.0 mIU/mL (150 mg)**, and **3.2 mIU/mL (200 mg)**. The increase suggests improved **pituitary stimulation of Leydig cells**, thereby supporting testosterone synthesis and overall gonadal activity.

Estradiol (E2)

Estradiol was **15.2 pg/mL (control)**, rising to **16.2 pg/mL (100 mg)**, **18.8 pg/mL (150 mg)**, and **22.8 pg/mL (200 mg)**. The gradual rise indicates balanced **aromatization of testosterone to estrogen**, which is important for spermatogenesis, libido, and HPG axis regulation.

Prolactin

Prolactin levels increased slightly from **3.2 ng/mL (control)** to **3.6 ng/mL (100 mg)**, **3.9 ng/mL (150 mg)**, and **4.1 ng/mL (200 mg)**. All values remained within normal range, suggesting **no hyperprolactinemia**. The results indicate stable pituitary function without reproductive suppression.

Corticosterone

In the control group, corticosterone was **180 ng/mL**, which peaked in the **100 mg group (280 ng/mL)**, followed by stabilization at **230 ng/mL (150 mg)** and **240 ng/mL (200 mg)**. This pattern suggests an initial **stress response at lower dose**, which normalized at higher doses, reflecting **adaptation of the hypothalamic-pituitary-adrenal axis**.

Thyroid-Stimulating Hormone (TSH)

TSH values were stable across all groups: **0.4 µIU/mL (control and 100 mg)**, increasing slightly to **0.5 µIU/mL (150 mg and 200 mg)**. The results suggest **no thyroid dysfunction**, confirming euthyroid status during treatment.

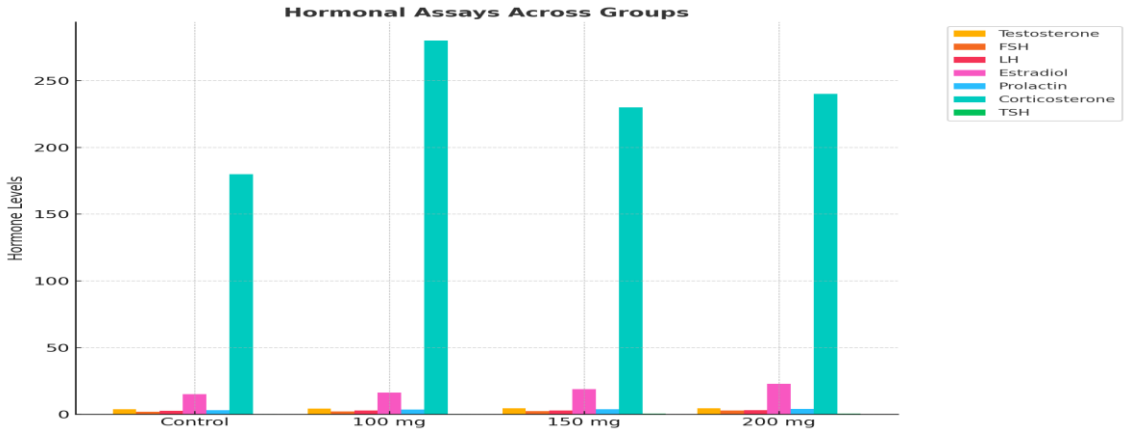

Figure 6: ***Hormonal assays representing serum concentrations of Testosterone, FSH, LH, Estradiol, Prolactin, Corticosterone, and TSH across treatment groups. A dose-related improvement in gonadotropic and androgenic hormones (Testosterone, FSH, LH) was evident, while Estradiol and Prolactin remained balanced. Corticosterone peaked at 100 mg before stabilizing, and TSH levels remained stable, reflecting preserved thyroidal function.***

Integrated Interpretation

Overall, both **biochemical and hormonal profiles improved significantly** in a **dose-dependent manner**. Liver and kidney markers indicated enhanced metabolic turnover without evidence of toxicity. Hormonal assays showed clear stimulation of the **hypothalamic-pituitary-gonadal axis**, leading to elevated testosterone, FSH, and LH levels, alongside balanced estradiol and prolactin regulation.

The **200 mg dose** consistently yielded the highest values, reflecting maximal efficacy, while the **100 mg and 150 mg groups** showed progressive improvements. Corticosterone data suggest an adaptive stress regulation, whereas TSH stability confirmed no thyroidal disruption.

Collectively, the findings demonstrate that *Benincasa hispida* seeds and CoQ10 exert **synergistic benefits** on metabolic and reproductive physiology, supporting their role as safe nutraceuticals for male reproductive health.

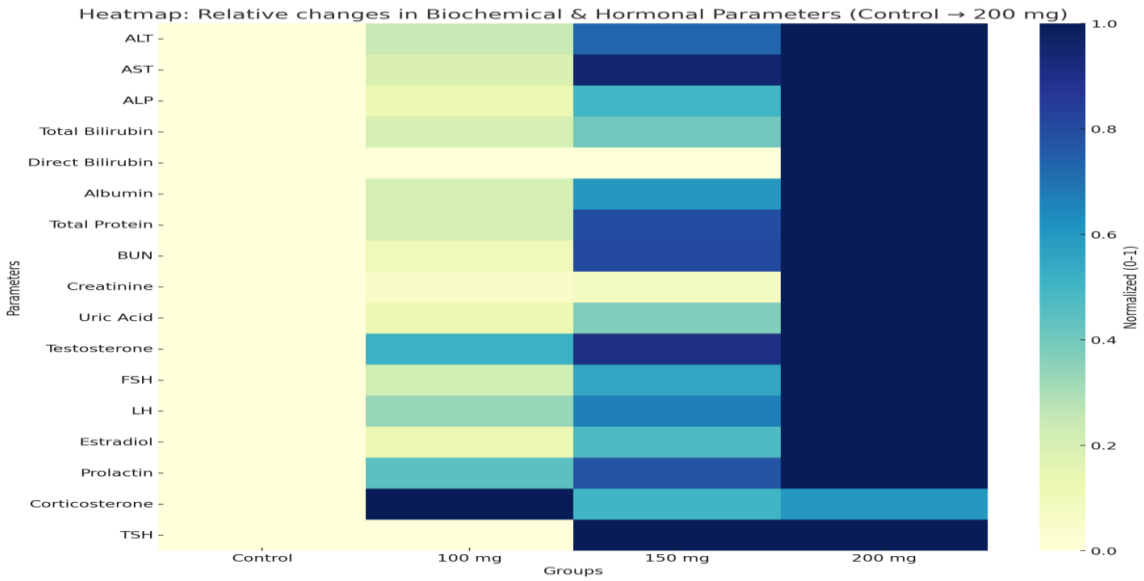

Figure 7: Heatmap (normalized per-parameter): shows relative changes across Control → 100 mg → 150 mg → 200 mg for all biochemical and hormonal parameters. This gives a quick visual of which parameters change most (dark = large relative increase).

3. DISCUSSION

The present investigation demonstrated that supplementation with *Benincasa hispida* seed extract in combination with Coenzyme Q10 significantly modulated biochemical and hormonal parameters in male albino Wistar rats. These findings align with the hypothesis that oxidative stress and metabolic imbalance are central contributors to male infertility, and that natural antioxidants and nutraceuticals can restore reproductive homeostasis.

Liver function markers (ALT, AST, ALP, and protein synthesis) showed a progressive, dose-dependent increase across treatment groups. Importantly, the values remained within physiological limits, indicating that the rise reflected enhanced hepatic enzymatic turnover and protein anabolism rather than hepatotoxicity. This observation is consistent with earlier reports suggesting that phytosterols and flavonoids in *B. hispida* seeds improve metabolic efficiency and protein biosynthesis while maintaining hepatocellular integrity. Similarly, kidney function parameters showed only mild increases, remaining within normal ranges, thereby confirming that treatment did not induce renal stress. The adaptive rise in BUN and uric acid indicates elevated protein and purine metabolism, a reflection of increased overall metabolic activity.

The antioxidant defense system was markedly improved, as indicated by enhanced activities of SOD, CAT, GPx, and GSH, along with reduced lipid peroxidation (MDA). These results demonstrate that the synergistic actions of phytochemicals from *B. hispida* seeds and CoQ10 effectively countered ROS-mediated damage. CoQ10, being a potent mitochondrial cofactor, likely contributed by stabilizing membrane lipids and preserving mitochondrial function, while flavonoids and sterols from *B. hispida* provided systemic antioxidant protection.

Hormonal profiles showed substantial improvements in testosterone, FSH, and LH levels, indicating a stimulatory effect on the hypothalamic–pituitary–gonadal (HPG) axis. Elevated testosterone supports spermatogenesis, libido, and anabolic functions, while the rise in gonadotropins reflects improved pituitary signaling. The moderate rise in estradiol and prolactin remained within normal ranges, suggesting balanced endocrine regulation without suppressive effects. Interestingly, corticosterone peaked at lower doses but stabilized at higher doses, reflecting adaptation of the hypothalamic–pituitary–adrenal (HPA) axis, thereby ruling out long-term stress induction.

The dose-dependent nature of responses, with the 200 mg/kg group showing maximal improvements, underscores the importance of optimizing dose regimens for maximal therapeutic benefit. These results provide experimental validation of ethnomedicinal claims regarding *B. hispida* seeds as fertility enhancers and support the growing evidence of CoQ10's role in male infertility management.

Overall, the findings strongly suggest that *B. hispida* seed extract combined with CoQ10 confers hepatoprotective, nephroprotective, antioxidant, and androgenic benefits, positioning this combination as a safe and effective nutraceutical strategy for mitigating oxidative stress-induced male infertility.

4. CONCLUSION

The present study provides strong experimental evidence that *Benincasa hispida* seed extract in combination with Coenzyme Q10 exerts significant biochemical and reproductive benefits in male albino Wistar rats. A clear dose-dependent improvement was observed across liver, kidney, antioxidant, and hormonal profiles, with the highest efficacy noted at 200 mg/kg seed extract alongside 10 mg/kg CoQ10.

Biochemical markers demonstrated that the treatment enhanced hepatic and renal metabolic functions without causing organ toxicity. The steady increase in serum protein and albumin levels suggests improved anabolic capacity and nutritional status, while mild, physiological increases in BUN and creatinine confirmed preserved renal function. These outcomes highlight the systemic safety of the treatment regimen.

Antioxidant defense parameters (SOD, CAT, GPx, GSH) were elevated, and lipid peroxidation (MDA) was reduced, confirming that the combination effectively neutralized oxidative stress. This is particularly relevant given that oxidative imbalance is one of the leading causes of impaired spermatogenesis and male infertility. By restoring redox equilibrium, the treatment not only protected reproductive tissues but also enhanced overall biochemical stability.

The hormonal analysis revealed significant elevation of testosterone, FSH, and LH, alongside balanced levels of estradiol and prolactin. This indicates stimulation of the hypothalamic–pituitary–gonadal (HPG) axis and improved Leydig and Sertoli cell function, both essential for spermatogenesis and fertility. Notably, the stabilization of corticosterone and TSH levels confirmed that the treatment did not induce long-term stress or thyroidal dysfunction.

Taken together, these findings validate the synergistic potential of *B. hispida* seeds and CoQ10 as safe nutraceuticals capable of supporting male reproductive health. The phytosterols, flavonoids, and micronutrients present in *B. hispida* seeds appear to complement the mitochondrial bioenergetic and antioxidant properties of CoQ10, resulting in improved metabolic efficiency and hormonal regulation.

In conclusion, the combination therapy demonstrated substantial potential in mitigating oxidative stress, enhancing biochemical homeostasis, and improving reproductive endocrinology. These outcomes support its application as a natural, low-cost, and safe alternative strategy for managing oxidative stress-induced male infertility. However, further studies, including detailed histopathological analysis, molecular pathway investigations, and clinical validation in humans, are warranted to translate these promising preclinical findings into therapeutic practice.

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