

Dose-dependent ameliorative effects of green tea (*Camellia sinensis*) leaf extract on physiological, biochemical, and reproductive impairments in male rats: an experimental study

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Highlights

- Green tea leaf extract showed a coherent dose-dependent protective pattern, probably linked to attenuation of oxidative stress and restoration of reproductive endocrine and sperm-quality endpoints.

Abstract

Background: Green tea (*Camellia sinensis*) is rich in catechin polyphenols and has been investigated as a dietary antioxidant with potential reproductive benefits. **Objective:** This study evaluated whether green tea leaf extract (GTE) could improve physiological, biochemical, oxidative-stress and reproductive outcomes in a male rat model of reproductive dysfunction. **Methods:** Seventy-two male rats were distributed into six groups (n=12/group): normal control (NC), reproductive dysfunction model (MOD), GTE 100 mg/kg (GTE_L), GTE 200 mg/kg (GTE_M), GTE 400 mg/kg (GTE_H), and vitamin E 100 mg/kg positive control (POS). Treatments were administered for 42 days. Endpoints included body-weight dynamics, food and water intake, relative reproductive organ weights, serum glucose, ALT, AST, urea, creatinine, MDA, SOD, CAT, GSH, reproductive hormones, sperm parameters and fertility indices. One-way ANOVA followed by Tukey post-hoc testing was used. **Results:** The MOD group showed lower final body weight, body-weight gain, reproductive organ weights, antioxidant enzymes, testosterone, gonadotropins, sperm quality and fertility indices, together with higher glucose, liver/kidney markers, MDA and sperm abnormality. GTE produced dose-dependent recovery across most outcomes, with the 400 mg/kg group approaching the NC and POS groups. For example, compared with MOD, GTE_H restored final body weight (243.76 ± 4.87 vs. 219.48 ± 7.69 g), reduced MDA (2.50 ± 0.52 vs. 5.81 ± 0.55 nmol/mg protein), increased testosterone (4.35 ± 0.42 vs. 2.02 ± 0.44 ng/mL), improved sperm concentration (72.02 ± 5.15 vs. 38.91 ± 7.48 million/mL), and increased fertility index (79.25 ± 8.39 vs. $38.83 \pm 5.51\%$). **Conclusion:** In this model, GTE showed a coherent dose-dependent protective pattern, probably linked to attenuation of oxidative stress and restoration of reproductive endocrine and sperm-quality endpoints.

Keywords: *Camellia sinensis*; green tea extract; male reproductive dysfunction; oxidative stress; sperm quality; testosterone; fertility index; antioxidant enzymes.

Introduction

Male reproductive dysfunction is often linked to oxidative stress, endocrine disruption, impaired spermatogenesis and altered accessory organ function[1, 2]. Spermatozoa are particularly vulnerable to reactive oxygen species because their membranes contain abundant polyunsaturated fatty acids and because excessive oxidative stress can damage sperm

motility, viability, morphology and DNA integrity[3]. Natural antioxidants are therefore frequently explored as supportive interventions in experimental models of reproductive injury[4].

Green tea, derived from *Camellia sinensis*, contains catechin polyphenols such as epigallocatechin gallate, epicatechin gallate, epigallocatechin and epicatechin[5, 6]. These compounds can scavenge reactive oxygen species and modulate endogenous antioxidant defense pathways. Prior experimental and review evidence suggests that green tea catechins may improve reproductive health through antioxidant and cytoprotective mechanisms, although the response is dose- and context-dependent [5, 7].

Animal studies have reported beneficial effects of green tea or green tea extract on sperm concentration, viability, motility, testosterone and oxidative stress markers under normal or toxicant-induced reproductive injury conditions [8]. However, high-dose green tea polyphenols can also show pro-oxidant or organ-toxic effects in specific models, underscoring the need to evaluate efficacy together with liver and kidney safety biomarkers [9].

This study evaluated the dose-dependent effects of green tea leaf extract on physiological, biochemical, oxidative stress and reproductive outcomes in a model of reproductive dysfunction.

Materials and methods

Preparation of Green Tea Extract

Dried leaves of *Camellia sinensis* (Luang Prabang Province, Lao PDR) were used as the source material for green tea extract preparation. The leaves were ground into powder and extracted with 70% ethanol (Merck, USA) as the extraction solvent. The mixture was macerated at room temperature with intermittent stirring, followed by filtration to remove plant residues. The filtrate was concentrated under reduced pressure and dried to obtain the crude green tea extract. Before administration, the extract was freshly dissolved in distilled water to prepare the required doses of 100, 200, and 400 mg/kg/day.

Study Design and Animals

A 42-day experimental study was conducted using 72 healthy adult male Wistar rats. The animals were randomly allocated into six groups, with 12 rats per group: normal control (NC), reproductive dysfunction model (MOD), low-dose green tea extract group (GTE_L), medium-dose green tea extract group (GTE_M), high-dose green tea extract group (GTE_H), and positive control group (POS). Reproductive dysfunction was induced in the MOD, GTE_L, GTE_M, GTE_H, and POS groups using cadmium chloride (CdCl₂; Sigma-Aldrich/Merck, St. Louis, MO, USA) at a dose of 1 mg/kg body weight/day by oral gavage for 42 consecutive days. Rats in the NC group received the corresponding vehicle only. Rats in the MOD group received CdCl₂ without treatment. Rats in the GTE_L, GTE_M, and GTE_H groups received CdCl₂ together with green tea extract at doses of 100, 200, and 400 mg/kg/day, respectively. Rats in the POS group received CdCl₂ together with vitamin E at 100 mg/kg/day as the positive control treatment. All treatments were administered orally once daily throughout the 42-day experimental period. All experimental procedures were reviewed and approved by the Ethics Committee of Souphanouvong University, Luang Prabang Province, Lao PDR (SU-EC-13/2026). The study was conducted in accordance with institutional guidelines for the care and use of laboratory animals and followed internationally accepted principles for animal research.

Treatment Groups

The animals were divided into six experimental groups. Rats in the NC group served as the normal control and received the vehicle only. Rats in the MOD group were subjected to the reproductive dysfunction model and received the vehicle. Rats in the GTE_L, GTE_M, and GTE_H groups were subjected to the same model and treated orally with green tea extract at doses of 100, 200, and 400 mg/kg/day, respectively. Rats in the POS group were subjected to the model and treated with vitamin E at 100 mg/kg/day as the positive control.

Physiological measurements

Body weight was recorded at baseline and at the end of the 42-day treatment period using a digital balance. Body-weight gain was calculated as the difference between final and initial body weight. Food intake and water intake were monitored throughout the experimental period and expressed as average daily intake per rat. At the end of the experiment, rats were euthanized under anesthesia. The testes and epididymides were carefully excised, cleaned of adherent fat and connective tissue, and weighed. Relative organ weights were calculated as organ weight/body weight $\times$ 100.

Serum biochemical analysis

At the end of the treatment period, blood samples were collected and centrifuged at 3,000 rpm for 10 min to obtain serum. Serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine levels were measured using commercial diagnostic kits according to the manufacturers' instructions. Glucose, ALT, AST, urea, and creatinine assay kits were purchased from Randox Laboratories Ltd. (Crumlin, UK). Absorbance was measured using a microplate reader, and the results were expressed in standard biochemical units.

Oxidative stress assessment

Testicular tissues were homogenized in cold phosphate-buffered saline and centrifuged to obtain the supernatant for oxidative stress assays. Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were measured using commercial colorimetric assay kits according to the manufacturers' protocols. MDA, SOD, CAT, and GSH assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). MDA levels were used as an index of lipid peroxidation, whereas SOD, CAT, and GSH were assessed as antioxidant defense markers. Protein concentration in tissue homogenates was determined using a bicinchoninic acid protein assay kit from Thermo Fisher Scientific (Waltham, MA, USA), and oxidative stress markers were normalized to protein content where appropriate.

Reproductive hormone analysis

Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) concentrations were measured using rat-specific enzyme-linked immunosorbent assay kits. Testosterone, LH, and FSH ELISA kits were purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). All assays were performed according to the manufacturer's instructions. Absorbance was read using a microplate reader, and hormone concentrations were calculated from standard curves generated for each assay.

Sperm parameter evaluation

The cauda epididymis was isolated and minced in pre-warmed physiological saline to allow sperm dispersion. Sperm concentration was determined using a hemocytometer and expressed as million sperm/mL. Sperm motility was assessed under a light microscope by calculating the percentage of motile spermatozoa. Sperm viability was evaluated using eosin–nigrosin staining solution from Sigma-Aldrich (St. Louis, MO, USA), with unstained sperm considered viable.

and stained sperm considered non-viable. Sperm abnormality was assessed using stained sperm smears under a light microscope, and spermatozoa with head, midpiece, or tail defects were recorded as abnormal.

Fertility assessment

For fertility evaluation, treated male rats were paired with untreated healthy adult female rats during the final phase of the experiment. The presence of sperm in vaginal smears or a vaginal plug was considered evidence of successful mating. Vaginal smears were prepared using sterile normal saline and examined under a light microscope. The mating index was calculated as the percentage of females showing evidence of mating. The fertility index was calculated as the percentage of mated females that became pregnant. Pregnancy rate was determined based on the proportion of females confirmed pregnant after pairing with males from each experimental group.

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation. Differences among groups were analyzed using one-way analysis of variance followed by Tukey's post-hoc test for multiple comparisons. In the figures, statistical annotations indicate Tukey-adjusted comparisons against the MOD group. A p value of less than 0.05 was considered statistically significant.

Results

GTE improved physiological outcomes and reproductive organ indices

The MOD group showed impaired growth compared with NC. Final body weight decreased from 241.81 ± 6.00 g in NC to 219.48 ± 7.69 g in MOD, whereas GTE_H restored this endpoint to 243.76 ± 4.87 g. Body-weight gain followed a similar pattern, increasing from 21.07 ± 9.07 g in MOD to 43.80 ± 6.20 g in GTE_H. Relative testis and epididymis weights were also reduced in MOD and improved progressively with GTE treatment, particularly at 400 mg/kg (Figure 1).

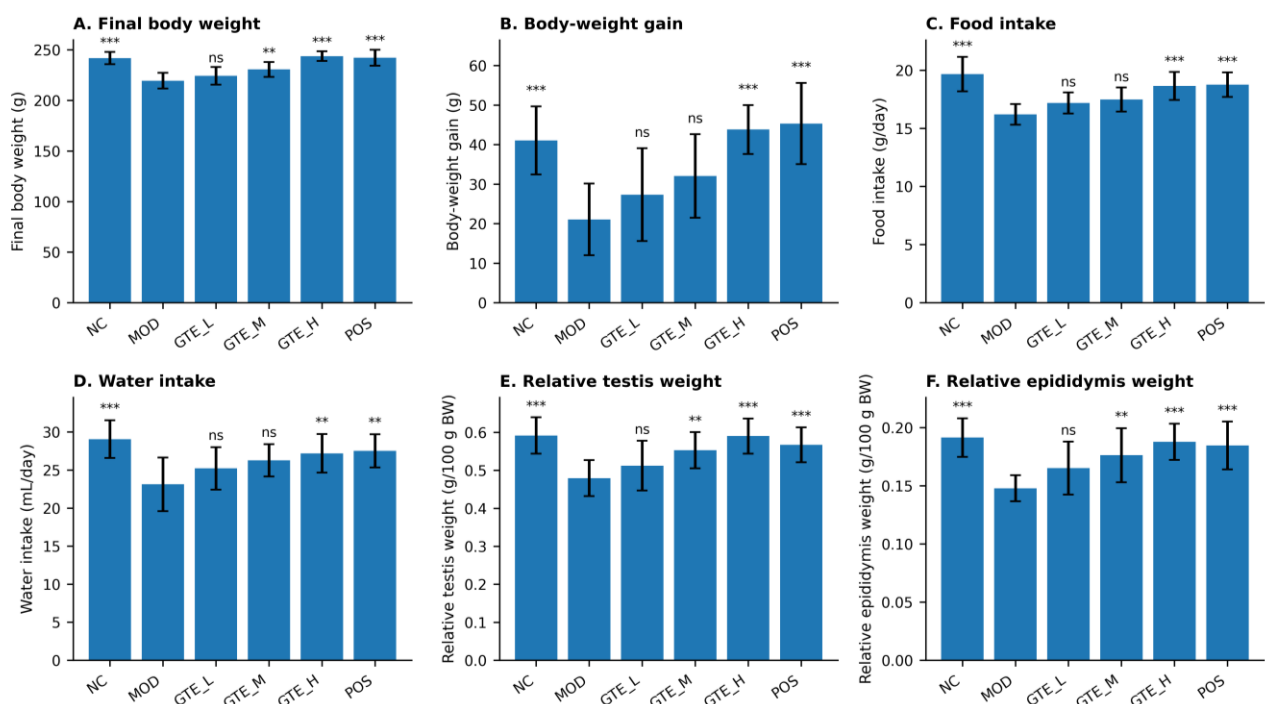


Figure 1. Physiological and reproductive organ outcomes. Bars represent mean $\pm$ SD (n=12/group). Asterisks indicate Tukey post-hoc comparison versus MOD.

GTE normalized biochemical markers related to metabolic, hepatic and renal status

The MOD group displayed elevated glucose, ALT, AST, urea and creatinine compared with NC. GTE produced a dose-dependent normalization of these markers. Serum glucose decreased from 121.60 ± 9.20 mg/dL in MOD to 102.32 ± 8.69 mg/dL in GTE_H. ALT declined from 66.27 ± 5.79 U/L to 40.32 ± 6.43 U/L, and AST declined from 146.91 ± 13.80 U/L to 106.41 ± 12.57 U/L. Urea and creatinine also shifted toward NC values in the medium- and high-dose GTE groups (Figure 2).

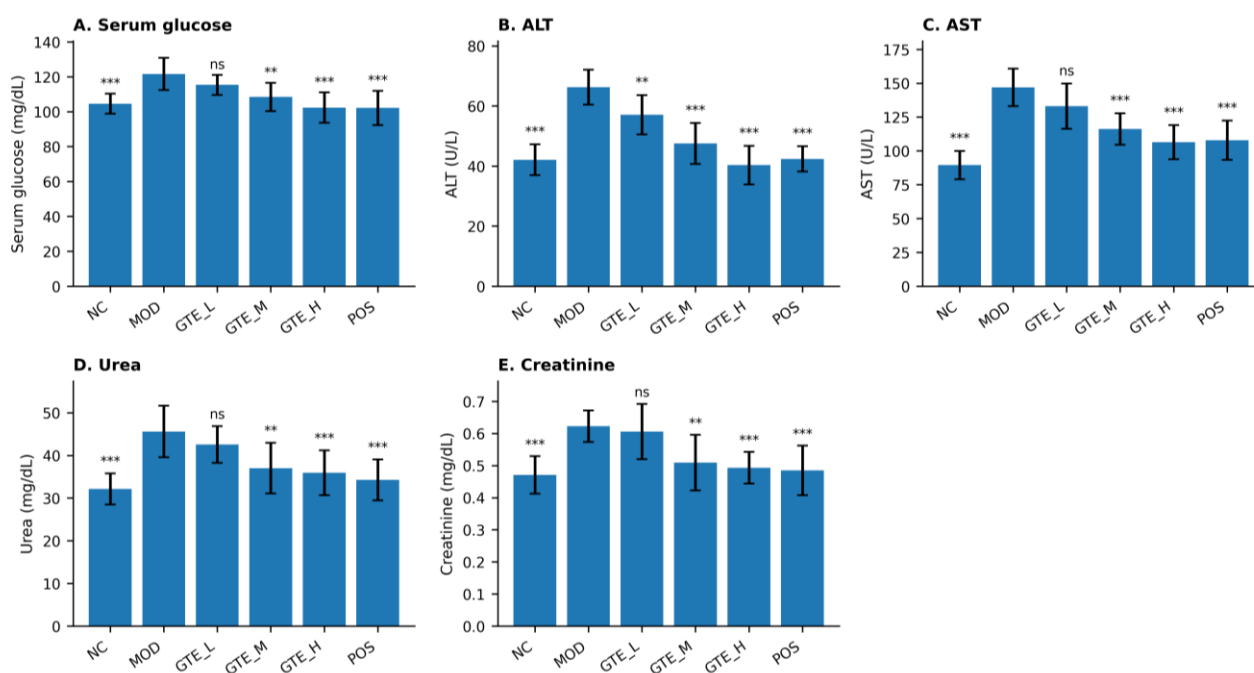


Figure 2. Serum biochemical and organ safety markers. Bars represent mean $\pm$ SD (n=12/group). Asterisks indicate Tukey post-hoc comparison versus MOD.

GTE attenuated oxidative stress and restored antioxidant defense

MOD markedly increased lipid peroxidation and suppressed antioxidant defenses. MDA increased to 5.81 ± 0.55 nmol/mg protein in MOD, while SOD, CAT and GSH decreased to 23.45 ± 3.59 U/mg protein, 13.59 ± 2.75 U/mg protein and 3.80 ± 0.56 μ mol/g tissue, respectively. GTE dose-dependently reduced MDA and enhanced SOD, CAT and GSH. GTE_H nearly normalized MDA (2.50 ± 0.52) and CAT (27.45 ± 1.98) relative to NC (Figure 3).

GTE improved reproductive endocrine status and sperm quality

The reproductive dysfunction model reduced testosterone, LH and FSH, together with sperm concentration, motility and viability, while increasing sperm abnormality. Testosterone rose from 2.02 ± 0.44 ng/mL in MOD to 4.35 ± 0.42 ng/mL in GTE_H. Sperm concentration increased from 38.91 ± 7.48 million/mL to 72.02 ± 5.15 million/mL, sperm motility increased from 38.88 ± 4.87 % to 75.33 ± 6.23 %, and sperm abnormality decreased from 24.03 ± 3.83 % to 9.17 ± 3.30 % (Figure 4).

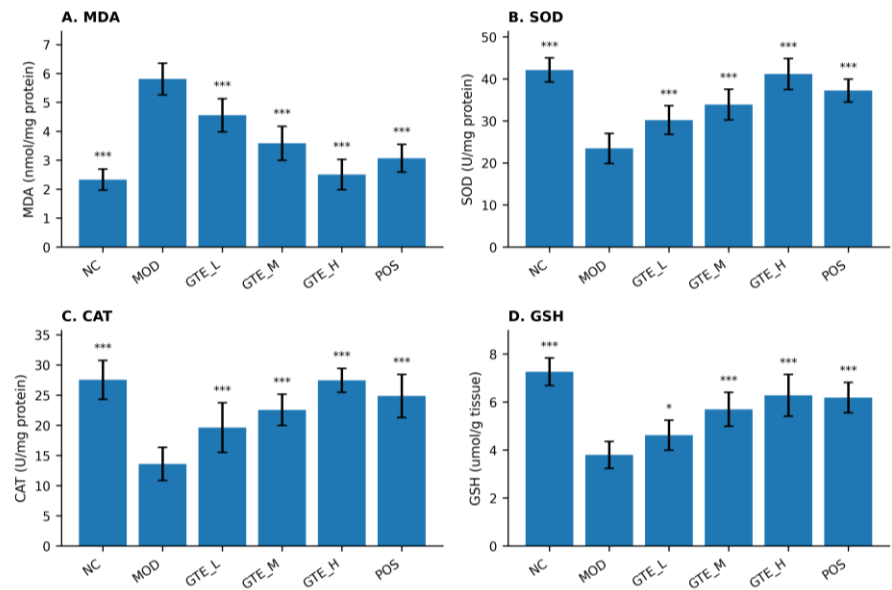


Figure 3. Oxidative stress and antioxidant defense. Bars represent mean $\pm$ SD (n=12/group). Asterisks indicate Tukey post-hoc comparison versus MOD.

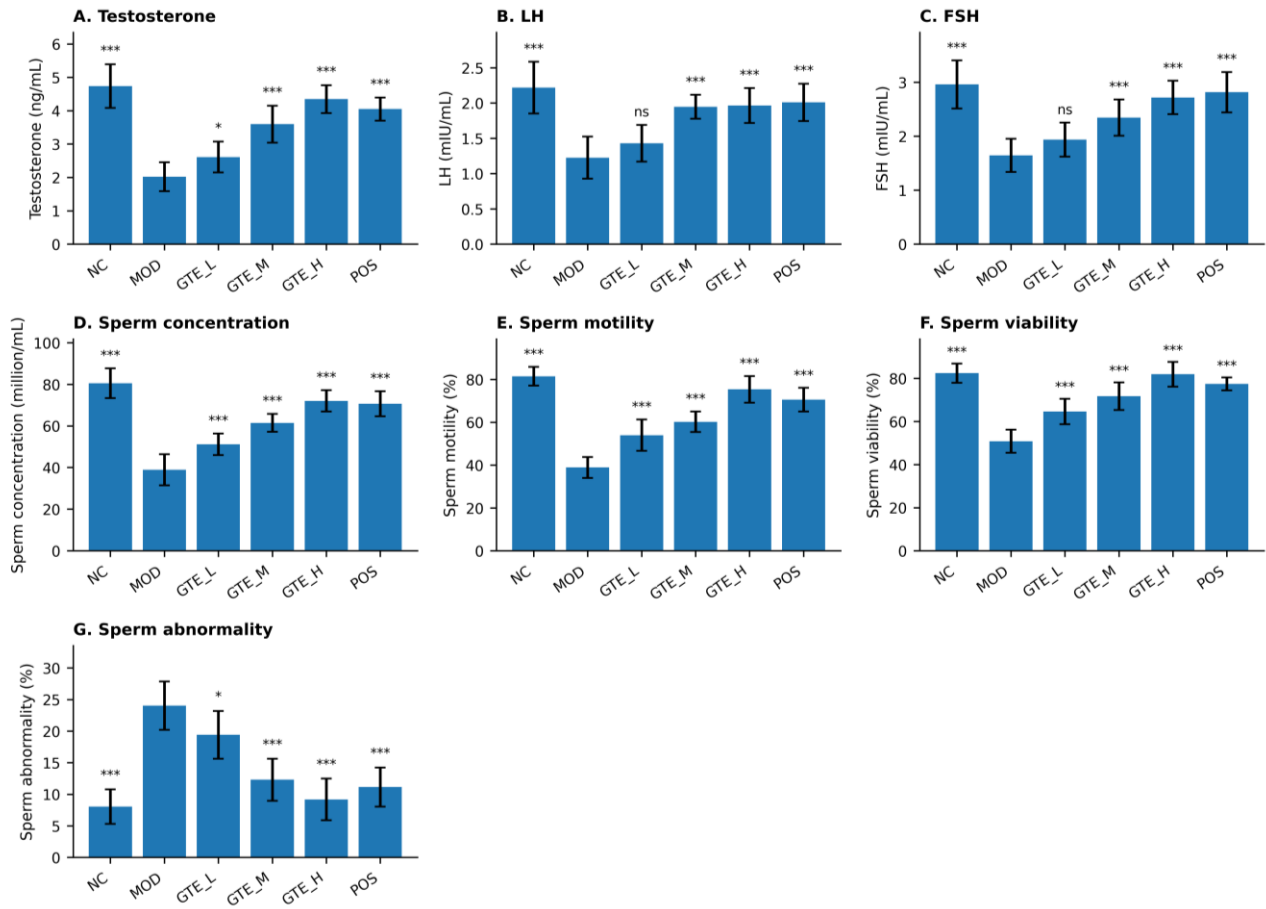


Figure 4. Endocrine and sperm quality outcomes. Bars represent mean $\pm$ SD (n=12/group). Asterisks indicate Tukey post-hoc comparison versus MOD.

GTE improved mating and fertility performance

Mating, fertility and pregnancy indices were substantially reduced in MOD. GTE improved these reproductive performance outcomes in a dose-dependent manner. Fertility index increased from 38.83 ± 5.51 % in MOD to 79.25 ± 8.39 % in GTE_H, while pregnancy rate increased from 34.92 ± 6.49 % to 74.50 ± 5.70 % (Figure 5).

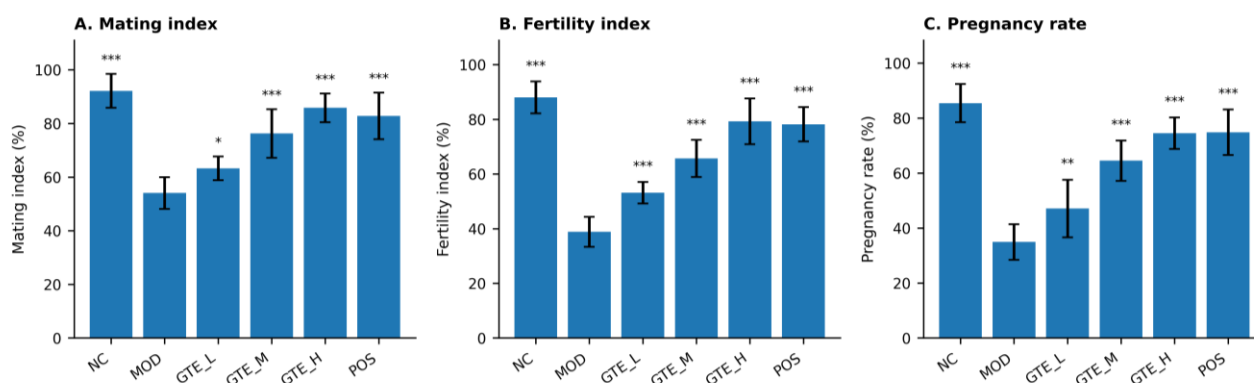


Figure 5. Mating and fertility performance. Bars represent mean $\pm$ SD (n=12/group). Asterisks indicate Tukey post-hoc comparison versus MOD.

Discussion

This study demonstrated that reproductive dysfunction was associated with impaired growth performance, systemic biochemical disturbances, oxidative stress, endocrine suppression, reduced sperm quality, and decreased fertility outcomes. Green tea extract (GTE) attenuated these alterations in a dose-dependent manner, with the 400 mg/kg dose producing the most consistent protective effects and showing outcomes comparable to those of the vitamin E positive-control group.

The first major finding was the restoration of antioxidant balance. The model group showed high MDA and low SOD, CAT and GSH, suggesting a shift toward lipid peroxidation and impaired endogenous antioxidant defense. GTE reversed these endpoints, supporting the hypothesis that green tea catechins may protect reproductive tissues by reducing oxidative damage. This interpretation is consistent with the known antioxidant actions of tea polyphenols and with reports that green tea extract can mitigate toxicant-induced oxidative injury in reproductive models [10, 11].

The second major finding was the improvement of reproductive endocrine parameters and sperm quality. Testosterone, LH and FSH were reduced in the model group, and their recovery after GTE suggests preservation of the hypothalamic-pituitary-gonadal axis or reduced oxidative injury within testicular steroidogenic compartments. The parallel improvement in sperm concentration, motility and viability, together with reduced sperm abnormality, is consistent with previous studies reporting that green tea consumption or extract administration can improve sperm parameters in male rats [12, 13].

The third major finding was the partial normalization of liver, kidney and metabolic markers. ALT, AST, urea, creatinine and glucose increased in the model group and shifted toward control values after medium- and high-dose GTE. These trends suggest that the reproductive benefit was not isolated but occurred alongside broader systemic recovery. Nevertheless, interpretation must remain cautious because green tea extracts are not universally protective at all doses. High-dose green tea polyphenols have been reported to aggravate hepatic or renal stress in certain experimental contexts [13, 14, 15].

The reproductive performance endpoints provide integrated functional support for the sperm and hormonal findings. Improved mating, fertility and pregnancy indices in the GTE groups suggest that biochemical and sperm-quality restoration translated into better reproductive output in this model. Among the tested doses, GTE_H produced the strongest overall effect, while GTE_M already showed meaningful recovery in several biochemical and reproductive endpoints. GTE_L produced partial but less consistent improvement, indicating a dose-response trend (Figure 4, 5).

These findings suggest that green tea leaf extract may improve reproductive dysfunction through antioxidant and endocrine-restorative mechanisms.

Conclusion

In this rat model, green tea leaf extract dose-dependently improved physiological status, serum biochemical markers, antioxidant defense, reproductive hormone levels, sperm quality, and fertility indices. The strongest protective effect was observed at 400 mg/kg, with several outcomes approaching those of the positive-control group. Overall, these findings support a potential antioxidant-mediated protective effect of green tea extract against reproductive dysfunction.

Data availability

The datasets analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics statement

All experimental procedures were reviewed and approved by the Ethics Committee of Souphanouvong University, Luang Prabang Province, Lao PDR (SU-EC-13/2026).

Conflict of interest

The authors declare no conflict of interest.

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Declaration of generative AI in scientific writing

Generative AI tools were used only to improve language clarity and readability. The authors reviewed and approved the final content and take full responsibility for the manuscript.

CRediT author statement

Khamphouy Phommachanh: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft. **Souksavanh Vongdala:** Methodology, Validation, Investigation, Resources, Writing – review & editing. **Manivone Sihavong:** Supervision, Project administration, Formal analysis, Writing – review & editing.

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