



## EFFECT OF EPIGALLOCATECHIN GALLATE ON THE SPERMATOZOA QUALITY OF RATS (*RATTUS NORVEGICUS*) EXPOSED TO 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE

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### Summary

Wahyudanto, D. P., Budiarto, S. Susilowati, Widjiati, S. P. Madyawati, K. Rachmawati, P. Nugraha, V. F. Hendrawan & D. Rahmawati, 2025. Effect of epigallocatechin gallate on the spermatozoa quality of rats (*Rattus norvegicus*) exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Bulg. J. Vet. Med.* (online first).

Environmental exposure to neurotoxic compounds is known to impair reproductive function, particularly by reducing sperm quality through oxidative stress and mitochondrial dysfunction. This study aimed to evaluate the protective effect of epigallocatechin gallate (EGCG) a major antioxidant compound in green tea, on sperm quality in rats exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Thirty-two Wistar rats (180–200 g) were randomly divided into four groups (n=8): control; MPTP only (16 mg/kg BW intraperitoneally); EGCG only (30 mg/kg BW/day orally), and MPTP + EGCG. EGCG was administered once daily for 7 consecutive days. On Day 8, sperm samples were collected from the cauda epididymis and analysed microscopically. The MPTP group showed a significant decrease in sperm motility, viability, and concentration compared to the control group (P<0.01), confirming reproductive toxicity. Rats treated with EGCG alone had significantly improved sperm parameters, indicating its beneficial role in enhancing male fertility. In the group receiving EGCG after MPTP exposure, a significant improvement in all sperm parameters was also observed (P<0.01), although at a slightly lower extent than in the EGCG-only group. The findings suggest that EGCG has potential therapeutic effects in mitigating MPTP-induced reproductive damage, most likely through its antioxidant and cytoprotective mechanisms. While the results are promising, further research is recommended to explore the underlying molecular pathways, including oxidative and hormonal markers, and to evaluate the long-term effects of EGCG treatment. This study supports the potential application of EGCG as a protective agent against chemically induced male infertility.

**Key words:** EGCG, MPTP, sperm concentration, sperm motility, sperm viability

## INTRODUCTION

Male infertility is a growing concern linked to environmental exposure to neurotoxic compounds, many of which impair the hypothalamic-pituitary-gonadal (HPG) axis. A well-established neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has been shown to disrupt dopaminergic neurons, reduce testosterone levels, and impair spermatogenesis (Gómez-Benito *et al.*, 2020; Xu *et al.*, 2022).

MPTP is metabolised into MPP<sup>+</sup>, which inhibits mitochondrial complex I and generates excessive reactive oxygen species (ROS), triggering oxidative stress, lipid peroxidation, and subsequent DNA damage in sperm (Risiglione *et al.*, 2020; Chen *et al.*, 2020). Include suppression of gonadotropin-releasing hormone (GnRH), luteinising hormone (LH) and follicle-stimulating hormone (FSH), Leydig cell dysfunction, and disruption of steroidogenic acute regulatory protein (StAR)-mediated steroidogenesis (Clara, 2025).

Oxidative stress is a central mechanism in testicular toxicity, with reductions in enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) being consistently observed. Several studies have shown that phytochemicals can restore this redox balance. For example, *Ceratonia siliqua L.* extract ameliorated lead-induced reproductive damage by improving sperm parameters and upregulating Nrf2 and antioxidant enzymes (Soleimanzadeh *et al.*, 2020). Similarly, *Quercus brantii* extract reversed oxidative stress and improved sperm concentration and motility in lead-exposed mice (Soleimanzadeh *et al.*, 2018).

Other plant-based antioxidants like *Allium sativum* have demonstrated comparable protective effects against busulfan-

induced testicular damage, acting through enhancement of antioxidant defense (Soleimanzadeh *et al.*, 2018). In another study, allicin and hesperidin prevented reproductive toxicity by stabilising sperm parameters, showing the potential of structurally related polyphenols in fertility protection.

Among natural antioxidants, epigallocatechin gallate (EGCG) a major catechin from *Camellia sinensis* (green tea) has been widely studied for its ability to activate Nrf2/ARE signaling, restore glutathione levels, reduce lipid peroxidation, and enhance antioxidant enzyme activities (Thangapandiyar & Miltonprabu, 2015). EGCG is also well recognised in cancer and toxicology research as a multi-target protective agent, particularly through modulation of oxidative and inflammatory pathways (Asma *et al.*, 2022). Although a study by Xu *et al.* (2022) demonstrated that 50 mg/kg/day EGCG can reverse MPTP-induced reproductive toxicity via Nrf2 activation, further investigation is needed to evaluate the efficacy of lower doses such as 30 mg/kg/day especially when administered over a short period.

Recent meta-analyses and *in vivo* studies also support the efficacy of EGCG in restoring sperm parameters and antioxidant enzyme activities in oxidative stress models and idiopathic male infertility cases (Hung *et al.*, 2022; Li *et al.*, 2022; Nguyen *et al.*, 2023; Chen *et al.*, 2020). Therefore, this study aimed to investigate whether oral administration of EGCG at 30 mg/kg BW/day can improve sperm quality (motility, viability, and concentration) in male rats exposed to MPTP. We hypothesised that EGCG would attenuate MPTP-induced sperm damage by partially restoring redox balance and preserving testicular function.

## MATERIALS AND METHODS

### *Ethical approval*

All animal experiments were approved by the Animal Care and Use Committee (ACUC) at the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia with ethical clearance number 2.KEH.061.04.2024, issued on April 23, 2024.

### *Animals and experimental design*

Thirty-two male Wistar rats (180–200 g) were acclimatised for 7 days under standard laboratory conditions ( $22 \pm 2^\circ\text{C}$ , 12-hour light/dark cycle) with ad libitum access to standard feed and water. Rats were randomly divided into four groups (n = 8 per group):

- K1: Control (no MPTP, no EGCG);
- K2: MPTP only (16 mg/kg BW, intraperitoneally);
- K3: EGCG only (30 mg/kg BW/day, orally for 7 days);
- K4: MPTP + EGCG.

MPTP administration followed the acute toxicity protocol by Chen *et al.* (2020), using four intraperitoneal injections of 4 mg/kg BW at 2-hour intervals (cumulative dose = 16 mg/kg BW in 1 day). This protocol mimics acute exposure and induces dopaminergic and reproductive toxicity.

EGCG ( $\geq 95\%$  purity, Sigma-Aldrich, USA; cat. no. E4143) was dissolved in distilled water and administered orally via gavage once daily for 7 consecutive days at a dose of 30 mg/kg BW/day. For group K4, EGCG administration started one day after MPTP injection and continued for 7 days. This post-exposure design evaluates EGCG's therapeutic (not preventive) effects. All animal handling and treatment procedures complied with international

guidelines for the ethical use of animals in laboratory research.

### *Sperm collection and evaluation*

On Day 8, rats were anaesthetised using a combination of ketamine (80 mg/kg BW) and xylazine (10 mg/kg BW), and then euthanised by cervical dislocation. The *cauda epididymis* was dissected and placed in 1 mL of 0.9% NaCl at  $37^\circ\text{C}$ , then minced to release spermatozoa into the suspension.

*Sperm motility.* A drop of epididymal suspension was placed on a pre-warmed microscope slide, covered with a coverslip, and observed under a light microscope at  $400\times$  magnification ( $37^\circ\text{C}$  stage). Five random fields per sample were examined. Motile spermatozoa were counted and expressed as a percentage of total sperm observed (Susilowati *et al.*, 2022; Wijayanti *et al.*, 2023).

*Sperm viability.* Viability was determined using eosin-nigrosin staining. A drop of semen was mixed with the stain, smeared on a glass slide, air-dried, and observed under  $400\times$  magnification. Live sperm remained unstained, while dead sperm stained pink. At least 200 sperm cells were counted per sample.

*Sperm concentration.* For concentration analysis, the sperm suspension was diluted with eosin solution to the 101 mark of a Thoma pipette. After mixing, one drop was placed into a Neubauer hemocytometer. Five diagonal large squares were counted under  $400\times$  magnification, and the final sperm concentration ( $\times 10^6 / \text{mm}^3$ ) was calculated as:

$$\text{Sperm concentration} \left( \times \frac{10^6}{\text{mm}^3} \right) = \frac{(A \times \text{dilution factor} \times 10)}{\text{number of counted squares}}$$

All sperm evaluations were performed by a blinded observer to avoid assessment bias.

*Statistical analysis*

Data were analysed using SPSS version 28 (IBM Corp., Armonk, NY, USA). Normality was assessed via the Shapiro-Wilk test, and homogeneity of variances with Levene’s test. Group comparisons were conducted using one-way ANOVA, followed by Duncan’s Multiple Range Test (DMRT) as post hoc analysis. Statistical significance was set at  $P < 0.05$ . Sample size ( $n=8$ ) per group was selected based on previous studies with similar sperm parameters, and provides  $>80\%$  power to detect a 20% difference between groups at  $\alpha = 0.05$ , as used in *Quercus brantii* antioxidant sperm studies (Soleimanzadeh *et al.*, 2018).

RESULTS

Group-wise comparisons of sperm motility, viability, and concentration are presented in Table 1 and Fig. 1.

The administration of MPTP resulted in a significant decline in all measured sperm parameters compared to the negative control group. Rats in the MPTP-only group (K2) exhibited markedly lower sperm motility, viability, and concentration ( $P < 0.01$  for all), indicating successful

induction of reproductive toxicity.

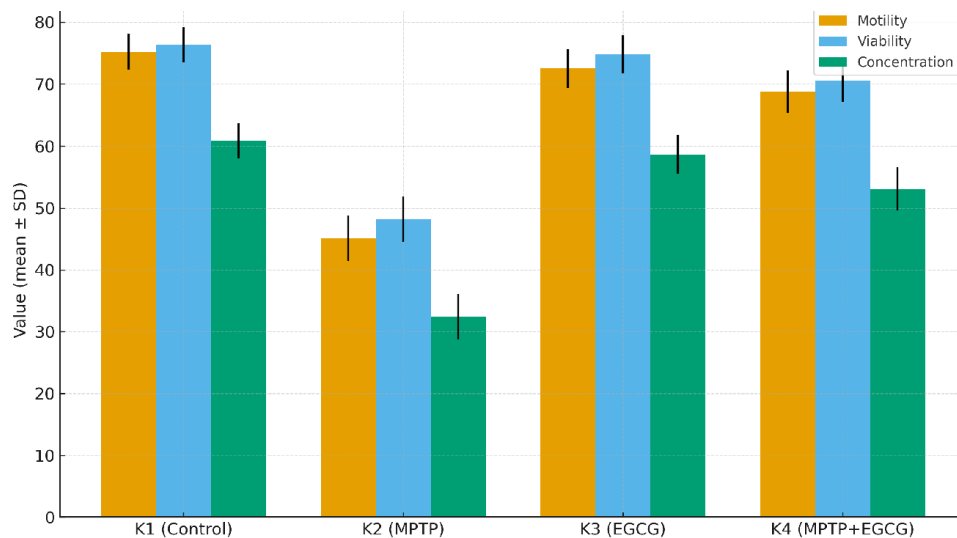
Significant differences in sperm motility were observed among experimental groups. The negative control group (K1) exhibited the highest motility ( $75.20 \pm 2.85\%$ ), while MPTP exposure (K2) caused a marked decrease ( $45.10 \pm 3.65\%$ ;  $P=0.004$ ). EGCG treatment significantly improved motility in both the EGCG-only group (K3) and the combined MPTP+EGCG group (K4) to  $72.50 \pm 3.10\%$  and  $68.75 \pm 3.45\%$ , respectively. These improvements were statistically significant compared to K2 ( $P=0.006$  for K3 and  $P=0.010$  for K4).

Sperm viability followed a similar trend. K1 showed  $76.40 \pm 2.15\%$  viable sperm, while viability dropped significantly in K2 ( $48.25 \pm 2.30\%$ ;  $P=0.003$ ). EGCG administration increased viability to  $74.80 \pm 2.05\%$  in K3 ( $P=0.005$ ) and  $70.53 \pm 1.69\%$  in K4 ( $P=0.008$ ), confirming the protective effect.

Sperm concentration was also significantly reduced by MPTP. K2 exhibited the lowest concentration ( $32.40 \pm 2.85 \times 10^6/\text{mm}^3$ ), significantly lower than the control group K1 ( $60.85 \pm 3.12 \times 10^6/\text{mm}^3$ ;  $P=0.002$ ). In contrast, K3 and K4 demonstrated concentrations of  $58.70 \pm 3.20 \times 10^6/\text{mm}^3$  and  $53.12 \pm 2.95 \times 10^6/\text{mm}^3$ ,

**Table 1.** Sperm motility, viability, and concentration in experimental groups of rats (mean  $\pm$  SD;  $n=8$ )

Group	Treatment	Motility (%)	Viability (%)	Concentration ( $\times 10^6/\text{mm}^3$ )
K1	Negative control (no MPTP, no EGCG)	$75.20 \pm 2.85$	$76.40 \pm 2.15$	$60.85 \pm 3.12$
K2	MPTP only (16 mg/kg BW)	$45.10 \pm 3.65$	$48.25 \pm 2.30$	$32.40 \pm 2.85$
K3	EGCG only (30 mg/kg BW/day)	$72.50 \pm 3.10$	$74.80 \pm 2.05$	$58.70 \pm 3.20$
K4	MPTP+EGCG (30 mg/kg BW/day)	$68.75 \pm 3.45$	$70.53 \pm 1.69$	$53.12 \pm 2.95$



**Fig. 1.** Sperm motility, viability, and concentration across treatment groups (mean ± SD; n=8).

respectively, both showing significant recovery compared to K2 (P=0.004 and P=0.007).

## DISCUSSION

The present study demonstrates that MPTP exposure at a cumulative dose of 16 mg/kg BW significantly impairs sperm quality, including motility, viability, and concentration in male rats. These findings are in line with the established toxicological profile of MPTP, which, through its active metabolite MPP<sup>+</sup>, inhibits mitochondrial complex I, generates reactive oxygen species (ROS), and induces dopaminergic neurotoxicity with systemic effects on the hypothalamic-pituitary-gonadal (HPG) axis (Chen *et al.*, 2020; Risiglione *et al.*, 2020).

In this model, the reproductive toxicity of MPTP likely results from oxidative stress, endocrine disruption, and direct mitochondrial injury to germinal cells. These mechanisms collectively impair

spermatogenesis and reduce semen quality, as confirmed by the sharply diminished sperm parameters in the MPTP-only group (K2). Although the present study did not measure oxidative stress biomarkers directly, the significant improvements in sperm parameters following EGCG administration suggest potential indirect antioxidant activity (Rahmani *et al.*, 2018).

EGCG, a major polyphenol in *Camellia sinensis* (green tea), is known to modulate the Nrf2/ARE signaling pathway, enhance mitochondrial function, and reduce lipid peroxidation (Thangapandiyan & Miltonprabu, 2015). Its protective role in male fertility has been reported previously in MPTP models (Xu *et al.*, 2022), and our findings further support its efficacy, even at a lower dose of 30 mg/kg BW/day. Interestingly, group K3 (EGCG-only) showed better sperm quality than the combined MPTP+EGCG group (K4), implying that preventive administration may be more effective than therapeutic

use. This is in line with the notion that phytochemicals offer superior benefits when used to maintain cellular redox balance prior to toxic insult (Nourian *et al.*, 2017). Our results agree with recent studies showing EGCG's antioxidant and cytoprotective effects on testicular tissue via upregulation of SOD, CAT, and GPx, as well as enhancement of mitochondrial function (Hung *et al.*, 2022; Pérez-Aguirre *et al.*, 2025). In idiopathic male infertility models, EGCG has also been shown to improve sperm motility and DNA integrity (Li *et al.*, 2022; Nguyen *et al.*, 2023), suggesting broader therapeutic potential.

Comparable results have been reported for other natural antioxidants. For instance, *Allium sativum* extract was shown to reverse oxidative damage in sperm and restore testicular function in mice exposed to busulfan (Soleimanzadeh *et al.*, 2018). Similarly, *Ceratonia siliqua L.* extract alleviated lead-induced reproductive toxicity by improving sperm parameters and upregulating Nrf2 and iNOS expression (Soleimanzadeh *et al.*, 2020). In another study, *Quercus brantii* extract restored sperm motility and viability using a nearly identical 7-day dosing protocol in lead-exposed mice (Soleimanzadeh *et al.*, 2018). Additionally, combinations such as allicin and hesperidin have demonstrated potent protective effects on spermatogenesis, further highlighting the importance of plant-derived polyphenols in reproductive toxicology (Asma *et al.*, 2022).

This study has several limitations. First, although EGCG improved sperm parameters, oxidative stress biomarkers (e.g., MDA, SOD, CAT, GSH) were not measured, making the mechanistic interpretation indirect and speculative. Second, no hormonal profile (e.g., testosterone, LH, FSH) was evaluated to confirm the

involvement of endocrine recovery. Third, testicular histology and molecular markers such as Nrf2, BAX, CASP3, or StAR were not assessed. Fourth, the study employed only a single EGCG dose and duration. A dose-response design would be necessary to identify optimal efficacy. Lastly, sample size justification was based on precedent, but no formal power analysis was included (Pérez-Aguirre *et al.*, 2025).

## CONCLUSIONS

This study demonstrates that epigallocatechin gallate (EGCG) at 30 mg/kg BW/day effectively mitigates the detrimental effects of MPTP exposure on sperm quality in male rats. The significant improvement in sperm motility, viability, and concentration observed in the EGCG-treated groups supports its potential role as a natural cytoprotective agent in chemically induced reproductive toxicity. While the findings are promising, the lack of oxidative stress, hormonal, and molecular data limits direct mechanistic interpretation. Therefore, further investigations incorporating biochemical and histological analyses are warranted to confirm the antioxidant and endocrine-modulating actions of EGCG in the context of male fertility preservation.

## ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Faculty of Veterinary Medicine, Universitas Airlangga and Universitas Brawijaya for institutional support. Special thanks to all laboratory staff and academic colleagues who provided technical assistance and guidance throughout the research and manuscript preparation.

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Paper received 30.09.2025; accepted for publication 21.11.2025

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