

Effect of the Ethanolic Extract of the Rhizome of *Gastrodia elata* on Spermatogenesis in Male Sprague-Dawley Rats

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ABSTRACT

Background: *Gastrodia elata* is a traditional Chinese herb used to treat neurological disorders. Gastrodin, its main bioactive component, has been extensively studied for pharmacological effects. This study aimed to evaluate the effect of the Ethanolic Extract of the rhizome of *G. elata* (EEGE) on spermatogenesis in male Sprague Dawley (SD) rats. **Materials and Methods:** The rhizome powder was extracted with ethanol. Healthy adult male SD rats were divided into six groups ($n=6$ per group) and treated daily for 28 days, viz., Control, Vitamin C (200 mg/kg), EEGE (100, 200, 400, and 800 mg/kg). Post-treatment, bilateral orchiectomy was performed, and sperm count and motility were assessed. Blood samples were collected at the end of the study for biochemical and haematological analyses. After sacrifice, the liver, kidneys, and testes were excised and weighed for organ weight analysis. **Results:** EEGE treatment significantly affected some liver function and haematological parameters compared to the control. No significant changes were observed in body weight, kidney function, lipid profile, electrolytes, sperm motility, or sperm count across EEGE-treated and Vitamin C groups. **Conclusion:** EEGE exhibited no adverse effects on body weight, renal function, or spermatogenesis in male SD rats; higher doses affected liver enzyme levels and some haematological parameters.

Keywords: Liver enzymes, Reproductive toxicity, Sperm count, Spermatogenesis.

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Received: 18-03-2026;

Revised: 04-05-2026;

Accepted: 22-06-2026.

INTRODUCTION

Global longitudinal data indicate a significant decline in male fertility, with research confirming a 50% to 60% reduction in sperm concentration in Western populations between 1973 and 2011 (Levine *et al.*, 2017). Currently, infertility affects approximately 15% of couples globally, with the male factor being solely responsible for 20% of cases and contributing to 30% to 40% of all infertility instances (Hull *et al.*, 1985; Thonneau *et al.*, 1991).

In male Sprague-Dawley rats, the reproductive system functions to produce sperm and essential hormones. The testes contain seminiferous tubules where spermatogenesis occurs. This complex process involves the transformation of primordial germ cells into haploid spermatozoa through three distinct stages: mitotic cell division for spermatogonia replication, meiosis to generate haploid cells, and spermiogenesis for the maturation of motile sperm cells (Dym, 1994). Rats are prolific breeders,

typically achieving sexual maturity between 6 and 12 weeks of age, and they possess an open inguinal canal, allowing for the migration of testes between the scrotum and the abdominal cavity (Zemunik *et al.*, 2003; Das *et al.*, 2023).

Gastrodia elata, known as Tianma, is a saprophytic perennial herb belonging to the family Orchidaceae, primarily distributed in Eastern Asia. The dried rhizome, *Rhizoma Gastrodiae*, has been utilized for over 2,000 years and was described in *Shennong's Classic of Materia Medica* as a "top-grade medicine" that can rejuvenate the body without toxicity (Liu *et al.*, 2018). Modern pharmacological studies have shown that *G. elata* and its primary active component, gastrodin, possess neuroprotective, antioxidative, anti-inflammatory, and circulatory system modulating effects (Wang *et al.*, 2025).

Gastrodin is rapidly absorbed and distributed to various organs, including the kidney, liver, and brain. Considering the global decline in sperm counts and the established pharmacological benefits of *G. elata* in reducing oxidative stress, its potential role in supporting reproductive health warrants investigation. Therefore, this study was designed to test the hypothesis that *G. elata* extract increases spermatogenesis in male Sprague-Dawley rats. The primary objective was to investigate the effect of the ethanolic extract of the rhizome of *G. elata* on spermatogenesis in male SD rats.



DOI: 10.5530/fra.20260002

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MATERIALS AND METHODS

Plant Material and Chemicals

The primary plant material consisted of dried rhizome decoction pieces of *G. elata* obtained from a Traditional Chinese Medicine (TCM) Pharmacy. Chemicals used in the study included 95% ethanol (HMBG, Malaysia), Vitamin C, 0.5% w/v Carboxymethyl Cellulose (CMC), and Biggers, Whitten, and Whittingham (BWW) medium.

Animals

Healthy adult male Sprague Dawley (SD) rats (180 ± 20 g) were obtained from the Central Animal House, AIMST University, Malaysia. The animals were housed in spacious polyacrylic cages at ambient room temperature with a 12 hr light/dark cycle and provided with a normal rat pellet diet and water *ad libitum*. The study protocol was approved by the AIMST University Human and Animal Ethics Committee (AUAEC/FOP/2024/05) and conducted in accordance with Animal Research Panel guidelines.

Extraction of *G. elata* Rhizome

The dried rhizome was ground into powder using an electric grinder. The powder was weighed and packed into a porous cellulose thimble within a Soxhlet extraction chamber. Extraction was performed using 95% ethanol at 80°C. The process continued for 3-4 cycles until the solvent became transparent. The resulting extract was evaporated using a rotary vacuum evaporator at 45°C to 50°C to form a dry mass concentrated to one-third or less of its original volume. This Ethanolic Extract of *G. elata* (EEGE) was stored in amber glass containers and refrigerated.

Experimental Design

The SD rats were divided into six groups ($n=6$ per group) and administered the following treatments orally once daily for 28 days:

- Group I: Control.
- Group II: Vitamin C (200 mg/kg).
- Group III: EEGE (100 mg/kg).
- Group IV: EEGE (200 mg/kg).
- Group V: EEGE (400 mg/kg).
- Group VI: EEGE (800 mg/kg).

Both Vitamin C and EEGE were suspended in 0.5% w/v CMC for administration. Body Weight (BW) and behaviour were monitored regularly.

Sperm Analysis

At the conclusion of the 28-day treatment period, the rats were subjected to bilateral orchiectomy. Sperm samples were collected

from the cauda epididymis and placed in BWW medium. Sperm motility was determined using the swim-up technique. An appropriate volume of spermatozoa was transferred to a preheated cell count plate, and the number of active cells was observed under a microscope to calculate motility from a total of 200 spermatozoa (He *et al.*, 2018).

Following motility assessment, the remaining suspension was heated in a 60°C water bath for 5-10 min to induce loss of motility. The spermatozoa were then added to a cell count plate. The sperm count was determined using the red blood cell counting method with a haemocytometer (He *et al.*, 2018).

Biochemical and Haematological Parameter Analysis

The blood sample was collected through retro-orbital plexus puncture and used for the analysis of biochemical and haematological parameters (performed by Gribbles Pathology Lab Sdn. Bhd., Malaysia).

Gross Pathology and Organ Weights

Animals were sacrificed under ether anaesthesia. Gross pathology was observed, and organs, including the brain, lungs, heart, liver, kidney, spleen, and testes, were harvested. Both absolute and relative organ weights were recorded.

Statistical Analysis

Data are presented as Mean $\pm$ Standard Error of the Mean (SEM). Statistical significance was determined using one-way ANOVA followed by Tukey's *post hoc* test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The rats administered Vitamin C or EEGE did not show any significant changes in body weight throughout the study when compared with that of the control (Figure 1).

The effect of EEGE on biochemical parameters in all groups was summarized in Table 1. The results showed that there were no significant changes in glucose, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, GGT, urea, creatinine and uric acid levels among all treated groups when compared with the control group. The group treated with 100 mg/kg EEGE showed a significant increase in AST and ALT levels when compared with the control group. The group treated with 200 mg/kg EEGE showed a significant increase in alkaline phosphatase, AST and ALT levels when compared with the control group. Similarly, animals administered EEGE at 400 and 800 mg/kg showed significant increases in ALT levels compared with the control group. However, no significant differences were observed in serum electrolyte levels when compared with those of the control group (data not presented).

The effect of EEGE on haematological parameters in all groups was summarized in Table 2. The group treated with 400 mg/kg EEGE showed a significant increase in haemoglobin and basophil levels when compared with the control group. The group treated with 800 mg/kg EEGE showed a significant increase in haemoglobin, MCV, MCH and basophil counts when compared with the control group. There were no significant changes in the haematological parameters of the group administered with Vitamin C, EEGE 100 and 200 mg/kg when compared with those of the control. However, no significant differences were observed in lipid profile when compared with those of the control group (data not presented).

In sperm analysis, the group treated with Vitamin C, 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg did not show any significant changes in the sperm motility and count when compared with that of the control (Figures 2 and 3). In organ weight analysis, Vitamin C, 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg did

not affect absolute and relative organ weights of the testes, liver, and kidneys when compared with control (data not presented).

DISCUSSION

In the present study, the administration of EEGE did not significantly affect the body weight of male SD rats over the study period. All groups, including the control and those treated with Vitamin C and various doses of EEGE, exhibited a progressive rise in body weight from Day 1 to Day 28. This finding implies that, at all doses tested, EEGE did not appear to have any appreciable negative impacts on total body growth or general health. According to research, oral treatment with *G. elata* extract significantly reduced the total body weight of male mice on a high-starch diet. In the group treated with *G. elata* extract, there was a significant decrease in liver weight, regional fatty tissues, blood and liver triglyceride levels (Lee *et al.*, 2023). Analysis of organ weight was used in toxicology studies for the identification of potentially harmful effects of chemicals. Absolute and relative

Table 1: Effect of ethanolic extract of rhizome of *Gastrodia elata* on biochemical parameters (liver functions) of rats.

	Glucose (mmol/l)	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Albumin/ Globulin ratio	Alkaline Phosphatase (U/L)	Total Bilirubin (μmol/L)	GGT (U/L)	AST (U/L)	ALT (U/L)	Urea (mmol/L)	Creatinine (μmol/L)	Uric Acid (mmol/L)
Control	4.62 ± 0.16	68.67 ± 0.88	44.50 ± 1.23	22.83 ± 1.54	1.99 ± 0.15	200.50 ± 11.05	2.00 ± 0.0	3.00 ± 0.0	146.67 ± 11.79	65.33 ± 1.71	5.87 ± 0.19	38.67 ± 2.38	0.16 ± 0.02
Vitamin C (200 mg/kg)	4.73 ± 0.18	68.50 ± 1.06	43.17 ± 1.45	24.50 ± 1.23	1.78 ± 0.11	216.33 ± 21.01	2.00 ± 0.0	3.00 ± 0.0	160.17 ± 11.60	93.17 ± 7.56	6.05 ± 0.29	37.17 ± 1.85	0.13 ± 0.01
EEGE (100 mg/kg)	4.68 ± 0.14	68.33 ± 0.56	44.83 ± 1.35	24.50 ± 1.48	1.87 ± 0.17	368.00 ± 48.65	2.00 ± 0.0	2.50 ± 0.22	237.33 ± 27.20**	112.67 ± 6.53**	5.80 ± 0.27	38.67 ± 2.73	0.13 ± 0.01
EEGE (200 mg/kg)	5.15 ± 0.13	68.83 ± 0.48	43.17 ± 2.10	25.83 ± 1.74	1.73 ± 0.21	393.00 ± 54.64*	2.00 ± 0.0	3.33 ± 0.33	221.83 ± 12.30*	106.33 ± 5.81**	5.98 ± 0.36	39.33 ± 4.28	0.13 ± 0.01
EEGE (400 mg/kg)	4.95 ± 0.13	68.67 ± 1.23	45.17 ± 1.11	24.17 ± 1.40	1.90 ± 0.14	377.67 ± 47.93	2.00 ± 0.0	3.33 ± 0.33	218.83 ± 12.76	99.17 ± 7.79*	6.02 ± 0.40	47.17 ± 3.45	0.12 ± 0.00
EEGE (800 mg/kg)	5.07 ± 0.24	68.33 ± 1.86	43.17 ± 1.14	23.67 ± 1.98	1.91 ± 0.21	366.17 ± 52.22	2.00 ± 0.0	3.17 ± 0.17	210.50 ± 20.32	94.33 ± 7.40*	6.17 ± 0.51	45.67 ± 1.71	0.12 ± 0.01

All the values are Mean ± SEM (n=6). * $p < 0.05$, ** $p < 0.01$ compared with that of control (One-way ANOVA followed by Tukey *post hoc* test).

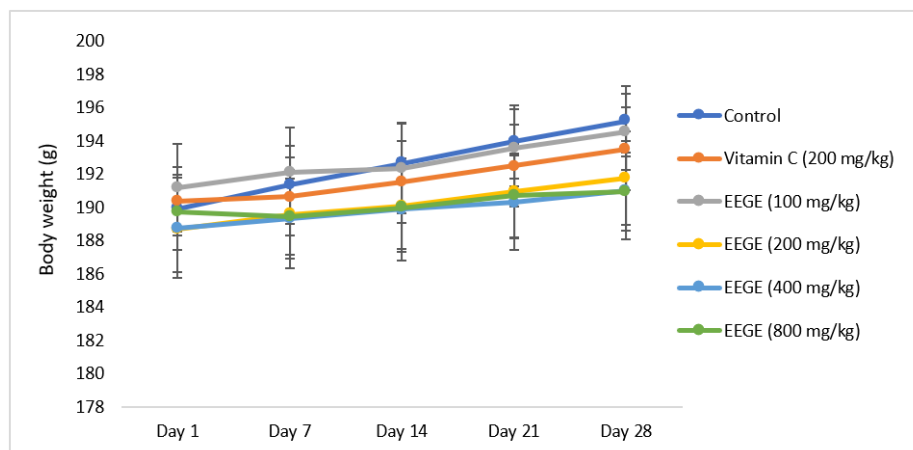


Figure 1: Effect of ethanolic extract of the rhizome of *Gastrodia elata* on the body weight of male rats. All the values are Mean ± SEM (n=6).

Table 2: Effect of ethanolic extract of rhizome of *Gastrodia elata* on haematological parameters of rats.

	Control	Vitamin C (200 mg/kg)	EEGE (100 mg/kg)	EEGE (200 mg/kg)	EEGE (400 mg/kg)	EEGE (800 mg/kg)
Haemoglobin (g/L)	123.17 ± 5.49	139.17 ± 4.74	136.17 ± 6.07	140.50 ± 3.82	151.67 ± 3.61**	155.17 ± 2.60***
RBC (x 10 ¹² /L)	8.27 ± 0.32	8.16 ± 0.39	8.05 ± 0.52	8.43 ± 0.19	8.73 ± 0.27	8.62 ± 0.11
PCV (L/L)	0.48 ± 0.03	0.48 ± 0.02	0.47 ± 0.02	0.49 ± 0.01	0.48 ± 0.02	0.50 ± 0.02
MCV (fL)	49.67 ± 2.44	50.67 ± 2.95	54.33 ± 4.23	57.17 ± 1.89	59.17 ± 1.08	61.67 ± 1.02*
MCH (pg)	15.33 ± 0.49	15.17 ± 0.31	16.83 ± 0.60	16.67 ± 0.49	16.83 ± 0.54	18.17 ± 0.31**
MCHC (g/L)	292.17 ± 5.14	292.33 ± 4.51	293.83 ± 2.32	292.67 ± 2.51	293.33 ± 5.06	295.67 ± 2.51
RDW (%)	18.65 ± 0.69	18.08 ± 0.56	17.90 ± 0.42	18.05 ± 0.56	18.42 ± 0.35	18.57 ± 0.24
White Cell Count (x 10 ⁹ /L)	8.02 ± 0.63	8.05 ± 0.71	7.92 ± 1.05	9.48 ± 1.30	8.70 ± 0.72	8.80 ± 0.54
Neutrophils (%)	23.50 ± 1.36	24.50 ± 2.26	25.70 ± 1.94	27.50 ± 1.45	26.45 ± 1.58	25.92 ± 1.73
Lymphocytes (%)	67.33 ± 0.99	65.50 ± 2.32	65.17 ± 2.02	64.50 ± 1.61	62.50 ± 1.54	64.33 ± 1.43
Monocytes (%)	5.83 ± 0.48	6.00 ± 0.26	5.83 ± 0.75	5.33 ± 1.63	6.01 ± 1.05	4.69 ± 0.48
Eosinophils (%)	3.17 ± 0.48	3.83 ± 0.31	3.17 ± 0.31	2.67 ± 0.67	2.85 ± 0.48	2.83 ± 0.40
Basophils (%)	0.17 ± 0.17	0.17 ± 0.17	0.17 ± 0.17	0.00 ± 0.00	2.16 ± 0.60***	2.20 ± 0.32***
Platelets (x10 ⁹ /L)	852.50 ± 59.54	897.33 ± 65.56	875.67 ± 66.06	891.50 ± 64.30	807.50 ± 77.16	693.83 ± 45.92

All the values are Mean ± SEM (n=6). **p*<0.05, ***p*<0.01, ****p*<0.001 compared with that of control (One-way ANOVA followed by Tukey *post hoc* test).

organ weights are utilized to account for differences in body weights across treatment groups. In this experiment, we have examined the effect of EEGE on the organ weight of the testes, liver and kidneys. Overall, there were no significant changes in the absolute and relative weight for most of the visceral organs in our study.

Serum samples were used to determine the biochemical parameters. The glucose level was tested to check the impairment of glucose metabolism. In this experiment, the results revealed that there were no significant changes among all groups of male SD rats. A study showed that high-fat diet-fed mice treated with EEGE exhibited a decrease in fasting blood glucose concentration (Wang *et al.*, 2022). Regarding electrolytes, there were no significant changes in the sodium, chloride, calcium, or potassium levels of male SD rats in all the groups. Similarly, lipid profiles of male SD rats in each group did not differ significantly. Our results disagree with the results conducted by Kho *et al.*, which revealed that the use of EEGE can decrease elevated triglyceride and cholesterol levels, as well as reducing hepatic fat accumulation and visceral fat, while increasing HDL cholesterol in animal models of metabolic syndrome (Kho *et al.*, 2014).

ALP, AST and ALT were used to check liver function. In this experiment, AST and ALT levels increased significantly at 100 mg/kg, 200 mg/kg, 400 mg/kg and 800 mg/kg EEGE doses, while ALP levels were elevated significantly in the group treated with 200 mg/kg EEGE compared to the control (*p*<0.05). These findings point to a dose-dependent effect on liver enzymes, which at greater EEGE concentrations may be indicative of hepatic stress or injury. A study conducted by Chen *et al.* showed that EEGE has hepatoprotective effects, including reducing elevated levels of liver enzymes like ALT and ALP, improving liver function, regulating lipid metabolism and alleviating oxidative stress and inflammation (Chen *et al.*, 2022).

Urea and creatinine are extremely helpful in determining how well the kidneys are functioning. In our study, urea, creatinine and uric acid levels were not substantially changed by EEGE across all treatment groups. This shows that under the investigated settings, EEGE does not significantly impair kidney function. However, in a study conducted by Seok *et al.*, in rats exposed to acetaminophen-induced liver and kidney damage, EEGE has been demonstrated to have protective benefits by lowering levels of albumin, total protein, urea nitrogen, uric acid and cholesterol (Seok *et al.*, 2018). The haematological parameters showed that haemoglobin levels were increased significantly in groups treated

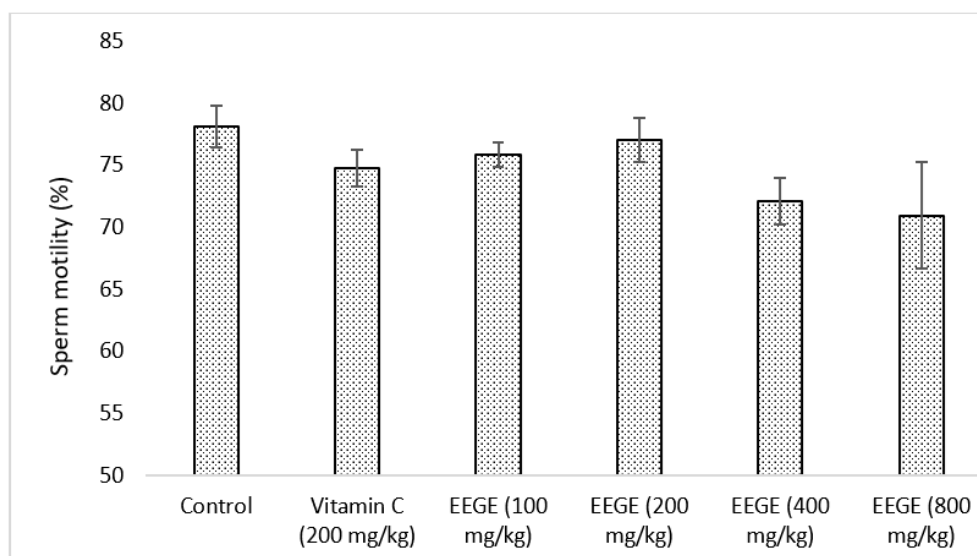


Figure 2: Effect of ethanolic extract of the rhizome of *Gastrodia elata* on sperm motility of rats. All the values are Mean $\pm$ SEM (n=6).

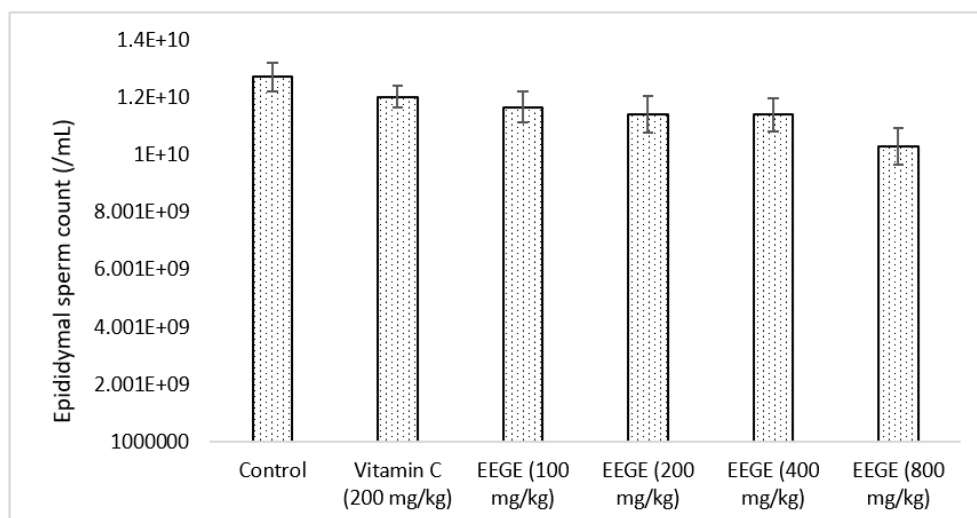


Figure 3: Effect of the ethanolic extract of the rhizome of *Gastrodia elata* on sperm count in rats. All the values are Mean $\pm$ SEM (n=6).

with 400 mg/kg and 800 mg/kg EEGE. MCV and MCH were elevated in the 800 mg/kg EEGE group compared to the control, indicating potential changes in the red blood cell morphology or production, which could be a compensatory response to some toxicity. Interestingly, basophil counts were markedly higher in the 400 and 800 mg/kg groups. The increase in basophils and reduction in platelet counts at higher EEGE doses may reflect immune modulation or haematopoietic effects of the extract.

In sperm analysis, sperm motility and sperm count of male SD rats in all the groups were not affected by EEGE at any of the doses tested. This suggests the extract does not have a direct toxic effect on sperm function. However, the anti-inflammatory and antioxidant activities of the n-butanol fraction of *G. elata*, which could potentially affect sperm motility by reducing oxidative stress and inflammation in the reproductive system (He *et al.*, 2020). While the extract does not show direct toxic effects on

sperm, the elevated liver enzymes raise concerns about potential indirect effects on fertility via liver toxicity.

CONCLUSION

The results of this study demonstrate that the Ethanolic Extract of the rhizome of *G. elata* (EEGE) possesses a complex biological activity profile. Administration of the extract resulted in no detrimental effects on body weight, renal function, lipid profile, electrolytes, or the spermatogenesis of male Sprague-Dawley rats across the tested doses. However, significant effects were observed on liver enzymes (AST, ALT, and ALP) and certain haematological measures (Haemoglobin, MCV, MCH, and Basophils), particularly at higher dosages. These findings underscore the importance of careful dosage optimization and thorough monitoring of liver and blood parameters in any future EEGE applications. To further clarify these results, a

histopathological examination of the liver would be necessary to establish the existence and severity of any potential hepatotoxicity. Additionally, mechanistic investigations are required to further understand the causes of the reported effects on liver and blood parameters.

ABBREVIATIONS

ALT: Alanine Aminotransferase; **AST:** Aspartate Aminotransferase; **BWW medium:** Biggers-Whitten-Whittingham Medium; **CMC:** Carboxymethyl Cellulose; **EEGE:** Ethanolic Extract of the Rhizome of *Gastrodia elata*; **GGT:** Gamma-Glutamyl Transferase; **HDL:** High-Density Lipoprotein; **MCH:** Mean Corpuscular Hemoglobin; **MCV:** Mean Corpuscular Volume; **SD rats:** Sprague-Dawley Rats; **SEM:** Standard Error of the Mean; **TCM:** Traditional Chinese Medicine.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Wei CZ, Jun CJ, Parasuraman S. Effect of the Ethanolic Extract of the Rhizome of *Gastrodia elata* on Spermatogenesis in Male Sprague-Dawley Rats. *Free Radicals and Antioxidants*. 2026;16(1):12-7.