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Sprague-Dawley 랫트를 이용한 잠사(Bombycis Faeces) 열수추출물의 단회경구투여 독성시험

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Single Oral Dose Toxicity Evaluation of the Aqueous Extract of Bombycis Faeces in Sprague-Dawley Rats

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ABSTRACT

Background: Bombycis feces (dried feces of silkworms; *Bombyx mori* L.) are traditionally used in Korean and East Asian medicine to treat conditions including sensory disturbances, itching, and headaches. Recent studies have demonstrated pharmacological activities including anti-inflammatory, antioxidant, and neuroprotective effects. However, toxicological evidence supporting its safety is currently sparse. We aimed to evaluate the acute oral toxicity of an aqueous extract of Bombycis feces in Sprague-Dawley rats, following OECD guidelines.
Methods and Results: Forty rats (20 males and 20 females) were randomly assigned to receive a single oral dose of the extract at 0 (control), 500, 1000, or 2000 mg/kg. The animals were observed for 14 days to assess clinical signs, mortality, body weight changes, and hematological parameters. No mortality or clinical signs of toxicity were observed in any group. Normal body weight gain occurred in both sexes, and macroscopic examination revealed no treatment-related organ abnormalities. Hematological parameters remained within physiological ranges in males. Although not clinically relevant, monocyte counts increased significantly in female rats in all treated groups ($p < 0.01$).
Conclusions: Aqueous extract of Bombycis Faeces was well tolerated at doses up to 2000 mg/kg, indicating negligible acute toxicity. Collectively, these findings provide foundational safety data to support further development of Bombycis Faeces-based pharmaceutical and nutraceutical products.

Key Words: *Bombyx mori*, Bombycis Faeces, LD_{50}, OECD Guideline, Single Oral Dose Toxicity

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INTRODUCTION

Bombyx mori L., commonly known as the silkworm, is a lepidopteran insect of immense economic importance, traditionally valued for its central role in the global silk industry. Notably, silkworms have emerged as versatile resources with diverse applications in the food and pharmaceutical sectors (Kim and Koh, 2022). Various parts of the silkworm, including its cocoon, pupae, feces (Bombycis Faeces), and stiffened larvae (*Bombyx Batryticatus*), have been used in Korean medicine and other Asian medical systems to treat stroke, convulsions, skin diseases, and sensory disturbances (Kwon *et al*., 2021; Kim and Koh, 2022; Rodriguez-Ortiz *et al*., 2024). These diverse traditional applications highlight the silkworm's significance as a valuable medicinal crop.

Importantly, the medicinal value of silkworms is strongly linked to their exclusive mulberry leaf diet. During digestion, silkworms efficiently absorb and biotransform secondary metabolites from mulberry leaves, such as flavonoids, amino acids, and fatty acids, into bioactive compounds (Li *et al*., 2021). These beneficial plant-derived compounds accumulate or are modified within the silkworm body. Given this unique pharmacological potential and historical medicinal use, silkworms are increasingly regarded as promising bioresources for the development of functional foods, herbal medicines, and biopharmaceutical products (Rodriguez-Ortiz *et al*., 2024; Aramani *et al*., 2025).

Bombycis Faeces, the faeces of silkworms, are a medicinal resource that has been used for centuries. Bombycis Faeces is used in Korean medicine to treat various diseases, including sensory disturbances and itching (Kwon *et al*., 2021). Recent studies have revealed the components and pharmacological efficacy of extracts of Bombycis Faeces. Bombycis Faeces demonstrated anti-migraine properties via phytol-mediated inhibition of Nav1.7 sodium channels in trigeminal ganglion neurons (Song *et al*., 2023). Additionally, Bombycis Faeces effectively ameliorated renal anemia in rats by regulating iron metabolism via hepcidin inhibition and promoting erythropoietin synthesis (Mei *et al*., 2021). Moreover, its anti-obesity (Lee *et al*., 2025) and antidiabetic (Matsuda *et al*., 2023) effects have been documented in the literature.

Overall, these diverse biological activities indicate that Bombycis Faeces has potential as a multi-target therapeutic agent for treating diseases. However, a comprehensive safety evaluation is necessary. Owing to the rapid increase in the use of natural products for health promotion, ensuring human safety has become increasingly important in recent years. Although traditional use provides valuable information, it differs from rigorous safety and efficacy validation according to modern scientific standards.

Therefore, in this study, we aimed to evaluate the single oral dose toxicity of aqueous extracts of Bombycis Faeces in rats according to the OECD guidelines. This study is expected to provide essential preclinical safety data to support the continued development of *B. mori*-based therapeutic agents, guide the establishment of safe dose ranges, and contribute to the scientific validation of this traditional medicine for modern pharmaceutical and nutraceutical applications.

MATERIALS AND METHODS

1. Test substances

Bombycis Faeces were purchased from Kwong-Mung-dang Company (Ulsan, Korea) and authenticated by Dr. Goya Choi of the Herbal Medicine Resources Research Center, Korea Institute of Oriental Medicine (Naju, Korea). A voucher specimen (medicinal ID: 2-18-0114) was deposited at the same institution. The Bombycis Faeces extract was prepared by refluxing in distilled water at 100 ± 2°C for 3 hours. The extract was subsequently filtered, concentrated using a rotary vacuum evaporator, and lyophilized (yield, 6.78%). Finally, the resulting powder was stored at 4°C until use (extract ID: 3-18-0044).

2. Animals

Male and female Sprague–Dawley (SD) rats (7-week-old) were purchased from Orient Bio (Seongnam, Korea) and acclimatized for 6 days. During the acclimatization and experimental periods, the animals had ad libitum access to tap water and standard rodent pellets (Teklad Certified Irradiated Global 18% Protein Rodent Diet; Envigo, USA). Rats were housed under controlled environmental conditions (temperature, 22 ± 2°C; relative humidity, 55 ± 10%; 12-h light/dark cycle). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Korea Conformity Laboratories (Incheon, Korea) in accordance with the Animal Protection Act of the Republic of Korea (approval no.: IA20-02462).

3. Experimental groups

Forty rats (20 males and 20 females) were randomly assigned

to four groups (n = 10 per group; 5 males and 5 females): 0 (vehicle control), 500, 1000, and 2000 mg/kg oral doses of Bombycis Faeces extract.

4. Treatment administration

After a 4-h fasting period, the animals were orally gavaged with bombycis fecal extract or vehicle at the designated doses (10 mL/kg body weight). Notably, the administration volume was calculated based on the individual body weights on the day of dosing. Oral administration was selected because it reflected the intended clinical route of administration.

5. Clinical observations and mortality

All animals were observed for clinical signs immediately after dosing, and at 30 min and 1, 2, 3, 4, 5, and 6 h after administration. Daily observations were conducted for 14 days to monitor clinical signs and mortality. Observations included posture, gait, coat condition, skin, eyes and pupils, mucous membranes, respiratory patterns, response to handling, and any convulsions, stereotypies, or abnormal behaviors.

6. Body weight measurement

Individual body weights were recorded immediately before administration and on days 1, 3, 7, and 14 after treatment.

7. Gross necropsy

On day 14, all surviving animals were anesthetized using isoflurane and euthanized by exsanguination of the abdominal aorta and posterior vena cava. A comprehensive macroscopic examination was performed on the external surface, cranial cavity, thoracic cavity, abdominal cavity, and their contents.

8. Hematological analysis

Blood samples were collected on day 14 under anesthesia for hematological analyses. Hematological parameters, including total white blood cell (WBC) count, differential WBC count, red blood cell (RBC) count, hemoglobin (Hb) level, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) count, were measured using an ADVIA 2120i hematology analyzer (Siemens Ireland, Dublin, Ireland).

9. Statistical analysis

Data on body weight and hematological parameters were analyzed using one-way analysis of variance (ANOVA) followed by post hoc comparisons, where appropriate. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$. All data are expressed as mean ± standard deviation (SD).

RESULTS

1. Mortality and clinical observations

No mortality was observed in any of the treatment groups during the 14-day observation period (Table 1). All animals (5/5 in each group of both sexes) survived until the scheduled termination date. Additionally, no abnormal clinical signs were detected in male or female rats at any dose (500, 1000, or 2000 mg/kg). All rats exhibited normal behavioral patterns, including normal posture, gait, coat condition, skin integrity,

Table 1. Mortalities and clinical signs of rats.

Summary of mortalities and clinical signs					Sex : Male
		Group(㎎/㎏)			
		G1(0)	G2(500)	G3(1,000)	G4(2,000)
Mortalities	No. of dead animals	0 / 5	0 / 5	0 / 5	0 / 5
	%	0	0	0	0
Clinical signs	No abnormalities detected	5 / 5	5 / 5	5 / 5	5 / 5
		Sex : Female			
		Group(㎎/㎏)			
		G1(0)	G2(500)	G3(1,000)	G4(2,000)
Mortalities	No. of dead animals	0 / 5	0 / 5	0 / 5	0 / 5
	%	0	0	0	0
Clinical signs	No abnormalities detected	5 / 5	5 / 5	5 / 5	5 / 5

eye appearance, mucous membranes, and respiratory patterns. Moreover, no instances of lacrimation, convulsions, piloerection, diarrhea, stereotypical behavior, or other abnormal movements were observed in any of the groups.

2. Body weight

Body weight changes are shown in Fig. 1 and Table 2. In male rats, initial body weights ranged from 234.17 ± 11.78 to 240.32 ± 6.88 g across all groups, with no significant baseline differences observed. At day 14, body weight range increased to 360.15 ± 13.50 to 374.82 ± 17.89 g, representing weight gains of 120.29 ± 16.63 to 138.86 ± 17.80 g. Notably, the percentage weight gains in the 500, 1000, and 2000 mg/kg groups were 96.09, 96.21, and 99.20%, respectively, compared with that in the control group, indicating no treatment-related effects on body weight gain.

In female rats, initial body weights ranged from 186.33 ± 5.95 to 188.29 ± 5.56 g. However, the final weights on day 14 ranged from 230.80 ± 15.10 to 244.57 ± 10.36 g, representing weight gains of 42.51 ± 12.20 to 58.23 ± 5.08 g. Importantly, the percentage weight gains in the 500, 1000, and 2000 mg/kg groups were 96.62, 102.38, and 101.22%, respectively, compared with that in the control group. Collectively, these results indicate that there were no significant differences in body weight or weight gain, regardless of treatment or sex.

3. Macroscopic examination

Table 3 summarizes the gross necropsy findings. Notably, no treatment-related macroscopic abnormalities were detected in any of the examined organs. All animals in the dose groups showed normal gross findings for all organs examined, including the external surface and cranial, thoracic, and abdominal cavities. Collectively, these results indicate the absence of overt organ toxicity following a single oral administration of the extracts.

4. Hematological analysis

Tables 4 and 5 present the hematological parameters of male and female rats, respectively. In male rats, no significant changes in any of the hematological parameters were observed across all treatment groups compared with the control group. All measured parameters, including total and differential WBC counts, RBC parameters, and platelet counts, remained within the normal range.

In female rats, statistically significant increases were observed in absolute monocyte counts at all dose levels (500, 1000, and 2000 mg/kg) compared with those in the control group ($p < 0.01$). Monocyte percentages were significantly elevated in all treatment groups ($p < 0.01$). Specifically, the absolute monocyte count increased from 0.13 ± 0.04 K/μL in the control group to 0.23 ± 0.04, 0.19 ± 0.05, and 0.20 ± 0.02 K/μL in the 500, 1000 and 2000 mg/kg groups, respectively. Additionally, the monocyte percentage increased from 1.3 ± 0.3% in the control group to 2.7 ± 0.8, 2.3 ± 0.2, and 2.2 ± 0.4% in the 500, 1000 and 2000 mg/kg groups, respectively.

All other hematological parameters in female rats, including total WBC count, neutrophil count, RBC parameters (hemoglobin, hematocrit, MCV, MCH, and MCHC), and platelet count, showed no significant differences compared with those in the

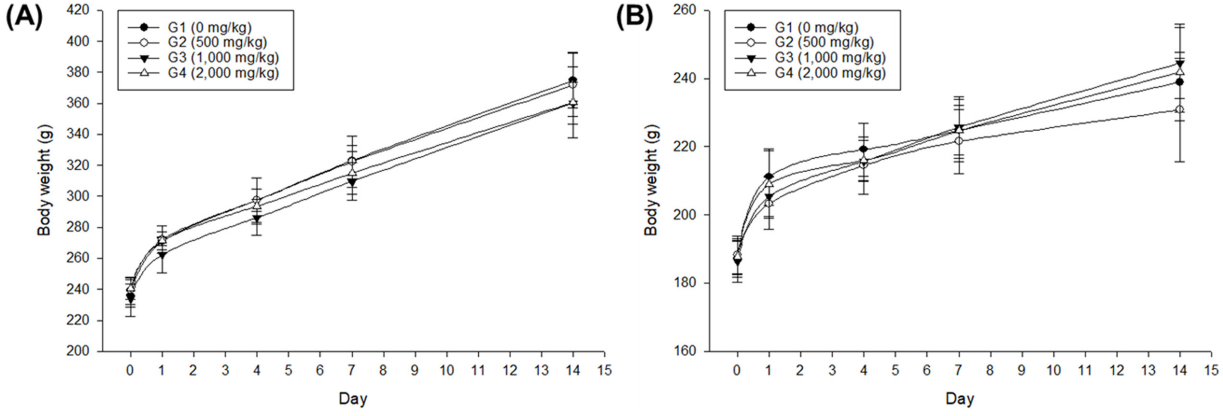

Fig. 1. Changes in body weight of male (A) and female (B) Sprague-Dawley rats after single oral administration of Bombycis Faeces aqueous extract. Animals were administered a single dose of the extract at 0 (control, G1), 500 (G2), 1000 (G3), or 2000 mg/kg (G4) and monitored for 14 days. Body weight was measured on days 0, 1, 3, 7, and 14. Data are expressed as mean ± standard deviation (SD), n=5 per sex per group. No statistically significant differences in body weight or weight gain were observed among treatment groups in either sex throughout the study period ($p > 0.05$).

Table 2. Body weights of rats.

Summary of body weights(g)				Sex : Male
Day	Group(㎎/㎏)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
0	235.96 ± 7.32 (5)	239.22 ± 8.75 (5)	234.17 ± 11.78 (5)	240.32 ± 6.88 (5)
1	270.77 ± 2.40 (5)	272.09 ± 8.98 (5)	262.38 ± 11.57 (5)	271.20 ± 5.83 (5)
3	297.56 ± 7.27 (5)	297.52 ± 14.22 (5)	286.27 ± 11.55 (5)	293.59 ± 11.26 (5)
7	323.08 ± 9.64 (5)	322.30 ± 16.45 (5)	309.69 ± 11.99 (5)	314.93 ± 13.83 (5)
14	374.82 ± 17.89 (5)	371.83 ± 20.35 (5)	360.15 ± 13.50 (5)	360.61 ± 22.91 (5)
Gains[a]	138.86 ± 17.80 (5)	132.61 ± 15.46 (5)	125.98 ± 10.27 (5)	120.29 ± 16.63 (5)
% to control		99.20	96.09	96.21
			Sex : Female	
Day	Group(㎎/㎏)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
0	187.27 ± 5.40 (5)	188.29 ± 5.56 (5)	186.33 ± 5.95 (5)	187.77 ± 5.21 (5)
1	211.15 ± 8.25 (5)	203.36 ± 7.62 (5)	205.38 ± 5.93 (5)	208.99 ± 9.87 (5)
3	219.16 ± 7.73 (5)	214.45 ± 8.43 (5)	215.76 ± 6.05 (5)	215.96 ± 5.81 (5)
7	224.83 ± 7.29 (5)	221.56 ± 9.41 (5)	225.64 ± 9.01 (5)	224.68 ± 9.17 (5)
14	238.89 ± 8.76 (5)	230.80 ± 15.10 (5)	244.57 ± 10.36 (5)	241.80 ± 14.18 (5)
Gains	51.62 ± 6.12 (5)	42.51 ± 12.20 (5)	58.23 ± 5.08 (5)	54.04 ± 9.78 (5)
% to control		96.62	102.38	101.22

Body weights and gains are presented as mean ± standard deviation (n = 5).

Table 3. Gross findings of rats.

Summary of gross findings					Sex : Male
Organs	Signs	Group(㎎/㎏)			
		G1(0)	G2(500)	G3(1,000)	G4(2,000)
All organs	No gross finding detected	5 / 5	5 / 5	5 / 5	5 / 5
			Sex : Female		
Organs	Signs	Group(㎎/㎏)			
		G1(0)	G2(500)	G3(1,000)	G4(2,000)
All organs	No gross finding detected	5 / 5	5 / 5	5 / 5	5 / 5

control group. Although statistically significant, the observed monocyte counts and percentages were within the normal physiological range in Sprague–Dawley rats.

DISCUSSION

To the best of our knowledge, this is the first study to comprehensively evaluate the single oral dose toxicity of aqueous extracts of Bombycis Faeces in Sprague–Dawley rats according to internationally recognized OECD guidelines. The extract was administered as single oral doses of 500, 1000, and 2000 mg/kg, and the animals were monitored for 14 days for clinical signs, body weight changes, gross pathological lesions, and hematological alterations. The extract did not induce mortality or any observable toxicological abnormalities up to the highest dose of 2000 mg/kg, indicating a favorable safety profile.

Table 4. Hematological values of male rats.

Summary of hematological tests				Sex : Male
Test item	Group(㎎/㎏)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
WBC[1](K/μL)	9.05 ± 2.72 (5)	10.53 ± 1.48 (5)	11.36 ± 2.36 (5)	11.16 ± 1.44 (5)
NE[2](K/μL)	1.59 ± 0.54 (5)	1.81 ± 0.36 (5)	2.61 ± 0.99 (5)	1.94 ± 0.70 (5)
EO[3](K/μL)	0.07 ± 0.02 (5)	0.11 ± 0.04 (5)	0.11 ± 0.03 (5)	0.09 ± 0.02 (5)
BA[4](K/μL)	0.00 ± 0.01 (5)	0.01 ± 0.01 (5)	0.01 ± 0.01 (5)	0.01 ± 0.00 (5)
LY[5](K/μL)	7.16 ± 2.20 (5)	8.29 ± 1.55 (5)	8.23 ± 1.39 (5)	8.86 ± 1.02 (5)
MO[6](K/μL)	0.16 ± 0.11 (5)	0.25 ± 0.11 (5)	0.32 ± 0.13 (5)	0.21 ± 0.05 (5)
LUC[7](K/μL)	0.06 ± 0.04 (5)	0.07 ± 0.02 (5)	0.07 ± 0.02 (5)	0.07 ± 0.02 (5)
NEP[8](%)	17.70 ± 2.90 (5)	17.40 ± 4.30 (5)	22.50 ± 4.50 (5)	17.20 ± 4.60 (5)
EOP[9](%)	0.80 ± 0.20 (5)	1.00 ± 0.30 (5)	1.10 ± 0.40 (5)	0.80 ± 0.10 (5)
BAP[10](%)	0.00 ± 0.10 (5)	0.10 ± 0.00 (5)	0.10 ± 0.10 (5)	0.10 ± 0.00 (5)
LYP[11](%)	79.10 ± 3.90 (5)	78.40 ± 5.30 (5)	73.00 ± 4.20 (5)	79.50 ± 4.20 (5)
MOP[12](%)	1.80 ± 0.80 (5)	2.40 ± 1.00 (5)	2.80 ± 0.70 (5)	1.90 ± 0.40 (5)
LUP[13](%)	0.60 ± 0.30 (5)	0.70 ± 0.20 (5)	0.60 ± 0.20 (5)	0.60 ± 0.20 (5)
RBC[14](M/μL)	6.64 ± 1.00 (5)	7.75 ± 0.22 (5)	7.89 ± 0.17 (5)	8.06 ± 0.22 (5)
Hb[15](g/dℓ)	12.80 ± 2.60 (5)	15.10 ± 0.20 (5)	15.30 ± 0.70 (5)	15.60 ± 0.40 (5)
RDW[16](%)	12.00 ± 0.60 (5)	12.30 ± 0.50 (5)	11.90 ± 0.40 (5)	11.80 ± 0.40 (5)
HCT[17](%)	40.60 ± 7.00 (5)	46.90 ± 0.90 (5)	47.20 ± 1.90 (5)	48.00 ± 1.50 (5)
MCV[18](fL)	61.00 ± 2.50 (5)	60.60 ± 2.30 (5)	59.80 ± 1.50 (5)	59.50 ± 1.70 (5)
MCH[19](pg)	19.20 ± 1.40 (5)	19.60 ± 0.70 (5)	19.40 ± 0.50 (5)	19.40 ± 0.60 (5)
MCHC[20](g/dℓ)	31.50 ± 1.10 (5)	32.30 ± 0.60 (5)	32.40 ± 0.10 (5)	32.50 ± 0.30 (5)
PLT[21](K/μL)	799 ± 384 (5)	1217 ± 90 (5)	1128 ± 104 (5)	1035 ± 165 (5)
MPV[22](fL)	8.70 ± 0.40 (5)	8.90 ± 0.30 (5)	8.80 ± 0.50 (5)	8.80 ± 0.40 (5)

Values are presented as mean ± standard deviation (n = 5). 1: White blood cell, 2: Neutrophil, 3: Eosinophil, 4: Basophil, 5: Lymphocyte, 6: Monocyte, 7: Large unstained cell, 8: Percent of neutrophil, 9: Percent of eosinophil, 10: Percent of basophil, 11: Percent of lymphocyte, 12: Percent of monocyte, 13: Percent of large unstained cell, 14: Red blood cell, 15: Hemoglobin, 16: Red cell distribution width, 17: Hematocrit, 18: Mean corpuscular volume, 19: Mean corpuscular hemoglobin, 20: Mean corpuscular hemoglobin concentration, 21: Platelet, 22: Mean platelet volume

No mortality or clinical signs of toxicity were observed even at the highest dose of 2000 mg/kg, indicating that the LD_{50} is greater than 2000 mg/kg according to OECD guidelines. According to the Globally Harmonized System (GHS) for the classification and labeling of chemicals, substances with oral LD_{50} values > 2000 mg/kg are categorized as Category 5 or remain unclassified. This classification suggests that the extract possesses low single oral dose toxicity under the conditions of this study.

Body weight gain, a sensitive indicator of general health and systemic toxicity, proceeded normally in all animals. There were no significant differences between treated and control groups, suggesting that the extract did not adversely affect nutrient absorption, metabolism, or growth. In addition, no gross pathological changes were observed at necropsy, indicating that the extract did not cause acute organ damage or structural abnormalities in the major organ systems.

Hematological parameters remained within the physiological limits across all groups. While male rats showed no significant hematologic changes, female rats exhibited a statistically significant increase in monocyte counts across all treated groups. However, these increases were not dose-dependent and remained within the normal reference range for Sprague–

Table 5. Hematological values of female rats.

Summary of hematological tests				Sex : Female
Test item	Group(㎎/㎏)			
	G1(0)	G2(500)	G3(1,000)	G4(2,000)
WBC[1](K/μL)	10.55 ± 2.76 (5)	8.97 ± 1.88 (5)	8.33 ± 1.84 (5)	9.30 ± 1.01 (5)
NE[2](K/μL)	1.19 ± 0.35 (5)	1.11 ± 0.27 (5)	1.40 ± 0.60 (5)	1.43 ± 0.43 (5)
EO[3](K/μL)	0.12 ± 0.03 (5)	0.13 ± 0.04 (5)	0.11 ± 0.04 (5)	0.14 ± 0.04 (5)
BA[4](K/μL)	0.01 ± 0.01 (5)	0.00 ± 0.00 (5)	0.00 ± 0.00 (5)	0.01 ± 0.00 (5)
LY[5](K/μL)	9.02 ± 2.43 (5)	7.42 ± 1.88 (5)	6.55 ± 1.31 (5)	7.46 ± 0.95 (5)
MO[6](K/μL)	0.13 ± 0.04 (5)	0.23** ± 0.04 (5)	0.19** ± 0.05 (5)	0.20** ± 0.02 (5)
LUC[7](K/μL)	0.07 ± 0.03 (5)	0.08 ± 0.04 (5)	0.07 ± 0.02 (5)	0.06 ± 0.03 (5)
NEP[8](%)	11.40 ± 1.90 (5)	12.90 ± 4.50 (5)	16.50 ± 4.60 (5)	15.30 ± 4.50 (5)
EOP[9](%)	1.20 ± 0.30 (5)	1.60 ± 0.60 (5)	1.40 ± 0.50 (5)	1.60 ± 0.50 (5)
BAP[10](%)	0.10 ± 0.10 (5)	0.00 ± 0.10 (5)	0.10 ± 0.10 (5)	0.10 ± 0.00 (5)
LYP[11](%)	85.40 ± 2.20 (5)	81.90 ± 5.70 (5)	79.00 ± 4.60 (5)	80.10 ± 3.80 (5)
MOP[12((%)	1.30 ± 0.30 (5)	2.70** ± 0.80 (5)	2.30** ± 0.20 (5)	2.20** ± 0.40 (5)
LUP[13](%)	0.70 ± 0.20 (5)	0.90 ± 0.30 (5)	0.80 ± 0.10 (5)	0.70 ± 0.30 (5)
RBC[14](M/μL)	8.08 ± 0.31 (5)	8.03 ± 0.25 (5)	8.19 ± 0.56 (5)	8.40 ± 0.52 (5)
Hb[15](g/dℓ)	15.10 ± 0.50 (5)	15.40 ± 0.50 (5)	15.60 ± 0.70 (5)	15.90 ± 0.80 (5)
RDW[16](%)	11.40 ± 0.60 (5)	11.00 ± 0.50 (5)	11.00 ± 0.40 (5)	11.00 ± 0.60 (5)
HCT[17](%)	45.50 ± 1.70 (5)	46.00 ± 1.30 (5)	46.80 ± 2.10 (5)	48.10 ± 2.30 (5)
MCV[18](fL)	56.40 ± 1.70 (5)	57.30 ± 1.60 (5)	57.20 ± 2.50 (5)	57.30 ± 2.20 (5)
MCH[19](pg)	18.70 ± 0.50 (5)	19.20 ± 0.60 (5)	19.10 ± 1.00 (5)	19.00 ± 1.10 (5)
MCHC[20](g/dℓ)	33.10 ± 0.40 (5)	33.50 ± 0.30 (5)	33.30 ± 0.30 (5)	33.10 ± 0.60 (5)
PLT[21](K/μL)	1089 ± 92 (5)	1100 ± 177 (5)	1158 ± 116 (5)	1093 ± 117 (5)
MPV[22](fL)	8.70 ± 0.30 (5)	8.30 ± 0.40 (5)	8.50 ± 0.20 (5)	8.30 ± 0.30 (5)

Values are presented as mean ± standard deviation (n = 5). 1: White blood cell, 2: Neutrophil, 3: Eosinophil, 4: Basophil, 5: Lymphocyte, 6: Monocyte, 7: Large unstained cell, 8: Percent of neutrophil, 9: Percent of eosinophil, 10: Percent of basophil, 11: Percent of lymphocyte, 12: Percent of monocyte, 13: Percent of large unstained cell, 14: Red blood cell, 15: Hemoglobin, 16: Red cell distribution width, 17: Hematocrit, 18: Mean corpuscular volume, 19: Mean corpuscular hemoglobin, 20: Mean corpuscular hemoglobin concentration, 21: Platelet, 22: Mean platelet volume. **: Significant difference compared with the control group value, one-way ANOVA, $p < 0.01$

Dawley rats (Han *et al*., 2010; He *et al*., 2017; Delwatta *et al*., 2018). Furthermore, no associated clinical symptoms or gross organ lesions were observed. Therefore, these changes were considered to be incidental biological variations rather than indicative of treatment-related toxicity or an adverse immunostimulatory response.

Previous studies have reported the pharmacological efficacy of Bombycis faecal extracts, including antioxidant, anti-inflammatory, hypolipidemic, and immunomodulatory properties (Ali *et al*., 2011; Wang *et al*., 2020; Zhang *et al*., 2020). However, toxicological validation of this extract remains limited. Our findings provide critical baseline data on the single oral dose toxicity of aqueous extracts of Bombycis Faeces and are consistent with previous studies reporting the low toxicity of sericin and fibroin-derived products (Yigit *et al*., 2021). Notably, the complex mixture of bioactive compounds in the whole extract, including flavonoids, sericin, and carotenoids, underscores the need to evaluate the full formulation rather than isolated components.

Limitations of this study include the single-dose design and the absence of histopathological examination, which restricts the depth of toxicological interpretation. Microscopic tissue

alterations may occur without overt gross lesions. Therefore, future repeated-dose toxicity studies incorporating comprehensive histopathological evaluations, genotoxicity, and reproductive toxicity assessments are necessary to confirm these findings and establish a definitive safety profile.

In conclusion, the absence of mortality, clinical toxicity, and major hematological or pathological abnormalities at doses up to 2000 mg/kg suggests that the aqueous extract of Bombycis Faeces has low single oral dose toxicity. Overall, this study provides essential toxicological evidence to support the future development of *Bombyx mori*-derived nutraceuticals and herbal medicinal products.

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