Safety Assessment of Water-Extract Sericin from Silkworm (Bombyx mori) Cocoons Using Different Model Approaches

Huiyan Qin,1 Jiehong Zhang,1 Hui Yang,1 Siyu Yao,1 Li He,1 Huili Liang,1 Yanwu Wang,1 Huafeng Chen,1 Peng Zhao,1 and Guangqiu Qin1,2

1Institute of Toxicology, Guangxi Center for Disease Prevention and Control, Nanning, China
2Department of Preventive Medicine, Guangxi University of Chinese Medicine, Nanning, China

Correspondence should be addressed to Guangqiu Qin; qinguangqiu@hotmail.com

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Sericin is a natural protein component of silks of silkworm (Bombyx mori) larvae. Natural silk consists of two main proteins, sericin and fibroin, with fibroin being the structural center and sericin being the surface coating surrounding it. Sericin is essential in the formation of a cocoon. However, since sericin affects silk fabric dyeing and feel of silk floss, it is removed during the degumming or refining of raw silk. In the silk of silkworms, sericin accounts for about 20-30% of the weight and fibroin accounts for the rest [1]. The molecular weight of sericin was between 10 and 400 kDa [2, 3]. A total of 18 kinds of amino acids have been found in silkworm sericin, among which polar amino acids account for about 78% (mainly serine and aspartic acid) and nonpolar amino acids account for 22% [4].

Silkworm cocoon and its boiled water (mainly sericin) are used as an important component in diet therapy regimens to treat hyperglycemia and hyperlipidemia. The results of modern biomedical studies showed that sericin in silkworm cocoons has multiple biological activities. For example, sericin was observed to activate collagen synthesis in skin tissue and play the role of antiwrinkle and antiaging through its collagen-promoting activity [5–7]. Sericin could stimulate cell proliferation in serum-free culture dishes [4, 8]. Other studies found that sericin reduced the serum lipid content and improved the glucose tolerance in rats fed with high-fat diet [9, 10] and had protective effects on alcohol-induced liver injury in mice [11]. These results suggest that sericin has a wide application potential and it has been applied in food-related products, cosmetics, and medical supplies [12].

Although the biological effects of sericin in silkworm cocoon have been widely recognized in public and confirmed by modern biomedical research, the understanding of its toxicological safety is still limited. Previous studies have
reported adverse effects of sericin such as immunologic stimulation and cytotoxicity [13, 14]. For example, it was reported that sericin extracted by the urea treatment was severely harmful to cells at concentrations > 100 μg/mL [5]. The objective of the present study was to evaluate the genotoxicity and subchronic toxicity of water-extract sericin from silkworm cocoon using different model approaches, so as to provide necessary information for safety assessment sericin in food-related products.

2. Materials and Methods

2.1. Sericin Extract and Its Characterization. Fresh Bombyx mori cocoons were provided by the Guangxi Institute for Product Quality Inspection (Nanning, China). A traditional high-temperature degumming technique was used to prepare heat-degraded silk sericin solution. Briefly, cocoons were cut into small pieces and immersed in deionized water (1:30, w:v) and degummed at 100°C for 3 h, without chemical additives. The silk sericin solution was filtered with an 18-mesh nonwoven filter to remove the silk fibroin, and the sericin solution was further boiled to concentrate the sericin. The concentrated solution was centrifuged (8000 rpm) for 10 min. The supernatant was collected and freeze-dried to obtain sericin extract. Sericin extract was dissolved in deionized water before used for the study.

The amino acid compositions of the water-extract sericin were examined in accordance with the standardized guidelines [15]. Briefly, the samples were hydrolyzed with 6 mol/L HCl (1:3, w:v) and 0.2 mL phenol at 110°C for 22 h under vacuum. Amino acids were analyzed with a Hitachi L-8500A amino acid analyzer (Tokyo, Japan).

2.2. Experimental Animals and Husbandry. Three-week-old specific pathogen-free Sprague-Dawley (SD) rats and adult Kunming mice (25-35 g) were provided by the Animal Experimental Center at Guangdong Academy of Medical Science (Gangzhou, China). The animals were kept in the laboratory animal facility with a controlled temperature at 23 ± 1°C, relative humidity at 60 ± 5%, and a 12 h light/dark cycle. The animals were offered conventional diets and sterilized tap water ad libitum. The animals were acclimated for one week before used for the experiments. All animal test protocols were approved by the Animal Experimentation Ethics Committee at Guangxi Center for Disease Prevention and Control (Nanning, China). The data of approval was 2017-05-08, and the approval number was 20170009.

2.3. Genotoxicity Studies. To evaluate the potential genotoxicity of water-extract sericin, the bacterial reverse mutation test, the mammalian erythrocyte micronucleus test, and the mouse spermatogonia chromosomal aberration test were conducted. The doses used in these studies were selected based on the maximum tolerated dose (MTD) for oral intake of water-extract sericin in the preexperiment and following the recommendation of standardized guidelines set by the Ministry of Health of China. These guidelines were developed based on the internationally recognized guidelines including the OECD Guidelines for the testing of chemicals and USFDA Redbook 2000 Toxicological Principles for the Safety Assessment of Food Ingredients [16, 17].

The bacterial reverse mutation test was conducted to examine the ability of water-extract sericin to induce the reverse mutation at five strains of Salmonella typhimurium (TA97a, TA98, TA100, TA102, and TA1535), following the standardized protocol described previously [16, 18]. A maximum dose of 5000 μg/plate for potentially low bacterial toxicity substances was used as the highest dose, followed by 1581, 500, 158, and 50 μg/plate, using a common interval ratio of √10. Rat liver S9 mix (MoltoxVR Molecular Toxicology, Inc., USA) was used as the exogenous metabolic activation, and five standard mutagens were used as the positive control, including 10 μg/plate 2-aminofluorene, 6 μg/plate daunorubicin, 50 μg/plate dexamethasone, 1.5 μg/plate sodium azide, and 50 μg/plate 1, 8-dihydroxyanthraquinone. The test solutions were autoclaved (0.103 MPa, 20 min) before used for the test. Using the plate mixing method, 0.1 mL of the test S. typhimurium solution, 0.1 mL of water-extract sericin, and 0.5 mL of S9 mixed solution (when metabolic activation was needed) were successively mixed with the top layer of culture medium and then poured into the hardened bottom culture medium. For solvent control, sterilized pure water and dimethyl sulphoxide (DMSO) were used instead of water-extract sericin, and other conditions were the same as those of the treatment groups. Three parallel dishes were made for each dose group. The bacteria were cultured at 37°C for 48 hours, and the number of colonies in each dish was counted. The bacterial reverse mutation test was repeated twice under the same experimental conditions.

The in vivo mammalian erythrocyte micronucleus test was conducted following the standardized method described previously [19, 20]. Briefly, 25-30 g Kunming mice were randomly assigned to 5 groups with 5 males and 5 females in

| Table 1: Amino acid contents of water-extract sericin. |
|--------------------------------------------------------|
| **Amino acids** | **Contents (% w/w)** |
|-----------------|----------------------|
| Aspartic acid (Asp) | 16.5 |
| Threonine (Thr) | 9.8 |
| Serine (Ser) | 26.0 |
| Glutamate (Glu) | 5.0 |
| Proline (Pro) | 1.5 |
| Glycine (Gly) | 9.1 |
| Alanine (Ala) | 3.6 |
| Cysteine (Cys) | 0.3 |
| Valine (Val) | 4.2 |
| Methionine (Met) | 1.2 |
| Isoleucine (Ile) | 1.9 |
| Leucine (Leu) | 2.8 |
| Tyrosine (Tyr) | 6.3 |
| Phenylalanine (Phe) | 1.7 |
| Lysine (Lys) | 3.6 |
| Histidine (His) | 1.4 |
| Arginine (Arg) | 5.0 |
| **Total** | **100.0** |
### Table 2: Results of the bacterial reverse mutation test for water-extract sericin.

| Groups       | TA97a -S9 | TA97a +S9 | TA98 -S9 | TA98 +S9 | TA100 -S9 | TA100 +S9 | TA102 -S9 | TA102 +S9 | TA1535 -S9 | TA1535 +S9 |
|--------------|-----------|-----------|----------|----------|-----------|-----------|-----------|-----------|------------|------------|
| Sericin      | 117 ± 17.7| 125 ± 17.5| 38.3 ± 4.5| 39.0 ± 4.4| 157.3 ± 21.7| 155.3 ± 18.6| 269.0 ± 29.5| 277.7 ± 22.0| 24.0 ± 5.3| 21.7 ± 5.5 |
| 5000 µg/plate| 131.3 ± 25.0| 127.3 ± 22.0| 39.7 ± 4.2| 42.3 ± 4.2| 150.7 ± 19.6| 156.0 ± 16.7| 280.0 ± 20.1| 273.0 ± 20.1| 18.3 ± 2.3| 22.3 ± 5.9 |
| 1581 µg/plate| 124.7 ± 19.7| 114.7 ± 18.7| 38.7 ± 7.2| 41.7 ± 6.5| 154.0 ± 19.7| 152.0 ± 20.5| 271.7 ± 22.1| 266.7 ± 23.9| 22.7 ± 3.2| 26.7 ± 9.2 |
| 500 µg/plate | 123.7 ± 21.2| 118.7 ± 23.7| 37.7 ± 4.7| 41.0 ± 4.4| 152.7 ± 17.8| 159.7 ± 24.0| 275.7 ± 22.0| 275.3 ± 21.2| 22.3 ± 7.1| 24.3 ± 4.2 |
| 50 µg/plate  | 125.7 ± 16.0| 120.3 ± 17.5| 43.3 ± 5.0| 43.0 ± 7.6| 151.3 ± 21.5| 156.7 ± 20.5| 267.7 ± 22.9| 275.7 ± 21.0| 22.7 ± 7.0| 25.0 ± 6.2 |
| Untreated control | 120.0 ± 13.2| 130.0 ± 10.5| 41.3 ± 5.5| 44.3 ± 6.7| 148.3 ± 22.3| 155.3 ± 25.6| 271.7 ± 21.2| 274.7 ± 21.0| 23.3 ± 6.8| 23.7 ± 4.5 |
| H₂O control  | 125.0 ± 15.6| 127.0 ± 19.3| 41.0 ± 5.0| 38.0 ± 4.6| 157.3 ± 19.7| 150.7 ± 19.4| 271.0 ± 20.5| 269.7 ± 19.8| 21.7 ± 8.3| 24.3 ± 5.8 |
| DMSO control | 124.3 ± 17.2| 122.3 ± 17.6| 36.0 ± 5.3| 38.0 ± 7.0| 157.7 ± 22.2| 158.0 ± 21.6| 279.3 ± 19.9| 271.3 ± 16.3| 20.0 ± 4.4| 25.3 ± 8.3 |
| Positive control | 2-AF 1785.3 ± 68.2| 4886.7 ± 210.1| 2886.7 ± 102.6 |
|              | DNR 2980.0 ± 111.4 |
|              | Dexon 2795.3 ± 60.5 |
|              | NaN3 3056.7 ± 156.3 |
|              | 1,8-DHAQ 864.3 ± 73.6 |
|              | CP 308.7 ± 13.3 |

Values represent the mean ± standard deviation of triplicates. -S9: without metabolic activation; +S9: with metabolic activation; 2-AF: 2-aminofluorene (10 µg/plate); DNR: daunorubicin (6 µg/plate); Dexon: dexon (50 µg/plate); NaN3: sodium azide (1.5 µg/plate); 1,8-DHAQ: 1, 8-dihydroxyanthraquinone (50 µg/plate); CP: cyclophosphamide (200 µg/plate).
was collected from the caudal vein and the peripheral blood of the animals twice with a 24 h interval, while the animals in the negative and positive controls were treated with distilled water and 40 mg/kg cyclophosphamide, respectively. After 6 h following the second gavage, the chromosomal aberrations including chromo-

| Groups   | Number of mice examined | Number of PCE examined | Number of micronucleated PCE observed | Micronucleated PCE (%) | Number of RBC examined | Number of PCE observed | PCE/RBC |
|----------|------------------------|------------------------|---------------------------------------|------------------------|------------------------|------------------------|---------|
| Male     |                        |                        |                                       |                        |                        |                        |         |
| 6660     | 5                      | 5 × 2000               | 13                                    | 1.3 ± 0.4              | 5 × 1000               | 232                    | 4.64 ± 0.92 |
| 3330     | 5                      | 5 × 2000               | 16                                    | 1.6 ± 0.7              | 5 × 1000               | 240                    | 4.80 ± 1.17 |
| 1665     | 5                      | 5 × 2000               | 15                                    | 1.5 ± 0.5              | 5 × 1000               | 237                    | 4.74 ± 0.80 |
| Negative | 5                      | 5 × 2000               | 15                                    | 1.5 ± 0.5              | 5 × 1000               | 223                    | 4.46 ± 0.72 |
| Positive | 5                      | 5 × 2000               | 219                                   | 21.9 ± 4.3**           | 5 × 1000               | 156                    | 3.12 ± 1.12 |
| Female   |                        |                        |                                       |                        |                        |                        |         |
| 6660     | 5                      | 5 × 2000               | 14                                    | 1.4 ± 0.5              | 5 × 1000               | 226                    | 4.52 ± 0.96 |
| 3330     | 5                      | 5 × 2000               | 14                                    | 1.4 ± 0.8              | 5 × 1000               | 241                    | 4.82 ± 1.02 |
| 1665     | 5                      | 5 × 2000               | 15                                    | 1.5 ± 0.5              | 5 × 1000               | 224                    | 4.48 ± 1.52 |
| Negative | 5                      | 5 × 2000               | 14                                    | 1.4 ± 0.5              | 5 × 1000               | 229                    | 4.58 ± 1.09 |
| Positive | 5                      | 5 × 2000               | 213                                   | 21.3 ± 3.1**           | 5 × 1000               | 164                    | 3.28 ± 1.02 |

PCE: polychromatoly erythrocyte; RBC: red blood cell. For micronucleated PCE and PCE/RBC, the data represent the mean ± standard deviation of 5 animals. ** Significant difference compared to the control at p < 0.01.

2.4. Subchronic Toxicity Study. The 90-day repeated-dose oral toxicity study was conducted in accordance with the test guidelines of the National Food Safety Standards of China described previously [22, 23]. The maximum tolerated dose (MTD) for oral intake of water-extract sericin was observed to be >1000 mg/kg in rats in the acute toxicity study. Therefore, 1000 mg/kg was selected as the high dose for the 90-day toxicity study.

Briefly, a total of 80 rats (60-80 g) were randomly divided into 4 groups with 10 males and 10 females in each group. The animals in the control group received deionized water at a dosing volume of 1 mL/100 g body weight (BW), while animals in the treatment groups received 1000, 500, and 250 mg/kg BW of sericin extract, respectively, for continuous 90 days. The animals were allowed free access to food and water during the exposure. Clinical signs and behavioral symptoms were recorded every day. Body weights were recorded weekly, and food consumption was recorded twice a week throughout the study. Food utilization rates were calculated as follows: Food utilization rate = body weight gain (g)/food intake (g) × 100%.

The ophthalmological examination was conducted before and at the end of the exposure. The cornea, lens, bulbar conjunctiva, and iris were observed with an ophthalmoscope.

After 90 days of the exposure, overnight fasted rats were anaesthetized with pentobarbital and sacrificed. Blood was collected immediately from the aorta abdominalis for hematological and biochemical examination, using a Sysmex XT-1800 automated hematological analyzer (Sysmex, Kobe, Japan) and an Olympus AU400 analyzer (Olympus, Tokyo, Japan), respectively. Urine samples were collected from the bladders for urinalysis using a Urit-500B urine chemistry analyzer (Urit, Guilin, China).

A complete gross necropsy was conducted on all rats. Absolute weight was measured, and relative organ weight (organ weight/body weight) was determined for the liver,
Table 4: Results of mouse spermatogonia chromosomal aberration test for sericin extract.

| Group (mg/kg) | Number of cells examined | Chromosomal number abnormality | Alteration of chromosomal structure | Number of cells with chromosomal aberration | Rate of cells with chromosomal aberration (%) |
|--------------|--------------------------|--------------------------------|-------------------------------------|---------------------------------------------|---------------------------------------------|
| 6660 (24 h)  | 100 × 5                  | 0                              | 36                                  | 28                                         | 14.6 ± 1.7                                  |
| 6660 (48 h)  | 100 × 5                  | 0                              | 36                                  | 22                                         | 14.2 ± 1.9                                  |
| 3330         | 100 × 5                  | 0                              | 33                                  | 23                                         | 13.4 ± 0.9                                  |
| 1665         | 100 × 5                  | 0                              | 35                                  | 32                                         | 12.2 ± 1.5                                  |
| Negative control | 100 × 5               | 0                              | 37                                  | 20                                         | 14.4 ± 1.8                                  |
| Positive control | 100 × 5                | 0                              | 37                                  | 47                                         | 61.8 ± 3.9**                                |

Rate of cells with chromosomal aberration (%) = \( \frac{(\text{number of cells with chromosomal aberration} - \text{number of cells with chromosomal gaps})}{\text{number of cells examined}} \times 100 \).
spleen, kidneys, testes, ovaries, brain, heart thymus adrenal, epididymis, and uterus. The samples of organs and tissues were taken for histopathological examination, including the brain, thyroid gland, liver, spleen, pancreatic gland, heart, kidneys, adrenal gland, stomach, mesenteric lymph nodes, small intestine, jejunum, ileum, prostate, bladder, testes, and ovaries. The tissue samples were fixed in 4% neutral buffered formaldehyde, embedded in paraffin, stained with Giemsa, and then examined under a Leica DM 6000B optical microscopy (Wetzler, Germany). The number of animals with histopathological lesions was recorded, and the degree of lesions was scored into four levels: normal (0), mild (1), moderate (2), and severe (3).

2.5. Statistical Analysis. The data of male and female rats were analyzed separately, using SPSS v16.0 (SPSS Inc., Chicago, Illinois, USA). The homogeneity of variances of data was checked by Bartlett’s test, and then, the data of the treatment groups were compared to those of the control by one-way ANOVA followed by Dunnett’s test. The significance level was set at $p \leq 0.05$.

3. Results

3.1. Characterization of the Water-Extract Sericin. The results of the amino acid analysis are shown in Table 1. The water-extract sericin contents were $1.46 \text{g/100 mL}$ of crude protein, $12.66 \text{g/100 mL}$ of free amino acids, and $1277.0 \text{g/100 mL}$ of hydrolyzed amino acids. Serine (26.0%), aspartic acid (16.5%), and threonine (9.8%) were the major amino acids found in the extract. The contents of tryptophan and taurine were below the detecting limits ($<1.3 \text{g/100 mL}$).

3.2. Genotoxicity Studies. The results of the bacterial reverse mutation test are shown in Table 2. Compared to the untreated group, the positive groups significantly increased the number of revertant colonies in the presence and absence of S9. At the same time, the numbers of revertant bacterial colonies in the untreated, solvent, and positive control were comparable to the historical data in our lab. On the other hand, sericin extract at doses of up to $5000 \text{mg/kg}$ was comparable to the historical data in our lab. The homogeneity of variances of data was checked by Bartlett’s test, and then, the data of the treatment groups were compared to those of the control by one-way ANOVA followed by Dunnett’s test. The significance level was set at $p \leq 0.05$.

3.3. Observations and Findings. The results of the mammalian erythrocyte micronucleus test showed that the micronucleated PCEs were significantly higher in the positive group ($p < 0.01$). In contrast, the micronucleate rate and PCEs/RBCs of mice were not affected by sericin extract, when compared to the negative control (Table 3).

For the mouse spermatogonia chromosomal aberration test, it was shown that sericin extract at all doses did not significantly affect spermatogonia chromosomal aberration in mice as compared to the negative group, while the rate of cells with the chromosomal aberration of the positive group was significantly higher than that in the negative group ($p < 0.01$, Table 4).
Table 6: Absolute organ weights of rats treated with sericin extract for 90 days.

| Dose (mg/kg) | Liver       | Kidneys     | Spleen      | Testes/ovaries | Brain      | Heart      | Thymus     | Adrenal    | Epididymis/uterus |
|--------------|-------------|-------------|-------------|----------------|------------|------------|------------|------------|-------------------|
| Male         |             |             |             |                |            |            |            |            |                   |
| 1000         | 13.83 ± 1.08| 3.532 ± 0.229| 0.939 ± 0.050| 3.715 ± 0.309  | 2.168 ± 0.066| 1.561 ± 0.104| 0.590 ± 0.078| 0.085 ± 0.016| 1.348 ± 0.112     |
| 500          | 13.54 ± 0.99| 3.479 ± 0.266| 0.946 ± 0.074| 3.673 ± 0.253  | 2.164 ± 0.078| 1.627 ± 0.124| 0.540 ± 0.067| 0.083 ± 0.013| 1.431 ± 0.120     |
| 250          | 14.05 ± 1.98| 3.599 ± 0.504| 0.961 ± 0.098| 3.728 ± 0.401  | 2.099 ± 0.075| 1.663 ± 0.200| 0.591 ± 0.074| 0.090 ± 0.026| 1.389 ± 0.185     |
| Control      | 13.92 ± 1.36| 3.573 ± 0.243| 0.925 ± 0.094| 3.742 ± 0.155  | 2.085 ± 0.098| 1.639 ± 0.129| 0.541 ± 0.068| 0.084 ± 0.016| 1.446 ± 0.175     |
| Female       |             |             |             |                |            |            |            |            |                   |
| 1000         | 7.74 ± 0.89 | 1.873 ± 0.135| 0.555 ± 0.075| 0.158 ± 0.023  | 1.899 ± 0.046| 1.015 ± 0.084| 0.433 ± 0.051| 0.075 ± 0.015| 0.594 ± 0.064     |
| 500          | 7.56 ± 0.45 | 1.909 ± 0.121| 0.507 ± 0.052| 0.159 ± 0.015  | 1.967 ± 0.079| 1.005 ± 0.101| 0.477 ± 0.057| 0.072 ± 0.010| 0.657 ± 0.077     |
| 250          | 7.93 ± 1.16 | 1.883 ± 0.173| 0.559 ± 0.046| 0.159 ± 0.029  | 1.896 ± 0.094| 1.006 ± 0.064| 0.458 ± 0.053| 0.076 ± 0.013| 0.609 ± 0.065     |
| Control      | 7.96 ± 1.08 | 1.965 ± 0.350| 0.571 ± 0.055| 0.155 ± 0.018  | 1.890 ± 0.070| 0.999 ± 0.148| 0.455 ± 0.085| 0.073 ± 0.012| 0.624 ± 0.091     |

Note: The values represent the mean ± standard deviation of 10 rats. The values of the treatment groups did not differ statistically from the control according to one-way ANOVA at $p < 0.05$. 

Table 7: Relative organ weights of rats treated with sericin extract for 90 days.

| Dose (mg/kg) | Liver   | Kidneys | Spleen  | Testes/ovaries | Brain   | Heart   | Thymus  | Adrenal | Epididymis/uterus |
|-------------|---------|---------|---------|----------------|---------|---------|---------|---------|-------------------|
| Male        |         |         |         |                |         |         |         |         |                   |
| 1000        | 2.562 ± 0.173 | 0.655 ± 0.051 | 0.174 ± 0.011 | 0.689 ± 0.057 | 0.402 ± 0.019 | 0.289 ± 0.018 | 0.109 ± 0.010 | 0.016 ± 0.002 | 0.250 ± 0.026     |
| 500         | 2.475 ± 0.118 | 0.636 ± 0.039 | 0.173 ± 0.016 | 0.673 ± 0.048 | 0.397 ± 0.023 | 0.298 ± 0.021 | 0.098 ± 0.009 | 0.015 ± 0.002 | 0.263 ± 0.030     |
| 250         | 2.488 ± 0.149 | 0.638 ± 0.052 | 0.171 ± 0.009 | 0.663 ± 0.045 | 0.375 ± 0.025 | 0.296 ± 0.026 | 0.105 ± 0.010 | 0.016 ± 0.004 | 0.247 ± 0.026     |
| Control     | 2.531 ± 0.133 | 0.652 ± 0.051 | 0.169 ± 0.016 | 0.684 ± 0.058 | 0.380 ± 0.018 | 0.299 ± 0.021 | 0.098 ± 0.010 | 0.015 ± 0.003 | 0.264 ± 0.036     |
| Female      |         |         |         |                |         |         |         |         |                   |
| 1000        | 2.696 ± 0.164 | 0.654 ± 0.041 | 0.193 ± 0.021 | 0.655 ± 0.009 | 0.665 ± 0.042 | 0.355 ± 0.031 | 0.151 ± 0.018 | 0.026 ± 0.004 | 0.208 ± 0.025     |
| 500         | 2.534 ± 0.157 | 0.640 ± 0.046 | 0.201 ± 0.021 | 0.054 ± 0.008 | 0.661 ± 0.053 | 0.337 ± 0.033 | 0.160 ± 0.016 | 0.024 ± 0.004 | 0.221 ± 0.030     |
| 250         | 2.688 ± 0.189 | 0.641 ± 0.040 | 0.191 ± 0.019 | 0.054 ± 0.007 | 0.648 ± 0.054 | 0.344 ± 0.03 | 0.156 ± 0.019 | 0.026 ± 0.004 | 0.209 ± 0.034     |
| Control     | 2.694 ± 0.121 | 0.663 ± 0.056 | 0.195 ± 0.018 | 0.053 ± 0.008 | 0.646 ± 0.051 | 0.340 ± 0.038 | 0.154 ± 0.022 | 0.025 ± 0.003 | 0.213 ± 0.035     |

Note: The values represent the mean ± standard deviation of 10 rats. Relative organ weight (%) = (absolute organ weight/fasting body weight) × 100%. The values of the treatment groups did not differ statistically from the control according to one-way ANOVA at \( p < 0.05 \).
3.3. 90 d Subchronic Toxicity Study. During the exposure, no treatment-related deaths or abnormal clinical changes were observed. Body weights, weight gains, food intake, and food utilization rates of the treated groups were comparable with those of the control for both sexes \((p > 0.05;\) Figure 1, and Table 5). The absolute and relative weight of major organs of rats in the treated groups were similar to those of the control in both sexes \((p > 0.05,\) Table 6 and Table 7). In the ophthalmological examinations, no abnormal symptoms of the eyes were observed before and after the exposure.

For blood hematolgy and serum biochemistry, the treatment of water-extract sericin did not induce significant changes in all parameters when compared with the control \((p > 0.05;\) Table 8 and Table 9).

In urinalysis, SG, pH, urine color, and clarity of the treatment groups were comparable to those of the control \((p > 0.05,\) Table 10). The positive values of WBC, PRO, BLD, Cr, Ca, and MA were observed in the treatment groups, but were considered to be spontaneous changes since the occurrences of these positive parameters were relatively low \((\leq 30\%);\) and comparable to those of the control.

In the gross necropsy, no apparent symptoms of pathological lesions were recorded in all animals. Therefore, histopathological examinations were conducted only in the 1000 mg/kg treatment group and the control group. In the histopathological examinations, several mild histopathological changes in the liver and kidneys of rats were observed, as listed in Table 11. Nevertheless, these histopathological changes were minor and comparable to those of the control therefore was considered to be spontaneous lesions. For other organs or tissue, no treatment-related histopathological changes were observed.

4. Discussion

Concerning the utilization of silk sericin, a number of studies have focused on the structural information, biological activity, and pharmacological effects \([24, 25]\). However, further application of sericin in food-related industries has been restricted by the limited knowledge of safety. In the present study, the toxicological safety of water-extract sericin from silkworm \((Bombyx mori)\) cocoons was evaluated using different model approaches, including bacterial reverse mutation test, in vivo mammalian erythrocyte micronucleus test, the mouse spermatogonia chromosomal aberration test, and a 90 d repeated-dose oral toxicity study.

The amino acid composition of sericin was reported to be dependent on the extraction method used \([26, 27]\). In the

### Table 8: Hematology examination of rats treated with sericin extract for 90 days.

(a)

| Sex     | Dose (mg/kg) | HGB (g/L)   | RBC \((10^{12}/L)\) | PLT \((10^{9}/L)\) | HCT (%) | PT (s) | APTT (s) |
|---------|--------------|-------------|----------------------|-------------------|---------|--------|----------|
| Male    | 1000         | 156.0 ± 6.5 | 8.54 ± 0.37          | 982.4 ± 119.9     | 44.4 ± 1.6 | 9.3 ± 0.6 | 15.6 ± 1.7 |
|         | 500          | 155.2 ± 3.8 | 8.34 ± 0.26          | 946.6 ± 138.1     | 44.0 ± 2.1 | 9.5 ± 0.5 | 15.6 ± 1.1 |
|         | 250          | 154.4 ± 6.4 | 8.36 ± 0.32          | 958.6 ± 132.2     | 43.2 ± 2.0 | 9.3 ± 0.5 | 16.3 ± 1.9 |
| Control | 155.8 ± 5.5  | 8.49 ± 0.31  | 966.6 ± 149.0        | 44.1 ± 2.5        | 9.2 ± 0.4  | 16.1 ± 1.2 |
| Female  | 1000         | 154.8 ± 8.0 | 8.18 ± 0.35          | 946.8 ± 126.4     | 44.5 ± 2.1 | 9.2 ± 0.6 | 15.8 ± 1.7 |
|         | 500          | 149.6 ± 7.8 | 7.88 ± 0.36          | 998.8 ± 110.4     | 42.5 ± 2.1 | 9.2 ± 0.5 | 16.0 ± 1.6 |
|         | 250          | 155.4 ± 6.0 | 8.33 ± 0.30          | 953.4 ± 157.3     | 44.6 ± 2.1 | 9.4 ± 0.6 | 15.9 ± 1.0 |
| Control | 151.6 ± 9.3  | 8.01 ± 0.43  | 985.4 ± 169.9        | 43.7 ± 2.0        | 9.3 ± 0.4  | 15.7 ± 1.2 |

(b)

| Sex     | Dose (mg/kg) | WBC \((10^9/L)\) | LYM (%)   | NEUT (%) | MONO (%) | EO (%) | BASO (%) |
|---------|--------------|------------------|-----------|-----------|----------|--------|----------|
| Male    | 1000         | 7.81 ± 1.09      | 74.6 ± 5.3 | 18.7 ± 4.8 | 4.90 ± 0.82 | 1.79 ± 0.47 | 0.02 ± 0.06 |
|         | 500          | 8.19 ± 1.37      | 73.5 ± 4.8 | 19.1 ± 4.2 | 5.05 ± 0.83 | 1.55 ± 0.60 | 0.04 ± 0.08 |
|         | 250          | 8.72 ± 1.74      | 74.3 ± 5.3 | 19.2 ± 5.6 | 4.89 ± 0.63 | 1.61 ± 0.47 | 0.02 ± 0.06 |
| Control | 8.49 ± 1.38  | 73.8 ± 4.4       | 19.2 ± 4.6 | 5.14 ± 0.62 | 1.89 ± 0.45 | 0.04 ± 0.08 |
| Female  | 1000         | 7.74 ± 1.19      | 75.3 ± 3.4 | 18.2 ± 3.9 | 4.40 ± 0.55 | 2.05 ± 0.52 | 0.02 ± 0.06 |
|         | 500          | 8.19 ± 0.86      | 76.1 ± 5.2 | 17.1 ± 5.0 | 5.02 ± 0.78 | 1.77 ± 0.80 | 0.02 ± 0.06 |
|         | 250          | 8.06 ± 1.35      | 74.4 ± 5.2 | 18.5 ± 5.1 | 4.97 ± 0.62 | 2.11 ± 0.54 | 0.03 ± 0.09 |
| Control | 7.76 ± 1.20  | 75.5 ± 4.6       | 17.8 ± 4.5 | 4.82 ± 0.52 | 1.82 ± 0.60 | 0.04 ± 0.08 |

Note: The values represent the mean ± standard deviation of 10 rats. HGB: hemoglobin concentration; RBC: red blood cell count; PLT: platelet count; HCT: hematocrit; PT: prothrombin time; APTT: activated partial thromboplastin time; WBC: white blood cell count; LYM: percent of lymphocytes; NEUT: percent of neutrophils; MONO: percent of monocytes; EO: percent of eosinophils; BASO: percent of basophils. The values of the treatment groups did not differ statistically from the control according to one-way ANOVA at \(p < 0.05\).
D-amino acids are mainly involved in protein synthesis and metabolism, while D-amino acids have different and specific functions in different organisms [29]. Studies have shown that D-serine had significant nephrotoxicity in rats, inducing polyuria, diabetes, proteinuria, creatinine accumulation, and histopathological changes in the kidneys, while L-serine with the same doses did not induce nephrotoxicity ([30], Hiroshi et al. 2019). In the present study, there were no significant differences in blood urea nitrogen (BUN) and creatinine (Cr), urine protein, urine glucose, urine creatinine, kidney weight, and kidney relative weight between the treatment groups and the negative control group. In the meantime, no treatment-related histopathological changes were observed in the kidneys. These results indicated that water-extract sericin had no nephrotoxicity in rats.

Previous studies indicated that silk might be allergens leading to sensitization in humans [13, 31, 32]. It was reported that sensitization to silk was an independent predictor of childhood asthma in rural China [13]. Other studies indicated that there was a high burden of sensitization to silk allergen and occupational asthma among silk farmers [31, 32]. Eosinophils (EO) are recognized as important cells in the process of immune response and allergic reaction. In the present study, the number of eosinophils in the rats of the treatment groups was not significantly different from that in the negative control group in the 90-d subchronic toxicity test, indicating a little sensitization effect of water-extract sericin on rats. To evaluate the genotoxicity of sericin extract, a
The NOAEL of water-extract sericin was determined to be 1 g/kg/day for both male and female SD rats. These results indicated that water-extract sericin was nonmutagenic and nongenotoxic both in vitro and in vivo. To further systematically evaluate the safety of water-extract sericin for human was determined to be 1 g/kg/day (i.e., 0.7 g/day for a 70 kg person).

battery of test including the bacterial reverse mutation test, the mouse bone marrow micronucleus test, and the mouse spermatogonia chromosomal aberration test were conducted. The results indicated that sericin extract was nonmutagenic and nongenotoxic in both in vitro and in vivo. To further systematically evaluate the safety of water-extract sericin, a 90 d repeated-dose subchronic toxicity study was conducted. The results of the present study showed that water-extract sericin at doses of 250, 500, and 1000 mg/kg did not induce significant changes in body and organ weight, food intake, blood hematology and serum biochemistry, urine index, and histopathology of rats in both sexes. Basing on these results, the NOAEL of the water-extract sericin was determined to be 1 g/kg/day for both male and female SD rats, by oral gavage for 90 days. Using a nominal 100-fold (10-fold for differences between species and another 10-fold for differences within species) safety factor [33], the safe dose of water-extract sericin for human was determined to be 10 mg/kg/day (i.e., 0.7 g/day for a 70 kg person).

In previous studies, sericin proteins were reported to influence glucose and lipid metabolism in experimental animals [1, 28]. It was reported that 0.8% dietary sericin could reduce blood glucose levels in type 2 diabetic mice by improving the antioxidant capacity, increasing the insulin sensitivity and glycogen synthesis, and reducing the glucose neogenesis and lipid synthesis [1]. Another study reported that sericin treatment increased lipid excretion in feces in obese mice, suggesting potential antiobesity effects [28]. However, in the current study, a little effect of sericin extract on blood glucose and obesity-related parameters (e.g., body weight, cholesterol, and triglyceride) was observed. One possible explanation of this discrepancy is that the healthy rats are probably more tolerant to the effects of sericin as compared to the disease model animals. This explanation is supported by the results of a previous study showing that sericin significantly influenced the fasting blood glucose of type 2 diabetic mice while it had a little effect on the blood glucose of normal mice [1].

### Table 10: Urinalysis of rats treated with sericin extract for 90 days.

| Dose (mg/kg) | Number of abnormal color/clarity | SG (mg/kg) | pH | WBC | KET | NIT | URO | BIL | PRO | GLU | BLD | Cr | Ca | MA |
|-------------|--------------------------------|-----------|----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|
| Male        |                                |           |    |     |     |     |     |     |     |     |     |    |    |    |
| 1000        | 0                              | 1.014 ± 0.003 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| 500         | 0                              | 1.014 ± 0.004 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| 250         | 0                              | 1.014 ± 0.003 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| Control     | 0                              | 1.013 ± 0.004 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| Female      |                                |           |    |     |     |     |     |     |     |     |     |    |    |    |
| 1000        | 0                              | 1.014 ± 0.003 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| 500         | 0                              | 1.014 ± 0.004 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| 250         | 0                              | 1.014 ± 0.003 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |
| Control     | 0                              | 1.013 ± 0.004 | 7.6 ± 0.4 | 0   | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1  | 1  | 0  |

Note: The values of SG and pH represent the mean ± standard deviation of 10 rats. SG: specific gravity; WBC: white blood cell; KET: ketone body; NIT: nitrite; URO: urobilinogen; BIL: bilirubin; PRO: protein; GLU: glucose; BLD: occult blood; Cr: creatinine; Ca: calcium; MA: microalbumin. The values of the treatment groups did not differ statistically from the control according to one-way ANOVA at p < 0.05.

### Table 11: Histopathology examination of rats treated with sericin extract for 90 days.

| Organs              | Histopathological changes | Male 1000 mg/kg | Male Control | Female 1000 mg/kg | Female Control |
|---------------------|---------------------------|-----------------|--------------|-------------------|---------------|
| Inflammatory cell infiltration in portal duct areas | 2 | 1 | 1 | 2 |
| Liver               | Mild spotty necrosis of hepatocytes | 1 | 1 | 1 | 1 |
| Kidneys             | Mild fatty degeneration of hepatocytes | 1 | 2 | 2 | 2 |
|                     | Cell infiltration in renal cortex | 1 | 2 | 2 | 1 |

Note: The values represent the number of rats with histopathological changes in 10 rats of each group.

5. Conclusion

This study evaluated the safety of water-extract sericin from silkworm (*Bombyx mori*) cocoons using different model approaches, including a test of contact allergy in guinea pigs, a battery of genotoxicity study, and a 90 d subchronic toxicity study. Our results showed that water-extract sericin was nonmutagenic and nongenotoxic both in vitro and in vivo. The NOAEL of water-extract sericin was determined to be 1 g/kg/day for both male and female SD rats. These results indicated that water-extract sericin was of low toxicity in the experimental conditions of the current study and had the potential for application in food-related products.
Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Authors’ Contributions

Huiyan Qin and Jiehong Zhang contributed equally to this work.

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References

[1] X. Dong, S.-X. Zhao, X.-L. Yin, H.-Y. Wang, Z. G. Wei, and Y. Q. Zhang, “Silk sericin has significantly hypoglycaemic effect in type 2 diabetic mice via anti-oxidation and anti-inflammation,” International Journal of Biological Macromolecules, vol. 150, pp. 1061–1071, 2020.

[2] N. Kato, S. Sato, A. Yamanaka, H. Yamada, N. Fuwa, and M. Nomura, “Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity,” Bioscience, Biotechnology, and Biochemistry, vol. 62, no. 1, pp. 145–147, 2014.

[3] Y. Takasu, H. Yamada, and K. Tsubouchi, “Isolation of three main sericin components from the cocoon of the silkworm, Bombyx mori,” Bioscience Biotechnology and Biochemistry, vol. 66, no. 12, pp. 2713–2718, 2014.

[4] K. Tsubouchi, Y. Igarashi, Y. Takasu, and H. Yamada, “Sericin enhances attachment of cultured human skin fibroblasts,” Bioscience, Biotechnology and Biochemistry, vol. 69, no. 2, pp. 403–405, 2005.

[5] P. Aramwit, S. Kanokpanont, T. Nakpheng, and T. Srichana, “The effect of sericin from various extraction methods on cell viability and collagen production,” International Journal of Molecular Sciences, vol. 11, no. 5, pp. 2200–2211, 2010.

[6] B. B. Mandal and S. C. Kundu, “Self-assembled silk sericin/po-loxamer nanoparticles as nanocarriers of hydrophobic and hydrophilic drugs for targeted delivery,” Nanotechnology, vol. 20, no. 35, p. 355101, 2009.

[7] Y. J. Ren, Z. Y. Zhou, B. F. Liu, Q. Y. Xu, and F. Z. Cui, “Preparation and characterization of fibroin/hyaluronic acid composite scaffold,” International Journal of Biological Macromolecules, vol. 44, no. 4, pp. 372–378, 2009.

[8] S. Terada, M. Sasaki, K. Yanagihara, and H. Yamada, “Preparation of silk protein sericin as mitogenic factor for better mammalian cell culture,” Journal of Bioscience and Bioengineering, vol. 100, no. 6, pp. 667–671, 2005.

[9] N. Limpeanchob, K. Trisat, A. Duangiai, W. Tiyaboontchai, S. Pongcharoen, and M. Sutheerawattanannoda, “Sericin reduces serum cholesterol in rats and cholesterol uptake into Caco-2 cells,” Journal of Agricultural and Food Chemistry, vol. 58, no. 23, pp. 12519–12522, 2010.

[10] Y. Okuzaki, S. Kakehi, Y. Xu et al., “Consumption of sericin reduces serum lipids, ameliorates glucose tolerance and elevates serum adiponectin in rats fed a high-fat diet,” Bioscience, Biotechnology, and Biochemistry, vol. 74, no. 8, pp. 1534–1538, 2014.

[11] Y.-G. Li, D.-F. Ji, S. Chen, and G.-Y. Hu, “Protective effects of sericin protein on alcohol-mediated liver damage in mice,” Alcohol and Alcoholism, vol. 43, no. 3, pp. 246–253, 2008.

[12] T.-T. Cao and Y.-Q. Zhang, “Processing and characterization of silk sericin from Bombyx mori and its application in biomaterials and biomedicines,” Materials Science and Engineering C: Materials for Biological Applications, vol. 61, pp. 940–952, 2016.

[13] J. C. Celedon, L. J. Palmer, X. Xu, B. Wang, Z. Fang, and S. T. Weiss, “Sensitization to silk and childhood asthma in rural China,” Pediatrics, vol. 107, no. 5, article e80, 2001.

[14] P. Thitivuthikhit, P. Aramwit, and S. Kanokpanont, “Effect of Thai silk sericin and its extraction methods on L929 mouse fibroblast cell viability,” Advanced Materials Research, vol. 93-94, pp. 385–388, 2010.

[15] China Food and Drug Administration, National Food Safety Standards: determination of amino acids in foods, China Food and Drug Administration, 2016.

[16] G. Qin, Y. Gao, P. Wen et al., “Evaluation of the genotoxicity and teratogenicity of xylan using different model approaches,” Drug and Chemical Toxicology, pp. 1–7, 2020.

[17] J. Zhang, X. Zhang, X. Jia, L. Zhang, and J. Wang, “Analysis of the current situation and management countermeasures on food standards of procedures and methods for toxicological assessment,” Journal of Toxicology, vol. 29, no. 2, p. 113, 2015.

[18] China National Health and Family Planning Commission, National Food Safety Standards: bacterial reverse mutation test, p. 4, 2014.

[19] China National Health and Family Planning Commission, National Food Safety Standards: chromosome aberration test of mammalian bone marrow cells, p. 6, 2014.

[20] P. Wen, P. Zhao, G. Qin et al., “Genotoxicity and teratogenicity of seabuckthorn (Hippophae rhamnoides L.) berry oil,” Drug and Chemical Toxicology, vol. 43, no. 4, pp. 391–397, 2020.

[21] China National Health and Family Planning Commission, National Food Safety Standards: mouse spermatogonia or spermatocytes chromosomal aberration test, p. 8, 2014.

[22] China National Health and Family Planning Commission, National Food Safety Standards: 90-day oral toxicity study, pp. 13, 2015.

[23] G. Qin, P. Wen, Y. Wang et al., “Safety assessment of xylan by a 90-day feeding study in rats,” Regulatory Toxicology and Pharmacology, vol. 85, pp. 1–6, 2017.

[24] T. Takechi, Z. I. Maekawa, and Y. Sugimura, “Use of sericin as an ingredient of salad dressing,” Food Science and Technology Research, vol. 17, no. 6, pp. 493–497, 2011.

[25] M. H. Wu, J. X. Yue, and Y. Q. Zhang, “Ultrafiltration recovery of sericin from the alkaline waste of silk floss processing and controlled enzymatic hydrolysis,” Journal of Cleaner Production, vol. 76, pp. 154–160, 2014.
[26] R. I. Kunz, R. M. C. Brancalhão, L. de Fátima Chasko Ribeiro, and M. R. M. Natali, “Silkworm sericin: properties and biomedical applications,” *BioMed Research International*, vol. 2016, 19 pages, 2016.

[27] A. Veiga, F. Castro, F. Rocha, and A. L. Oliveira, “Recent advances in silk sericin/calcium phosphate biomaterials,” *Frontiers in Materials*, vol. 7, p. 24, 2020.

[28] R. I. Kunz, A. N. Capelassi, A. C. P. Alegre-Maller et al., *Sericin as treatment of obesity: morphophysiological effects in obese mice fed with high-fat diet*, Einstein (São Paulo), 2019.

[29] G. L. Marcone, E. Rosini, E. Crespi, and L. Pollegioni, “D-amino acids in foods,” *Applied Microbiology and Biotechnology*, vol. 104, no. 2, pp. 555–574, 2020.

[30] Z. Chen, *Nephrotoxic Study of D-Serine*, Shanghai Jiao Tong University, Master Thesis, 2013.

[31] G. Gowda, N. Sarkar, A. G. Ashwathnarayana et al., “A study on occupational asthma among workers of silk filatures in South India,” *Indian Journal of Occupational and Environmental Medicine*, vol. 18, no. 2, pp. 64–67, 2014.

[32] G. Gowda, A. H. Shivalingaiah, A. M. Vijayendra, N. Sarkar, C. Nagaraj, and N. R. R. Masthi, “Sensitization to silk allergen among workers of silk filatures in India: a comparative study,” *Asia Pacific Allergy*, vol. 6, no. 2, pp. 90–93, 2016.

[33] A. Renwick, “Structure-based thresholds of toxicological concern—guidance for application to substances present at low levels in the diet,” *Toxicology and Applied Pharmacology*, vol. 207, no. 2, pp. 585–591, 2005.