Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 1403-1406

Received: 06-09-2020
Accepted: 17-10-2020

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# *In vivo* studies on wound healing activity of sericin using excision model

**Priyadharshini Pachiappan, Prabhu S, Thangamalar A and Maria Joncy A**

**Abstract**
The present study was investigated on wound healing activity of sericin in rats using excision model. The results indicated that both sericin 4% and sericin 8% improved wound healing activity than control on 7$^{th}$ day and 14$^{th}$ day. Wound contraction was significantly increased in wounds treated with sericin when compared to wounds treated with neosporin and untreated wounds, which indicated that sericin had shown more wound healing property than control. Also observed that there were no rashes on the skin treated with sericin when compared to control wound. Appearance of inflammation in wound areas were observed in all groups on first day of treatment. But the inflammation in sericin treated group was gradually reduced from 4$^{th}$ day onwards which proved that sericin is a very good biomaterial agent for wound healing. Histological analysis revealed that the tissue section of wound control showed only re epithelialization and there was no proliferation of fibroblasts and evenness of epidermis was seen in control when compared to sericin treated wounds which showed evenness of epidermis and increased number of fibroblasts.

**Keywords:** Sericin, biomaterial, wound contraction, fibroblast

## Introduction
Fibroin and sericin are the proteins produced by silkworm, *Bombyx mori*, belongs to the order Lepidoptera and family, Bombycidae family. The fibroin is reeled into raw silk and used in the manufacture of different types of yarns and silk fabrics [1, 2]. The sericin is usually discarded by the reeling industry into surrounding areas and create many environmental issues. It is estimated that 50,000 tons of sericin is mostly discarded through reeling water [3], which generates a high chemical, biological oxygen demand and contamination of water [4].
Sericin comprises 25 to 30% of the cocoon weight [5, 6]. Structurally, sericin is a globular protein consisting of random coil and $\beta$-sheets. Changes in random coil structure for $\beta$-sheet easily occur in response to mechanical stretching properties, moisture absorption, and temperature, where the sol-gel transition occurs. At lower temperatures, the solubility is reduced and the random coil structure is converted into $\beta$-sheets, resulting in the formation of a gel. Macromolecule of hydrophilic character of sericin is composed of 18 amino acids with strong polar groups such as hydroxyl, carboxyl, and amino groups [7] capable of forming crosslinks, copolymerizations, and combinations with other polymers [8]. Its organic composition is given by 46.5% carbon 31% oxygen, 16.5% nitrogen, and 6% hydrogen. Sericin consists of 30% serine content and approximately 15% glycine, 15% aspartic acid and 6% threonine content [9, 10].
The molecular weight of sericin ranges from 10 to 400 kDa depending on the extraction and processing methods [11]. For instance, *B. mori* silk sericin has 36.1% random coils structure that predominates other conformations such as turns (35.1%) and helix (28.8%). It has been proven that sericin does not cause immunological reaction with materials that have more widespread acceptance in biological applications. In addition, it has some unique characteristics, such UV resistance, antioxidation, moisture absorbance, and biocompatibility [12-16].
It has been determined that sericin decreases the peroxyl radical activity and tyrosinase[15] and suppresses lipid peroxidation[16]. Based on this back ground, the present study was investigated on wound healing activity of sericin in rats using excision model.

## Materials and Methods

Healthy, adult albino rats were procured from the central animal house PSG College of Pharmacy, Coimbatore, were used to study the various parameters of wound repair. The body weight of these animals varied from 100 to 200 gms and the age ranged from 8-10 months. All procedures of this study including animals were approved (Approval Number :442/IAEC/2019) by the Institutional Animal Ethical Committee, PSG College of Pharmacy, Coimbatore, Tamil Nadu, India. Five days prior to the experimentation procedures and animals were acclimatized by housing five rats per cage at , 24 °C ± 1 °C and 55% ± 5% humidity. Animals were allowed free access to food and water. The animals were anaesthetised with an intraperitoneal (i.p.) injection of cock tail preparation of anaesthesia (ketamine & Xylazine). A 2 × 2 $cm^2$ (~400 $mm^2$) open excision-type wound was created to the depth of loose subcutaneous tissue. The animals, after recovery from anaesthesia, were housed individually in properly disinfected cages and divided into the following four groups:

| Groups | Animals |
|---|---|
| Control + Wound | 6 |
| Wound + Neosporin | 6 |
| Wound + 4% Sericin | 6 |
| Wound + 8% Sericin | 6 |

Photographs of the wound area in each group of rats were captured on days 1, 7 & 14. The surface area of the wound was measured using Image J software on days 1, 4, 7& 14 in all groups. The area ($mm^2$) within the boundaries of each tracing was determined.

$$\text{Percentage Wound area} = \frac{\text{Unhealed wound area}}{\text{Day 0 wound area}} \times 100$$

### Histopathological examination

After fixation of the skin biopsy samples in 10% buffered formalin for 24 h, routine paraffin wax embedding procedures were used and samples were blocked. About 5-µm sections were cut and then stained with both hematoxylin and eosin. Sections were investigated at a magnification of 40× and examined the thickness of their epithelium.

### Determination of hydroxy proline

### Preparation of standard and sample

10µL of the 1mg/ml hydroxyproline standard solution was diluted with 90µL of water to prepare a 0.1mg/mL standard solution. 0,2,4,6,8 and 10µL of the 0.1mg/mL hydroxyproline standard solution was added into a 96 well plate, generating 0(blank),0.2,0.4,0.6,0.8 and 1.0µg/well standards.10mg of sericin was homogenized in 100µL of water and transferred to a pressure-tight vial with PTFE lined capor 2mL polypropylene tube and 100µL of concentrated HCL(12M)was added and hydrolyzed at $120^0$ C for 3 hrs. 10-50µL of supernatant was collected and transferred to 96 well plate. Both sericin and standards well were evaporated to dryness under vacuum and placed plates in a $60^0$ C oven to dry samples.

### Assay reaction

The following two assay reagents are stable for 2-3 hrs after preparation, and should be prepared after sample preparation, just prior to the start of the assay.100µL of chloramine T oxidative buffer mixture was added to each well and incubated at room temperature at 5 mins. Similarly, 100 µL of DMAB was added to each plate and mixed. Incubated the mixture for 90mins at 60 $^0$C and measured the absorbance at 550nm. ($A_{560}$). The calculation are as follows.

Concentration of hydroxy proline C = Sa/Sv

Sa = Amount of hydroxy proline in unknown sample(µg) from standard curve

Sv = Sample volume (µL) added into the wells

C = Concentration of hydroxy proline in sample

### Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean ± standard deviation (SD). Two way ANOVA using SPSS software was used to compare the mean values of each treatment. Significant differences ($p<0.05$) among the means were determined by using Duncan's multiple range Test.

## Results and Discussion

The percentage of wound in untreated and sericin treated groups were measured and the results are shown in Fig. 1. There was a significant difference in the healing of wound between wounds treated with sericin, standard Neosporin and control. The results indicated that both sericin 4% (77.0 ± 5.431) and sericin 8% (72.6 ± 1.140) improved wound size reduction than control group (81.0 ± 5.700) on 7$^{th}$ day. Similarly, 8% sericin showed wound reduction of 100 ± 0.00 per cent to 5.2 ± 2.588 on 14$^{th}$ day. Wound contraction was significantly increased in wounds treated with sericin when compared to wounds treated with neosporin (32.4 ± 5.727) and untreated wounds (51.6 ± 5.50), which indicated that sericin showed more wound healing property than control (Fig 2).

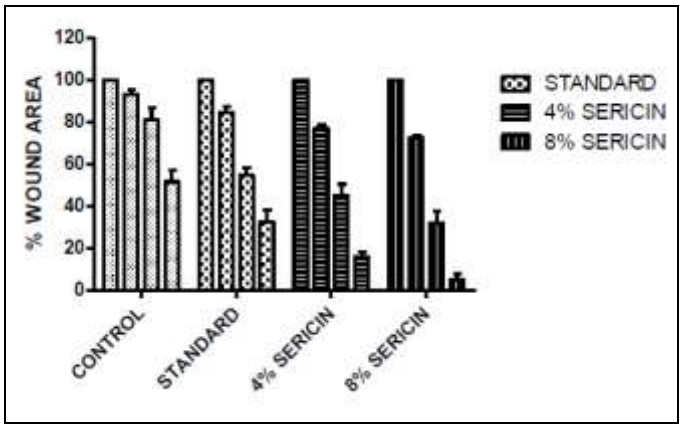


**Fig 1:** Percentage of wound reduction by sericin

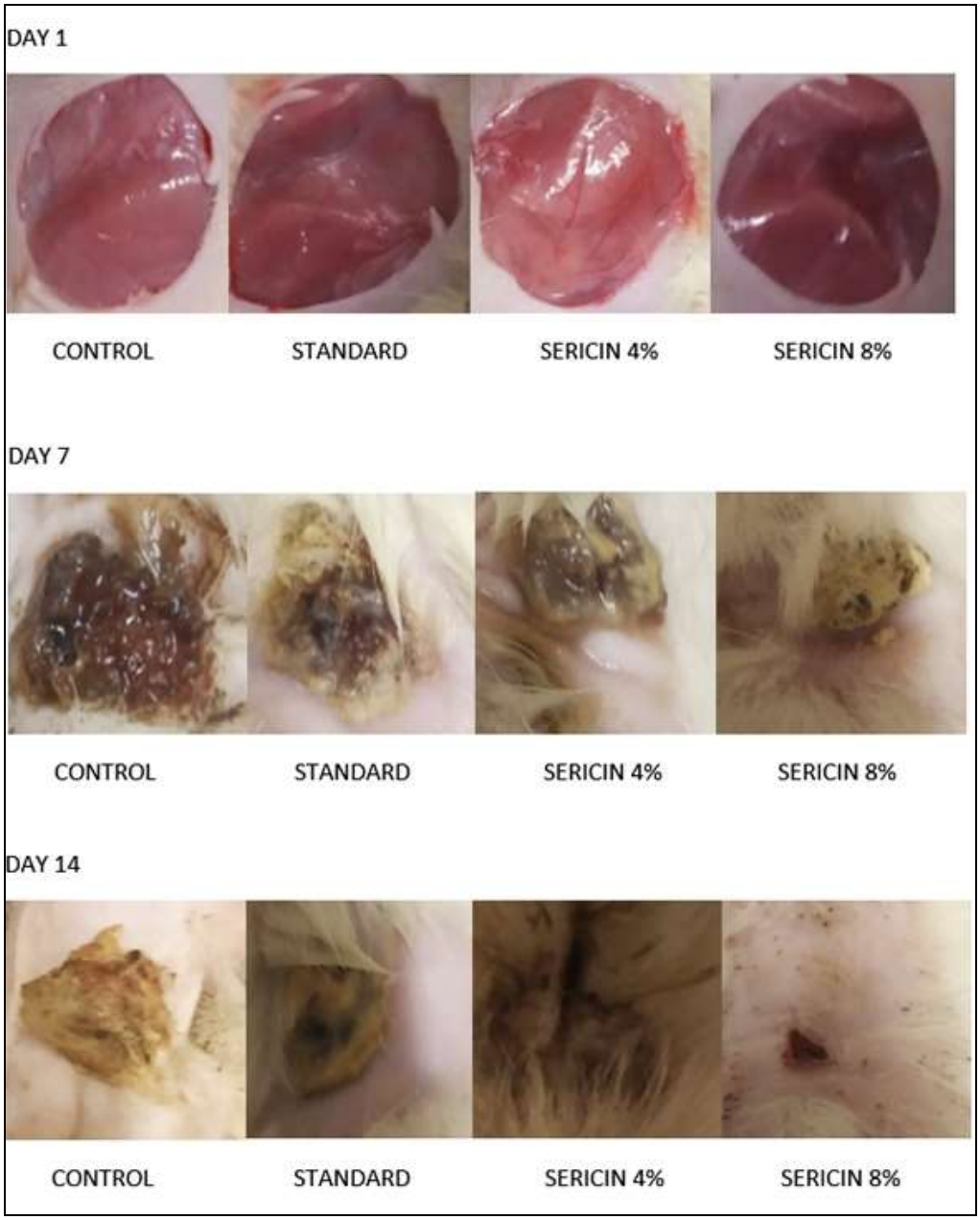


**Fig 2:** Wound size reduction of sericin

Aramwit and Sangcakul [17] evaluated the effect of sericin on wound healing and wound size reduction in rats. The results indicated that wound size was reduced in sericin treated groups on 15th day. *In vitro* and *in vivo* studies of biocompatibility and antioxidant potential of sericin demonstrated that sericin is immunologically inert and have proven the safety and open wide possibility of applications of sericin in biomedicine, such as cryopreservation, wound healing, antitumour effect and various metabolic effects in organic systems [18, 19].

In the present study, it was also observed that there were no rashes on the skin treated with sericin when compared to control wound. Appearance of inflammation in wound areas were observed in all groups on first day of treatment. But the inflammation in sericin treated group was gradually reduced from 4th day onwards which proved that sericin is a very good biomaterial agent for wound healing. The tissue section of wound control showed only re epithelialization**.** There was no proliferation of fibroblasts and evenness of epidermis was seen in control when compared to sericin treated wounds which showed evenness of epidermis and increased number of fibroblasts. Best recovery from the tissue damage was observed in 8% sericin.

The mitogenic effect of sericin on mammalian cells is well-established on fibroblasts and keratinocytes, which are majorly involved in the wound healing process [20]. Qi *et al.* [21] reported that materials containing sericin rich in serine residues with a higher number of hydrophilic groups might help in preventing prolonged inflammation of chronic wounds. Also, it is responsible for natural moisturization of skin and keeping the wound bed moist for accelerated healing outcomes. In another studies, it has been shown that sericin helps in fibroblast proliferation and inducing collagen secretion, thereby aiding in wound contraction at a faster rate [22].

**Hydroxyproline content**

Data were expressed as mean ± SD(n=5). Statistics were made using ONEWAY ANOVA followed by Tukey's Multiple Comparison Test. The hydroxyproline content was significantly increased ($P<0.001$) by both standard and sericin 8%w/w treatment group compared to diabetic control. There

was no statistical significance between hydroxyproline levels of standard and sericin 8% w/w, which represents the proportionate level of hydroxyproline between these groups.

## Conclusion

Sericin, natural biopolymer and glycoprotein is produced by the silkworm insect showed fast wound healing activity in rats. Histological examination also clearly revealed that more evenness of epidermis, proliferation of fibroblast and collagen secretion in sericin treated wounds when compared to control wounds. All these effects showed that sericin can be used as a compatible biomaterial in pharmaceutical industries.

## Acknowledgement

The authors are very thankful to Tamil Nadu Agricultural University for funding of this project work.

## References

1. Mondal M, Trivedy K, Kumar SN. The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn., - a review, Caspian Journal of Environmental Sciences. 2007;5(2):63-76.
2. Padol AR, Jayakumar K, Mohan K, Manochaya S. Natural biomaterial silk and silk proteins: applications in tissue repair. International Journal of Materials and Biomaterials Applications 2012; 2(4):19-24.
3. Gulrajani ML. "Sericin: a bio-molecule of value," in *Proceedings of the Souvenir 20th Congress of the* International Sericulture Commission. Bangalore, India. 2005, 21-29.
4. Fabiani C, Pizzichini M, Spadoni M, Zeddita G. Treatment of waste water from silk degumming processes for protein recovery and water reuse, Desalination 1996; 105(1):1-9.
5. Sehnal F, Akai H. Insect silk glands: their types, development and function, and effects of environmental factors and morphogenetic hormones on them. International Journal of Insect Morphology and Embryology; 1990;19(2):79-132.
6. Michaille JJ, Garel A, Prudhomme JC. The expression of five middle silk gland specific genes is territorially regulated during the larval development of *Bombyx mori*. Insect Biochemistry. 1989;19(1):19-27.
7. Zhang YQ. Applications of natural silk protein sericin in biomaterials, Biotechnology Advances 2002;20(2):91-100.
8. Dash B, Mandal B, Kundu SC. Silk gland sericin protein membranes: fabrication and characterization for potential biotechnological applications, Journal of Biotechnology. 2009;144(4):321-329.
9. Sorour J, Larik O, Ramadan A, Shalaby A, Osman S. Anatomical, histological and electron microscopy studies on the salivary glands of larvae of *Spodoptera littoralis* Boids. (Lepidoptera: Noctuidae). *Zoologische Jahrbucher. Abteilung f ¨ur Anatomie ¨ und Ontogenie der Tiere* 1990;120:251-271.
10. Yang M, Shuai Y, Zhou G, Mandal N, Zhu L, Mao C. Tuning molecular weights of *Bombyx mori* (*B. mori*) silk sericin to modify its assembly structures and materials formation, ACS Applied Materials & Interfaces 2014;6(16):13782-13789.
11. Kumar JP, Mandal BB. Antioxidant potential of mulberry and non-mulberry silk sericin and its implications in biomedicine, Free Radical Biology and Medicine 2017;108:803-818.
12. Kato N, Sato S. Yamanaka A. Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. Bioscience, Biotechnology, and Biochemistry 1998;62:145-47.
13. Dash R, Acharya C, Bindu PC, Kundu SC. Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts. BMB Reports 2008;41:236-41.
14. Manosroi A, Boonpisuttinant K, Winitchai S. Free radical scavenging and tyrosinase inhibition activity of oils and sericin extracted from Thai native silkworms (*Bombyx mori*). Pharmaceutical Biology 2010; 48:855-60.
15. Takechi T, Wada R, Fukuda T. Antioxidant activities of two sericin proteins extracted from cocoon of silkworm (*Bombyx mori*) measured by DPPH, chemiluminescence, ORAC and ESR methods. Biomedical Reports 2014;2:364-69.
16. Chlapanidas T, Faragò S, Lucconi G. Sericins exhibit ROS-scavenging, anti-tyrosinase, anti-elastase, and *in vitro* immunomodulatory activities. International Journal of Biological Macromolecules 2013;58:47-56.
17. Aramwit P, Sangcakul A. The effects of sericin cream on wound healing in rats. Bioscience, Biotechnology, and Biochemistry 2007;71:2473-77.
18. Sehnal F. Prospects of the practical use of silk sericins. Entomological Research 2008;38(1):1-8.
19. Lamboni L, Gauthier M, Yang G, Wang Q. Silk sericin: a versatile material for tissue engineering and drug delivery. Biotechnology Advances 2015;33(8):1855-1867.
20. Gilotra S, Chouhan D, Bhardwaj N, Nandi SK, Mandal BB. Potential of silk sericin based nanofibrous mats for wound dressing applications, Materials Science and Engineering 2018;90:420-432.
21. Qi C, Xu L, Deng Y, Wang G, Wang Z, Wang L. Sericin hydrogels promote skin wound healing with effective regeneration of hair follicles and sebaceous glands after complete loss of epidermis and dermis, Biomaterials Science 2018;6:2859-2870.
22. Aramwit P, Kanokpanont S, De-Eknamkul W, Srichana T. Monitoring of inflammatory mediators induced by silk sericin, Journal of Bioscience and Bioengineering 2009;107:556-561.