Current Trends in Pharmaceutical Research, 2022
Vol 8 Issue 2
ISSN: 2319-4820 (Print)
2582-4783 (Online)

Review article

A REVIEW ON PHYSICOCHEMICAL, STRUCTURAL AND BIOMEDICAL PROPERTIES OF SILK WORM SERICIN

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Abstract

The silkworm, Bombyx mori, produces sericin that surrounds and holds together the two fibroin filaments that make up the thread of the cocoon. A major advantage of reusing sericin that would otherwise be discarded by the textile industry is that it considerably decreases environmental issues and has a high scientific and commercial value.. The molecule's physicochemical properties are crucial in numerous biomedical applications. Sericin's molecular weight and amino acid concentration vary depending on the method used to extract the molecule and the silkworm's ancestry. Food and cosmetics can benefit from the antioxidant and hydrophobic amino acids included in sericin. In addition, sericin is plausibly used for wound healing, cell proliferation, protection from the harmful effects of sunlight and formulation of lotions and shampoos and moisturising agents. Low digestibility of sericin, together with the antioxidant properties, increases the use in the medical sector, such as the treatment of cancer, antibiotic, and anti-inflammatory agent.Computational studies on structural identification of sericin led to determine its functional activities.Additionally, sericin can be used for tissue engineering, cryopreservation, and cell growth, as well as a useful cryoprotectant.

Keywords: Sericin; biomedical; physicochemical; computational; biomedicine; tissue engineering.

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Introduction

Silk is a natural polymer with distinct physicochemical and mechanical properties, making it an appealing biomaterial for biomedical and pharmaceutical applications [1]. Silk is made up of two kinds of proteins: silk fibroin and sericin. Sericin accounts for approximately 20-30% of total cocoon weight. Serine and 18 amino acids are found in high concentrations in sericin, which includes essential amino acids [2]. Sericin is a major byproduct in the silk industry, and it is discarded in large quantities. However, studies have revealed that it is essential for biochemical activity. However, studies have discovered that it is essential for biochemical activity [2]. Sericin helps cells proliferate when it is used as a constituent of serum-free media for cell culture. The protein sericin, when utilised in its pure form and/or when blended in matrices, increases cell adhesion and proliferation [3]. The physicochemical properties of the molecule are responsible for a wide range of applications in biomedicine, and they are impacted by the extraction process and silkworm lineage, which can result in changes in the molecule's molecular weight and amino acid content. The presence of highly hydrophobic amino acids and their antioxidant potential enable sericin to be used in the food and cosmetic industries. Its moisturizing ability allows it to be used as a therapeutic agent for wound healing, stimulating cell proliferation, preventing ultraviolet radiation and making creams [4]. Anand et al. in 2009 explained the cocoonase and sericin phylogenetic study evidenced for homology between the protein structures although their origin is different [5].

In the Japanese mulberry silkworm, silk gland in the abdomen produces sericin, which is named for the fact that it is generated from silk of silkworm. *B. mori* has an undeveloped gland from the first to the fourth instar. During that time, it produces tiny amounts of silk, which is produced and utilised to attach the insect's tegument to the substrate. Weight of the insect might be anywhere between 20% and 40% of the overall weight at this stage of development. During the last stage of larval development, the silkworm *B. mori* produces large amounts of silk protein. The middle silk gland holds these proteins. After the fifth instar, the proteins are released through the anterior duct and spinneret, and then expelled through the anterior leg. Both fibroin and sericin are macromolecular proteins, each comprised of several chains and connected by disulfide bonds, making up the bulk of silk cocoons.

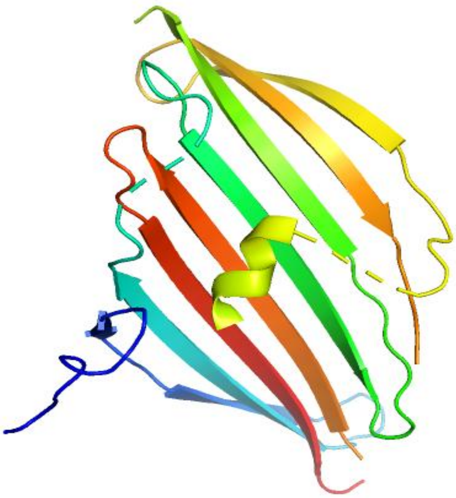

Figure 1. Silk fibroin heavy chain A (source: PDB ID: 3UA0, protein data bank).

The Sericin and Fibroin families are two separate protein families. The secreted glycoprotein has a heavy (H) chain of 350 kDa and a light (L) chain of 25 kDa, together with the relatively large polypeptide P25 of 27 kDa (Figure 1). The L and H genes are found on the 14th and 25th chromosomes. In this way, fibroin has a double structure; it has both microfibrils and fibrils grouped into microfibrils organised into fibrils bundles, all of which combine to create a single silk thread. The two filaments originate from silk glands, each with a sericin layer around it, and they combine to create the silk thread [6].

Biophysical properties of sericin

The fibroin fibres in sericin are held together by another protein known as the "gluelike" family, which connects to the central protein and binds to the fibres together. Fibre silk is stiff and harsh while in the presence of sericin, but turns soft and glossy when the sericin is removed. The spherical protein called sericin is made up of randomly coiled and β-sheet structures. Molecular motion, humidity, and temperature influence the random coil shape of β-sheet, causing the sol-gel transition to take place. If the water is hotter than 50–60°C, the protein becomes soluble. Solubility is decreased at lower temperatures, leading to random coil formation, and then into β-sheets, which leads to the development of a gel.

Complex hydrophilic macromolecule is formed of 18 amino acids with polar groups such as hydroxyl, carboxyl, and amino, and also has amine and other types of amide functional groups that are capable of crosslinking, polymerizing, and joining with other polymers. The carbon to oxygen ratio is 46.5 percent while the ratio of nitrogen to hydrogen is 16.5 percent. Sericin possesses several biological qualities such as biocompatibility, antibacterial activity, antioxidant, and moisturising, all of which are related to its biochemical characteristics.

Secondary structure analysis by Lee and co-workers in 2003 of silk sericin using circular dichroism, Fourier transform infrared spectroscopy, and X-ray diffraction have all been used on multiple occasions for indepth analysis [7]. Methyl alcohol dramatically changed the SS morphology from a sheet-like structure to a spherical fine particle type. Spectroscopic analyses revealed that molecular orientation and conformation of SS were not altered by the addition of methyl alcohol, X-ray diffraction patterns and infrared spectra revealed that the configuration of the molecule was unchanged [8]. Sericin is hydrophilic in nature due to the presence of large amount of polar amino acids along with hydroxyl groups which plays a major role in modifying the structure of sericin and key factors for biological activities [7]. Computational studies revealed that silk has outstanding mechanical properties that can be fully explained by structural effects, which involve geometric confinement of beta-sheet nanocrystals, combined with highly extensible semi-amorphous domains [9]. Silk sericin is a natural *Bombyx mori* macromolecular protein. Sericin may be recovered for other applications throughout several phases of the production of raw silk and textiles. The recovery of sericin also decreases the environmental effect of silk production. Because of its characteristics, sericin protein is helpful. The protein resists oxidation, is UV-resistant, and readily absorbs and releases humidity. Sericin protein can be cross-linked, copolymerized or fused with other macromolecular materials, in particular artificial polymers, in order to generate better materials. The protein is also used to enhance or coate natural and artificial fibres, textiles and products. The changed materials are helpful in degradable biomaterials, biomedical materials, article polymers, functional membranes, fibres and textiles.

Composition of sericin fibroin protein

The main characteristics of fibroin amino acid composition are that glycine, alanine, and serine have large amounts, accounting for over 80% of the total, and that phenylalanine is lacking, as phenylalanine has a polar side chain. Additionally, interesting is that the amino acid makeup of fibroin appears to be species-specific, making it possible to distinguish fibroin according to its origin. The main sequence plays an essential role in the definition of fundamental materials. Although its main structure is quite different, the heavy chain of *B. mori* fibroin and spider spidroins have essential commonalities. The two feature huge central core modular repeating units (Figure 4), bordered by non-repetitive amino-(NRN) [10, 11] and carboxy terminal domains (NRC) [12]. (Figure 3). *B. mori* fibroin's light chain contains a regular composition of amino acid and a non-repeating sequence. It merely plays a minor part in the fiber [13]. As can be seen in the various protein sequences, the arrangement of the repeating modular units can be considerably diverse. The whole amino acid sequence of the *B. mori* fibroin heavy chain, as a significant component of *B. mori* fibroin, consists of a highly repeated sequence (Gly-Ala)n motif and rich in tyrosine [14]. Molecular diversity in composition of silk fibroin is observed evidentially. In modular unit composition of a subset of the sequence motifs (Ala)n followed by multiple GGX motifs, where X represents a variable amino acid. The GGX motif is substituted in other cases by the GPGXX motif, which has more proline residues [15, 16]. The modular units are repeated hundreds of times in the central core of *B. mori* fibroin heavy chain and spider spidroins, determining the macroscopic characteristics of the fibres. For fibre formation, the highly conserved sequence of nonrepetitive amino- and carboxy-terminal domains is believed to be functional and is necessary.[17-21]. Furthermore, hydropathicity examination of these fibroins revealed a pair of hydrophobic and hydrophilic equivalents. The protein's core portion is mostly hydrophobic, but the nonrepetitive amino- and carboxy-terminal regions are more hydrophilic [22]. The firm threads are indicative of well-oriented β-sheet, the main secondary structure in silk fibres [23-25]. The first Raman spectrum of *B. mori* silk fibre has clearly shown the predominance of β-sheet, matching the results previously obtained from other techniques [26]. The overall quantity of β-sheet is about 50 percent for *B. mori* silk, which matched the percentage of the (Gly-Ala)n pattern [27, 28, 29]. Therefore, it is commonly believed that the *B. mori* fibroin is constituted of a highly repetitive (Gly-Ala)n sequence motif adopting antiparallel β-sheet shape, specifically silk II of the crystalline form. The β-sheet crystallite is the molecular network created by crosslinking β-sheet conformation of the molecular structures inside multiple adjacent silk protein molecules [30]. Aspartic acid, glycine, and serine are the three most prevalent amino acids in sericin, which is a highly hydrophilic protein. [31]. Sericin's high hydrophilicity is attributable in part to its high content of polar hydroxyl amino acids (45.8%), particularly serine (32%), which makes up the majority of its amino acid profile. [32]. Sericin's secondary structure fluctuates depending on the environment. Sericin is more commonly found in an amorphous random coil than in a structured β-sheet structure. Random coil configurations can easily be transformed into a β-sheet structure due to repeated moisture absorption and mechanical stretching [33]. Normally, sericin is partially unfolded, with 63 percent random coil and 35% β-sheet, without α-helix content [34].

Biomedical Applications of sericin

Some investigations have shown that the silk proteins [35–37] are activated in front of the immune system and, throughout history, hypersensitivity responses have been linked to sericin [4]. However, further research revealed that sericin has a differential immunological function. An intriguing discovery was provided by Panilaitis et al. [38] addressing the immune responses to silk and evaluated the inflammatory potential of intact silk fibres and their in vitro extracts. Silk fibres and soluble sericin have been identified as immunologically inert in murine macrophage cell cultivations, while insoluble fibroin parts can cause substantial TNF-α (tumournecrosis factor-α) release. Even while sericin does not activate the immune system itself, a strong macrophage in bacterial lipopolysaccharide is detected when it coats fibroin fibres. The authors therefore confirmed the minimal inflammatory potential of silk fibres and made them viable candidates. Tissue engineering is a technique that seeks to assist in the healing of injured tissue by the bio integration of cells inside porous materials. Such porous structures function as scaffolds, guiding the proliferation of new tissue and, as a result, assisting the cells in the process of regeneration [12]. Investigation on the effects of a 30 percent sericin supplementation in the diet of an animal model of colon carcinogenesis on the development of colon tumours [39]. The use of sericin for 115 days had no effect on body weight or food intake; nevertheless, there was a reduction in the incidence of colon adenoma as a result of the treatment. The antitumor action of sericin is accompanied by a decrease in cell proliferation rate, a drop in the production of oncogenes, and a reduction in oxidative stress. Sasaki et al. [40] discovered a comparable impact with sericin supplementation in a colon cancer model as they did with sericin supplementation. According to this study, supplementation with 3 percent of sericin for 5 weeks decreased the frequency of aberrant crypt foci, demonstrating the antitumor activity of sericin. Zhaorigetu et al. [41] supplemented the food of an animal model of colon cancer with 3 percent sericin for 28 days in order to better understand the mechanism involved in the antitumor activity of sericin in colon cancer. The administration of sericin decreased the number of intestinal aberrant crypt foci and the amount of lipid peroxidation in colonic mucosa in these mice by 36 percent and 34 percent, respectively, in these animals. The authors also discovered that the sericin is not digested and, as a result, the significant antioxidant capacity of the sericin that has not been digested and is present in the colon results in less oxidative stress and cancer in the organ.

***Insilico* studies on sericin protein**

Insilico study of sericin from silkworm is yet to unveiled thoroughly. In a study carried out by Patel and Panchal, 2013, depicted the modeling of sericin protein from the primary sequence. They have followed homology modeling based on template and structural validation using various web tools to exhibit the 3D shape of the sericin protein. In another study, it has been demonstrated in insilico molecular docking studies, sericin has a strong binding ability for COX-2 as well as ASIC-1a, MMP-2, and MMP-9 with exceptionally high binding energy, and that it has neuroprotective potential benefits against cerebral ischemia when administered intravenously [42]. Aside from that, it also has a strong antioxidant function and is organically derived, thus it has no or little adverse effects when used. The administration of sericin by dietary supplements as well as intravenously using drug delivery devices may thus have significant therapeutic promise in the treatment of cerebral stroke and the oxidative stress-mediated damage that results from the stroke. Its ability to function as a biomaterial scaffold in the field of tissue engineering and regenerative medicine for organ development demonstrates its usefulness in these fields [42]. To determine the optimum binding site for a tiny fragment of graphene on the N-termini domain of *Bombyx mori* fibroin, a molecular dynamics simulation-based docking simulation was performed by Tran et al., 2018. As an example, they presented the optimum binding position, for which the binding free energy is 54.8 KJ/mol, which indicates that there is a strong binding.

Xie et al., 2018, reported novel SEDSSEVDIDLGN sericin peptide (SP-GI) with α-d-glucosidase inhibitory action. SP-GI showed substantial inhibitor activity of 2.9 ± 0.1 μmol/L with an IC50 reversible and non-concurring inhibition of 1.0 ± 0.1 μmol/L. An interaction investigation with SP-GI has showed that it is bundled to α-d-glucosidase at a single location, resulting in changes of the secondary structure of α-d-glucosidase. This resulted in a static dampening of the inherent α-d-glucosidase fluorescence. The results of molecular docking revealed that the SP-GI binding site on d-glucosidase varied from the acarbose binding site, with hydrogen bonding and van der Waals forces serving as the primary drivers of binding.

Conclusion

Sericin is a naturally occurring *Bombyx mori* macromolecular protein that represents 25-30 percent of the total protein composition of silk. Silk protein is a kind of protein. From *B. mori*, silk gland, produces and secretes sericin, a natural polymer that is found in many plants and animals. Sericin is a water-soluble glycoprotein that accounts for 25 to 30% of the weight of the cocoon; it is distinguished by the presence of 18 amino acids, all of which have strong polar side groups (hydroxyl, carboxyl, and amino groups), as well as a high concentration of serine, aspartic acid, and glycine, which results in a hydrophilic protein. Sericin is produced by the digestion of serine, aspartic acid, and glycine. Due to the physicochemical properties of sericin, which are heavily influenced by the method of sericin isolation and the lineages of the silkworm, sericin's functional properties are affected, and it has the potential to be used in biomedical applications due to the fact that it is a biocompatible material. On the other hand, biomolecule Sericin has very precious antibacterial, UV resistant, oxidative and moisturising properties. Silk sericin is a natural, macromolecular silkworm, *Bombyx mori* that makes up 25-30 percent of the silk protein. However, sericin contains antibacterial, UV-resistant, oxidative resistant and moisturising characteristics, and is a biomolecular of significant significance.

Conflicts of Interest: Authors declare no conflicts of interest

Funding statement: This work has not been supported by any internal and external funding source

References

1. Kundu, Subhas C., et. al.; "Natural protective glue protein, sericin bioengineered by silkworms: potential for biomedical and biotechnological applications." *Progress in Polymer Science* 33.10,2008: 998-1012.
2. Mondal M, Trivedy K, NIRMAL KS. The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn.,-a review.
3. 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 Oct 2;2(4):19-24.
4. Altman GH, Diaz F, Jakuba C, Calabro T, Horan RL, Chen J, et. al.; Silk-based biomaterials. Biomaterials. 2003 Feb 1;24(3):401-16.
5. Nagaraju J, Goldsmith MR. Silkworm genomics–progress and prospects. Current Science. 2002 Aug 25:415-25.

6. Joseph B, Raj SJ. Therapeutic applications and properties of silk proteins from *Bombyx mori*. Frontiers in Life Science. 2012 Dec 1;6(3-4):55-6
7. Gulrajani ML. Sericin: A bio-molecule of value, Souveni 20th Congress of the international sericultural commission. Bangalore, India 15-18th Dec-2005. 21-29.
8. Fabiani C, Pizzichini M, Spadoni M, Zeddita G. Treatment of waste water from silk degumming processes for protein recovery and water reuse. Desalination. 1996 Jun 1;105(1-2):1-9.
9. Aramwit P, Kanokpanont S, De-Eknamkul W, Srichana T. Monitoring of inflammatory mediators induced by silk sericin. Journal of bioscience and bioengineering. 2009 May 1;107(5):556-61.
10. Kato N, Sato S, Yamanaka A, Yamada H, Fuwa N, Nomura M. Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. Bioscience, biotechnology, and biochemistry. 1998;62(1):145-7.
11. Wu JH, Wang Z, Xu SY. Preparation and characterization of sericin powder extracted from silk industry wastewater. Food chemistry. 2007 Jan 1;103(4):1255-62.
12. 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 Jan 1;19(2):79-132.
13. R. F. Chapman, *The Insect Structure and Function*, American Elsevier Publishing, New York, NY, USA, 1998.
14. Dhawan S, Gopinathan KP. Cell cycle events during the development of the silk glands in the mulberry silkworm *Bombyx mori*. Development genes and evolution. 2003 Sep;213(9):435-44.
15. SOROUR J. Anatomical, histological and electron microscopy studies on the salivary glands of larvae of Spodoptera littotalis Boisd.(Lepidoptera: Noctuidae). Zool. Jb. Anat.. 1990;120:251-71.
16. Akai H. The structure and ultrastructure of the silk gland. Experientia. 1983 May;39(5):443-9.

17. Zhang CD, Li FF, Chen XY, Huang MH, Zhang J, Cui H, et. al.; DNA replication events during larval silk gland development in the silkworm, *Bombyx mori*. Journal of insect physiology. 2012 Jul 1;58(7):974-8.
18. Tashiro Y, Morimoto T, Matsuura S, Nagata S. Studies on the Posterior silk gland of the silkworm, *Bombyx mori*: I. Growth of posterior silk gland cells and biosynthesis of fibroin during the fifth larval instar. The Journal of cell biology. 1968 Sep 1;38(3):574-88.
19. Ichimura S, Mita K, Zama M, Numata M. Isolation of the giant ramified nuclei of the posterior silk glands of *Bombyx mori*. Insect biochemistry. 1985 Jan 1;15(2):277-83.
20. Liu X, Zhang KQ. Silk fiber—molecular formation mechanism, structure-property relationship and advanced applications. Oligomerization of chemical and biological compounds. 2014 Jun 18;3:69-102.
21. Chen F, Porter D, Vollrath F. Structure and physical properties of silkworm cocoons. Journal of the Royal Society Interface. 2012 Sep 7;9(74):2299-308.
22. Matsuura S, Morimoto T, Nagata S, Tashiro Y. STUDIES ON THE POSTERIOR SILK GLAND OF THE SILKWORM, *BOMBYX MORI*: II. Cytolytic Processes in Posterior Silk Gland Cells During Metamorphosis from Larva to Pupa. The Journal of Cell Biology. 1968 Sep 1;38(3):589-603.
23. Kimura K, Oyama F, Ueda H, Mizuno S, Shimura K. Molecular cloning of the fibroin light chain complementary DNA and its use in the study of the expression of the light chain gene in the posterior silk gland of*Bombyx mori*. Experientia. 1985 Sep;41(9):1167-71.
24. Padamwar MN, Pawar AP. Silk sericin and its applications: A review.
25. 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 Jan 1;19(1):19-27.

26. 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 Jan 1;19(1):19-27.
27. SEHNAL F. Prospects of the practical use of silk sericins. Entomological Research. 2008 Nov;38:S1-8.
28. Okamoto H, Ishikawa E, Suzuki Y. Structural analysis of sericin genes. Homologies with fibroin gene in the 5'flanking nucleotide sequences. Journal of Biological Chemistry. 1982 Dec 25;257(24):15192-9.
29. Gamo TA. Biochemical genetics and its application to the breeding of the silkworm. JARQ (Japan). 1983.
30. Garel A, Deleage G, Prudhomme JC. Structure and organization of the *Bombyx mori* sericin 1 gene and of the sericins 1 deduced from the sequence of the Ser 1B cDNA. Insect Biochemistry and Molecular Biology. 1997 May 1;27(5):469-77.
31. Takasu Y, Yamada H, Tamura T, Sezutsu H, Mita K, Tsubouchi K. Identification and characterization of a novel sericin gene expressed in the anterior middle silk gland of the silkworm *Bombyx mori*. Insect Biochemistry and Molecular Biology. 2007 Nov 1;37(11):1234-40.
32. Michaille JJ, Garel A, Prudhomme JC. Cloning and characterization of the highly polymorphic Ser2 gene of *Bombyx mori*. Gene. 1990 Feb 14;86(2):177-84.
33. Kludkiewicz B, Takasu Y, Fedic R, Tamura T, Sehnal F, Zurovec M. Structure and expression of the silk adhesive protein Ser2 in *Bombyx mori*. Insect biochemistry and molecular biology. 2009 Dec 1;39(12):938-46.
34. Kataoka K, “The solubility of sericina in water,” *Nippon Sanshigaku Zasshi*, vol. 46, no. 3, pp. 227–230, 1977.
35. Zhu LJ, Yao J, Youlu L. Structural transformation of sericina dissolved from cocoon layer in hot water. Zhejiang Nongye Daxue Xuebao. 1998;24(3):268-72.
36. ZHU LJ, ARAI M, HIRABAYASHI K. Sol-gel transition of sericin. The Journal of Sericultural Science of Japan. 1996 Aug 27;65(4):270-4.

37. Zhang YQ. Applications of natural silk protein sericin in biomaterials. Biotechnology advances. 2002 May 1;20(2):91-100.
38. Dash BC, Mandal BB, Kundu SC. Silk gland sericin protein membranes: fabrication and characterization for potential biotechnological applications. Journal of Biotechnology. 2009 Dec 1;144(4):321-9.
39. Rajput SK, Singh MK. Sericin-a unique biomaterial. IOSR Journal of Polymer and Textile Engineering. 2015;2(3):29-35.
40. Aramwit P, Keongamaroon O, Siritientong T, Bang N, Supasyndh O. Sericin cream reduces pruritus in hemodialysis patients: a randomized, double-blind, placebo-controlled experimental study. BMC nephrology. 2012 Dec;13(1):1-1.
41. Sasaki M, Yamada H, Kato N. Consumption of silk protein, sericin elevates intestinal absorption of zinc, iron, magnesium and calcium in rats. Nutrition Research. 2000 Oct 1;20(10):1505-11.
42. Zhaorigetu S, Sasaki M, Watanabe H, KATo N. Supplemental silk protein, sericin, suppresses colon tumorigenesis in 1, 2-dimethylhydrazine-treated mice by reducing oxidative stress and cell proliferation. Bioscience, biotechnology, and biochemistry. 2001 Jan 1;65(10):2181-6.
43. Maurya K, Pandey AK. Molecular docking study for evaluation of neuroprotective potential of sericin against cerebral stroke and exploring its biomaterial properties. Biomedical Research Journal. 2019 Jan 1;6(1):17.

How to cite this article:

Das P. A Review on Physicochemical, Structural and Biomedical Properties of Silk Worm Sericin, *Curr Trends Pharm Res*,2022;8 (2): 81-93.