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| Research Article

Study of the Chemistry and Biological Activity of Bombyx Mori Cocoons

Samandarov Doston Ishmukhammat ogli
PhD, docent, Tashkent State Technical University, Republic of Uzbekistan, Tashkent

Safarov Jasur Esirgapovich, Sultanova Shakhnoza Abduvakhitovna
DSc, prof., Tashkent State Technical University, Republic of Uzbekistan, Tashkent

Usenov Azamat Bakir ogli
PhD, Tashkent State Technical University, Republic of Uzbekistan, Tashkent

Abstract
Silk cocoon, naturally produced by silkworms, scientific name Bombyx mori L. (Lepidoptera, Bombycidae), is one of the well-known medicinal products with multiple therapeutic activities. The present study aims to review various aspects of silk cocoon, including chemical composition, traditional uses, biological and biotechnological activities, and toxicological issues, to provide scientists with a scientific source. For this purpose, electronic databases including PubMed, Scopus, Google Scholar, Web of Science, and traditional literature were searched as of December 2021. Silk is composed of 75–83% fibroin, 17–25% sericin, and 1–5% non-sericin components, including secondary metabolites, wax, pigments, carbohydrates, and other impurities. In recent years, the biological properties of silk cocoon, especially its major proteins, namely fibroin and sericin, have attracted special attention.

Keywords: Bombyx mori L., sericin, fibroin, therapeutic properties.

Introduction. Silk is a natural fiber produced by arthropods such as spiders, silkworms, and scorpions. Among these, the most widely used is silk obtained from the domesticated silkworm Bombyx mori L. (Lepidoptera, Bombycidae) [1]. According to historical records obtained in Northern China, commercial sericulture, which has existed for 5,000 years, is considered one of the oldest agricultural discoveries of mankind [2]. In addition, silk has traditionally been widely used in various cultures as a natural therapy or in yarn production [3].

The silk cocoon layer is composed of two main proteins: fibroin and sericin, where fibroin, a fibrous protein, is the central part, and sericin, a globular protein, is the adhesive part that coats the fibers and binds them together. In addition, impurities such as carbohydrates, salts, and waxes, known as "non-sericin" components, give the silk cocoon its water-repellent properties [4]. It is estimated that from the approximately 1 million tons of fresh cocoons produced worldwide, approximately 400,000 tons of dry cocoons are obtained, from which 50,000 tons of sericin are obtained [5].

Similarly, silk, especially silk fibroin, has been used in medical products, food supplements, new drug delivery methods, and the development of tissue engineering scaffolds for various organs due to its biocompatibility and a wide range of outstanding physicochemical properties [6, 7].

Ander Reizabal et al. [7] conducted scientific research on silk fibroin as a sustainable advanced material: material properties and characterization, processing and applications. Other scientific works were also studied at a high level and analyzed in depth in terms of chemical structure. Raw SF is a semi-crystalline protein (having a 65% crystalline region and a 30% amorphous region) [7], and its function is to provide structural stability and mechanical strength to the cocoons (Fig. 1). SS is an amorphous protein coating that binds the SF filaments together and acts as an adhesive to maintain the integrity of the cocoon. SF and SS are bound by specific interactions dominated by Van der Waals dispersion forces and H-bonds [7].

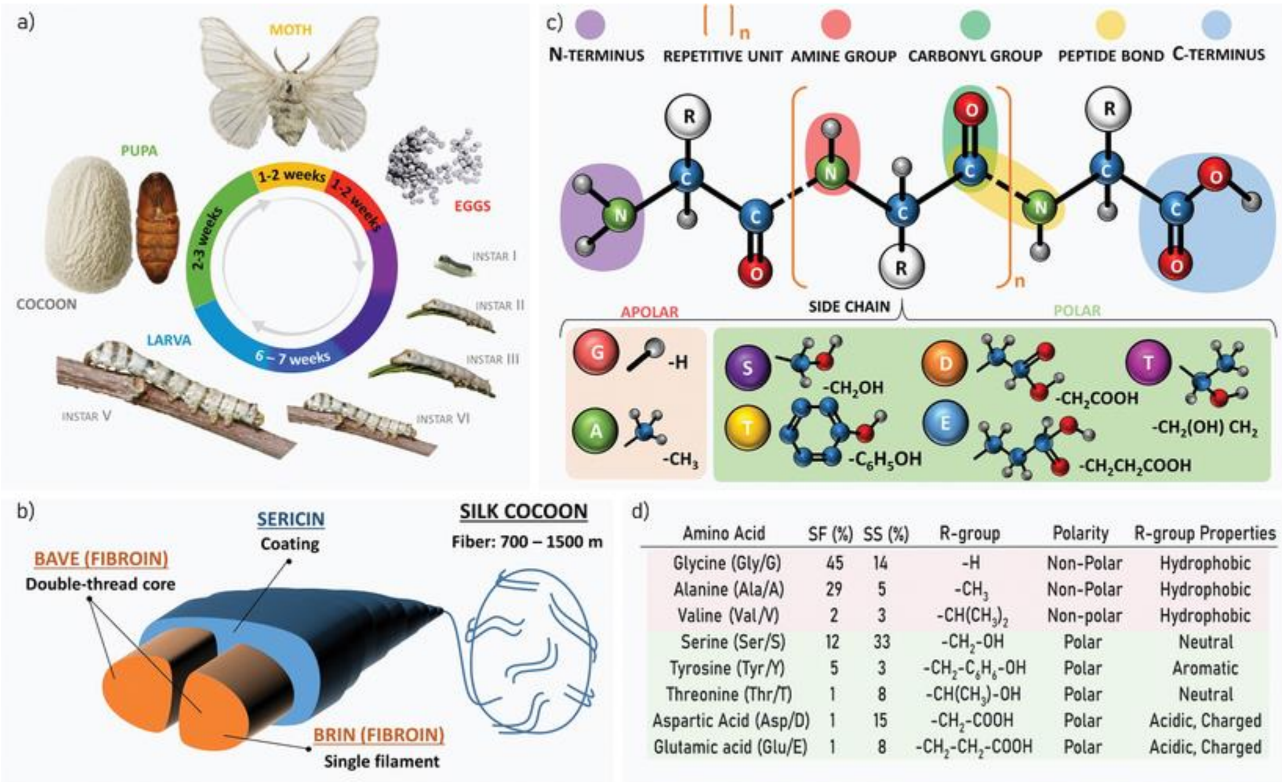

Fig.1. Structure of the cocoon.

a) Schematics of Bombyx mori lifecycle, including the time required to grow a silkworm and obtain a cocoon, the main resource from which silk fibroin is extracted. b) Bombyx mori cocoons graphical representation and main components (fibroin and sericin) arrangement to form the fibers leading to the cocoon. c) Graphical representation of silk fibroin (SF) molecular structure based on peptidic units repetition, and the main side chains (R-groups) providing specific functionality to the material. d) Silk fibroin (SF) and sericin (SS) main amino acid composition and properties. Adapted with permission

Materials and methods. A search of databases including PubMed, Scopus, Google Scholar, Web of Science, and traditional literature was conducted. Data were collected from 1966 to 2021 (through December). There were no language restrictions. The reference lists of retrieved articles were also reviewed for additional relevant studies. All published articles, as well as abstracts presented at conferences, were assessed.

Chemical composition of the silk cocoon. Regarding different types of silkworms in terms of food sources and component extraction methods, the cocoon is mainly composed of fibroin, sericin and other impurities (e.g. pigments, waxes, carbohydrates and phytochemicals), accounting for 75–83, 17–25 and about 1–4% of the cocoon components, respectively [7]. The amino acid residues found in silk proteins can be functionally summarized into three classes, including charged (aspartic acid), polar (serine) and hydrophobic (glycine) amino acids [8]. Silk fibroin isolated from the posterior labile gland of silkworms is composed of three proteins, namely H chain, low chain and P25 glycoprotein with molecular weights of 350, 26 and 30 kDa, respectively [9]. It is constructed from 18 of the 20 known amino acids, of which non-polar amino acids such as glycine, alanine, and valine make up 76% of the protein, while others, including polar amino acids, especially serine, make up only about 24% [4]. Silk sericin is a highly hydrophilic macromolecular family of glycoproteins produced by the middle silk gland, which makes up 25-30% of the cocoon weight [2]. The molecular weight of sericins ranges from 24 to 400 kDa, and like fibroin, it is composed of 18 amino acids possessing a significant number of polar functional groups such as hydroxyl, carboxyl, and amino groups, which allows for cross-linking, copolymerization, and coupling with various polymers [10]. Despite the amino acid composition of fibroin, silk sericin is primarily composed of polar amino acids such as serine and aspartic acid. Moreover, sericin exists in various secondary configurations, namely, β-sheet or random helix, which are easily changed by factors such as stretching forces, hydrolytic cleavage, and/or temperature [2]. However, despite studies aimed at understanding the composition and structure of proteins, there is still no consensus [11]. Phytochemicals are naturally occurring secondary metabolites with a wide range of varieties and health-promoting properties that are ubiquitous in nature [12]. In this study, various types of flavonoids, especially quercetin and to a lesser extent kaempferol, in both free and glycosylated forms [13], alkaloids [14] and coumarin derivatives [15], as well as aromatic esters, namely 3,4-dihydroxyphenyl-n-pentanyl ester and 2,3,4-trihydroxypenyl-n-pentanyl ester [16], were isolated from silk cocoons. Furthermore, two natural flavonoids containing an amino acid fragment were isolated for the first time from the cocoon husk of the silkworm B. mori L. [17]. Fig. 2 presents some of the main chemical components of silk cocoons.

Silk in Traditional Medicine and Pharmacological Effects. Historically, sericulture is considered an ancient agricultural discovery by humans. It existed 5,000 years ago in northern China, from where it spread to other parts of the world [2]. However, archaeological evidence suggests that silk originated more than a millennium earlier in South Asia, particularly in the Indus region, than in China. It is believed that Bombyx mandarina, a wild silkworm, was domesticated in China and evolved into the well-known B. mori.

Silk is a well-known polymer which has been used as a suture material since ancient times [3]. In Persian medical literature, the silk cocoon is called abrisham (abre sham), which is commonly known as abrisham muqriz (muqriz means to cut). Abrisham is one of the 60 natural remedies mentioned by Avicenna in his treatise on cardiovascular diseases. Abrisham with various ingredients from natural sources prepared in various famous Unani polypharmaceutical formulations; namely, Hameer-e-Abrisham Sada, Hamireh Abresham Hakim Arshad Wala, etc., are used to treat various cardiac and nervous diseases [16, 18]. Regarding ethnomedicinal use in South India, the ash and cocoon of silkworm have been used as a rejuvenating tonic and aphrodisiac [19]. In traditional Persian medicine, silk is widely used in the treatment of respiratory and cardiovascular diseases [18]. Silkworm cocoons of Mulberry carbonisata as a charcoal-based medicine in traditional Chinese medicine, used to relieve pain and stop bleeding, were first described in the Peaceful Sacred Benevolent Prescriptions, an official medical book of the early Chinese imperial Song Dynasty (960–1127 CE). For centuries, due to their exceptional biological activity and safety, carbonized silkworm cocoons have been widely used to relieve the symptoms of diseases such as skin ulcers, fever, fatigue, etc. [20]. In addition, the potential of silk cocoons for the health of biological systems has been noted, such as lowering blood pressure and heart palpitations, dilating bronchi and relieving asthma symptoms, and treating eye catarrh [16].

In a preclinical study by Mahmood T. et al. [21], the effects of pretreatment with different formulations of cocoon silk extract, particularly Hamira Abresham Sada and Hamira Abresham Hakim Arshad Wala, were examined in an in vivo model in Wistar rats suffering from isoproterenol-induced cardiotoxicity. The results showed that the silk formulations were able to suppress cardiac injury by reducing the levels of cardiac marker enzymes (such as CK-MB and troponin), heart weight to body weight ratio, the degree of myonecrosis and inflammatory cell filtration, as well as improving cardiac repair, cardiac antioxidant activity and lipid peroxidation. Similarly, the cardioprotective effect of pretreatment with ethanol extracts of silkworm cocoons (Abresham) in isoprenaline-induced myocardial infarction (ISO) was demonstrated by reduced inflammation, erythema, capillary dilation, and scar formation as assessed by histopathological examination compared to the ISO group. Improvements in various cardiac enzymes and the heart-to-body weight ratio were also reported. Furthermore, in Wistar albino rats administered doxorubicin, a potentially cardiotoxic drug, pretreatment significantly attenuated cardiac-related enzymatic changes and tissue damage, and increased antioxidant activity.

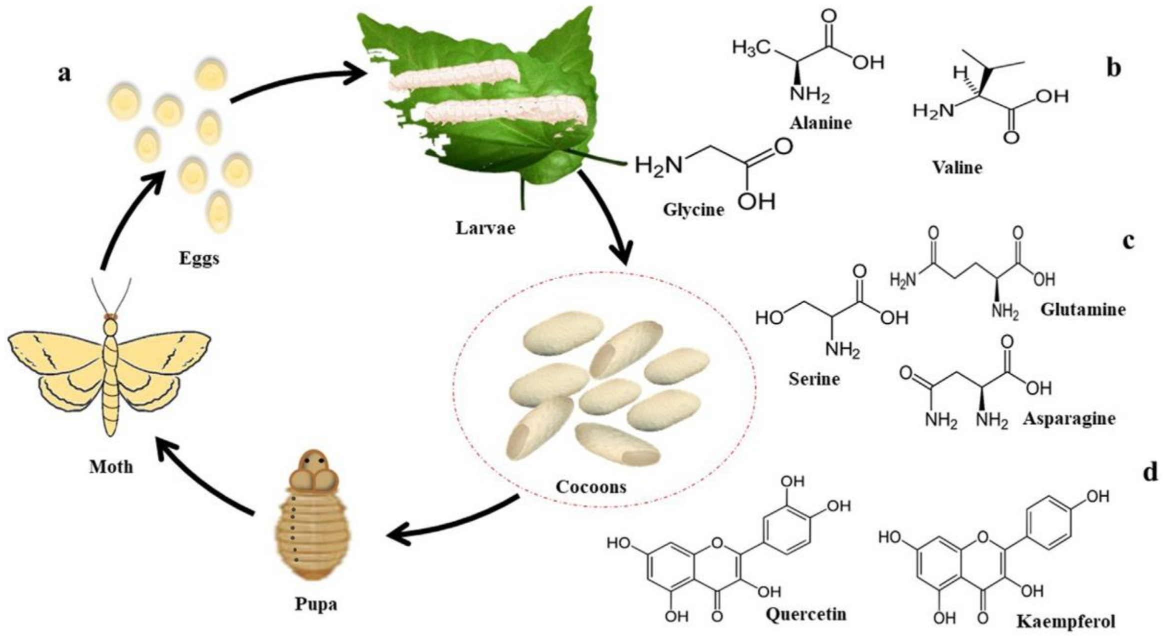

Fig. 2. The main chemical components of silkworm cocoons. a - Life cycle of silkworm; b - Essential amino acids found in silk fibroin; c - Silk sericin; d - Secondary metabolites found in silk cocoons.

Sericin has antihyperlipidemic and fat-burning effects, making it a potential therapeutic natural protein against obesity. Silk proteins with varying fibroin-to-silk ratios exhibited altered hyperlipidemia, atherogenic index, and body fat in mice fed a high-fat diet by increasing fecal lipid excretion, inhibiting lipogenesis, and regulating adipokine production [19]. In a study by Mir Mahdi A. et al. [20], the effect of B. mori cocoon extract was examined in a rabbit model of hyperlipidemia and atherosclerosis. The results demonstrated a significant improvement in lipid profile, a decrease in atherosclerotic plaque size, and an increase in body weight.

Sericin has long been known as an antioxidant. It has been shown to suppress lipid peroxidation in the intestine due to its antioxidant potential; thus, it can be used to suppress the occurrence and spread of colon tumors.[14] In a study using a mouse model of alcoholic liver injury, You-Gui et al. highlighted the ability of sericin to restore the reduced activities of antioxidant enzymes such as GSH, GSH-PX, CAT, and SOD to normal levels.[21] Furthermore, sericin also demonstrated strong hydroxyl, superoxide, and DPPH radical scavenging capacity, as well as antioxidant activity against linoleic acid peroxidation.[22] Similarly, sericin demonstrated antioxidant effects against hydrogen peroxide-induced oxidative stress in a feline fibroblast cell line by reducing the activities of catalase and thiobarbituric acid reductase (TBARS).

Conclusion. Silk cocoon is a well-known natural remedy with numerous therapeutic properties. These effects are sometimes similar to those suggested in folk medicine. One of the most important properties of silk cocoon in traditional medicine is its pronounced effect on the heart and its support for cardiovascular health. New research has also confirmed these effects and demonstrated silk's cardioprotective activity. In traditional medicine, silk cocoon is also known as a liver tonic, alleviating liver failure and liver weakness. Modern research also confirms its protective effect on the liver. Silk is used in traditional medicine as a tonic for general weakness, which, if scientifically proven, may be due to the protein matrix and the variety of amino acids found in sericin.

In the gastrointestinal system, silk cocoon is cited as a digestive aid. Recent studies have shown that silk can improve the condition of the gastric mucosa, but no studies have been conducted on its effects on digestion. Silk is said to heal wounds inside the eye, improve vision, and effectively support eye health. Silk's effect on corneal wound healing, mediated by the protein sericin, has been confirmed.

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