



Review article

## Identification and applications of neuroactive silk proteins: a narrative review

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### Abstract

In traditional medicine, natural silk is regarded as a cognitive enhancer and a cure for ameliorating the symptoms of heart disease, atherosclerosis, and metabolic disorders. In this review, general characteristics of both silk proteins, fibroin and sericin, extracted from silkworm *Bombyx mori* and their potential use in the neuronal disorders was discussed. Evidence shows that silk proteins exhibit neuroprotective effects in models of neurotoxicity. The antioxidant, neuroprotective, and acetylcholinesterase inhibitory mechanisms of silk proteins could prove promising in the treatment of neurodegenerative diseases. Owing to their excellent neurocompatibility and physicochemical properties, silk proteins have been used as scaffolds and drug delivery materials in the neuronal tissue engineering. These data support the potential of silk proteins as an effective complementary agent for central and peripheral neurological disorders.

**Keywords:** Fibroin; Neuroprotection; Sericin; Silk proteins

### Highlights:

- Silk proteins may hold promising alternatives for the treatment of neurodegenerative diseases.
- Silk proteins diminish oxidative stress damage and enhance enzymatic antioxidant activity.
- Silk proteins have potential to be used in neuroregeneration.

## Introduction

Silkworm, the larva or caterpillar of the domesticated silkworm (*Bombyx mori*), commercially viable in the production of silk. Silkworms depend upon the leaves of the mulberry trees as food (Ahsan et al., 2018). Excellent physical properties of silk, such as lightweight, high mechanical strength, and flexibility, make it appropriate to produce new biomaterials with application in tissue repair and drug delivery (Florczak et al., 2014). Silk has been commonly used for centuries as a suture material for wound treatment. Besides the vast application of silk in the textile industry, it has been widely used in traditional medicine as a home-made remedy for improving a broad range of health-related complications, particularly in the cardiac and nervous system (Nazmi et al., 2011). In Unani medicine, the silk is known as Abresham muqriz (muqriz means cut). Its cocoons are broadly used as an ingredient for several Unani formulations including Khamira Abresham Hakeem

Arshad Wala (KAHAW), Khameer-E-Abresham Sada, and Khameere Abresham Ood Mastagi Wala (Ahsan et al., 2018). Avicenna introduced silk (*Abresham*) as a strong exhilarant, cardiogenic, and cardioprotective substance (Hameed, 1983; Sina, 1995). Further, the Food and Drug Administration (FDA) has approved silk medical devices for sutures and as a support structure during reconstructive surgery. Also, silk has recently gained attention in developing degradable electronic and photonic implantable medical devices (Huang et al., 2018; Preda et al., 2013). Silk proteins have antidiabetic, anti-hypercholesterolemic and metabolic modulator, cardioprotective, anti-tumor, antioxidant, anti-bacterial, wound healing, cell proliferation, UV protection, and cryopreservation (Ahsan et al., 2018; Padamwar and Pawar, 2004).

The silkworm cocoon is a single continuous and lustrous raw silk strand with approximately 700–1500 m length, which protects silkworms against invasion by natural predators and environmental damage enhancing survival chance of silkworm (Zhang et al., 2013). Cocoon silk shows structural stability over

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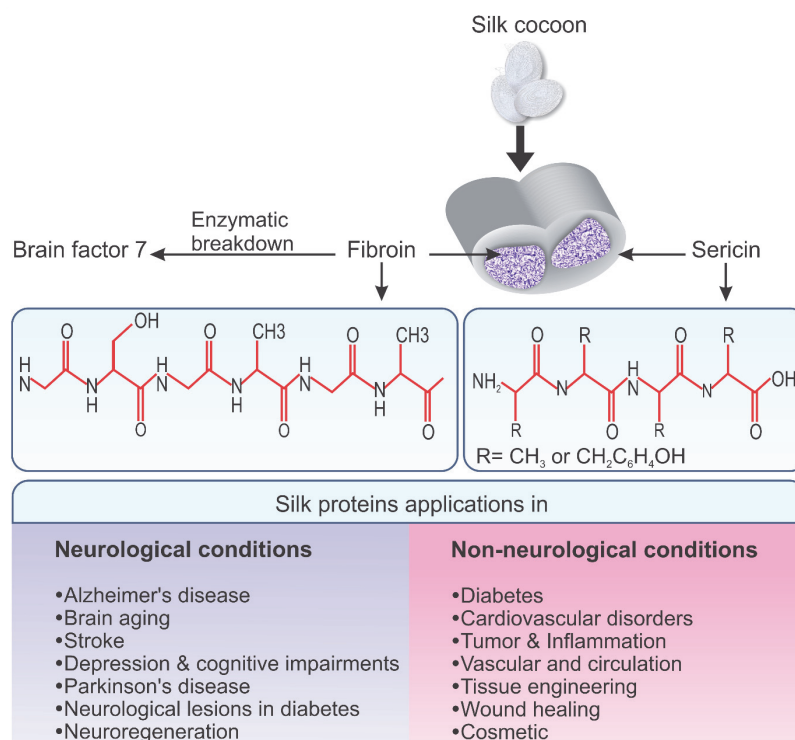
ing to the abundance of anti-protease proteins that improve the silk resistance (Zhang et al., 2017).

In spite of the extensive use of silk proteins, there is no comprehensive review regarding its neurological activities and applications. In this review, firstly, we will discuss the features of silk proteins, including fibroin and sericin, then their applications in the treatment of different neurological disorders and their possible mechanisms of action.

### Silk proteins

The silk is a natural fiber composed of two essential proteins, mainly fibroin and sericin. Other minor natural impurities of

raw silk include carbohydrate, inorganic salts, wax, and pigment (Mondal, 2007). Silk fibroin, the structural center of the silk, is double strand insoluble protein, linked by disulfide bonds, has a semi-crystalline structure that consists 70% of the cocoon shell of *B. mori*. The fibroin shell constitutes the core of silk and is covered by successive sticky layers of sericin (Fig. 1). The structure of fibroin mainly consists of the repeated amino acid sequence  $(\text{Gly-Ser-Gly-Ala-Gly-Ala})_n$  that form antiparallel beta sheets (Qi et al., 2017). The high glycine content (45%) contributes to the unique structure of silk and its resistant to breaking.



**Fig. 1.** A schematic theme of the review

Silk fibroin is biologically degraded by proteolytic enzymes such as chymotrypsin, actinase, and carboxylase. Fibroin is extracted from the silkworm cocoon by removal of sericin mainly by a thermo-chemical procedure known as degumming. Most of the sericin is removed in the course of raw silk production, increasing the softness, luster, smoothness, and whiteness of silk fibers (Gupta et al., 2013). Fibroin filament is composed of both amorphous and crystalline domains. Brain factor-7 (BF-7), a natural peptide extracted from *B. mori*, is prepared by enzymatic degradation of silk fibroin protein, in which raw silk is exposed to filtration and purification processes and proteolysis. Briefly, silk cocoon is washed, and the outer sericin protein is removed. To open up protein sheet, the remaining fibroin is heated in the presence of calcium chloride so protease enzymes can digest fibroin protein into smaller proteins (Chae et al., 2004; Kim et al., 2009). BF-7 is a natural brain-nourishing peptide extract in silk with a molecular weight range of 500–5000 daltons. BF-7 is a protein hydrosylate with a  $\beta$ -sheet structure rich in glycine and alanine, which are crucial amino acids for proper neurotransmitter function and cellular energy production. It has good solubility in water and other aque-

ous solutions. Due to its small size, BF-7 can readily cross the blood-brain barrier, where it can affect the brain cell and promote cognitive function (Kang et al., 2018).

Sericin (20–310 kDa) is a hydrophilic and globular glycoprotein that constitutes 20–30% of total cocoon weight. Indeed, sericin is a glue-like protein that joins two fibroin filaments to form silk yarn (Mondal, 2007). It contains 18 amino acids, 8 of them are essential for human, and a high content of hydrophilic amino acids, namely serine (30%) and glycine. Sericin has abundant polar side chains made of hydroxyl, carboxyl, and amino groups. Sericin exists mainly in a random coil and to a lesser extent in a  $\beta$ -sheet structure and is insoluble in cold water (Padamwar and Pawar, 2004). Its organic composition include 46.5% carbon, 31% oxygen, 16.5% nitrogen, and 6% hydrogen. The water solubility of sericin decreases by transforming from random coil to  $\beta$ -sheet structure. Isolation methods of sericin from silk thread affect its solubility, molecular weight, and gelling properties. Formerly, silk sericin was considered as a waste material of silk industry, but recent studies have proven its effectiveness in the pharmacological, biotechnological, and cosmetic applications such as wound

healing, antiwrinkle and antiaging (Mondal, 2007). Sericin is classified into three fractions upon their solubility and position within the layer of the cocoon, including sericin A, B, and C. These fractions build up the layers found on the silk fibroin. Sericin A composed the outer layer and is more soluble in warm water. Sericin B is the intermediate layer, and sericin C is the innermost layer, or the closest to the fibroin (Larin, 1951).

Several methods are used for extraction of silk sericin such as hot water, gel electrophoresis, enzymes (trypsin, alkaline protease or alkylase), spray-drying method, and aqueous urea. The physicochemical properties of sericin rely on the technique of sericin isolation (Padamwar and Pawar, 2004). Silk proteins can be processed in aqueous environments into various material formats, including films, sponges, electrospun mats, and hydrogels (Cao and Zhang, 2016; Lamboni et al., 2015). In

contrast to earlier studies (Celedón et al., 2001; Zaoming et al., 1996), recent studies showed that soluble sericin is immunologically inert and safe (Chlapanidas et al., 2013; Panilaitis et al., 2003; Terada et al., 2005), proposing it a promising candidate for various biomedical applications. Given its unique properties such as antioxidant, antidiabetic, antihyperlipidemic, anti-inflammatory, skin moisturizing, healing, antibacterial, antimicrobial protection, and antitumour (Table 1) (Aramwit et al., 2012; Kato et al., 1998b; Kunz et al., 2016), sericin has been widely used in biomedical, tissue engineering, regenerative medicine, and pharmaceutical and food industries. Sericin by cross-linking and mixing with other polymers can form a desirable scaffold with application in the pharmaceutical and cosmetic preparations (Aramwit et al., 2012).

**Table 1.** Summary of important non-neurological properties of silk proteins

Effects	Description	Protein type	References
	No nutritional effect		
Antidiabetic effects	Reduce blood glucose and glycosylated hemoglobin (HbA1c) levels, improve glucose tolerance and increase insulin levels Increase pancreatic $\beta$ cell mass Increase serum levels of the insulin-like growth factor-1 (IGF-1), growth hormone (GH) receptor, and adiponectin levels Decrease leptin and resistin levels Reduce the body fat content, triglyceride, and total cholesterol levels, atherogenic index, free unsaturated fats, phospholipids, very low-density lipoproteins (VLDL), low-density lipoprotein (LDL), and free fatty acid level	Fibroin and Sericin	Chen et al., 2013 Do et al., 2012 Okazaki et al., 2010 Rattana et al., 2017 Seo et al., 2011
Cardioprotection	Protect against atherosclerosis Protect against doxorubicin (Dox)-induced cardiotoxicity Prevent isoprenaline-induced myocardial damage and hypertrophy Improve cardiac functional recovery after myocardial infarction	Sericin	Ali and Arumugam, 2011 Goyal et al., 2010 Khan et al., 2014 Srivastav et al., 2013
Antitumor and anti-inflammatory effects	Decrease cell proliferation rate and oncogenes expression Induce apoptosis of tumor cells by triggering the activity of pro-apoptotic proteins Very low immunogenicity, reduce the release of interferon gamma (IFN- $\gamma$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and interleukin (IL)-1 $\beta$ Reduce percentage of CD8a and CD80 cells, and increase activity of natural killer (NK) cells	Sericin and Fibroin	Aramwit et al., 2009 Chlapanidas et al., 2013 Keawkorn et al., 2012, 2013 Promphet et al., 2014 Sasaki et al., 2000 Zhaorigetu et al., 2001
Vascular and circulation effects	Anticoagulant Vasodilation and relaxation of the smooth muscle of the artery wall	Sericin	Onsa-Ard et al., 2013 Tamada et al., 2004
Tissue engineering	Act as chief ingredient for bioimaging, drug delivery, tissue regeneration, and scaffold for tissue regeneration	Sericin and Fibroin	Aramwit et al., 2015 Kurland et al., 2014 Panico et al., 2018 Xiong et al., 2017 Zhang et al., 2015
Wound healing	Improve skin repair and collagen production Increase proliferation and migration of keratinocytes and fibroblasts into the injured area	Sericin	Aramwit and Sangcakul, 2007 Aramwit et al., 2010b Liang et al., 2007
Cosmetic effects	Increase hydration and elasticity of the skin, antiaging and antiwrinkle, and skin conditioner, cleaning with less irritation Protect surface hair from damage Protect nail from drying and fragility	Sericin	Ahsan et al., 2018 Oshika and Naito, 1998 Wong et al., 2014

### Application of silk proteins in neurological disorders

#### Alzheimer's disease

Studies on neurodegenerative disorders, such as aging and Alzheimer's disease (AD), appreciated the cognitive enhancing properties of sericin. As the most common type of dementia, the AD is characterized by the formation of neurofibrillary tangles and amyloid plaques, brain atrophy, and loss of neurons resulting in memory impairments (Arborelius et al.,

1999). Cognitive impairments in AD are generally associated with reduced synthesis of the acetylcholine (ACh) and overproduction of reactive oxygen species (ROS) in the cerebral cognitive-related structures, such as the hippocampus and cerebral cortex. Studies in AD have supported the importance of the antioxidants and acetylcholinesterase (AChE) inhibitor regimens for lessening of AD-related cognitive symptoms (Peera and Yellamma, 2015).

Pro-cognitive effect of BF-7 has been reported in both animal and human studies. Chae et al. (2004) have shown that BF-7 protects against amyloid-beta ( $A\beta$ )-induced apoptosis through the attenuation of ROS overproduction and inhibition of caspase-3 activity in the human neuronal cell. Pretreatment of neuroblastoma cells with milk fortified with fibroin BF-7 for seven days decreased  $A\beta$  neurotoxicity and increased cell survival rate (Choi et al., 2008). BF-7 was also shown to protect the human neuronal cell against scopolamine-induced neurotoxicity *in vitro*, and attenuate memory impairments induced by scopolamine *in vivo* (Iborra et al., 2004). Moreover, BF-7 (5 and 10 mg/kg/day for two weeks) increased ACh concentrations in the brain of rats treated with  $A\beta$  (Kim et al., 2005). Fibroin BF-7 has also been demonstrated to improve learning and memory impairments in  $A\beta$  induced AD model and to attenuate  $A\beta$ -induced apoptosis through decreasing intracellular calcium levels and ROS production, as well as inhibition of mitochondrial dysfunction and caspase activity (Lee et al., 2007).

Ethylcholine mustard aziridinium ion (AF64A) is a presynaptic cholinergic neurotoxin that causes alterations in mRNA expression and activity of choline acetyltransferase (ChAT), a necessary enzyme for ACh synthesis, resulting in a decrease of ACh release leading to memory and learning deficits (Walsh and Opello, 1994). Cha et al. (2017) have demonstrated that silk peptides treatment for five weeks in AT64A-induced AD model animals enhanced the expression of ChAT mRNA and increased ACh in the cerebrospinal fluid associated with improvement of cognitive deficits and suppression of hippocampal astrocytic activation.

#### Brain aging

D-galactose can induce many features of brain aging and has been widely applied to study the mechanisms of brain aging in rodents (Sadigh-Eteghad et al., 2017). Several studies have demonstrated that cognitive dysfunction, along with aging arises from oxidative stress and mitochondrial dysfunction. Research shows that sericin can reverse the memory impairments in D-galactose induced AD model through increasing ACh levels, inhibition of AChE activity, scavenging ROS, and the improvement of the antioxidant defense system in the hippocampus and cerebral cortex (Yellamma, 2014). It was reported that pretreatment (for five weeks prior to D-galactose injection) with two enzymatically modified silk peptides in a D-galactose induced aging model showed that silk peptides effectively inhibit AChE activity in the brain while enhancing the expression of the ChAT *in vitro*. Moreover, these silk peptides exhibited cognition-improving activities in both learning and memory tasks accompanied by the increase of ACh release and decreased the number of hippocampal activated astrocytes (Park et al., 2011). Moreover, two months administration of silk sericin (200 mg/kg/day) has been shown to improve learning and memory impairments in the Morris water maze in D-galactose-induced AD model in rats (Peera and Yellamma, 2013). Peera and Yellamma (2015) also reported that silk sericin improved D-galactose induced memory impairment through inhibition AChE activity and ultimately increasing ACh contents in the hippocampus and cerebral cortex, suggesting neuroprotective effects of silk protein on the cholinergic system. Another study showed positive effects of silk sericin on cognitive functions in D-galactose-induced AD model (Peera and Yellamma, 2016). Chen et al. (2011) have also demonstrated that sericin treatment improves learning and memory impairment induced by diabetes through attenuation of hippocampal nitric oxide (NO) contents and inhibition the nitric oxide synthase expression.

#### Anxiety and depression

Depression and anxiety are common psychiatric disorders worldwide. Preclinical and clinical evidence shows that neuroinflammation and oxidative stress in the structures involved in the regulation of mood and cognitive functions, hippocampus (HIP) and prefrontal cortex (PFC), play causative roles in the pathophysiology of these mood disorders (Banagozar Mohammadi et al., 2019; Haapakoski et al., 2015; Salehpour et al., 2019).

Recently, our group showed that sericin administration (100, 150, and 200 mg/kg/day, for 21 days) attenuated anxiety-and depressive-like behaviors induced by restraint stress accompanied by reduced serum corticosterone concentration in mice. Moreover, sericin could markedly diminish ROS and lipid peroxidation levels, restore mitochondrial membrane potential, and enhanced enzymatic antioxidant defense in the HIP and PFC. Furthermore, these results were associated with suppression of neuroinflammatory response and mitochondrial-dependent apoptosis in the HIP and PFC (Banagozar Mohammadi et al., 2019).

#### Stroke

Interruption of cerebral blood flow by a stroke can induce oxidative damage and neuroinflammation triggering brain dysfunction and cognitive impairments (Juurlink and Sweeney, 1997; Leker and Shohami, 2002). The protective effect of BF-7 on learning and memory deficits was also shown in the transient middle cerebral artery occlusion induced focal cerebral ischemia, which was associated with decreased size of infarcted areas and hippocampal neuronal cell death (Lee et al., 2005).

#### Nootropic

Chae et al. have reported that administration of BF-7 for one month improves learning and memory in healthy high school students (Chae et al., 2004). Clinical studies also showed that BF-7 improves short-term and long-term memories, cognitive flexibility, and attention in healthy school children (Kim et al., 2009; Kim et al., 2010a). A double-blind, placebo-controlled study reported that three weeks administration of BF-7 (200 and 400 mg) improved cognitive function in healthy adults in a dose-dependent manner (Lee et al., 2004). Silk fibroin BF-7 (400 mg/day for three weeks) was shown to enhance cognition through increasing glucose metabolism and blood supply to the brain structure involved in learning and memory including parahippocampal gyrus and medial temporal areas (Lee et al., 2004). Interestingly, a recent human study has found that three weeks administration of silk fibroin enzymatic hydrolysate (0, 280, 400, and 600 mg/day) dose-dependently improved verbal and visual memory and executive function with no adverse effects in healthy adults (Kang et al., 2018).

#### Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with progressive loss of dopaminergic neurons mainly in the substantia nigra, thereby in dopamine depletion, and the accumulation of  $\alpha$ -synuclein (Moore et al., 2005). L-3,4-dihydroxyphenylalanine (L-DOPA) is a dopamine precursor which has been used as a standard therapeutic for PD which reliefs movement impairments. However, long-term L-DOPA therapy can induce cytotoxicity by autooxidation to reactive species such as dopamine quinone, and overproduction of ROS and 6-hydroxydopamine (6-OHDA) (Asanuma et al., 2003). Tyrosinases are copper-containing enzymes that are involved in cancer and neurodegenerative diseases such as

PD. Over-expression of tyrosinase induces cell death by triggering ROS production in neuroblastoma cells (Hasegawa et al., 2008). Evidence also shows that tyrosinase is involved in both the hydroxylation of tyrosine to L-DOPA and the subsequent oxidation of L-DOPA to dopamine quinone, and thereby exacerbates L-DOPA neurotoxicity (Asanuma et al., 2003; Greggio et al., 2005; Sánchez-Ferrer et al., 1995). Previous studies have reported that silk sericin has an antityrosinase activity (Aramwit et al., 2010a; Kato et al., 1998b). Moreover, silk peptides were shown to protect against 6-OHDA-induced apoptotic neuronal death (Kim et al., 2011a). Interestingly, Kim et al. (2011b) have demonstrated that silk amino acid improves motor impairments in 6-OHDA induced PD model through the preservation of the dopaminergic neurons against 6-OHDA-induced neurotoxicity, and thus recovery of dopamine concentration.

Monoamine oxidases (MAO) are a family of enzymes that control the breakdown of monoamines such as dopamine, serotonin, and norepinephrine in the brain. MAO inhibitors prevent the removing of these neurotransmitter from the brain, which makes these brain chemicals more available. MAO-A inhibitors have been widely used in the treatment of depression and psychiatric disorder, and MAO-B inhibitors in the treatment of PD (Youdim and Riederer, 2004; Youdim et al., 2006). Interestingly, administration of silkworm extract (20 mg/kg/day for 14 days) in mice was reported to inhibit MAO-A activity, a dopamine-degrading enzyme, in the whole brain, cerebral cortex, and substantia nigra and MAO-B in the cerebellum and substantia nigra (Kang et al., 2005). Another study has shown that silkworm extract attenuated N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopamine depletion in the substantia nigra accompanied by inhibition of the activity of MAO-B in the substantia nigra, cerebellum, and whole brain (Kang et al., 2010). Therefore, based on above, it seems that silk peptides could be a promising candidate for the improvement of PD symptoms.

#### *Neurological lesions in diabetes*

Diabetes is a metabolic disorder that is associated with impaired glucose tolerance, hyperglycemia, insulin resistance, and dyslipidemia ultimately initiating inflammation and oxidative stress in different tissues, particularly in the brain (Tangvarasittichai, 2015). A growing body of preclinical and clinical evidence linked diabetes to neurodegeneration disorders and cognitive impairment (Verdile et al., 2015). The hippocampus is known to be more prone to deleterious effects of the chronic hyperglycemia associated with diabetes. Indeed, chronic hyperglycemia associated with diabetes can result in apoptotic cell death of hippocampal neurons and abnormal volumetric changes in this structure, which ultimately lead to cognitive dysfunction (Chen et al., 2012; Mijnhout et al., 2006). On the other hand, agents with antidiabetic effects can protect the brain against diabetes-induced neurodegeneration and learning and memory impairments (Alagiakrishnan et al., 2013; El-Mir et al., 2008; Patrone et al., 2014). In this regard, previous studies have confirmed antidiabetic effects of silk proteins, evident by reducing glucose levels and HbA1c, improving glucose tolerance and lipid profile, and increasing plasma insulin and adiponectin levels (Do et al., 2012; Jung et al., 2010; Kim et al., 2010b; Okazaki et al., 2010). Moreover, administration of sericin (2.4 g/kg/day for 35 days) can also reduce hippocampal neuronal apoptosis through promoting Akt signaling pathway and inhibition of pro-apoptotic BAD protein and its mRNA expression in type 2 diabetic rats (Chen et al., 2012).

The growth hormone (GH)/insulin-like growth factor 1 (IGF-1) axis is implicated in the growth and development of the CNS. Moreover, hippocampal expression of IGF-1 contributes to neuroprotection, synaptic regeneration, myelin formation, and neuroregeneration following hippocampal injury (Shangguan and Shi, 2007). In diabetes owing to GH resistance and reduced IGF-1 levels and expression of GH receptor, GH/IGF-1 axis undergoes abnormal changes, which results in abnormal blood glucose levels and accordingly aggravate the disease progression and complications (Chiarelli et al., 2004; Kim et al., 2006). Given that IGF-1 has an anti-apoptotic effect, a decrease in IGF-1 expression in the diabetic brains may induce hippocampal neuronal apoptosis and cognitive impairments (Cardona-Gómez et al., 2001; Feldman et al., 2002; Wine et al., 2009). However, administration of sericin (2.4 g/kg/day for 35 days) has been shown to attenuate hippocampal damage in diabetic rats through the improvement of the GH/IGF-1 axis (Chen et al., 2013). Furthermore, chronic administration of sericin has been demonstrated to decrease overexpression of heme oxygenase-1 in the hippocampal CA1 subfield and cerebral cortex of diabetic rats (He et al., 2011). In addition, chronic silk sericin can decrease nitric oxide (NO<sup>-</sup>) contents in the hippocampus of diabetic rats (Li et al., 2012). Sericin has also been reported to protect against diabetes-induced peripheral nerve injury, namely sciatic nerve, through increasing the expression of nerve growth factor and neurofilament protein expression, the primary marker identifying the function of synapses, in L<sub>4-6</sub> spinal ganglion and anterior horn cells. Moreover, sericin ameliorates nerve tissue ischemia and hypoxia induced by vasoconstriction and inhibition of neurotransmitter release due to excessive neuropeptide Y in diabetic rats (Song et al., 2013; Zhao et al., 2012).

#### *Antioxidant potential*

Oxidative stress damage is caused by an imbalance between free radicals formation and detoxification capacity of cells (Fridovich, 1986; Halliwell and Gutteridge, 2015), resulting in DNA fragmentation, lipid peroxidation, and protein oxidation and thereby cell death (Maes et al., 2009a, b, 2011). Due to large amounts of polyunsaturated fatty acids, high oxygen demand, and limited antioxidant capacity, brain tissue is vulnerable to oxidative stress disrupting neurotransmission and neuronal functions (Cobley et al., 2018; Hulbert et al., 2007; Salim, 2017; Shichiri, 2014). Oxidative stress plays a causative role in the pathophysiology of common neurodegenerative diseases, especially in PD and AD. Therefore, agents with the antioxidant property have been proposed for the prevention and treatment of neurodegenerative diseases (Kim et al., 2015).

The many medicinal advantages of silk can be attributed to its high antioxidant potential that confers protection against free radicals and oxidative damage. The mechanism underlying antioxidant property of sericin is related to its amino acid sequence and high content of hydroxyl amino acids such as serine and threonine, which enable chelating of free radicals and ROS. Importantly, the extraction methods may significantly affect the antioxidant activity of silk by affecting its molecular and functional properties such as contents of amino acids and polyphenol, molecular weight, and peptide length (Kumar and Mandal, 2017). In this regard, silk sericin extracted by urea displays the maximum antityrosinase activity, while alkali-degraded silk sericin cannot inhibit mushroom tyrosinase (Aramwit et al., 2010a). Kato et al. (1998a) have reported antioxidant effects of 0.3% sericin in the rat brain homogenate that was shown by decreased lipid peroxidation

assessed by thiobarbituric acid reactive substances (TBARS). Moreover, sericin can increase enzymatic antioxidant activity (Dash et al., 2008; Kumar and Mandal, 2017). Sericin at dose 0.25 g/kg has also been shown to protect the brain against oxidative stress induced by high-cholesterol diet in rats through attenuation the level of protein carbonylation and augmentation of the glutathione (GSH) levels in the brain tissue (Deori et al., 2016). Among the different mechanisms involved in A $\beta$ -induced neurotoxicity and apoptosis, ROS accumulation had received more attention (Hureau and Faller, 2009; Zawia et al., 2009). BF-7 was shown to attenuate A $\beta$ -induced apoptosis through decreasing ROS generation *in vitro* (Chae et al., 2004; Kim et al., 2005). Moreover, silk sericin was reported to enhance the antioxidant activity of superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) enzymes in the cerebral cortex and hippocampus of D-galactose received rats (Peera and Yellamma, 2016). A recent *in vitro* study has found that silk fibroin hydrosylate protected SH-SY5Y neuroblastoma cells and primary hippocampal neurons against A $\beta$ <sub>25-35</sub> induced apoptotic cell death and increased cell viability. The proposed mechanisms for its neuroprotection effects were attenuation of ROS accumulation, restoration of mitochondrial membrane potential, up-regulation of gene expression of SOD, inhibition the cleavage of pro-caspase 3 and the activation of MAPKs cascade, and suppression of protein phosphatase 2A (PP2A) phosphorylation and thereby inhibition of tau hyperphosphorylation (Xu et al., 2018). NO<sup>-</sup> is another cause for neuronal cell damages and its reaction with O<sub>2</sub> forms peroxynitrite (ONOO<sup>-</sup>) that is highly reactive (Brieger et al., 2012). Li et al. (2012) have reported that silk sericin can decrease nitric oxide content in the hippocampus of diabetic rats. BF-7 (10  $\mu$ M) was shown to protect neuroblastoma cell against FeSO<sub>4</sub>-induced apoptosis through attenuation of ROS accumulation (Lee et al., 2004). Based on the above, it seems that silk proteins have potential applications in the treatment of AD.

#### Neuroregeneration

The central or peripheral nerve injuries can lead to life-long disability, substantial work leave, and health care expenses. Therefore, nerve regeneration remains a major challenge in the clinic. Neural tissue engineering is a rapidly expanding research field aiming at nerve repair and regeneration (Gurgo et al., 2002; Ishihara et al., 2011).

Given their particular properties such as biocompatibility, low immunogenicity, slow degradation, flexibility, optimal mechanical strength, compatibility with sterilization methods, and FDA approval, silk proteins exhibit attractive potential applications in the neural tissue engineering and drug delivery (Hopkins et al., 2013; Li et al., 2013). Wang et al. (2015) used pure sericin hydrogel for ischemic neuronal tissue repair and demonstrated that sericin has neurotrophic and neuroprotective functions and prevents hypoxia and low glucose-induced cortical neuron death by modulation of the Bcl-2/BAX protein ratio and promotes the growth of primary neurons. In an *in vitro* study silk fibroin hydrolysate has been shown to protect neuronal cells against hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induced neurotoxicity by increased cell viability, which was attributed to its aromatic amino acids contents such as tyrosine and phenylalanine (Yeo et al., 2006). Moreover, silk fibroin has been proposed as an appropriate biomaterial for neural tissue engineering in the peripheral and central nervous system (CNS) therapies due to its good neurobiocompatibility. An *in vitro* study showed that administration of silk fibroin fiber to the cultured rat dorsal root ganglia (DRG) supports cell outgrowth

from DRG and silk fibroin extract fluid improves survival of Schwann cells from rat sciatic nerves with no remarkable cytotoxicity (Yang et al., 2007a). Three-dimensional (3D) silk fibroin with uniaxial multichannels directs the axonal adhesion and elongation of primary hippocampal neurons (Zhang et al., 2012). Furthermore, silk fibroin-based nerve graft has been shown to promote peripheral nerve regeneration in a rat model of sciatic nerve injury (Yang et al., 2007b). Tang et al. have also demonstrated that silk fibroin fibers promote the survival and growth of rat hippocampal neurons and administration of silk fibroin extract into the cell culture medium exerts no cytotoxic effects on the hippocampal neurons phenotype or functions (Tang et al., 2009). The tolerability of the implantation of silk fibroin hydrogels into the mice striatum has also been reported without inducing cell death or cognitive and sensorimotor deficits (Fernández-García et al., 2016). It has been demonstrated that electrospun silk fibroin nanofibers promote the growth and expansion of the Schwann cells, suggesting that silk fibroin can promote the regeneration of axons following axotomy and nerve-crush traumas (Hu et al., 2012). Moreover, in an *in vitro* model of traumatic optic neuropathy, electrospun silk fibers loaded with growth factor were reported to promote survival and neurite outgrowth of the retinal ganglion cells (Wittmer et al., 2011). A recent *in vivo* study has also shown that topical sericin combined with swimming exercise improved sciatic nerve injury in Wistar rats (Debastiani et al., 2019).

## Conclusions

Based on above, silk proteins play an effective role in the improvement of the brain function and provide protection against many neurodegenerative disorders. Moreover, silk proteins have biocompatibility, easy availability, and negligible immunogenicity, which make them a cost-effective and promising biomaterial in neuroregenerative medicine with vast potential (Fig. 1). Therefore, silk proteins can be a useful complementary strategy for physiological protection and functional recovery of the central and peripheral nervous system. Despite the long-established application of silk proteins as biomaterials and scaffolds, more *in vivo* studies are needed to verify the full neuroactive potentials of silk proteins. The fundamental and applied research will further elaborate the application of silk proteins in the neuroscience.

#### Conflict of interest

The authors have no conflict of interests to declare.

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