



# Hepatoprotective effects of sericin on aging-induced liver damage in mice

Yasin Bagheri<sup>1</sup> · Saeed Sadigh-Eteghad<sup>1</sup> · Ezzatollah Fathi<sup>2</sup> · Javad Mahmoudi<sup>1</sup> · Abdollah Abdollahpour<sup>3</sup> · Nasim Jalili Namini<sup>3</sup> · Zahra Malekinejad<sup>3</sup> · Kiarash Mokhtari<sup>3</sup> · Alireza Barati<sup>3</sup> · Soheila Montazersaheb<sup>4</sup>

Received: 29 March 2021 / Accepted: 14 September 2021 / Published online: 4 October 2021  
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

## Abstract

Aging is a physiological process in which there is a progressive decline of function in multiple organs such as the liver. The development of natural therapies, such as sericin, for delaying age-associated diseases is of major interest in this regard. Twenty-seven mice were divided into three groups of nine, including young control group (8 weeks, received normal saline), aged control group (24 months, received normal saline), and sericin-treated aged mice (24 months, received sericin at dose 100 mg/kg/day) via oral administration for 14 days. The liver enzymes in serum and oxidative stress markers in liver tissue were evaluated using spectrophotometric/ELISA methods. Apoptotic proteins, pro-inflammatory cytokines, COX2, JNK, and P-38 levels were assessed by western blot analysis.  $\beta$ -galactosidase expression was determined by a qRT-PCR method. The findings showed that 100 mg/kg of sericin reduced liver enzymes in aged mice. Antioxidant capacity in treated aged mice showed an improvement in all indexes in the liver tissue. Also, sericin administration declined pro-inflammatory markers to varying degrees in aged-treated mice. Sericin also increased the expression level of Bcl-2 and decreased the expression level of Bax and cleaved caspase-3. In addition, treatment with sericin suppressed protein expression of p-JNK and p-JNK/JNK. Collectively, these findings would infer that sericin administration may have a hepatoprotective effect in aging-induced liver damage in mice.

**Keywords** Sericin · Aging · Pro-inflammatory · Mice · Apoptosis · Liver

## Introduction

Aging is a natural process associated with a progressive decline in organ functions and accompanied by the development of age-related disease (Fathi and Farahzadi 2016). Some of the aging hallmarks include genomic and epigenetic alterations, telomere shortening, mitochondrial dysfunction, deregulated nutrient signaling, cellular senescence, and altered intercellular interactions (López-Otín et al. 2013;

Fathi, et al. 2019; Ebrahimi 2020; Farahzadi et al. 2016). The aging process increases the risk of inflammatory-related diseases, cancers, and neurodegenerative diseases in humans (Kirkwood 2005; Farahzadi et al. 2020; Fathi et al. 2019). Accordingly, delaying the aging process can hinder the onset and the progression of related disorders (Kaeberlein 2013; Mobarak et al. 2017). According to the overview of the literature, aging is associated with a deterioration of liver function (Mehdizadeh et al. 2017). It has been well documented that the liver has a key role in metabolic homeostasis via regulation of systemic energy metabolism, molecular biosynthesis, and clearance of xenobiotics and endobiotics (Rui 2011). Age-related alteration in liver function is closely linked to systemic susceptibility to age-related diseases (Kim et al. 2015; Basso et al. 1998). It has also been reported that structural and functional alterations in the liver are correlated with a dramatic reduction in mitochondria numbers (Wu et al. 2014). Mitochondria have a fundamental role in producing ATP for cellular demands.

✉ Soheila Montazersaheb  
smontazersaheb@gmail.com; montazersahebs@tbzmed.ac.ir

<sup>1</sup> Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>2</sup> Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

<sup>3</sup> Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

<sup>4</sup> Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Besides, mitochondria decline basal metabolic rate in the aging process (Jang et al. 2018).

It has been well established that the liver has superior role in the redox status by producing 90% of the circulating glutathione (GSH) (Jones 2008). In addition to this, aged hepatocytes are strongly associated with an increased reactive oxygen species (ROS) production (Kroemer et al. 2007; Green et al. 2011; Rezabakhsh, et al. 2017). Noteworthy, ROS can activate/inactivate a variety of signaling pathways and related mediators (Feizy et al. 2016; Saliani et al. 2017). All mentioned events result in progressive mitochondrial dysfunction, inflammatory state, and steatosis in the liver (Bejma et al. 2000). On the other hand, the liver has a crucial role in inflammatory status due to a reservoir of the resident macrophages such as Kupffer cells (Szabo and Csak 2012). According to several studies, aged hepatocytes can produce a higher level of cytokines such as interleukin 6 (IL-6), tumor necrosis factor 1- $\alpha$  (TNF $\alpha$ ), and interleukin-1 $\beta$  (IL-1 $\beta$ ) (Lasry and Ben-Neriah 2015), which ultimately lead to the development and progression of oxidative stress through ROS-mediated activity. This is mainly accompanied by activation and nucleus translocation of NF- $\kappa$ B as well as regulation of some genes such as COX2 (Iorio, et al. 2003; Wunderlich et al. 2010; Williams et al. 2008). Besides, TNF- $\alpha$  has ability to activate c-Jun amino-terminal kinase (JNKs) as a member of the mitogen-activated protein kinases (MAPKs) family (Kyriakis and Avruch 2001; Roux and Blenis 2004). Activation of JNK can stimulate several pathways which leads to phosphorylation of c-Jun, as a stimulator of inflammatory status (Schwabe and Brenner 2006). Furthermore, evidence declared that the impairment of the autophagosomal–lysosomal networks and  $\beta$ -galactosidase ( $\beta$ -Gal) can be predisposing factor in the onset and progression of many age-related diseases (Rajawat et al. 2009; Carmona-Gutierrez et al. 2016). Therefore,  $\beta$ -Gal has been used as an important biomarker to detect the aging process (Morgunova et al. 2015).

Growing evidence has shown that naturally derived products have benefits on the aging process via modulating biological and cellular processes (Ding et al. 2017). Sericin is a natural protein produced by the silkworm *Bombyx mori*. It has been demonstrated that sericin has ROS scavenging abilities as well as strong antioxidant capacity that inhibit lipid peroxidation activity (Deori et al. 2016; Seyedaghamiri 2021).

Although the anti-inflammatory, antioxidant, anti-cancer, and anti-tyrosinase activity of sericin has been well documented in many studies (Kunz, et al. 2016; Barajas-Gamboa et al. 2016), its effect on liver aging has not been established. In the present work, we study the possible hepatoprotective effect of sericin in parameters involved in apoptosis, inflammation, oxidative stress, and JNK/p38 signaling networks in aged mice.

## Materials and methods

### Animals

Nine male young (2 months) and eighteen aged mice (2 years) were used in the study. All experimental groups were housed in the cages under standard conditions ( $25 \pm 2$  °C, 70% humidity, and 12/12 h light/dark photoperiod schedule) and given free access to food and tap water. This study was confirmed by the ethics committee of Tabriz University of Medical Sciences (ethical code No. IR.TBZMED.VCR.REC.1399.334). All experimental procedures were conducted according to the guidelines of the National Institutes of Health (NIH) (publication No. 85–23, revised 1985).

### Treatment

The animals were divided into three experimental groups of nine individuals each, including young control (received normal saline, 2 ml/kg/day), aged control (received normal saline, 2 ml/kg/day), and sericin-treated aged mice (received sericin, 100 mg/kg/day). Sericin was purchased from Xian Lyphar Biotech Co. Ltd., Xian, China. Sericin was dissolved in normal saline just before treatment and administered orally for 14 days.

### Sampling

At the end of the treatment period, the animals were anesthetized by ketamine and xylazine (90 and 10 mg/kg, i.p., respectively), followed by blood sampling from the heart. Blood samples were centrifuged (3000 rpm for 10 min at 4 °C), and serum was separated and frozen until analyzed. The livers of animals were then cautiously removed, and samples were put into cryo-tubes and were frozen immediately in liquid nitrogen. The samples were kept at  $-70$  °C for further analysis.

### Liver functional assays

To evaluate liver function, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were examined in the serum sample using the commercial kits (Pars Azmoon, Iran) by spectrophotometry method (Olympus AU-600, Tokyo, Japan) based on the previously published study (Bagheri, et al. 2020).

### Evaluation of antioxidant capacity and lipid peroxidation

To investigate the antioxidant activity of sericin, the activity of superoxide dismutase (SOD), catalase (CAT), and

glutathione peroxidase (GPx), the levels of glutathione (GSH), and total antioxidant capacity (TAC) were determined in the liver tissues. Also, malondialdehyde (MDA) levels were assessed in the liver tissues using commercial kits (Zellbio, Biocore, Germany). The procedure was similar to the previous study by this group (Bagheri et al. 2021; Rahimi, et al. 2021).

### Liver protein extraction and western blot analysis of selected proteins

The western blotting analysis was used to determine the level of apoptosis (Bcl-2, Bax, and Caspase-3), pro-inflammatory markers (IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and Cox-2), and JNK and P38 in the liver tissues. Briefly, the liver tissues were homogenized with lysis buffer (RIPA with protease inhibitor). Then, protein concentration was measured by the Bradford method, and equal amounts of samples were subjected to SDS polyacrylamide gel electrophoresis (12.5%). After that, the bands were transferred onto PVDF membranes (PVDF) (Roche, UK). After blocking the blots, the membranes were incubated with primary antibodies overnight at 4 °C, according to the manufactures' instructions. Finally, after being washed with PBS, the membranes were exposed with secondary antibody (rabbit polyclonal) at room temperature for 2 h, visualized by chemiluminescence, an ECL Plus kit (Amesham Life Science Inc., Buckinghamshire, UK).  $\beta$ -Actin was served as a control for normalizing the optical density of the bands. The intensity of bands was calculated using ImageJ software (version 1.62, National Institutes of Health, Bethesda, MD, USA) (Montazersaheb et al. 2019; Tarhriz et al. 2019; Fathi et al. 2021).

### Evaluation of mRNA level by real-time PCR analysis

In order to evaluate  $\beta$ -Gal mRNA level, the qRT-PCR method was carried out. In brief, RNAs were extracted (Trisol) from all experimental groups and quantified by Picp-Drop (ND-1000, Thermo Scientific, USA). Due to the variation of RNA concentration in each sample, the concentrations were normalized and adjusted before cDNA synthesis. RNAs were subjected to reverse transcription using the RevertAid H Minus First-strand cDNA Synthesis Kit (Takara). After making cDNAs, samples were kept at -20 °C. The sequences of used primers are listed in Table 1. Real-time PCR was performed in a total volume of 20  $\mu$ L, consisting of SYBR green master mix 10  $\mu$ L (Takara), 1  $\mu$ L of each of the forward and reverse primers, 1  $\mu$ L of cDNA, and 6  $\mu$ L of nuclease-free water. The amplification profile was as follows: 94 °C for 4 min followed by 35 cycles of 94 °C for 10 s, 59 °C for 34 s, and 72 °C for 10 min. To quantify gene expression, we normalized the  $\beta$ -Gal gene to GAPDH as a housekeeping gene. Data were analyzed using

**Table 1** Primer sequences used for real-time PCR

Primer set	Sequence	T <sub>m</sub>
$\beta$ -Gal	Forward primer, CCCACTGCC	59
	Reverse primer, GCGGGGTAT CCGGAAGTAATG	
GAPDH NM_001256799	Forward primer, GTCTCCTCT	59
	Reverse primer, ACCACCCTG TTGCTGTAGCCAA	

ABI plus one to determine CT values and then calculated in relation to GAPDH CT by the  $2^{-\Delta\Delta CT}$  formula. The samples were run in triplicates (Tarhriz et al. 2018; Fathi, et al. 2020; Fathi, et al. 2020).

### Statistical analysis

Data are presented as mean  $\pm$  SEM. All results were analyzed by one-way ANOVA followed by a post hoc Tukey using GraphPad Prism 6.01 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was considered  $p$  value  $< 0.05$ .

## Results

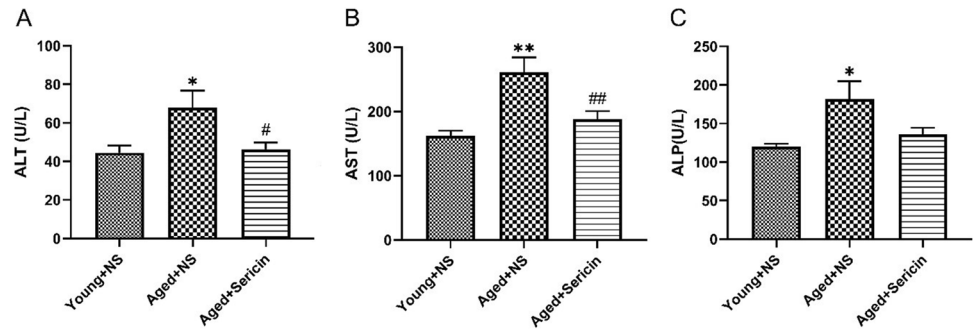
### The impact of sericin administration on liver function

The levels of liver function parameters such as AST, ALT, and ALP were measured in the serum samples of the animals. The results showed that the activity of ALT, AST, and ALP in the aged control was higher than young control ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.05$ , respectively). Compared to the aged control, sericin treatment significantly reduced ALT and AST levels ( $p < 0.05$  and  $p < 0.01$ , respectively). However, no significant alteration was observed in the level of ALP (Fig. 1) (Supplementary file 1).

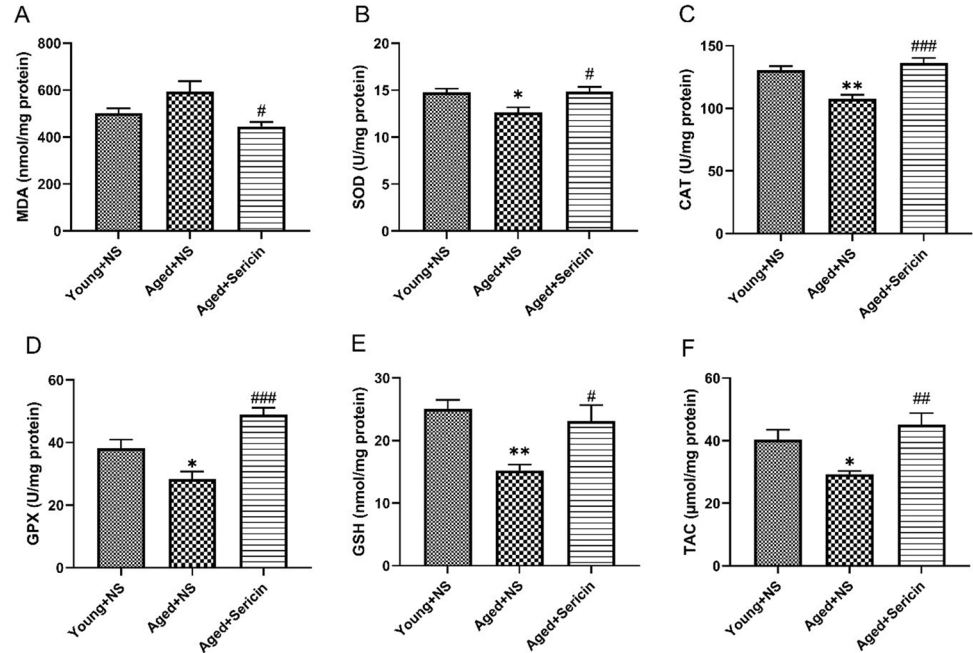
### Sericin administration improved antioxidant capacity in aged mice

As shown in Fig. 2, there was no significant change in MDA level between aged control and young control; however, sericin administration decreased the level of MDA compared to the aged control ( $p < 0.05$ ). The low expression level of SOD, CAT, GPX, GSH, and TAC was found in the aged controls when compared to the young controls ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.05$ , respectively); also sericin administration significantly increased the activity

**Fig. 1** The effect of sericin on liver function. The level of ALT (A), AST (B), and ALP (C) was determined in all experimental groups. Results are expressed as mean  $\pm$  SEM. Data were analyzed using one-way ANOVA. \* $p < 0.05$  and \*\* $p < 0.01$  compared with young controls and # $p < 0.05$  and ## $p < 0.01$  compared with age controls



**Fig. 2** The effect of sericin on antioxidant capacity in aged mice. The level of MDA (A), SOD (B), CAT (C), GPX (D), GSH (E), and TCA (F) was determined in all experimental groups. Results are expressed as mean  $\pm$  SEM. Data were analyzed using one-way ANOVA. \* $p < 0.05$  and \*\* $p < 0.01$  compared with young controls and # $p < 0.05$  and ## $p < 0.01$  and ### $p < 0.001$  compared with age controls



level of antioxidative markers in comparison to the aged control mice ( $p < 0.05$ ,  $p < 0.001$ ,  $p < 0.001$ ,  $p < 0.05$ , and  $p < 0.01$ , respectively). These findings imply that sericin treatment could improve the oxidative stress biomarkers to a varying degree in the liver of aged-treated mice (Supplementary file 1).

### Administration of sericin improved inflammation-related markers in aged mice

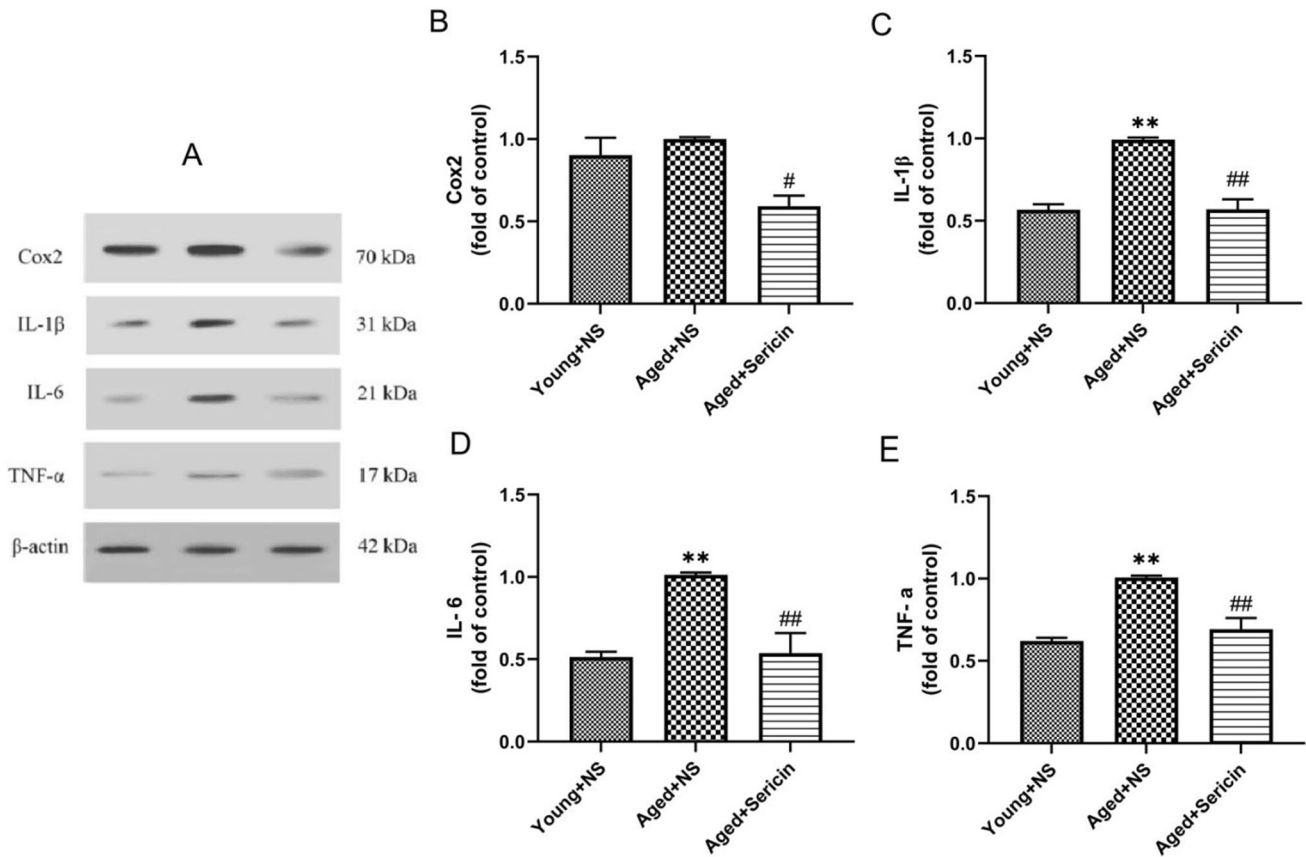
The expression levels of pro-inflammatory markers were analyzed by western blotting analysis in all experimental groups. As depicted in Fig. 3, the expression of Cox-2 as an inflammatory mediator was nearly the same in both young and aged controls; however, the level of this parameter was significantly reduced in sericin-treated mice ( $p < 0.05$ ). As expected, our analysis revealed a high level of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in aged controls when compared to young controls. However,

sericin administration could markedly suppress the expression level of pro-inflammatory markers compared to the age-matched control ( $p < 0.01$ ). The results presented here indicate that sericin administration effectively improves inflammatory biomarkers in the liver of aged mice (Supplementary file 2).

### Administration of sericin changed apoptotic-related proteins in aged mice

The impact of sericin administration on pro-apoptotic and anti-apoptotic proteins was identified by western blotting analysis. Compared with the young control, the expression level of Bax (Bcl-2-associated  $\times$  protein) and cleaved caspase-3 was higher in aged control ( $p < 0.05$ ), while the expression level of Bcl-2 showed a slight decrease in the aged control. Following the administration of sericin in aged mice, the protein levels of Bax and cleaved caspase-3 declined ( $p < 0.05$  and  $p < 0.01$ , respectively), and the protein level





**Fig. 3** Sericin improved the inflammation-related proteins in the age group. Representative western blotting is shown in **A**. The expression level of Cox2 (**B**), IL-1 $\beta$  (**C**), IL-6 (**D**), and TNF- $\alpha$  (**E**) in the liver tissue of all experimental mice. \*\* $p < 0.01$  compared with young con-

trols and ## $p < 0.01$  compared with age control. Data were analyzed using one-way ANOVA. The data were presented as mean  $\pm$  SEM from three independent experiments

of Bcl-2 went up ( $p < 0.05$ ) in comparison with age-matched controls (Fig. 4). The present results infer that sericin treatment declines Bax and cleaved caspase-3 expression and upregulates Bcl-2 expression (Supplementary file 3).

### Administration of sericin affected JNK activation in aged mice

Compared with the young control, the JNK level showed a tendency to increase in the aged control. There was no significant change in aged-treated mice when compared to the age-matched control. However, there was an enhancement in the level of p-JNK expression in aged control compared to young control ( $p < 0.001$ ). Comparing the relative density of p-JNK/JNK between aged control and young control showed this ratio is significantly higher in the aged control ( $p < 0.001$ ). Collectively, sericin treatment could effectively reduce p-JNK/JNK ratio in the aged-treated group as compared to the age control ( $p < 0.001$ ) (Fig. 5) (Supplementary file 4).

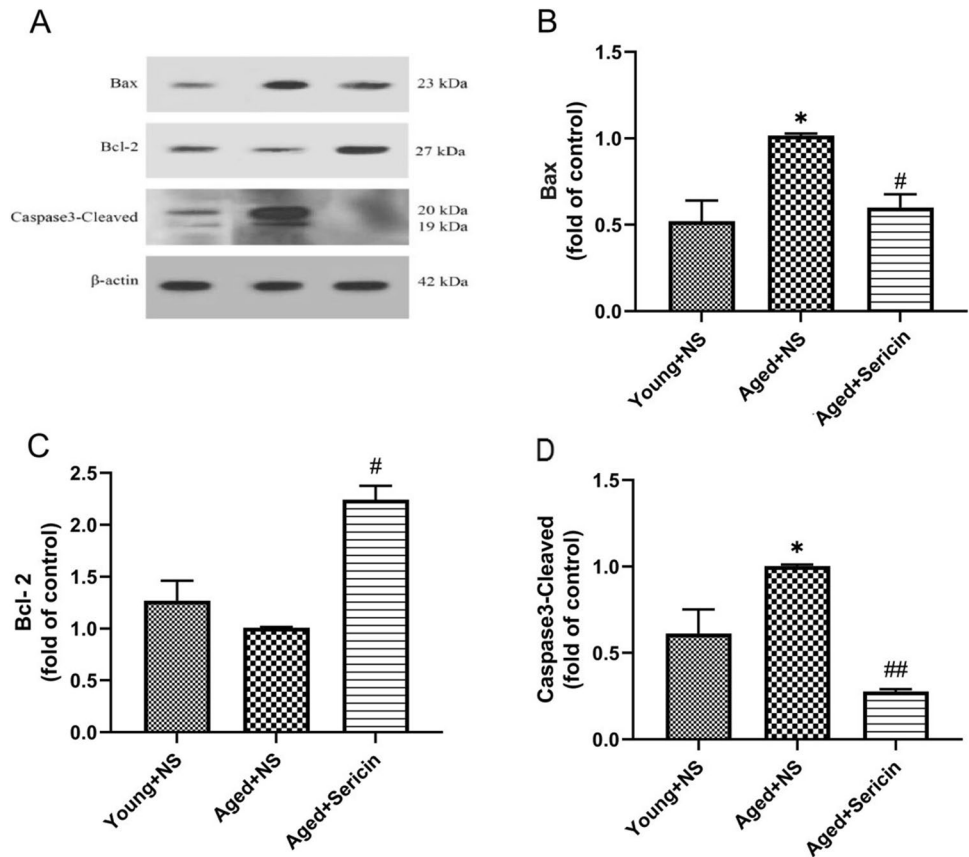
### Effect of sericin treatment on p-38 protein expression

p-38 expression was evaluated by western blot analysis. As presented in Fig. 6, the expression level of p-38 did not differ between both young and aged control. Moreover, there was no difference in the protein expression of p-38 in mice treated with sericin and aged control. There was no significant variation in the expression level of phospho p-38 in both controls. Treatment with sericin decreased the expression level of phospho p-38; however, it was not statistically significant in comparison to aged control. The ratio level of phospho p-38/p-38 notably decreased in sericin-treated mice compared to aged control ( $p < 0.001$ ) (Supplementary file 5).

### Effect of sericin on mRNA level of $\beta$ -Gal

As presented in Fig. 7, the results exhibited no statistically significant alterations in the mRNA level of the  $\beta$ -Gal in all experimental groups. In other words, sericin treatment did

**Fig. 4** Evaluation of pro- and anti-apoptotic proteins in mice subjected to sericin administration. Representative western blotting is shown in **A**. The expression level of Bax (**B**), Bcl-2 (**C**), and cleaved caspase-3 (**D**) was detected by western blotting. \* $p < 0.05$  compared with young controls, # $p < 0.05$  and ## $p < 0.01$  compared with age control. Data were analyzed using one-way ANOVA. The data were presented as mean  $\pm$  SEM from three independent experiments



not affect  $\beta$ -Gal mRNA level in treated mice (Supplementary file 6).

## Discussion

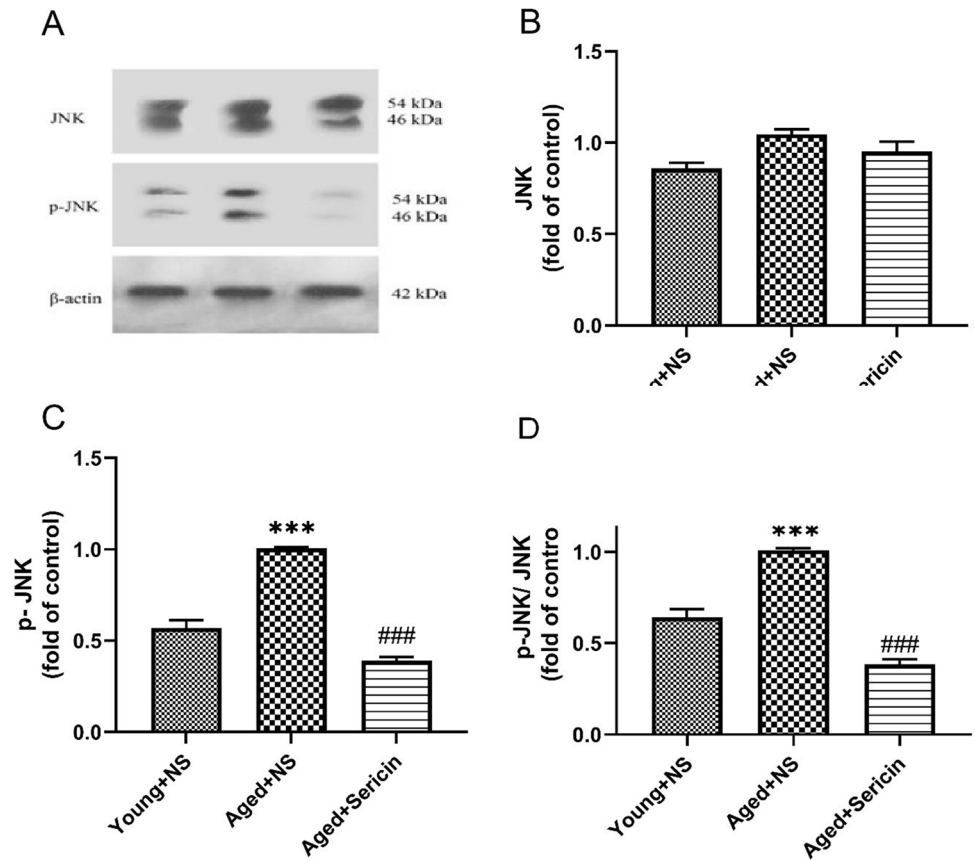
In this study, the administration of sericin showed protective effects on the liver of aged mice in terms of antioxidant and anti-inflammatory activities. Higher levels of antioxidants, as well as a reduction in MDA level, indicated the protective role of sericin against lipid peroxidation and ROS generation in aged animals. A significant reduction in the liver enzymes also indicated improvement of the liver function in comparison with aged controls. It seems that the hepatoprotective effects of sericin were mediated by restoring the normal indices of antioxidant enzymes and preventing ROS generation in the liver. The antioxidant properties of sericin could be related to the high serine and threonine content, whose hydroxyl groups' act as a chelating agent for trace elements such as copper and iron (Kato et al. 1998). Parsons concluded that the presence of polyphenols and flavonoids in sericin is responsible for its antioxidant activity (Prasong 2011). Furthermore, Kato et al. showed that sericin inhibits lipid peroxidation in rat brain homogenate (Kato et al. 1998). The lipid peroxides, derived from polyunsaturated fatty acids, are unstable and may decompose

into malondialdehyde (Yagi 1998; Armstrong and Browne 1994), whose levels are associated with cardiovascular risk factors, hypertension, diabetes, and hyperlipidemia (Walter et al. 2004).

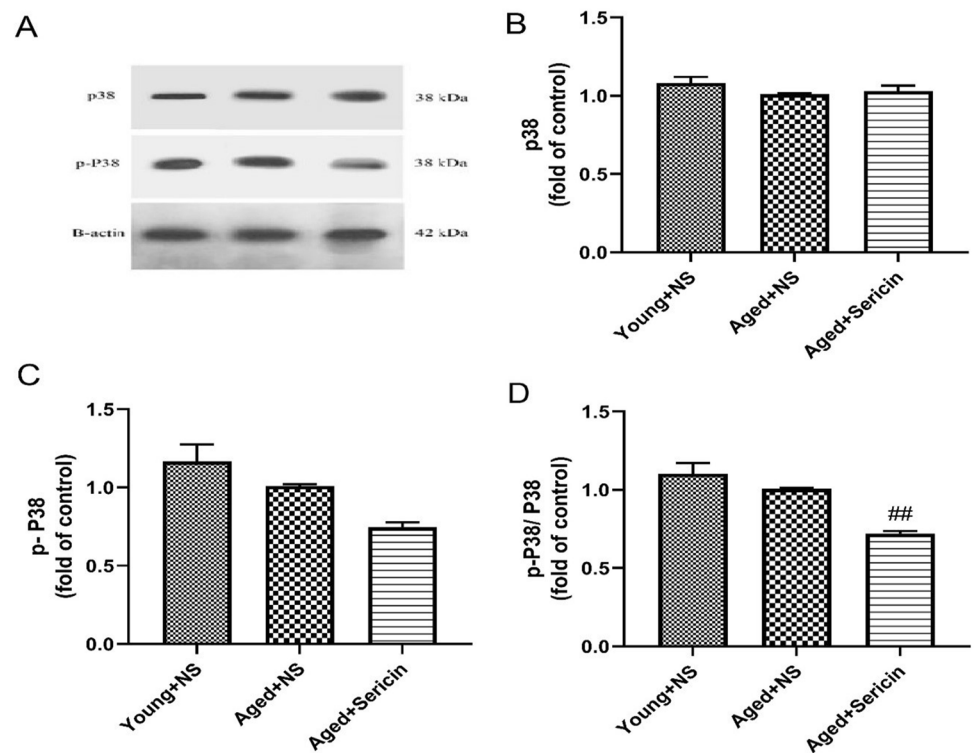
In this study, sericin decreased the expression level of the pro-inflammatory mediator production COX-2 and IL1 $\beta$ , IL6, and TNF- $\alpha$  in aged-treated mice. It can be assumed that the anti-inflammatory effect of sericin is possibly mediated through the suppression of ROS generation and ROS-activated mitochondrial apoptotic pathway. Consistently, Chlapanidas et al. found that sericin has anti-proliferative activities in peripheral blood mononuclear cells stimulated in vitro and reduces the release of IFN- $\gamma$  without having effects on the release of IL-10 and TNF- $\alpha$  (Chlapanidas et al. 2013). Besides, Aramwit et al. investigated the inflammatory mediators induced by sericin in vitro and in vivo (Aramwit et al. 2009).

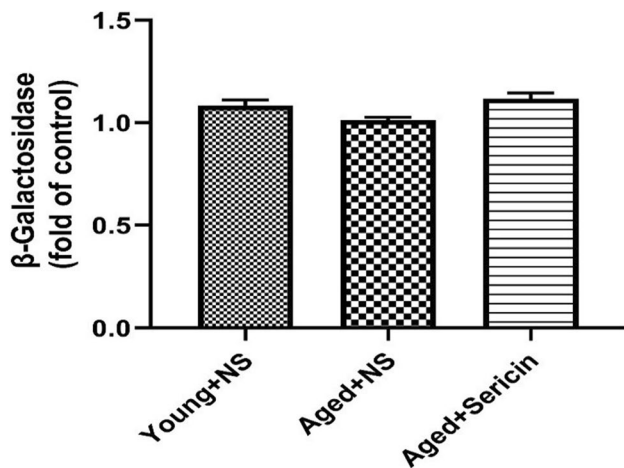
In the current study, the determination of Bcl-2 (an anti-apoptosis signal) and other pro-and anti-apoptotic proteins (Bax and cleaved caspase-3) showed that sericin could significantly stimulate the expression of these apoptotic-related proteins in the aged-treated group when aged control. This is supported by the findings by Dash et al. (2008) that pre-treatment with sericin could upregulate the expression level of Bcl-2 and downregulate Bax

**Fig. 5** Evaluation of JNK protein expression in mice subjected to sericin administration. Representative western blotting is shown in **A**. The expression levels JNK (**B**), p-JNK (**C**), and p-JNK/JNK (**D**) was detected by western blotting. \*\*\* $p < 0.001$  compared with young controls and ### $p < 0.001$  compared with age control. Data were analyzed using one-way ANOVA. The data were presented as mean  $\pm$  SEM from three independent experiments



**Fig. 6** Evaluation of p-38 protein expression in mice subjected to sericin administration. Representative western blotting is shown in **A**. The expression levels p-38 (**B**), phospho p-38 (**C**), and phospho p-38/p-38 (**D**) were detected by western blotting. ## $p < 0.01$  compared with aged control. Data were analyzed using one-way ANOVA. The data presented as mean  $\pm$  SEM from three independent experiments





**Fig. 7** Impact of sericin administration on the mRNA level of beta-galactosidase. Data were analyzed using one-way ANOVA. The data were presented as mean  $\pm$  SEM from three independent experiments

expression (Dash et al. 2008). These findings imply the anti-aging and anti-apoptotic benefits of sericin in the liver of aged mice. In this regard, the induction of pro-apoptotic agents results in the reduced activity of MAPK. Five groups of MAPK have so far been identified of which JNK and p38 MAPK are involved in the stress-related response of the cell (Pearson et al. 2001). The roles of JNK and p38 MAPK in aging invertebrates are not fully understood. A similar role of these kinases in mammals would open up the possibility of therapeutic prolongation of cell viability by inhibiting these kinases. However, the validity of this hypothesis remains to be verified (Sadler et al. 2004). In the present study, we demonstrated the effect of JNK and p38 MAPK inhibition on aging. Phosphorylation of tyrosine and threonine is necessary for the activation of JNK and p38 MAPK (Tarín et al. 2001). Comparing the results in each group showed that the values of JNK and P38 independently did not change significantly in each group, although the increase in P-JNK and decrease in p-P38 were seen in the aged control. Sericin treatment leads to a significant reduction in P-JNK, while no significant change was observed in the level of p38 in the treated group. Generally, the low ratios of p-JNK to JNK and P-p38 to p-38 in the aged-treated group than aged control indicate an association between sericin and increasing in the activity of P-38 and JNK.

It is worth mentioning that young mice were used for confirmation of aging. In other words, the aging of mice was validated by comparison of corresponding parameters in aged mice with young mice. Accordingly, the comparison of aged-treated mice with young untreated mice has not been related to the aim of the current study.

There are some limitations to this study. We did not evaluate the effect of sericin on the body weight or the liver tissues in all experimental groups; thereby, the impact of sericin on body and/or tissue weight is still unclear. However, according to our literature review, sericin treatment did not affect body weight (Qin, et al. 2020; Kunz 2016; SASAKI, M., et al. 2000).

## Conclusion

The beneficial effect of sericin is most likely mediated through the modulation of the series of events, including inflammation, antioxidant, and apoptosis pathways in the liver tissue. Based on the current findings, sericin would be a successful hepatoprotective agent for ameliorating liver dysfunctions in aged mice. In fact, antioxidant and anti-apoptotic properties of sericin may be a plausible way to alleviate the detrimental effects of aging in the liver tissues. Further studies are needed to elucidate the exact molecular mechanisms.

## Supplementary information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00210-021-02160-9>.

**Author contribution** BY and S-SE conceptualized and designed the experiment. BY and MJ conducted experiments. BY, AA, JN-N, MZ, and MK contributed new reagents and analyzed data. MS, S-SE, and FE supervised the project. MS, S-SE, and FE wrote, reviewed, and edited the manuscript. All listed authors have read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

**Funding** The financial sponsorship of this work was provided by the Molecular Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran (Pazhoohan ID., 65705; ethical code No., IR.TBZMED.VCR.REC.1399.334).

**Availability of data and materials** The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethics approval** This ethical code of this project is IR.TBZMED.VCR.REC.1399.334.

**Consent to participate** This is an animal study.

**Consent for publication** All authors agree to publish.

**Competing interests** The authors declare no competing interests.



## References

- Fathi E, Farahzadi R (2016) Isolation, culturing, characterization and aging of adipose tissue-derived mesenchymal stem cells: a brief overview. *Braz Arch Biol Technol* 59
- López-Otín C et al (2013) The hallmarks of aging. *Cell* 153(6):1194–1217
- Fathi E, et al (2019) Telomere shortening as a hallmark of stem cell senescence. *Stem Cell Investig* 6
- Ebrahimi V et al (2020) Epigenetic modifications in gastric cancer: focus on DNA methylation. *Gene*. 742:144577
- Farahzadi R et al (2016) L-carnitine effectively induces hTERT gene expression of human adipose tissue-derived mesenchymal stem cells obtained from the aged subjects. *Int J Stem Cells* 9(1):107–114
- Kirkwood TB (2005) Understanding the odd science of aging. *Cell* 120(4):437–447
- Farahzadi R, Fathi E, Vietor I (2020) Mesenchymal stem cells could be considered as a candidate for further studies in cell-based therapy of Alzheimer's disease via targeting the signaling pathways. *ACS Chem Neurosci* 11(10):1424–1435
- Fathi E, Sanaat Z, Farahzadi R (2019) Mesenchymal stem cells in acute myeloid leukemia: a focus on mechanisms involved and therapeutic concepts. *Blood Research* 54(3):165–174
- Kaerberlein M (2013) Longevity and aging. *F1000prime Rep* 5
- Mobarak H et al (2017) L-carnitine significantly decreased aging of rat adipose tissue-derived mesenchymal stem cells. *Vet Res Commun* 41(1):41–47
- Mehdizadeh A et al (2017) Liposome-mediated RNA interference delivery against Erk1 and Erk2 does not equally promote chemosensitivity in human hepatocellular carcinoma cell line HepG2. *Artif Cells, Nanomed Biotechnol* 45(8):1612–1619
- Rui L (2011) Energy metabolism in the liver. *Compr Physiol* 4(1):177–197
- Kim H, Kisseleva T, Brenner DA (2015) Aging and liver disease. *Curr Opin Gastroenterol* 31(3):184
- Basso A et al (1998) Reduced DNA synthesis in primary cultures of hepatocytes from old mice is restored by thymus grafts. *J Gerontol A Biol Sci Med Sci* 53(2):B111–B116
- Wu I-C et al (2014) Oxidative stress and frailty: a closer look at the origin of a human aging phenotype. *Aging*. Elsevier, pp 3–14
- Jang JY et al (2018) The role of mitochondria in aging. *J Clin Investig* 128(9):3662–3670
- Jones DP (2008) Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* 295(4):C849–C868
- Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87(1):99–163
- Green DR, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy–inflammation–cell death axis in organismal aging. *Science* 333(6046):1109–1112
- Rezabakhsh A et al (2017) Effect of hydroxychloroquine on oxidative/nitrosative status and angiogenesis in endothelial cells under high glucose condition. *BioImpacts*. 7(4):219
- Feizy N et al (2016) Morphine inhibited the rat neural stem cell proliferation rate by increasing neuro steroid genesis. *Neurochem Res* 41(6):1410–1419
- Saliani N, Montazersaheb S, Kouhsari SM (2017) Micromanaging glucose tolerance and diabetes. *Adv Pharm Bullet* 7(4):547
- Bejma J, Ramires P, Ji L (2000) Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and liver. *Acta Physiol Scand* 169(4):343–351
- Szabo G, Csak T (2012) Inflammasomes in liver diseases. *J Hepatol* 57(3):642–654
- Lasry A, Ben-Neriah Y (2015) Senescence-associated inflammatory responses: aging and cancer perspectives. *Trends Immunol* 36(4):217–228
- Di Iorio A et al (2003) Serum IL-1 $\beta$  levels in health and disease: a population-based study. 'The InCHIANTI study.' *Cytokine*. 22(6):198–205
- Wunderlich FT et al (2010) Interleukin-6 signaling in liver-parenchymal cells suppresses hepatic inflammation and improves systemic insulin action. *Cell Metab* 12(3):237–249
- Williams LM et al (2008) Rac mediates TNF-induced cytokine production via modulation of NF- $\kappa$ B. *Mol Immunol* 45(9):2446–2454
- Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 81(2):807–869
- Roux PP, Blenis J (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 68(2):320–344
- Schwabe RF, Brenner DA (2006) Mechanisms of liver injury. I. TNF- $\alpha$ -induced liver injury: role of IKK, JNK, and ROS pathways. *Am J Physiol-Gastrointest Liver Physiol*. 290(4):G583–G589
- Rajawat YS, Hilioti Z, Bossis I (2009) Aging: central role for autophagy and the lysosomal degradative system. *Ageing Res Rev* 8(3):199–213
- Carmona-Gutierrez D et al (2016) The crucial impact of lysosomes in aging and longevity. *Ageing Res Rev* 32:2–12
- Morgunova G et al (2015) Senescence-associated  $\beta$ -galactosidase—a biomarker of aging, DNA damage, or cell proliferation restriction? *Mosc Univ Biol Sci Bull* 70(4):165–167
- Ding A-J et al (2017) Current perspective in the discovery of anti-aging agents from natural products. *Nat Prod Bioprospect* 7(5):335–404
- Deori M et al (2016) Antioxidant effect of sericin in brain and peripheral tissues of oxidative stress induced hypercholesterolemic rats. *Front Pharmacol* 7:319
- Seyedaghamiri F, et al (2021) Sericin modulates learning and memory behaviors by tuning of antioxidant, inflammatory, and apoptotic markers in the hippocampus of aged mice. *Mol Biol Rep* 1–12
- Kunz RI, et al (2016) Silkworm sericin: properties and biomedical applications. *BioMed Res Int* 2016
- Barajas-Gamboa JA et al (2016) Sericin applications: a globular silk protein. *Ingeniería y Competitividad* 18(2):193–206
- Bagheri Y, et al (2020) Protective effects of gamma oryzanol on distant organs after kidney ischemia-reperfusion in rats: a focus on liver protection. *Human Exp Toxicol* 0960327120979014
- Bagheri Y et al (2021) Comparative study of gavage and intraperitoneal administration of gamma-oryzanol in alleviation/attenuation in a rat animal model of renal ischemia/reperfusion-induced injury. *Iran J Basic Med Sci* 24(2):175–183
- Rahimi M, et al (2021) Renoprotective effects of prazosin on ischemia-reperfusion injury in rats. *Human Exp Toxicol*. 0960327121993224
- Montazersaheb S et al (2019) Downregulation of TdT expression through splicing modulation by antisense peptide nucleic acid (PNA). *Curr Pharm Biotechnol* 20(2):168–178
- Tarhriz V et al (2019) Transient induction of Cdk9 in the early stage of differentiation is critical for myogenesis. *J Cell Biochem* 120(11):18854–18861
- Fathi E, Farahzadi R, Valipour B (2021) Alginate/gelatin encapsulation promotes NK cells differentiation potential of bone marrow resident C-kit(+) hematopoietic stem cells. *Int J Biol Macromol* 177:317–327
- Tarhriz V et al (2018) CDK9 regulates apoptosis of myoblast cells by modulation of microRNA-1 expression. *J Cell Biochem* 119(1):547–554

- Fathi E, et al (2020) L-carnitine extends the telomere length of the cardiac differentiated CD117+-expressing stem cells. *Tissue and Cell* 101429
- Fathi E et al (2020) Cardiac differentiation of bone-marrow-resident c-kit+ stem cells by L-carnitine increases through secretion of VEGF, IL6, IGF-1, and TGF- $\beta$  as clinical agents in cardiac regeneration. *J Biosci* 45(1):1–11
- Kato N et al (1998) Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. *Biosci Biotechnol Biochem* 62(1):145–147
- Prasong S (2011) Screening of antioxidant activity of some *Samia ricini* (Eri) silks: comparison with *Bombyx mori*. *J Biol Sci* 11(4):336–339
- Yagi K (1998) Simple assay for the level of total lipid peroxides in serum or plasma. *Free radical and antioxidant protocols*. Springer, pp 101–106
- Armstrong D, Browne R (1994) The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds related to oxidative stress as applied to the clinical chemistry laboratory. *Free radicals in diagnostic medicine*. Springer, pp 43–58
- Walter MF et al (2004) Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study. *J Am Coll Cardiol* 44(10):1996–2002
- Chlapanidas T et al (2013) Sericins exhibit ROS-scavenging, anti-tyrosinase, anti-elastase, and in vitro immunomodulatory activities. *Int J Biol Macromol* 58:47–56
- Aramwit P et al (2009) Monitoring of inflammatory mediators induced by silk sericin. *J Biosci Bioeng* 107(5):556–561
- Dash R et al (2008) Silk sericin protein of tropical tasar silkworm inhibits UVB-induced apoptosis in human skin keratinocytes. *Mol Cell Biochem* 311(1–2):111–119
- Pearson G et al (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 22(2):153–183
- Sadler KC et al (2004) MAP kinases regulate unfertilized egg apoptosis and fertilization suppresses death via Ca<sup>2+</sup> signaling. *Mol Reprod Dev* 67(3):366–383
- Tarín JJ, Pérez-Albalá S, Cano A (2001) Cellular and morphological traits of oocytes retrieved from aging mice after exogenous ovarian stimulation. *Biol Reprod* 65(1):141–150
- Qin H, et al (2020) Safety assessment of water-extract sericin from silkworm (*Bombyx mori*) cocoons using different model approaches. 2020
- Kunz RI, et al (2016) *Silkworm sericin: properties and biomedical applications*. 2016
- SASAKI M, et al (2000) A resistant protein, sericin improves atropine-induced constipation in rats. 6(4): 280–283

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.