

Original Article

Effects of exposure to sericin on serum GH, IGF-1 and antioxidant levels following parturition in mice

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ABSTRACT

This study aimed to determine the effects of exposure to sericin on serum GH, IGF-1 and antioxidant levels following parturition in mice. Forty pregnant female NMRI mice were allocated into four groups. In the control group, pregnant mice were given water as a placebo, whereas pregnant female mice in groups 2-4 were orally administered sericin (112.5, 225, and 450 mg/kg) on different days of gestation (5, 8, 11, 14, and 17). Following parturition, the open field test (OFT), forced swimming test (FST), tail suspension test (TST) and open field tests were used to evaluate depressive-like and antidepressant activity of sericin. At the end of the study, serum growth hormone (GH), insulin-like growth factor-1 (IGF-1), malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD), and total antioxidant status (TAS) levels were determined. According to the results, sericin administration during pregnancy decreased immobility time in FST and TST, and increased the number of crosses in the OFT compared to the control group ($P < 0.05$). Exposure to sericin significantly decreased serum MDA, while it increased SOD, GPx, and TAS levels compared with the control group ($P < 0.05$). Also, sericin exposure during gestation significantly increased serum GH and IGF-1 levels compared to the control group ($P < 0.05$). These results suggested pre-partum administration of sericin has antidepressant effect in postpartum mice.

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1. Introduction

Depression is the most common type of mental illness globally. Major depression is identified by alterations in both emotional state and physical functioning, along with a diminished interest in one's environment (1). Increasingly, there are reports showing higher rates of depression in both men and women, which significantly impacts society's health (2). The period after childbirth involves significant physical and emotional changes in mothers, in order to care for and support their newborns. Nevertheless, various psychiatric disorders may emerge during this period. The hypothalamic–pituitary–adrenal (HPA) axis is a key endocrine adapter against stressors and plays an important role in the pathophysiology of stress-related psychiatric diseases such as depression and anxiety disorders (2).

Previous studies showed hyperactivity of the HPA axis in depressive patients (3), implicating glucocorticoids, the final output of the HPA axis, in depression. However, details in the action of this hormone remain unclear. Postnatal mood and anxiety disorders impact the mental health of both mother and baby, as well as increase the risk of developing psychiatric disorders later in life, like postnatal depression (PPD), postnatal anxiety, and postnatal psychosis (4). Various animal models, such as stress-induced, high-fat diet-induced, and pup separation models, are employed to create experimental postpartum depression (4). Increasingly, new antidepressant medications are being reported to offer important insights into the neuropathology underlying depression.

Silk sericin is a natural macromolecular protein derived from the silkworm *Bombyx mori*. During the various stages of producing raw silk and textiles, sericin can be recovered for other uses. Also, sericin recovery reduces the environmental impact of silk manufacture. Sericin has exhibited neuroprotective effects in a rat model of diabetes mellitus by inhibiting cortical and hippocampal heme oxygenase-1 expression. There is consistent evidence that a range of psychosocial stressors lead to elevated microglial activity in the hippocampus, and good evidence that this is also the case in other brain regions. These effects were seen with early-life/prenatal stress, as well as stressors in adulthood (5). The materials modified with sericin and sericin composites are useful as degradable biomaterials, biomedical materials, polymers for forming articles, functional membranes, fibers, and fabrics.

The effects of sericin administration on anxiety- and depressive-like behaviors were assessed using the OFT and FST in mice. Sericin increased the number of crossings in OFT, while it reduced immobility time in TST and FST (6). Findings indicate that sericin has potential therapeutic effects on anxiety- and depressive-like behaviors in mice. Sericin is known as a biological antioxidant, and it decreased reactive oxygen species (ROS) and MDA levels and enhanced enzymatic antioxidant activities (6). Sericin treatments decreased ROS and lipid peroxidation levels, restored MMP, and enhanced total antioxidant capacity and enzyme activity of the GPx and SOD in brain regions, providing evidence for the protective effect of sericin therapy against psychopathological and behavioral changes induced by restraint stress (6).

Sericin administration showed a decrease in serum corticosterone and hippocampal MDA levels and enhanced hippocampal antioxidant defense in mice exposed to heat stress. This indicates a protective role of sericin against heat stress-mediated cognitive dysfunction and anxiety-like behavior through suppressing oxidative stress and apoptosis (7). In mice subjected to acute sleep deprivation, sericin pre-treatment prevented cognitive impairment, decreased hippocampal MDA levels, and enhanced the activity of SOD, GPx, and total antioxidant capacity (8). Sericin showed protective effects against alcohol-induced hepatic injury in mice by preventing lipid peroxidation and enhancing the activity of antioxidant enzymes in the serum and liver (9). Maternal IGF-1 has been shown to impact fetal growth in mice (10). Sericin has a key role in GH/IGF-1 axis in rats with type 2 diabetes (11).

Due to the protective role of sericin in oxidation, anxiety, and depressive-like behaviors, and the lack of information on its effect on parturition depression, this study aimed to determine the effects of exposure to sericin during pregnancy on depressive-like behaviors, serum antioxidant activity, and GH, IGF-1 levels following parturition in mice.

2. Materials and Methods

2.1. Animals and groups

Sixteen male and 40 virgin female NMRI mice, all weighing 28-30 g and aged 8-10 weeks, were provided and kept in a laboratory environment, which included a temperature of 22±2°C and a 12-hour light/dark cycle, as

well as unlimited access to standard chow pellets and fresh water. Next, the female mice were paired with fertile male mice in cages. Every morning, the female mice were checked to see if they had sperm or a vaginal plug, in order to determine if they were pregnant.

The pregnant mice were distributed randomly among four groups. In the control group, pregnant mice were given water as a placebo, whereas pregnant female mice in groups 2-4 were orally administered sericin (112.5, 225, and 450 mg/kg) on different days of gestation (5, 8, 11, 14, and 17) (12). After giving birth, behavioral tests and serum biochemical analyses were conducted.

2.2. Behavior tests

2.2.1. TST

TST is frequently used to evaluate the antidepressant-like effects in mice. The TST was conducted according to the procedure outlined by Cryan et al. (2005). In short, the mice were distant from the nearest objects and were isolated both acoustically and visually, preventing them from seeing or interacting with each other. Next, they were hung 50 cm above the ground with sticky tape located about 1 cm from the tip of the tail, ensuring they couldn't flee or grab onto surrounding surfaces. The duration of immobility was measured over a 6-minute period. Mice were considered motionless if they showed no vigorous body shaking or limb movement while hanging passively and remaining completely inactive (13).

2.2.2. FST

FST was conducted in mice according to the previously described protocol by Ueno et al. (2022). Every mouse was placed into a glass cylinder (measuring 25 cm in height and 15 cm in diameter) filled with 10 cm of water at a temperature of $25 \pm 1^\circ\text{C}$ for a duration of 15 minutes (pre-test session). Twenty-four hours later, the mouse was once again put in the cylinder and allowed to remain there for a period of 6 minutes (test session). The mouse's immobility was observed when it stopped resisting and stayed still while floating in the water, only moving slightly to keep its head above the water. The amount of time without movement during the final 4 minutes of the 6-minute testing period was recorded (14).

2.2.3. OFT

OFT was employed to evaluate the potential impacts of hesperidin on locomotion and exploration behaviors. A $45 \times 45 \times 30$ cm cage was utilized for conducting the open field test. Masking tape markers divided the open field cage floor into 3×3 squares. Each animal was positioned

alone in the center of the device and observed for 6 minutes to document its movement (number of segments crossed with all four paws) (15).

2.3. Biochemical analysis

Blood samples were collected to measure cardiac and serum MDA, SOD, GPx, TAS, GH, and IGF-1 levels, which were determined using assay kits (16).

2.4. Statistical analysis

Data were analyzed using one-way ANOVA and presented as mean $\pm$ SE (standard error) using SPSS version 22.0. For treatments showing significant differences by ANOVA, between-group evaluations were performed using the Tukey post hoc test ($P < 0.05$).

3. Results

According to Figure 1, exposure to sericin during pregnancy resulted in decreased immobility time in FST compared to the control group ($P < 0.05$). In light of Figure 2, exposure to sericin during gestation resulted in decreased immobility time in TST compared to the control group ($P < 0.05$). As seen in Figure 3, exposure to sericin during pregnancy increased the number of crosses in OFT compared to control group ($P < 0.05$). In this study, maternal exposure to sericin (112.5, 225, and 450 mg/kg) significantly decreased serum MDA levels in mothers of mice compared with the control group ($P < 0.05$) (Figure 4). Additionally, exposure to sericin (112.5, 225, and 450 mg/kg) during pregnancy significantly increased serum SOD levels in the mothers of mice compared with the control group ($P < 0.05$) (Figure 5).

Sericin (112.5, 225, and 450 mg/kg) exposure during gestation significantly amplified the serum GPx levels compared with the control group ($P < 0.05$) (Figure 6). Figure 7 illustrates sericin administration (112.5, 225, and 450 mg/kg) during pregnancy significantly increased TAS levels in treated mice compared to the control group ($P < 0.05$). Furthermore, Figure 8 shows that mice exposed to sericin (112.5, 225, and 450 mg/kg) had highest GH levels in serum compared with the control group ($P < 0.05$). Additionally, administration of sericin (112.5, 225, and 450 mg/kg) during gestation significantly increased serum levels of IGF-1 compared with the control group ($P < 0.05$) (Figure 9).

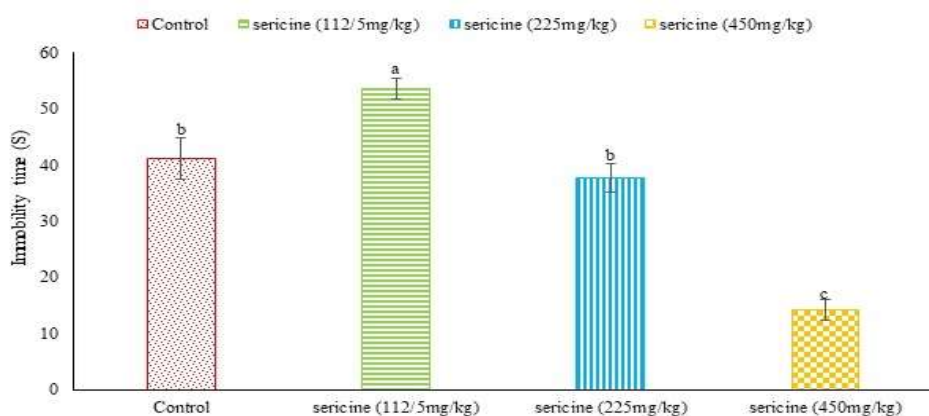


Figure 1. Effects of exposure to sericin on immobility time on FST following parturition in mice. The non-equivalent letters (a-c) indicate significant disparities between the experimental groups ($p < 0.05$).

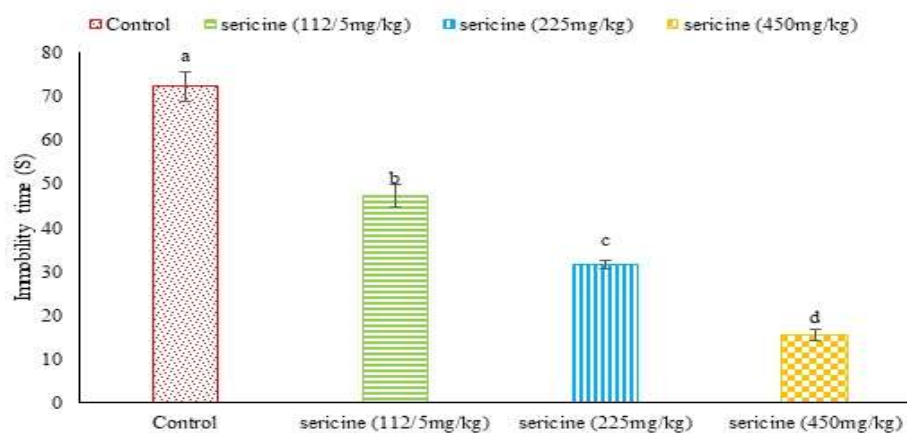


Figure 2. Effects of exposure to sericin on immobility time on TST following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

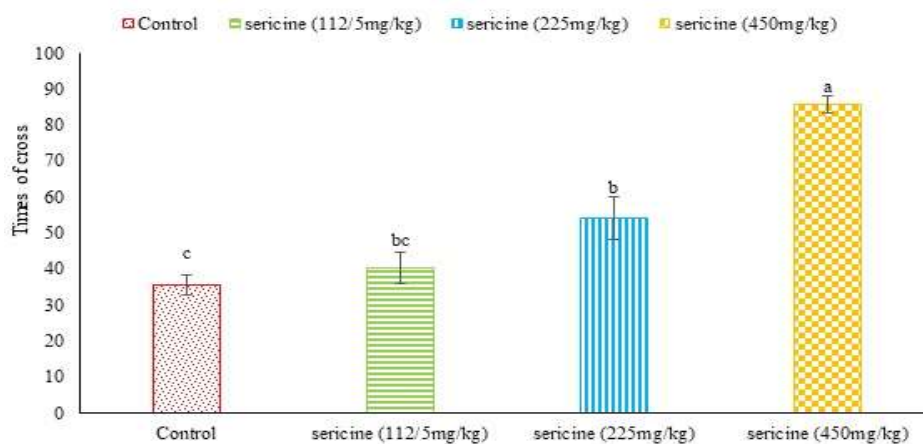


Figure 3. Effects of exposure to sericin on number of cross on OFT following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

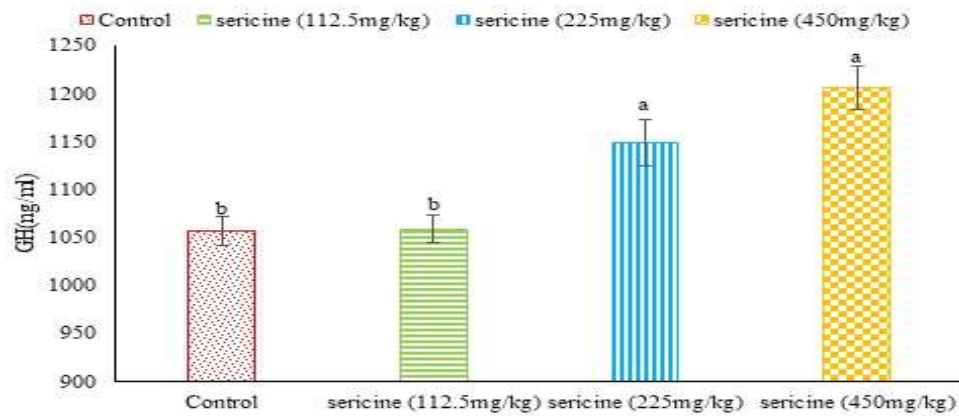


Figure 4. Effects of exposure to sericin on serum GH levels following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

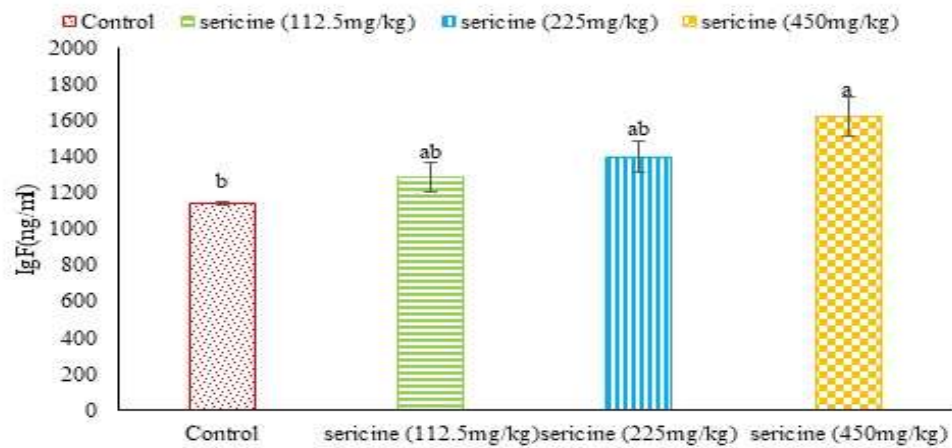


Figure 5. Effects of exposure to sericin on serum IgF levels following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

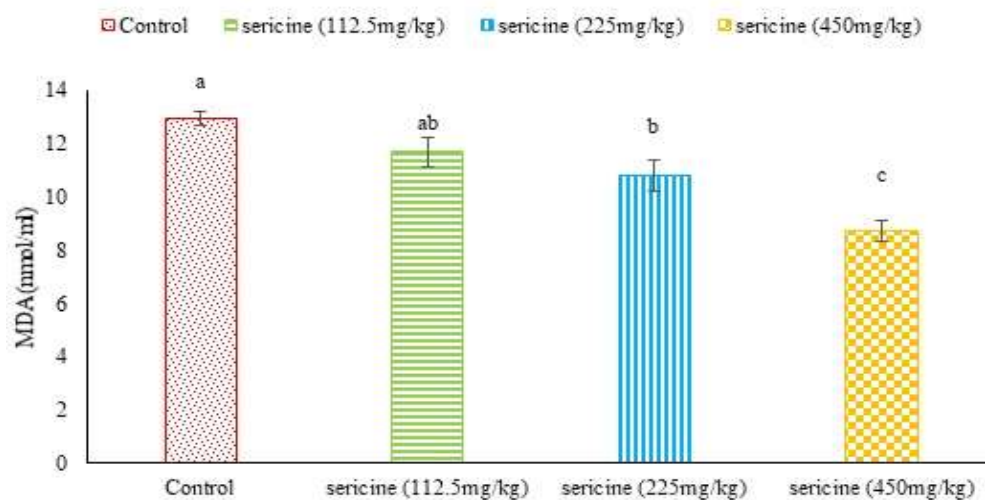


Figure 6. Effects of exposure to sericin on serum MDA levels following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

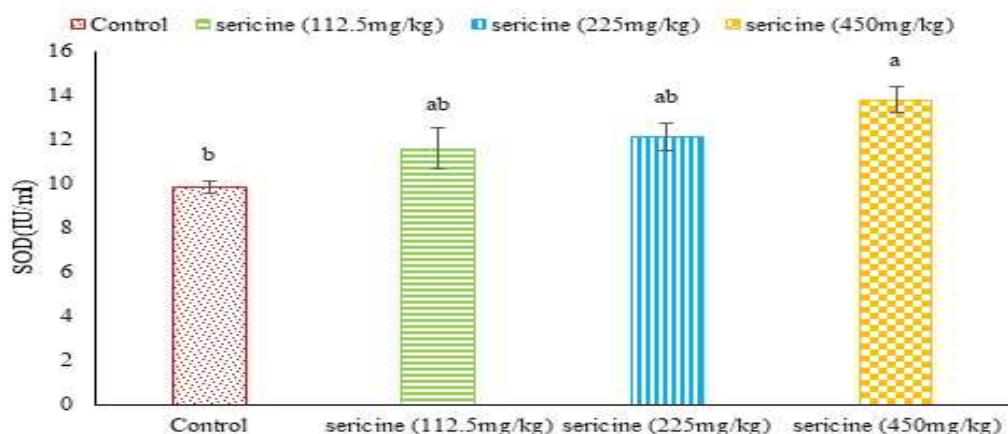


Figure 7. Effects of exposure to sericin on serum SOD levels following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

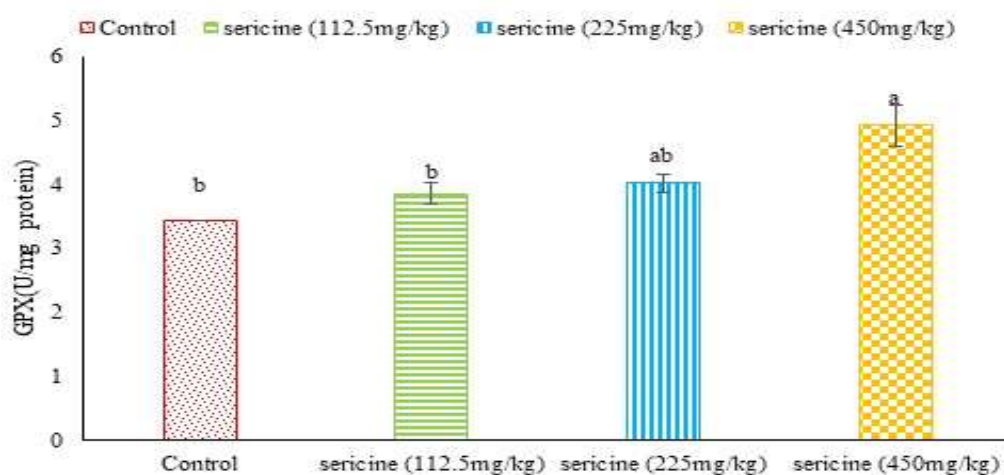


Figure 8. Effects of exposure to sericin on serum GPx levels following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

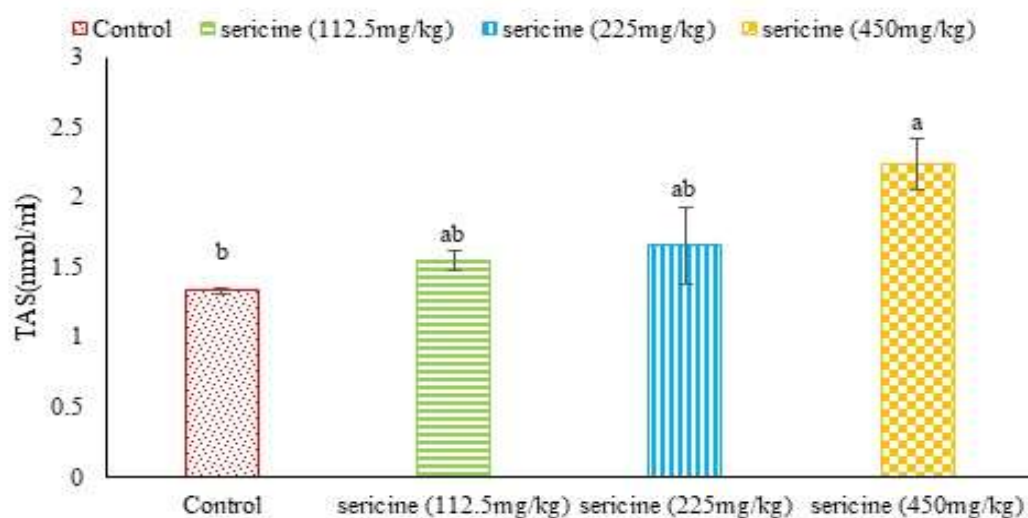


Figure 9. Effects of exposure to sericin on serum TAS levels following parturition in mice. The non-equivalent letters (a-c) represent significant differences between the experimental groups ($p < 0.05$).

4. Discussion

Depression is a prevalent, long-lasting condition that often recurs and can have serious consequences. Even though numerous research studies have been conducted on the physiological mechanisms of this disorder, the brain regions responsible for it remain poorly understood. Postpartum depression is a severe mood disorder that occurs immediately following childbirth and is characterized by feelings of sadness and anxiety in mothers (17).

Findings of the current study on the effects of sericin administration during pregnancy revealed that sericin exposure decreased signs of depression and anxiety. Also, sericin administration improved the activity of antioxidant enzymes. Mobility duration in the FST reflects a feeling of hopelessness and emotional distress. Changes in behavior resembling depression caused by stress are commonly assessed in rodents using the TST and FST. The duration of immobility in the TST and FST indicates a behavioral state of hopelessness that resembles depression in humans (18). There have been reports of variations in the neurochemical pathways involved in the performance induced by the FST and TST. Superficially, these two tests seem nearly identical; however, the variation in strength is influenced by pharmacokinetic and pharmacodynamic factors (19).

Moreover, sericin decreased immobility time in the FST and TST tasks in the stress-subjected groups. These findings strongly suggest anxiolytic and antidepressant effects of sericin in this model, and our finding was in agreement to this report. The study highlights the importance of developing monitoring and psychological programs for pregnant mothers to address mental health issues and improve maternal and infant behaviors. This can also help in coping with psychological stress during pregnancy and enhance tolerance and coping skills during this critical stage (20).

MDA is known as a biochemical marker for measuring oxidative stress, and elevation in MDA levels indicates lipid peroxidation and oxidative damage. SOD and GP_x are both enzymes that play important roles in antioxidant defense. SOD helps reduce superoxide levels in cells, while GP_x works to neutralize hydrogen peroxide and lipid peroxides (21). Additionally, a study on aging-induced liver damage in mice showed that sericin administration improved antioxidant capacity in the liver tissue (22).

Similarly, sericin normalized sleep deprivation-induced reduction in hippocampal activity of GP_x, indicating its potential to counteract cognitive impairments associated with oxidative stress. Sericin supplementation enhanced GP_x levels in the hippocampus, further supporting its role in modulating oxidative stress and improving memory and social behaviors in aged mice (8). Sericin administration decreased oxidative stress markers and increased endogenous antioxidants in peripheral tissues in rats that received high-cholesterol diet (23). The positive role of the antioxidant effects of antidepressants in the treatment of major depressive disorder is well documented. Antioxidant activity may be mediated by O²⁻ and modulation of CAT and GP_x activity. Previous reports revealed that hesperidin prevents cell membrane damage in neurodegenerative diseases (24).

An increase in SOD and GP_x enzyme levels in response to sericin exposure may be the result of the activation of signaling pathways related to oxidative stress. Activation of the transcription factor Nrf2, which plays a key role in the expression of antioxidant genes, is a likely hypothesis. Nrf2 can translocate to the cell nucleus and increase the expression of genes required for the synthesis of SOD and GP_x (6, 7). In a similar study, it was reported that an increased cardiac TAS level—an indicator of the body's overall antioxidant capacity—reflects an overall improvement in the body's ability to cope with free radicals and prevent oxidative damage. This increase is considered a defense mechanism to deal with the increased oxidation load resulting from stress (25).

As observed in the current study, sericin administration during pregnancy increased postpartum GH and IGF-1 levels in mice. In a similar study, it was reported that increased heart levels of GH and IGF, which play key roles in tissue growth and repair, are indicative of metabolic stimulation and modification of growth and rehabilitation systems. This response is likely to occur in order to cope with cellular stress caused by exposure to serotonin. Increased levels of GH and IGF help improve protein synthesis, reduce catabolism, and improve tissue repair capabilities. This process is mediated by activation of the PI3K/Akt signaling pathway, which plays an important role in the anabolic effects of GH and IGF. These pathways can also help regulate glucose metabolism and protein synthesis, both of which are essential for the health and growth of the fetus (23). It has

also been found that sericin, by modulating oxidative stress, neuroinflammation, and apoptosis in the prefrontal cortex and hippocampus, reduces depression and anxiety-like behaviors caused by stress. In addition, sericin has been shown to reduce behavioral and molecular changes caused by cognitive impairment and depression by modulating synaptic and apoptotic proteins in the hippocampus (6, 20).

The increase in antioxidant activity and growth hormones may be considered a protective response against the stresses caused by exposure to sericin. The results obtained from this study emphasize the importance of understanding the biological mechanisms affected by exposure to chemical compounds such as sericin. Sericin has been shown to possess anti-apoptotic properties in the hippocampal CA₁ region of diabetic rats through modulation of the Akt signal transduction pathway. It is reported that sericin reduced serum growth hormone levels, downregulated growth hormone expression, increased serum IGF-1 levels, and upregulated testicular growth hormone receptor and IGF-1 expression. Our finding on the role of sericin on serum IGF-1 levels was in agreement with this report (6, 20).

Additionally, the epigenetic consequences of biochemical and hormonal changes that occur in response to exposure to sericin can have generational effects, meaning that not only the individual directly exposed but also future generations may be affected. A deeper understanding of the biological mechanisms activated by exposure to sericin can help develop interventional strategies to reduce unwanted side effects. For example, if sericin is found to specifically activate a pathway such as Nrf₂, other drugs or interventions can be designed to regulate this pathway and prevent adverse outcomes. These results suggest that pre-partum administration of sericin has an antidepressant effect in postpartum mice. However, in this study, we were not able to compare the results with previous reports. We believe further research is needed to determine additional cellular and molecular mechanism underlying the protective role of sericin in postpartum mice.

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Authors' Contribution

Study concept and design: S.H

Acquisition of data: S.H

Analysis and interpretation of data: S.H

Drafting of the manuscript: S.F

Critical revision of the manuscript for important intellectual content: S.H, A.E

Statistical analysis: S.F

Administrative, technical, and material support: S.H, A.E.

Ethics

The Animal Ethics Committee of the Science and Research Branch of Islamic Azad University in Tehran, Iran approved all experimental procedures. The experimental procedures were followed according to the Guide for the Care and Use of Laboratory Animals to investigate experimental pain in animals.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

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Data Availability

Data is available by request after publish

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