Running title: Effects of sericin following parturition in mice

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

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Abstract

This paper aimed to determine 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) at different

days of gestation (5, 8, 11, 14, and 17). Following postpartum, open field test (OFT), forced swimming test (FST), tail suspension test (TST) and open field tests were used to evaluate depressive-like 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 number of cross in OFT compared to control group ($P<0.05$). Exposure to sericin significantly decreased serum MDA while 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.

Keywords: Sericin, GH, IGF-1, Antioxidant, Parturition, Mice

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 impact 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 adaptor 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 the 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 increasing 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 silkworm *Bombyx mori*. During the various stages of producing raw silk and textile, 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 oxygenase1 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 number of cross 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 known as biological antioxidant and decreased reactive oxygen species (ROS) and MDA levels and enhances 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 for 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 activity of the SOD and 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 key role on GH/IGF-1 axis in rats with type 2 diabetes (11).

Due to protective role of the sericin in oxidation, anxiety and depressive-like behaviors and lack of information on its effect on parturition depression, this study aimed to determine effects of

exposure to sericin during pregnancy on depressive-like behaviors, serum antioxidant activity and GH, IGF-1 levels following parturition in mice.

Material and Methods

Animals

Sixteen male and 40 virgin female NMRI mice, all weighing 28-30 g and aged 8-10 weeks were provided and kept at 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 pellet 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) at different days of gestation (5, 8, 11, 14, and 17) (12). After giving birth, behavior tests and serum biochemical analyses were conducted. 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 the experimental pain in the animals .

Behavior tests

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, hanging 50 cm above the ground with sticky tape located about 1 cm from the tip of the tail, ensuring it can't flee or grab onto surrounding surfaces. The duration of immobility was measured for 6 minutes. Mice were deemed motionless if they showed no vigorous body shaking or limb movement while hanging still and completely inactive (13).

FST

FST was conducted in mice according to the previously described protocol by Ueno et al. in 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 ± 1 °C for a duration of 15 minutes (pre-test session). 24 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 in the final 4 min of the 6 min testing period was recorded (14).

OFT

OFT was employed to evaluate potential impacts of hesperidin on locomotion and exploration behaviors. The 45×45×30 cage was utilized for conducting the open field test. Masking tape markers divided the open field cage flour into 3x3 squares. Every animal was positioned alone in the middle of the device and watched for 6 minutes to document their movement (amount of segments crossed with all four paws) (15).

Biochemical analysis

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

Statistical analysis

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

Results

According to figure 1, exposure to sericin during pregnancy resulted in decreased immobility time in FST compared to control group ($P < 0.05$).

In light of figure 2, exposure to sericin during gestation resulted in a decreased immobility time in TST compared to control group ($P < 0.05$).

As seen in figure 3, exposure to sericin during pregnancy increased number of cross 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 production 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 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 mice compared to the control group ($P < 0.05$).

Furthermore, figure 8 shows that the mice exposed to sericin at (112.5, 225, and 450 mg/kg) had highest GH levels in serum as compared to 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 ($P < 0.05$) (Figure 9).

Discussion

Depression is a prevalent, long-lasting condition that often returns 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 an intense 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 effects of sericin administration during pregnancy revealed that sericin exposure decreased signs of depression and anxiety. Also, sericin administration improved activity of antioxidant enzymes. Mobility duration in FST mirrors 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 TST and FST indicates a behavioral state of hopelessness that resembles depression in humans (18). There have been reports of variances 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 anti-depressant 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 enhancing tolerance and coping skills during this critical stage (20)**.** MDA knowns as a biochemical marker for measuring oxidative stress and elevation in MDA levels indicates lipid peroxidation and oxidative damage. SOD and GPX levels SOD and GPX are both enzymes that play important roles in antioxidant defense. SOD helps reduce superoxide levels in cells, while GPX 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 tissu (22). Similarly, sericin normalized sleep deprivation-induced reduction in hippocampal activity of GPx, indicating its potential to counteract cognitive impairments associated with oxidative stress. Sericin supplementation enhanced GPx 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 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 O^{2-} and modulation of the CAT and GPx activity. Previous reports revealed hesperidin prevents cell membrane damage in neurodegenerative diseases (24). Increase in SOD and GPX 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 GPx (6, 7). In a similar study it is reported increased cardiac TAS level, an indicator that represents the body's overall antioxidant capacity, indicates an overall improvement in the body's overall ability to cope with free radicals and prevent oxidative damage, this increase is considered as a defense mechanism to deal with the increased oxidation load resulting in the face of stress (25).

As observed, sericin administration during pregnancy increased postpartum GH and IGF-1 levels in mice. a similar study it is reported increased heart levels of GH and IGF, which play key roles in tissue growth and repair, have been indicative of metabolic stimulation and modification of growth and rehabilitation systems, which is likely to occur in order to cope with cellular stress

caused by facing serotonin. Increased levels of GH and IGF help improve protein synthesis, reduce catabolism, and improve tissue repair capabilities. This process is mediated by activating the PI3K/Akt signaling pathway, which plays an important role in the anabolic effects of GH and IGF. These paths 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, neuro-inflammation and apoptosis in the prefrontal cortex and hippocampus, it 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 as 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 introduced to be of anti-apoptotic properties in the hippocampal CA_1 region of diabetic rats through modulation of the Akt signal transduction pathway. It is reported 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 and our finding on role of the sericin on serum levels of the IGF-1 was in agreement to this report.

Additionally, the epigenetic consequences of biochemical and hormonal changes that occur in response to exposure to sericin can have generational effects. have, which means that not only the person who is 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 Nrf_2, other drugs or interventions can be designed to regulate this pathway and prevent adverse outcomes. These results suggested pre-partum administration of sericin has antidepressant effect in postpartum mice. However, in this study we were not able to compare results of the current study with previous reports. We think further research's needed to determine more cellular and molecular mechanism for protective role of the sericin in postpartum mice.

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