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Anti-Apoptotic Effects of Sericin in Lumbar Canal Stenosis-Induced Underactive Bladder Model

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ABSTRACT

Purpose: Underactive bladder (UAB) is a challenging urological condition lacking effective pharmacological treatments. While silk sericin has demonstrated anti-apoptotic properties in other tissues, its application in bladder dysfunction remains unexplored. This study investigated the effect of silk sericin on apoptotic markers and urodynamic outcomes in a lumbar canal stenosis (LCS)-induced rat model of UAB, with the novelty centered on evaluating sericin's therapeutic potential specifically in bladder tissue.

Methods: Thirty-six female Wistar rats were randomized into six groups: control, sham, UAB model (LCS+normal saline), and LCS+sericin (200, 250, or 300 mg/kg/day, orally for 14 days). UAB was induced via LCS by insertion of silicone rubber at L5–L6. Bladder function was assessed by awake urodynamics, bladder weight, and Western blotting of apoptotic proteins (Bax, Bcl-2, caspase-3, caspase-9). Outcome assessments were performed blinded to treatment group. Representative urodynamic tracings were obtained for each group.

Results: Urodynamic evaluation showed significant improvement limited to the intercontraction interval (ICI), which significantly improved with sericin 300 mg compared with LCS ($p=0.041$). No significant improvements were observed in other urodynamic parameters, including peak pressure, contraction amplitude, or contraction frequency. Bladder weight was significantly increased in LCS rats (indicating possible hypertrophy or edema) and was significantly reduced in the sericin 300 mg group versus LCS ($p=0.004$). Apoptotic markers Bax and cleaved caspase-3/procaspase-3 decreased significantly with sericin, whereas Bcl-2 expression increased. The most pronounced effects were observed at 300 mg/kg.

Conclusion: Sericin demonstrated anti-apoptotic and partial functional protective effects (limited to ICI) in the LCS-induced UAB model, particularly at 300 mg/kg. However, dissociation between apoptotic modulation and functional recovery indicates that anti-apoptotic mechanisms alone may be insufficient to restore detrusor contractility. These preliminary findings suggest sericin as a candidate requiring further investigation, though extensive mechanistic, pharmacokinetic, and long-term studies with histological validation are warranted before translational consideration.

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Introduction

Detrusor underactivity (DU) and underactive bladder (UAB) are prevalent yet poorly understood conditions that significantly impair quality of life in patients with lower urinary tract symptoms (LUTS).[1,2] UAB is commonly associated with aging, diabetes mellitus, neurological disorders, and pelvic surgery.[3,4] Complications include recurrent urinary tract infection, overflow incontinence, bladder stones, renal impairment, and pelvic floor dysfunction,[5] Current therapeutic approaches for UAB, including parasympathomimetics, alpha antagonists, botulinum toxin injection, and surgical interventions, are limited and often fail to restore effective bladder contractility. This underscores the need for novel pharmacological strategies.[1,4,6] Sericin, a natural silk protein, possesses antioxidant, anti-inflammatory, neuroprotective, and anti-apoptotic properties. Prior studies indicate that sericin enhances neuronal survival, attenuates oxidative stress, and modulates apoptotic signaling. However, its effects on bladder dysfunction remain largely unexplored.[7-10] The novelty of this study lies in applying silk sericin to an LCS-induced UAB model, as sericin's anti-apoptotic properties, while established in other tissues (e.g., hippocampus, neural tissue), have not previously been investigated in the context of bladder dysfunction. This study aimed to evaluate the therapeutic potential of sericin in an LCS-induced rat model of UAB, with a focus on apoptotic regulation and functional bladder outcomes.

Material and Methods

All stages of this research were performed in accordance with the guidelines of the National Institutes of Health (NIH; Publication No. 85-23, revised 1985). The local ethics committee approved experiments of this study (IR.TBZMED.VCR.REC.1398.469).

Animals and experimental design

In the present study, a total of 36 female Wistar rats (12-13 weeks and a weight of 220-270 g), were enrolled. Animals were housed in the Neuroscience Research Center's animal shelter at 25 ° C with a 12-hour light / dark cycle and unrestricted access to food and drinking water.

Rats were randomly divided into six groups (n=6 per group) as follows: 1. Control, 2. sham-operated (sham), 3. UAB model using Lumbar Canal Stenosis Model (LCS) as negative control group, 4. LCS group that received sericin 200 (200 mg/kg sericin/daily/P.O), 5. LCS+ sericin 250 (250 mg/kg sericin/daily/P.O), and 6. LCS+ sericin 300 (300 mg/kg sericin/daily/P.O). Sample size was based on previous similar studies; no a priori power calculation was performed, which represents a limitation.

Animal Model Preparation

To induce the UAB model, we used the LCS model, which was previously established by Sekido et al.[11]. All animals except control (LCS rats, n = 24; sham rats, n =6) were anesthetized with isoflurane (animals were placed in a closed box, gassed with isoflurane 5% in pure oxygen (1 L/min). After the induction of anesthesia, the drug levels were maintained at the appropriate level by a small mask with 0.8–1 L/min oxygen and 1–1.5% isoflurane. Under anesthesia, the L5-L6 vertebral arches were exposed, a small hole (1.5-2 mm in diameter) was drilled at the fifth vertebral arch, and a rectangular piece (3.5 mm * 5.0 mm * 0.5 mm) of silicone rubber was inserted into the epidural space. The layers of skin, subcutaneous and muscle were closed using sutures (size 3–0). The urinary bladder was compressed twice daily for emptying in the next day’s post-procedure, and an antibiotic (ciprofloxacin (1 mg/kg, i.p.) was administered.

The sham group underwent the same procedure, except that the silicone rubber was not placed in the epidural space.

Intervention

The control and sham groups, as well as the negative control group of LCS, received 10 ml/kg of normal saline by gavage daily for 14 days. Other LCS groups received the following interventions for up to 14 days: sericin 200 (200 mg/kg sericin/day orally), sericin 250 (250 mg/kg sericin/day orally), and sericin 300 (300 mg/kg sericin/day orally). Due to the solubility of sericin in normal saline (N.S.), the solution was prepared immediately before administration.

Awake urodynamic assessment

Urodynamic evaluations were performed using a custom-designed and fabricated animal urodynamic system developed by Tabriz University of Medical Sciences. The system consisted of a micro-infusion pump for continuous saline infusion, a pressure transducer connected to a signal amplifier, and a data acquisition interface linked to a computer running proprietary recording software. Before each experiment, the system was calibrated against a standard water column to ensure accuracy of pressure measurements. All urodynamic parameters, including baseline pressure, peak pressure, intercontraction interval (ICI; time between two consecutive voiding contractions), contraction frequency (number of contractions per minute), and contraction amplitude (calculated as peak pressure minus baseline pressure), were recorded and analyzed using the system's dedicated software. The custom device allowed for simultaneous monitoring of multiple animals and provided stable, reproducible recordings throughout the experimental period. Urodynamic assessments, bladder weight measurements, and Western blot analyses were performed by investigators blinded to treatment group allocation.

The animals underwent urodynamic assessment 14 days after the intervention.[12,13] For this purpose, the rats were anesthetized using inhaled isoflurane to insert two polyethylene (PE-20 and 50) tubes. First, the animal's abdomen was shaved and swabbed with 70% ethanol solution, and a low midline incision was done to expose the bladder. Using non-absorbable sutures, 6-0 purse-string sutures were surgically inserted in the dome of the bladder. Then, one end of both catheters became bell-shaped under mild heat and was inserted through a tiny incision in the dome of the bladder. To ensure the lack of leakage, saline was infused through the catheter.

The other end of the catheter was passed through the subcutaneous tissue and exited through the skin of the area between the two shoulders to prevent catheter manipulation. After the abdominal incision was closed by suturing the muscle and skin, the rats were placed in a restraining cage for 5-6 hours (including 2 hours for recovery following anesthesia). One of the catheters was connected to an infusion pump (for the continuous room temperature physiological saline infusion at a constant rate of 5 ml/h), and another was connected to the pressure transducer (perfusion compact, B/ BRAUN Melsungen AG. Typ 8714827). For each group, representative urodynamic tracings were recorded, and at least 5-6 stable voiding cycles were averaged per animal.

Bladder weight

Animals were euthanized with an overdose of ketamine/xylazine (Ketamine 300 mg/kg + xylazine 30 mg/kg) intraperitoneally after performing awake urodynamic measurements, and catheters were withdrawn from the bladder. The bladder was then removed, placed on a sheet of filter paper to collect any extra fluid (urine or injected saline), and its weight was calculated using a digital scale (Precisa XB 220A, Switzerland). The bladder tissue was then moved into the liquid nitrogen and put back together in the -80 freezer for Western blotting analysis.

Western blotting analysis

Western blotting was performed to quantitatively assess apoptotic protein expression, including Bax, Bcl-2, procaspase-3, cleaved caspase-3, procaspase-9, and cleaved caspase-9 in bladder tissue homogenates. Bladder tissues were homogenized in cold RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail (Complete™, Roche Diagnostics). Protein concentrations were determined using the Bradford method (Bio-Rad Laboratories,

Hercules, CA, USA). Equal amounts of protein (30 μg per lane) were separated by 10–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. Following electrophoresis, separated proteins were transferred electrophoretically onto polyvinylidene difluoride (PVDF) membranes (Millipore, Burlington, MA, USA). Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 hour at room temperature and then incubated overnight at 4°C with primary antibodies. The following primary antibodies were used: anti-Bax (mouse monoclonal, clone B-9, catalog No. sc-7480, Santa Cruz Biotechnology, Dallas, TX, USA) at 1:200 dilution (range 1:100–1:1000 as recommended by the manufacturer); anti-Bcl-2 (rabbit polyclonal, clone N-19, catalog No. sc-492, Santa Cruz Biotechnology) at 1:200 dilution (range 1:100–1:1000); anti-procaspase-3 (mouse monoclonal, clone E-8, catalog No. sc-7272, Santa Cruz Biotechnology) at 1:200 dilution (range 1:100–1:1000); anti-cleaved caspase-3 [32 kDa] and cleaved fragments [17/11 kDa]; anti-cleaved caspase-9 ([catalog No. 14697, Cell Signaling Technology]; anti-procaspase-9; and anti-β-actin (mouse monoclonal, clone C4, catalog No. sc-47778, Santa Cruz Biotechnology) at 1:200 dilution (range 1:100–1:1000) as loading control. After primary antibody incubation, membranes were washed three times with TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-mouse IgG or anti-rabbit IgG, 1:5000 dilution, Santa Cruz Biotechnology) for 1 hour at room temperature. Protein bands were visualized using enhanced chemiluminescence (ECL) detection system (Amersham Biosciences, Piscataway, NJ, USA) and exposed to radiographic films. Band densities were quantified using Image J software (National Institutes of Health, Bethesda, MD, USA). All densitometric values were normalized to β-actin. Expected molecular weights: Bax ~23 kDa, Bcl-2 ~26 kDa, procaspase-3 ~32 kDa, cleaved caspase-3 ~17/11 kDa, and β-actin ~43 kDa. Positive controls for each antibody, as recommended by the manufacturers, include: Jurkat whole cell lysate for Bax (sc-2204, Santa Cruz), Jurkat or U-937 for Bcl-2 (sc-2204 or sc-2239), CCRF-CEM or SUP-T1 for procaspase-3 (sc-2225 or sc-364796), and HeLa or DU145 whole cell lysate for β-actin (sc-2200 or sc-2268). Representative Western blot images are provided in Figure 2.

Statistical Analysis

Statistical analysis was performed using SPSS 21 software (SPSS Inc., Chicago, Illinois, USA). Results were expressed as the mean ± SEM, and one-way ANOVA and Tukey post-hoc tests was used for evaluations. $p<0.05$ was considered statistically significant.

Results

The body weight measurement between the groups in the first stage of the experiment did not show a significant difference between the studied groups ($p = 0.58$). The mean ± SEM bladder weight of rats in the control group was 148.50 ± 0.71 mg, in the sham group 150.50 ± 0.70, in the LCS + N.S. group, it was 217.50 ± 3.54; in the LCS + Sericin 200 group 204.01 ± 8.49 mg, in the group of LCS + sericin 250 was equal to 184.00 ± 1.41 and in the group of LCS + sericin 300, this amount was 174.01 ± 1.42 mg. The results show that compared to the control or sham groups, the weight of the bladder increased significantly in the LCS groups ($p<0.001$). After the treatment, the weight of the bladder in the group receiving sericin decreased compared to the groups without treatment. However, the results of Tukey's post-hoc tests showed that this difference was significant only in the LCS + sericin 300 group compared to the LCS + N.S. group ($p=0.004$). Without histological analysis (e.g., H&E staining or Masson's trichrome), it remains unclear whether this weight reduction reflects decreased edema, reduced smooth muscle hypertrophy, or other tissue changes.

Urodynamic assessment

Representative urodynamic tracings from each group are shown in Figure 1. These tracings visually demonstrate the prolonged intercontraction interval in LCS rats and its partial normalization following sericin treatment.

Urodynamic evaluations showed that the basal mean (SD) pressure values in the control and sham groups were 12.02 (2.50) and 13.04 (3.17) cmH_2O, respectively, which decreased to 6.73 (3.72) after model induction and increased after treatment with different doses of sericin. However, the results of the One-way ANOVA test showed that this difference was not statistically significant between the studied groups (P=0.357). Also, the results of Tukey's post-hoc tests did not show significant differences between the studied groups (P>0.05). Peak pressure decreased after LCS and increased after different doses of Sericin. However, the results of Tukey posthoc analysis showed that this difference was statistically significant between the model and control group.

The results of the One-way ANOVA test showed that inter contraction interval (ICI) was increased in the LCS group to 224.12 (0.87) seconds compared to the control and sham rats (P<0.05). Also, the results of Tukey's post-hoc tests showed that treatment with different doses of sericin reduced this interval; however, compared to rats receiving normal saline, it was statistically significant in Sericin 300 mg compared to the LCS group (P=0.041) (Figure 1, Table 1). Importantly, no significant improvements were observed in peak pressure, contraction frequency, or contraction amplitude, indicating that sericin's functional effects were limited to ICI.

The remaining urodynamic parameters (contraction, and amplitude also did not show statistically significant differences between the studied groups (Table 1).

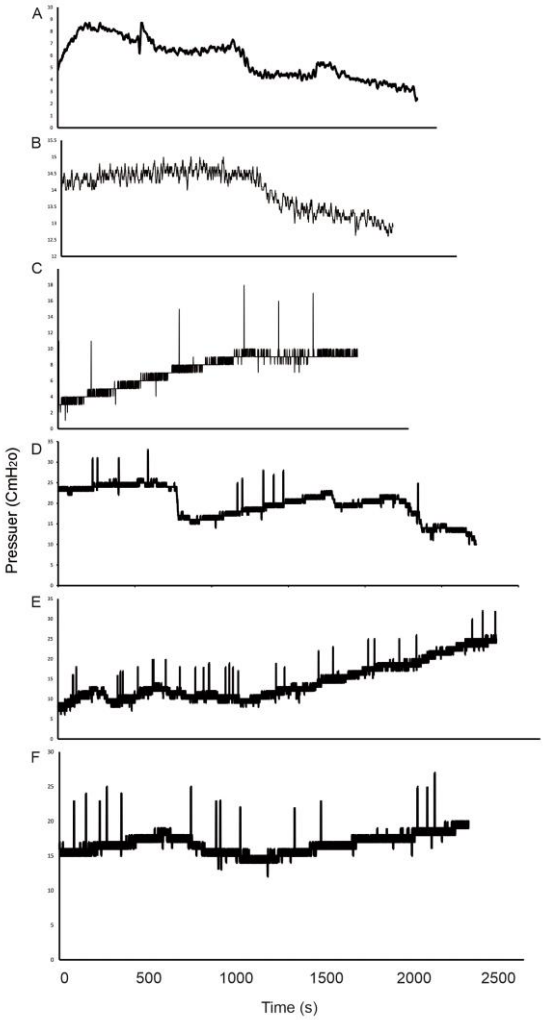

Figure 1. Representative urodynamic tracings from each study group. (A) Control; (B) Sham; (C) UAB (LCS + normal saline); (D) UAB + Sericin 200 mg/kg; (E) UAB + Sericin 250 mg/kg; (F) UAB + Sericin 300 mg/kg. Tracings demonstrate prolonged intercontraction interval in the UAB group (C) and progressive normalization with increasing sericin doses, particularly at 300 mg/kg (F).

Table 1 Urodynamic parameters (cystometry) in experimental groups

Groups	Baseline pressure (cmH_2O)	Peak pressure (cmH_2O)	*ICI (second)	*contractions/min	*Amplitude (cmH_2O)
Control	12.02 (2.50)	#32.25 (12.41)	#83.65 (26.85)	2.37 (1.72)	18.99 (4.09)
Sham	13.04 (3.17)	25.16 (5.18)	#85.53 (16.38)	1.73 (0.71)	17.76 (2.58)
UAB	6.73 (3.72)	#12.50 (3.53)	#224.12 (0.87)	0.24 (0.01)	13.75 (2.75)
UAB+ Sericin 200	10.76 (3.82)	19.50 (2.88)	110.60 (26.15)	1.21 (0.57)	16.99 (3.53)
UAB+ Sericin 250	12.29 (2.76)	26.75 (9.84)	94.49 (17.75)	1.64 (0.53)	15.27 (1.42)
UAB+ Sericin 300	14.17 (7.14)	25.00(4.69)	‡81.19 (30.93)	1.71 (0.52)	17.76 (1.87)

Data presented as Mean (SEM);*ICI: intercontraction interval.
#Tukey post-hoc analysis showed a statistically significant difference in the UAB group compared to the control group ($p < 0.05$).
‡$p = 0.041$ compared to control and sham groups (Tukey post-hoc).
Note: Functional improvements with sericin treatment were limited to ICI only; no significant changes were observed in peak pressure, contraction frequency, or amplitude.

Apoptosis-related factors in bladder tissue

The study's findings demonstrated that the apoptosis-related factors including Bax, and the ratio of cleaved caspase3/procaspase3 and cleaved caspase9/procaspase9 in bladder tissue were increased following the model induction and reduced after treatment. However, it was only statistically significant for dose 300 mg for Bax ($P<0.05$), and in all doses of sericin for the ratio of cleaved caspase9/procaspase9, and in dose 250, and 300 mg for cleaved caspase3/procaspase3 ($P<0.001$). In terms of Bcl-2 expression, our results revealed that the expression of Bcl-2 in the LCS groups was significantly reduced compared to the control and sham groups ($P<0.001$). Treatment with different doses of Sericin increased the amount that was highest in the group receiving sericin 300 compared to the LCS group receiving normal saline ($P <0.01$) (Figure 2). Despite these anti-apoptotic effects, functional improvements in detrusor contractility were not observed, suggesting that apoptotic modulation alone may be insufficient to restore bladder contractile function in this model.

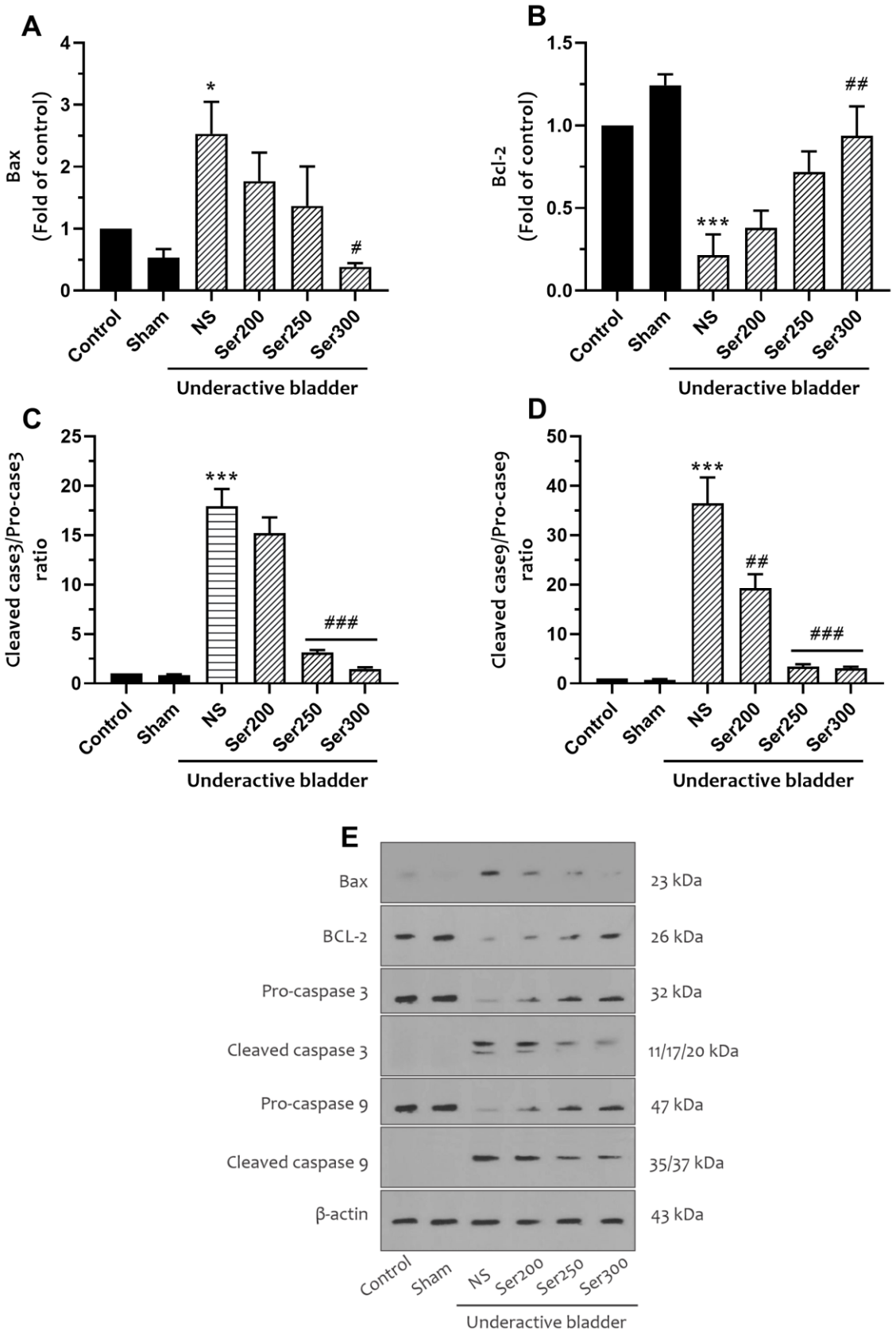

Figure 2. Western blot analysis of apoptotic factors in bladder tissue. Bar graphs show relative protein expression (normalized to β-actin) for (A) Bax, (B) Bcl-2, (C) ratio of cleaved caspase-3/procaspase-3, and (D) ratio of cleaved caspase-9/procaspase-9. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus control group; #p < 0.05, ##p < 0.01 versus UAB (LCS + normal saline).

Discussion

This study demonstrated that oral sericin exerted protective effects in an LCS-induced rat model of UAB, but functional improvements were strictly limited to the intercontraction interval, with no significant improvement in peak pressure, contraction amplitude, or contraction frequency. The key findings were: (1) sericin attenuated the increased bladder weight at the highest dose; (2) sericin improved ICI at 300 mg/kg but did not improve detrusor contractility parameters; and (3) sericin modulated apoptosis by downregulating Bax and caspases while upregulating Bcl-2. . These results are consistent with prior evidence showing sericin's neuroprotective and anti-apoptotic roles in models of diabetes, neurodegeneration, and oxidative stress.[14-16]

Current UAB treatments mainly focus on symptom relief and bladder emptying facilitation, with no definitive method to restore detrusor contractility.[6] This underscores the urgent need for further research and development of targeted therapies to manage UAB effectively.

The relationship between apoptosis and detrusor underactivity remains incompletely understood. Apoptosis may contribute to UAB through loss of smooth muscle cells, damage to afferent nerves, or impairment of the interstitial cells of Cajal that modulate bladder contractility. Studies have demonstrated that apoptosis of detrusor smooth muscle cells occurs in bladder dysfunction models, with macrophage migration inhibitory factor (MIF) promoting smooth muscle death in the obstructed bladder.[17] Regarding neuronal involvement, ischemia/reperfusion injury has been shown to cause both intramural bladder nerve dysfunction and a 14-fold increase in detrusor cell apoptosis, suggesting a link between nerve damage and apoptotic cell death.[18,19] Furthermore, injury to spinal micturition centers can increase neuronal apoptosis via altered Bax/Bcl-2 expression, similar to the apoptotic markers examined in the present study.[19] In the LCS model, chronic compression of cauda equina nerves may induce apoptosis in both neuronal and bladder wall tissues, contributing to functional impairment.[20] Our finding that sericin modulates apoptotic markers in bladder tissue suggests a potential protective mechanism, though whether this directly translates to improved contractile function requires further investigation, and our data suggest that this translation may be limited or require longer treatment duration. The observed anti-apoptotic activity aligns with sericin's known capacity to inhibit ROS accumulation and preserve mitochondrial integrity. In bladder tissue, where ischemia and neuronal damage contribute to UAB, such effects could theoretically help preserve detrusor function. Although urodynamic improvements were modest, the significant normalization of ICI at 300 mg indicates potential for partial functional recovery, particularly in sensory or afferent pathways. However, the lack of significant improvement in peak pressure and contraction amplitude which are more direct measures of detrusor contractility represents a critical limitation of this study. ICI prolongation in LCS rats likely reflects impaired sensory function or altered bladder afferent activity, and its normalization by sericin suggests effects on afferent pathways or central micturition control rather than direct enhancement of detrusor contractility.[21] The short intervention period (14 days) may have been insufficient to allow structural or functional recovery of the detrusor muscle itself, even though apoptotic markers were favorably modulated. Future studies should incorporate longer treatment durations (e.g., 4-8 weeks) and in vitro bladder strip preparations to directly assess the effects of sericin on smooth muscle contractility, as well as investigation of afferent signaling mechanisms. The dissociation between anti-apoptotic effects and the lack of improved detrusor contractility requires careful explanation. Several possibilities arise from the literature:

First, ICI prolongation in LCS rats likely reflects impaired sensory (afferent) function or altered bladder afferent activity rather than primary detrusor muscle dysfunction. Sekido et al. (2012)[11] demonstrated that LCS-induced cauda equina compression damages both afferent and efferent spinal nerves, but afferent dysfunction may dominate the early phenotype. Normalization of ICI by sericin without improvement in peak pressure suggests

that sericin may exert effects on afferent pathways or central micturition control rather than directly enhancing detrusor contractility. This is consistent with observations in other UAB models where prostaglandin E2 failed to restore voiding despite increasing afferent activity.[22]

Second, the 14-day intervention period may have been sufficient to modulate apoptotic signaling but insufficient to allow structural or functional recovery of the detrusor muscle itself. Oshiro et al. (2014)[23] showed that age-related detrusor underactivity is associated with downregulation of connexin43 (Cx43) gap junction proteins, leading to impaired intercellular communication between smooth muscle cells. Bladder contractility depends not only on individual myocyte health but also on coordinated electrical coupling via gap junctions. Anti-apoptotic effects alone may preserve myocyte viability but cannot rapidly restore the complex network of gap junctional intercellular communication once disrupted.

Third, UAB pathophysiology involves multiple parallel mechanisms: apoptosis, oxidative stress, chronic ischemia, fibrosis, and impaired neurotransmitter release. Shimizu (2024)[24] and Wang et al. (2015)[22] highlighted that in both metabolic syndrome-associated DU and LCS-induced DU, bladder underactivity is associated with downregulation of agrin (involved in acetylcholine receptor clustering) and upregulation of M2 muscarinic receptors as a compensatory but insufficient response. Sericin's anti-apoptotic effects may address only one component of a multifactorial pathology, leaving other deficits (e.g., impaired purinergic transmission, detrusor fibrosis, or smooth muscle atrophy) unaddressed.

Fourth, as noted by Sekido et al. (2012), LCS rats exhibit preserved responsiveness to carbachol but altered responses to electrical field stimulation, particularly at lower frequencies, suggesting a purinergic component deficit. Sericin's lack of effect on peak pressure and contraction amplitude may indicate that it does not restore the purinergic or cholinergic signaling deficits that underlie reduced detrusor contractility in this model.

The anti-apoptotic effects observed in this study, downregulation of Bax and caspases with upregulation of Bcl-2 are consistent with sericin's known capacity to inhibit ROS accumulation and preserve mitochondrial integrity in neural and diabetic models. However, the translation of these cellular effects into functional bladder recovery appears limited. This contrasts with studies in other tissues (e.g., hippocampus, pancreas) where anti-apoptotic effects correlated more directly with functional outcomes, suggesting that bladder detrusor contractility may have greater reserve capacity but also greater dependence on non-apoptotic factors such as gap junction integrity and extracellular matrix composition.

Notably, the increased bladder weight in LCS rats and its partial reduction with sericin 300 mg/kg without histological confirmation, could reflect decreased edema, reduced inflammation, or attenuated smooth muscle hypertrophy. However, without Masson's trichrome staining or α-SMA immunostaining, we cannot determine whether sericin reduced fibrosis or improved smooth muscle organization. Given that fibrosis is a key feature of DU in both aging and metabolic syndrome[24,25], this represents a critical gap.

An important consideration regarding oral sericin administration is its bioavailability. As a protein, sericin would be expected to undergo digestion in the gastrointestinal tract, raising questions about whether intact sericin or its peptide fragments mediate the observed effects. Emerging evidence suggests that sericin-derived peptides may resist complete degradation and exert biological activities.[14] Sericin is rich in serine (approximately 30-40%), and its unique repetitive amino acid sequence may yield bioactive peptides with antioxidant properties even after partial hydrolysis. Studies have demonstrated that orally administered sericin increases antioxidant enzyme activity in multiple organs, including the brain, suggesting that either absorbed peptides or sericin-induced metabolic effects (e.g., via gut microbiota modulation) may underlie its systemic actions. Furthermore, sericin has been shown to bind metal ions and scavenge free radicals directly, properties that may persist in smaller peptide

fragments. However, the present study did not validate sericin bioavailability or tissue distribution; future studies should investigate the pharmacokinetics of sericin-derived peptides and whether they reach bladder tissue in biologically relevant concentrations.

Limitations

This study has several important limitations that temper the conclusions and translational significance. First and foremost, the functional improvements observed with sericin treatment were strictly limited to the intercontraction interval, with no significant improvements in peak pressure, contraction amplitude, or contraction frequency. These latter parameters are key indicators of detrusor contractility and bladder emptying capacity; therefore, claims of therapeutic potential for improving voiding function must be qualified accordingly. Second, a notable dissociation was observed between anti-apoptotic effects and functional recovery. Despite significant modulation of Bax, Bcl-2, and caspase proteins, these cellular changes did not translate into improved detrusor contractility. This finding suggests that either anti-apoptotic mechanisms alone are insufficient to restore bladder function, that the 14-day treatment period was too short for functional recovery despite cellular protection, or that other pathophysiological pathways, such as oxidative stress, inflammation, gap junction dysfunction, or fibrosis, are more directly relevant to functional impairment. Third, mechanistic evidence in this study remains incomplete. While anti-apoptotic effects were demonstrated, we did not assess oxidative stress markers (such as MDA, SOD, and catalase), inflammatory mediators (including IL-1β, TNF-α, and NF-κB), or gap junction proteins like connexin43, all of which have been implicated in detrusor underactivity in prior studies. Fourth, histopathological analysis was not performed. The absence of hematoxylin and eosin staining, Masson's trichrome staining for fibrosis, and immunostaining for smooth muscle markers such as α-SMA severely limits interpretation of the observed bladder weight changes, as it is impossible to distinguish between true detrusor hypertrophy, edema, inflammation, or fibrosis. This represents a major limitation, and future studies must include histological validation. Fifth, the sample size of six animals per group with no priori power calculation means the study may be underpowered to detect modest but clinically relevant differences, and marginal significance values should be interpreted cautiously. Sixth, the oral bioavailability of sericin was not validated. As a protein, sericin would be expected to undergo digestion in the gastrointestinal tract, and without pharmacokinetic or tissue distribution evidence demonstrating that sericin or its bioactive peptides reach bladder tissue in sufficient concentrations, the translational rationale remains weakened. Seventh, the intervention duration was relatively short at only 14 days, which may have been insufficient to allow full structural or functional recovery of the detrusor muscle; longer-term studies of at least four to eight weeks are needed. Eighth, our analysis focused exclusively on bladder tissue, without assessing supraspinal or spinal micturition centers, despite their likely involvement in UAB pathophysiology. Ninth, the dose-response relationship beyond 300 mg/kg remains unexplored, and higher doses might yield greater functional improvements. Finally, we did not assess afferent or efferent nerve function directly. Given that ICI improvements suggest sensory pathway modulation, direct assessment of nerve function such as nerve conduction studies or afferent recording, would strengthen mechanistic conclusions.

Future directions

Based on these limitations, several directions for future research are recommended. Longer treatment durations of at least four to eight weeks should be employed to assess whether extended sericin administration can translate the observed anti-apoptotic effects into meaningful functional recovery, with intermediate time points to track the temporal relationship between cellular protection and functional improvement. Histological validation is critically needed; future studies must include hematoxylin and eosin staining to assess overall tissue architecture, Masson's trichrome staining to evaluate fibrosis, and immunostaining for smooth muscle markers such as α-SMA to

distinguish between true detrusor hypertrophy, edema, and fibrotic changes. Given the important role of gap junction intercellular communication in coordinated detrusor contraction, as highlighted by Oshiro and colleagues, future investigations should assess connexin43 expression and localization in bladder tissue following sericin treatment. Pharmacokinetic assessment of oral sericin bioavailability is essential, including measurement of sericin-derived peptides in plasma and bladder tissue to confirm that systemically relevant concentrations are achieved. In vitro bladder strip contractility studies should be performed to directly assess the effects of sericin on detrusor smooth muscle contractility independent of neural influences, using both cholinergic and purinergic receptor agonists. A more comprehensive mechanistic evaluation should include oxidative stress markers (such as MDA, SOD, and catalase), inflammatory mediators (including IL-1β, TNF-α, and NF-κB), and assessment of afferent versus efferent nerve function through nerve conduction studies or pelvic nerve recording. Finally, testing sericin in other UAB models, such as diabetic, ischemic, or post-obstructive models, would help validate the generalizability of these findings and determine whether the dissociation between anti-apoptotic effects and functional recovery is specific to the LCS model or represents a broader limitation of targeting apoptosis alone in UAB.

Conclusion

Sericin demonstrated anti-apoptotic effects in bladder tissue and partially improved ICI (but not detrusor contractility parameters: peak pressure, contraction amplitude, or contraction frequency) in an LCS-induced UAB model, with the strongest effects observed at 300 mg/kg. These findings are preliminary, and the therapeutic claims must be tempered given the dissociation between apoptotic modulation and functional recovery. The lack of significant improvements in peak pressure and contraction amplitude, the most direct measures of detrusor contractility, indicates that anti-apoptotic mechanisms alone are insufficient to restore bladder emptying function in this model. Sericin remains a candidate requiring further investigation, but extensive additional studies with longer treatment durations, histological validation, pharmacokinetic assessment, and evaluation of alternative mechanisms (gap junctions, purinergic signaling, fibrosis) are essential before any translational consideration.

Authors' Contribution

Hanieh Salehi-Pourmehr and Sakineh Hajebrahimi: Conceptualization, Data curation, Writing – review & editing.
Javad Mahmoudi: Data curation, Analysis, Writing – review & editing
Nasrin Abolhasanpour and Hanieh Salehi-Pourmehr, Leila Hosseini: Performing the experiments of the study, Writing – original draft, Writing – review & editing.
Hadi Mostafaei: Formal statistical analysis, Writing – review & editing.
Fateme Tahmasbi: Writing – original draft

Declaration of Competing Interest

The authors declare that they have no known competing interests.

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