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Research Article

The Therapeutic Effect of Ginsenoside Rb1 against Mechanical Trauma in a Rat Model of Postpartum Stress Urinary Incontinence

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Aims. The aim of this study was to confirm the repairing effect of ginsenoside Rb1 (GS-Rb1) on mechanical trauma to periurethral tissues caused by childbirth and to explore its potential preventive mechanisms for mechanical trauma-induced stress urinary incontinence. *Methods*. 48 healthy adult female Sprague–Dawley (SD) rats were randomly divided into four groups: normal control, SUI groups, L-GS-Rb1 groups, and H-GS-Rb1 groups, with 12 rats in each group. The histopathological examinations of the urethral were performed to detect the morphological changes after repair of periurethral traumatic tissue. The TGF-β1/Smad and NRF2/ARE signaling pathways related to periurethral tissue trauma repair were determined by RT-PCR and western blot. The bladder capacity and LPP were examined in rats. *Results*. GS-Rb1 significantly decreased the number of fragmented and disorganized elastic and muscle fibers in the urethra and anterior vaginal wall of SUI rats. GS-Rb1 also increased the collagen content and reduced damage to the structural integrity of the periurethral myofibers. Furthermore, GS-Rb1 promoted expressions of TGF-β1, Smad2, Smad3, Smad7, p-Smad3, p-Smad2, and collagens I and III. It also increased the protein levels of Nrf2, GPX1, and MnSOD. The bladder capacity and LPP of rats in the L-GS-Rb1 group were close to those of rats in the normal groups. *Conclusions*. Ginsenoside Rb1 promotes the repair of periurethral tissue trauma in the postpartum period and has a preventive effect on the occurrence of stress urinary incontinence.

1. Introduction

Stress urinary incontinence (SUI) is a highly prevalent condition, particularly among women. Globally, 200 million or more people suffer from urinary incontinence [1, 2]. The etiology, like other pelvic floor disorders (PFDs), is related to pelvic floor weakening and/or tears, usually due to obstetric trauma. The anatomical factors affecting the urethra include changes to the thickness of the urethral mucosa and muscular layer and a decrease in its elasticity that are related to the onset of SUI [3]. Histological findings in periurethral tissues of women with SUI consistently show abnormal collagen remodeling, loss of functional elastic fiber networks, and muscle fiber rupture [4]. At present, treatment for SUI occurs after a diagnosis of SUI is made which results in a high long-term recurrence rate [5]. Existing conservative treatments, as well as pelvic floor muscle exercise [6], show that intervention in the early postpartum stage is very beneficial for reducing SUI-related morbidity. However, early postpartum pharmacotherapy for pelvic floor tissue damage caused by mechanical trauma remains limited.

Continued research in this field has confirmed that the Transforming Growth Factor-β1 (TGF-β1)/Smad pathway mediates ECM remodeling that is associated with SUI pathology. TGF-β1 regulates ECM structure by modulating fibronectin, collagen, and elastin due to the phosphorylation of Smad2 and Smad3 [7]. SUI is also associated with oxidative damage resulting from

mechanical trauma [8]. Many studies have shown that activation of the antioxidant response elements Nuclear factor erythroid2-related factor 2 (Nrf2)/Antioxidant Response Elements (ARE) and the TGF-β1/Smad signaling pathway affect the treatment of SUI [9, 10].

Ginsenoside Rb1 (GS-Rb1), a crucial monomeric active constituent extracted from *Panax ginseng*, *Notoginseng radix* [11, 12], has attracted widespread attention because of its antioxidant and anti-inflammatory properties, as well as its ability to promote burn wound healing, reduce apoptosis, and induce cytokine production [13–16]. GS-Rb1 has also been shown to modulate the expression and activity of numerous proteins and pathways, including the TGF-β1/Smad, PI3K/mTOR, Nrf2/ARE, and MAPK/NF-κb pathways [16].

Based on these findings, it is plausible that GS-Rb1 may mitigate oxidative damage and promote matrix and tissue repair associated with mechanical-trauma-induced SUI. Thus, we hypothesized that GS-Rb1 inhibits damage to the urethral sphincter, elastic fibers, and other tissues, and promotes tissue repair. This study was conducted to evaluate the effects of GS-Rb1 on the early treatment of mechanical trauma of postpartum periurethral tissue and the prevention of SUI, together with an exploration of the potential underlying mechanisms.

2. Materials and Methods

2.1. SUI Model and Experimental Design. Forty-eight Sprague–Dawley (SD) rats (two-month-old females, weighing 220–250 g) were obtained from the animal experimental center of Shanxi Medical University. The rats were housed in cages of four at 25°C ± 2°C and a 12 h light/dark cycle, with free access to food and water. All animal procedures were performed per the Guide for the Care and Use of Laboratory Animals and approved by the Animal Experiment Ethics Committee of Shanxi Cancer Hospital (Approval Number 2021001, Shanxi, China). GS-Rb1 doses were obtained from the China Yunnan Teana Pharmaceutical Co. Ltd. (Yunnan, China). Based on the clinical dosage of GS-Rb1 prescribed to Chinese patients (60–180 mg/day), the equivalent dose for rats was calculated using the body surface area conversion method (coefficient 0.018), according to the following formula: Rat dosage = human dosage (mg/day) × 0.018/0.2 kg = 5.4/16.2/mg/kg/day. Using this dose as the theoretical reference, the GS-Rb1 concentrations of 4.5 (low dose) and 18 (high dose) mg/day, dissolved in saline, were selected for the low- and high-dose treatment groups, respectively. The rats were divided into four groups ($n = 12$ per group): control; SUI; SUI + H-GS-Rb1 (18 mg/kg); SUI + L-GS-Rb1 (4.5 mg/kg). The animals were anesthetized by intraperitoneal administration of 3% pentobarbital sodium (30 mg/kg) into the cavum abdominis and placed on their backs. A balloon containing 3 mL of saline was inserted into the vagina with a transurethral 12F catheter and left in place for 3 hours. Dysfunctional voiding was apparent in all rats following the procedure. GS-Rb1 (4.5 mg/kg and 18 mg/kg in saline) was administered intragastrically to the SUI + GS-Rb1 group after 24 hours and daily thereafter for one week. In the control and SUI groups, the rats were administered the same volume of saline. After drug withdrawal for 24 hours, the bladder capacity and LPP of the rats were measured, and the rats were euthanized thereafter. The urethral and anterior vaginal walls of the rats were collected for further analysis. The urethral and anterior vaginal wall tissues of six rats randomly selected from each group were fixed in 10% buffered formalin (pH 7.4), while the remaining tissue samples were frozen in liquid nitrogen.

2.2. Assessment of Urodynamics. Rats were anesthetized as above and intravesicular catheterization was performed via suprapubic cystostomy using a PE-50 polyethylene tube with a flared end as an anchor. The bladder was suctioned until it was empty through an inserted epidural catheter, and methylene blue saline at 37°C was injected at a rate of 10 mL/h. The end of the injection coincided with the appearance of the first drop of saline at the urethral meatus. The amount of saline injected corresponded to the bladder capacity and was injected using a PE-50 polyethylene tube connected to 50 ml syringe. The syringe was raised slowly and when the first drop of saline appeared at the urethral meatus, the height of the syringe was measured and was used to calculate the leak point pressure (LPP). This procedure was performed five times on each rat, and the average LPP was determined. After euthanasia, the urethral and anterior vaginal walls of the rats were harvested.

2.3. Histological and Immunohistochemical Analyses. The harvested tissues were fixed with 10% formalin, paraffin-embedded, and sectioned. Masson's trichrome was applied to determine the integrity of the urethral muscle system. Picrosirius red stains for collagen detection and Hart's stain for elastin detection were applied using standard protocols. Immunohistochemistry was performed per protocol (BIOS Biology Co., Ltd., China). Image J software was used to analyze the collagen content, expressed as the collagen volume fraction and calculated by the formula: collagen volume fraction = (collagen-positive blue area/total tissue area) × 100%.

2.4. RT-PCR. Total RNA was extracted using TRIzol (HaDa Biotech, Taiyuan, China) and reverse-transcribed to cDNA using the Revert Aid First Strand cDNA Synthesis Kit (HaDa Biotech). Quantitative PCR was performed on a Real-Time PCR platform (Applied HaDa Biotech) per the provided directions. GADPH was used for normalization, and relative expression was determined by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for the Real-Time PCR are provided in Supplemental Table 1 (Supplementary Material: primer sequence of TGF-β1, Smad3, Smad7, and collagens I and III).

2.5. Protein Extraction and Western Blotting. The harvested tissues were homogenized in RIPA lysis buffer. Twenty micrograms of protein per lane were separated on 10%

SDS-PAGE and transferred to PVDF (Bioss, Beijing, China). After blocking, the blots were sequentially probed with primary and secondary antibodies (1 : 10000). The antibodies used were anti-collagen I (bs-10423R) and anti-collagen III (bs-0549R) (Bioss, Beijing, China), anti-TGF-β1 (BD-PT4632), anti-Smad2 (BD-PT4331), anti-Smad3 (BD-PT4334), anti-Smad7 (BD-PN2330), anti-p-Smad2 (BD-PP0584), anti-p-Smad3 (BD-PP1501), anti-Nrf2 (BD-PT3189), anti-SOD-2 (BD-PT5575), anti-GPX-1 (BD-PN2008) (Biodragon, Beijing, China), and anti-β-Actin (Santa Cruz Biotechnology, CA, USA). The membranes were subsequently washed for 10 minutes three times in TBST, followed by incubation for 2 hours with a goat antirabbit horseradish peroxidase-conjugated antibody (1 : 2000) and visualized via chemiluminescence. Densitometry was used to quantify the results.

2.6. Statistical Analysis. All data were expressed as means ± SD and analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Data were analyzed by analysis of variance (ANOVA), and P values <0.05 were considered significant. GraphPad Prism 8.0.1.244 (GraphPad Software, San Diego, CA, USA) was used for figure construction.

3. Results

3.1. Effect of GS-Rb1 on the Bladder Capacity and LPP of Rats. SUI was successfully induced in the rat models using the VD method. The LPP was 35.4 ± 1.4 cm H_2O in control rats, 30.7 ± 2.4 cm H_2O in L-GS-Rb1 rats, 27.1 ± 2.8 cm H_2O in H-GS-Rb1 rats, and 24.5 ± 3.5 cm H_2O in SUI model rats (Figure 1(a)). Thus, the LPP was lower in SUI rats than in the control or GS-Rb1 rats. The bladder capacities were abnormally increased in SUI rats (0.95 ± 0.19 ml) relative to the controls (0.53 ± 0.03 mL) and SUI + GS-Rb1 rats (0.56 ± 0.07 ml) but were reduced in the SUI + H-GS-Rb1 rats (0.41 ± 0.07 mL) (Figure 1(b)).

3.2. Pathological Effects on Periurethral Tissue in Rats. The urethral striated muscles of the control rats were observed to be compact and circumferential on Masson staining. However, the muscle bundles of SUI rats had specifically changed and showed splitting of the muscle fibers (Figure 2(a)). In addition, the collagen content in tissues obtained from GS-Rb1-treated rats had increased compared with the SUI rats. Picrosirius red staining indicated thinning of the vaginal wall and reduced collagen content of the urethra and vaginal walls in the SUI group compared with the other groups (Figure 2(b)). The percentage of collagen in the urethral and anterior vaginal walls of the SUI group was significantly lower than in other groups (Figure 2(c)). Elastic fibers were tightly connected to the muscle bundles of smooth muscle with organization in control rats that also lined up with the vaginal wall tissue, whereas, in SUI rats, the fibers appeared fragmented and disorganized (Figure 3).

3.3. Effect of GS-Rb1 on the Expression of Factors and Proteins Associated with the TGF-β1/Smad3 Signaling Pathway. The role of the TGF-β1/Smad3 pathway in the recovery from trauma induced by VD was investigated. Gene expression profiles of ECM in the tissue surrounding the urethra tissues were analyzed. Compared with the SUI group, GS-Rb1 treatment resulted in a significant increase in the mRNA levels of TGF-β1, Smad3, Smad7, and collagens I and III (Figure 4(a)). Western Blot results further confirmed that the protein levels of the TGF-β1, Smad2, Smad3, Smad7, collagens I and III, and Smad2 and Smad3 phosphorylation were also increased in GS-Rb1-treated groups (Figures 4(b) and 4(c)).

3.4. Effects of GS-Rb1 on the Expression of Proteins Associated with the Nrf2/ARE Axis. Significant increases in levels of Nrf2, GPX1, and Mnsod proteins were observed in GS-Rb1-treated groups (Figure 5(a)). In the H-GS-Rb1 group, the levels of these proteins were higher than in the L-GS-Rb1 group (Figures 5(b)–5(d)). The results indicated that GS-Rb1 treatment may be associated with the stimulation of Nrf2/ARE signaling and inhibition of further tissue damage after mechanical trauma caused by VD.

4. Discussion

Vaginal delivery can cause traumatic injury to the pelvic floor tissues, potentially leading to stress urinary incontinence due to damage to the nerves, muscles, and connective tissues responsible for maintaining continence. There is evidence that injury to the connective tissue injury is involved in the development of SUI [17]. In the present study, we established a mechanical-trauma-induced rat model of postpartum stress urinary incontinence using the VD [18]. The structural changes of urethral and periurethral tissues were investigated. SUI model rats showed visible disruption of the urethral muscle fibers, together with reduced connective tissue and collagen expression. After GS-Rb1 treatment, the urethral microstructure recovered significantly relative to rats with untreated SUI. GS-Rb1 mitigated muscle fiber damage and increased collagen concentrations. The urethral wall is rich in loose connective tissue, elastic fiber, collagen, and other components, which render the urethral wall with great elasticity and flexibility. Under the action of external forces, the urethral wall can effectively close the urethra and ensure tightness of the urethral closure [19, 20]. The histological assessment showed that both low and high doses of GS-Rb1 had excellent therapeutic efficacy on SUI rats. To further evaluate the effect of GS-Rb1 on SUI, bladder capacity and LPP were measured in all rats after 7 days. LPP improved in the GS-Rb1 treatment group. However, the bladder capacity of rats in the SUI group was higher than that of rats in other groups. We speculate that short-term trauma causes edema of urethral tissue, which further causes urinary retention and increases bladder capacity in SUI rats.

TGF-β1 is known to be involved in injury repair processes through its role in cell proliferation, differentiation,

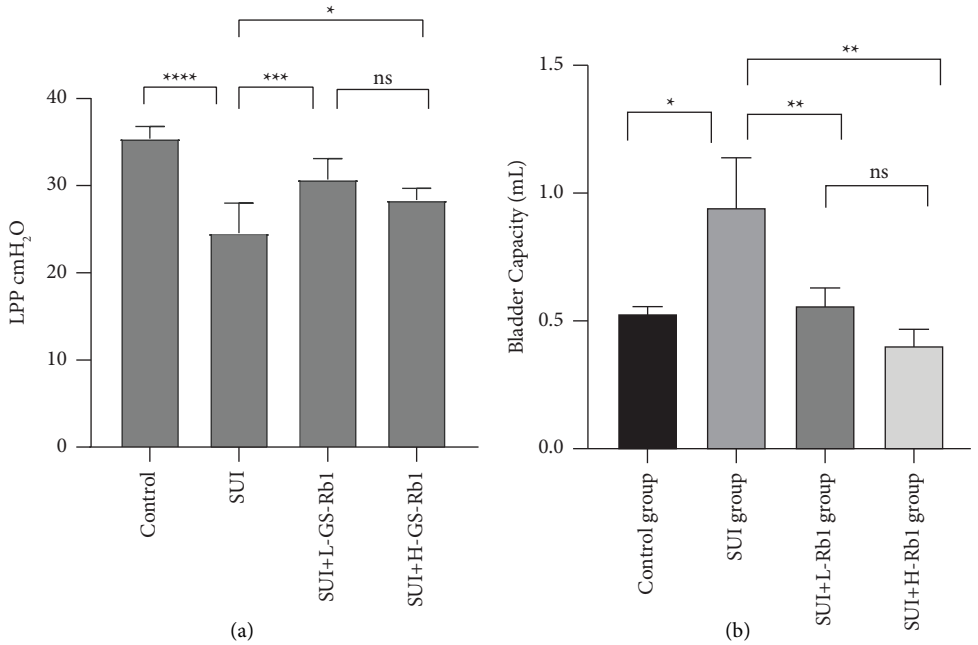


Figure 1: Comparison of LPP and bladder capacity in four groups of rats. SUI in rats was induced by VD, then treated with different concentrations of GS-Rb1 or saline for one week. The LPP and bladder capacity in rats was measured. Data are presented as mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$, ns: $P > 0.05$.

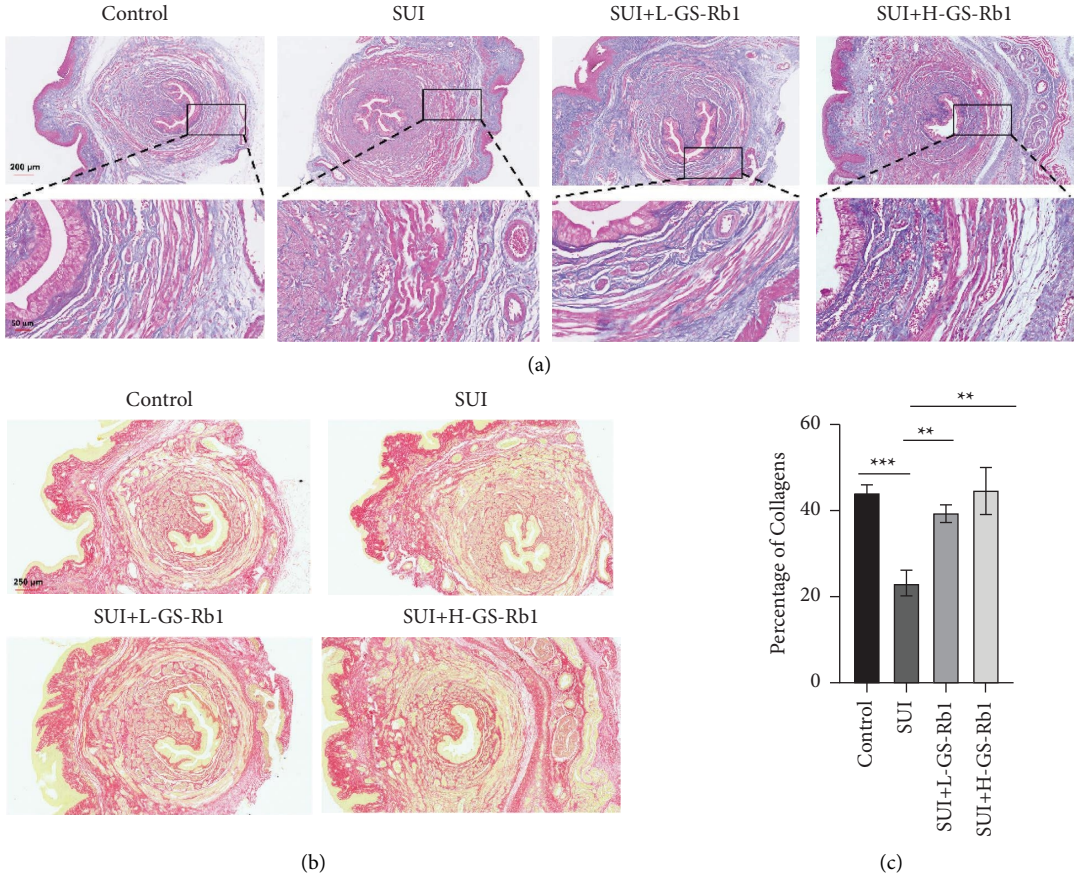


Figure 2: GS-Rb1 recovered urethral sphincter muscle structure and collagen concentrations. Urethral sphincter muscle structure morphology in rats was detected by Masson's trichrome stain. (b, c). The collagen content of urethral and anterior vaginal wall tissues in rats was demonstrated by Picrosirius red staining (b), and the percentage of collagen was analyzed (c). Data are presented as mean ± SD, $n = 6$. $^{**}P < 0.01$, $^{***}P < 0.001$.

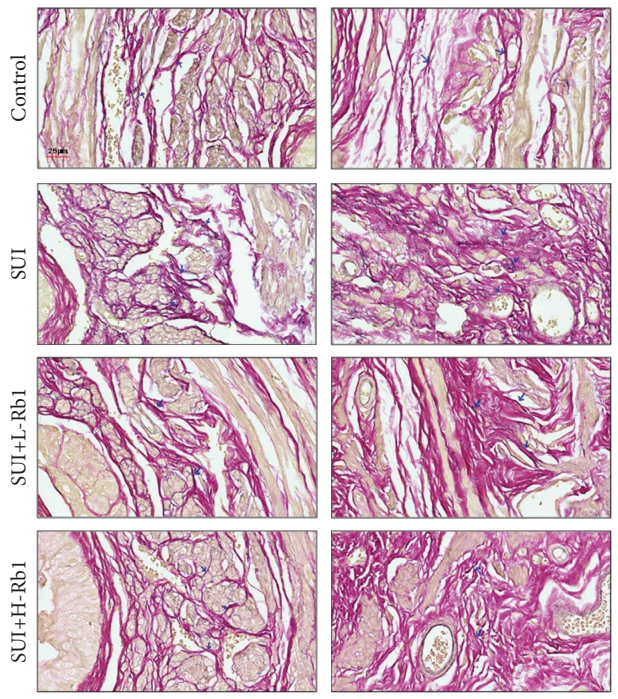
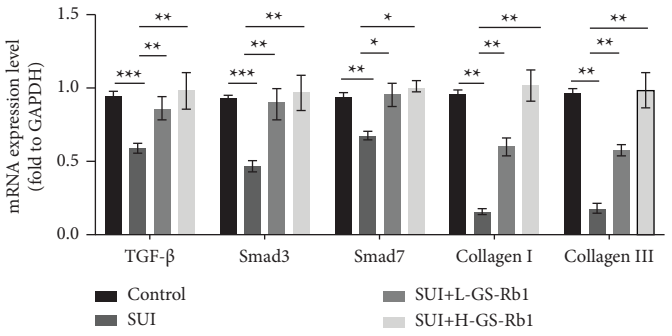
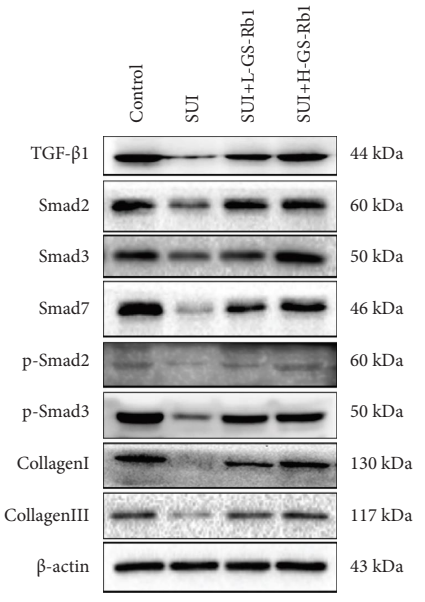


Figure 3: GS-Rb1 promotes the repair of elastic fibers in the urethra and vagina. Analysis of the morphology of the elastic fibers of the urethra and vaginal walls by Hart's stain. The blue arrow indicates the elastic fiber.



(a)



(b)

Figure 4: Continued.

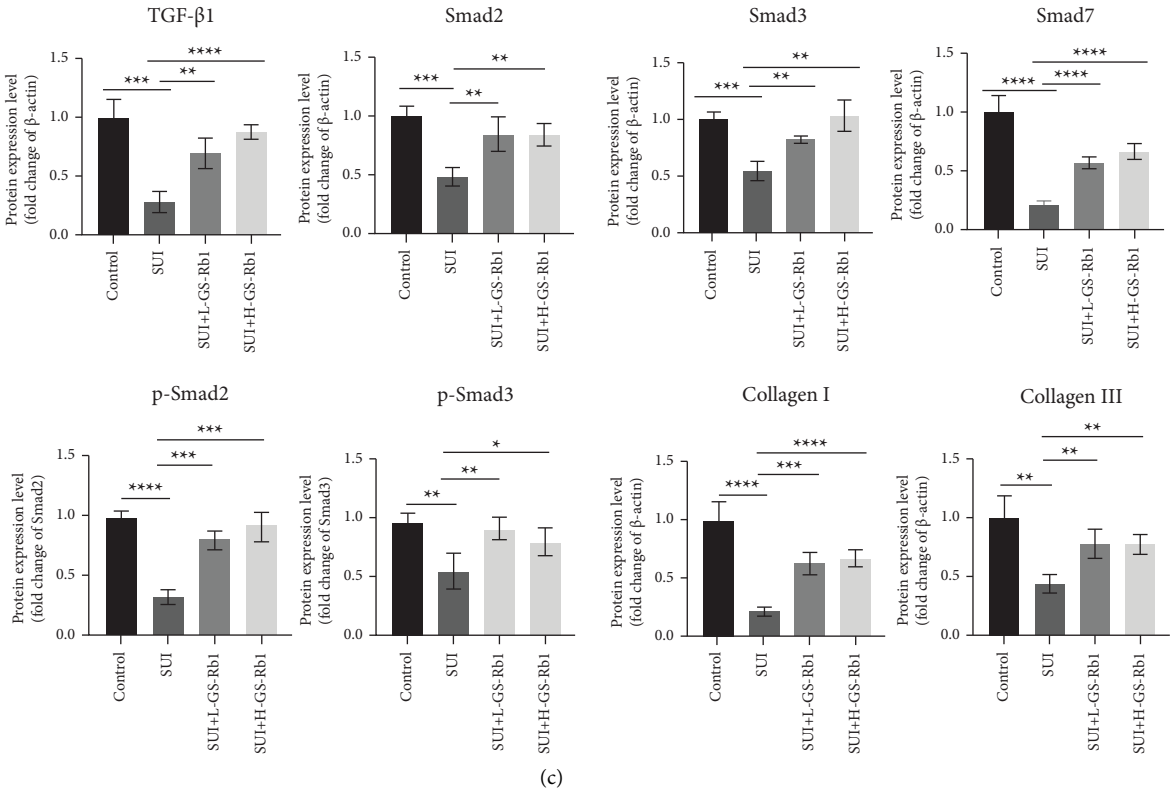
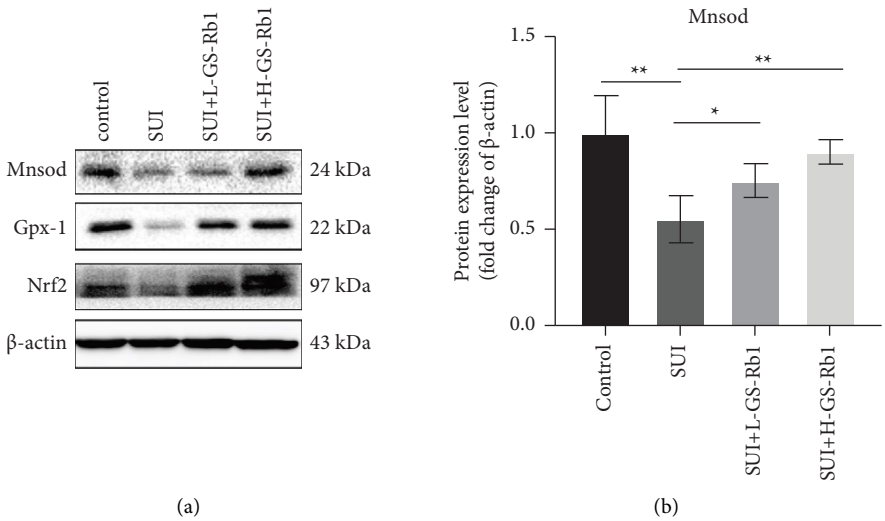


Figure 4: Expression of factors and proteins associated with the TGF-β1/Smad3 signaling pathway in rat urethral tissues. (a) Real-time RT-PCR was done using quantity one mRNA levels of TGF-β1, Smad3, Smad7, and collagens I and III in urethral tissues. (b) and (c) Western blotting was performed to detect the protein expression levels of TGF-β1, Smad2, Smad3, Smad7, collagen I and collagen III, and Smad2 and Smad3 phosphorylation in the urethra walls of rats in four groups (b), and the quantification was analyzed (c). Data are presented as mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.



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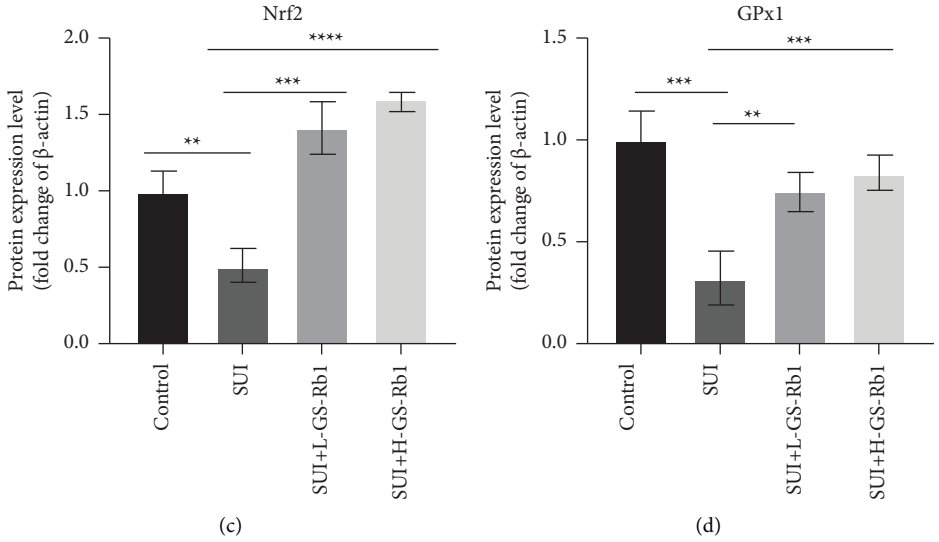

Figure 5: GS-Rb1 stimulated Nrf2/ARE signaling in urethral tissues. (a–d). Western blotting showed the expressions of Nrf2/ARE signaling pathway-related proteins in the urethra tissues in all four groups (a), the quantified data were analyzed (b–d). Data are presented as mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

and survival [20]. It plays a crucial role in regulating the ECM, where it phosphorylates Smad2 and Smad3 to stimulate the expression of ECM components such as collagen, fibronectin, and elastin [21]. TGF-β1 has also been demonstrated to play a key role in the pathogenesis of SUI, resulting from mechanical trauma [9, 21] and has been suggested as a potential target for SUI treatment [22, 23]. Therefore, we investigated the effects of GS-Rb1 on TGF-β1 and its associated proteins. It was found that the urethral tissue of SUI rats showed reduced levels of TGF-β1, Smad3, p-Smad3, and collagens I and III compared to both the control and GS-Rb1-treated rats. In view of these results, we conclude that TGF-β1/Smad3 signaling may play a critical role in GS-Rb1-mediated repair of tissue subjected to mechanical trauma.

Vaginal distension reduces blood flow to the urogenital organs responsible for continence, resulting in hypoxia and suggesting that ischemia and/or reperfusion injury may be responsible for the resulting damage [24]. Nrf2 modulates cellular response to oxidative stress by promoting the transcription of antioxidant genes carrying AREs in their promoter regions [23]. In the presence of oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, where it promotes the transcription of antioxidant genes, such as GPx1, MnSOD, CAT, and HO-1 [25]. It has been suggested that mechanical trauma can induce oxidative damage to both the urethral sphincter and the vaginal wall, causing ischemic and hypoxic injury to the pelvic floor tissue and leading to breakages in muscle and elastic fibers and reducing the ECM, resulting in SUI or pelvic floor dysfunction (PFD) [26]. Addressing oxidative damage may be critical for the prevention and treatment of SUI [9, 10]. Tang et al. [7] suggested that mechanical injury-induced ECM remodeling might be associated with the suppression of Nrf2/ARE signaling, leading to inhibition of the TGF-β1/Smad3 signaling pathway. Our study demonstrated that GS-Rb1 treatment reduced tissue injury in SUI rats with increased Nrf2/ARE activation. The protein levels of Nrf2 were assessed by Western blotting, showing increased expression in SUI + GS-Rb1 rats compared with control rats. Meanwhile, the structure of the damaged tissue was found to be restored in the GS-Rb1-treated groups. These findings suggest that GS-Rb1 may have a protective effect against tissue damage through its antioxidant actions and may promote tissue repair.

There are several limitations to this study. First, the adverse effects of GS-Rb1 were not evaluated. Second, pharmacokinetic analysis of ginsenoside Rb1 was not performed in the rats, and thus effective levels of the drug in the blood were not evaluated. In addition, as VD is usually temporary following childbirth, we only undertook a short-term study, and there are no further data from different time points after mechanical trauma. We propose to investigate this topic in depth in our next study to obtain direct evidence of this hypothesis.

5. Conclusion

In conclusion, GS-Rb1 may mitigate oxidative damage and promote matrix and tissue repair associated with mechanical-trauma-induced SUI. Thus, we speculated that GS-Rb1 attenuates oxidative damage and promotes matrix and tissue repair associated with mechanical trauma-induced SUI. GS-Rb1 may prevent SUI through early treatment of mechanical trauma to postpartum periurethral tissue.

Data Availability

All data generated or analyzed during this study are included in this article and are available from the corresponding author upon request.

Ethical Approval

The animal study was reviewed and approved by the Institutional Animal Experimental Ethics Committee of Shanxi Cancer Hospital, China. All the animal experiments were conducted in accordance with the guidelines of the institutional bioethical committee. The study was reported in accordance to ARRIVE guidelines (https://arriveguidelines.org).

Disclosure

The manuscript was already published as a preprint based on the link https://www.researchsquare.com/article/rs-3504789/v123 [27].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S.C. contributed to the study conception and design. S.C., B.W., S.Z., and R.H. performed material preparation and data collection and analysis. The first draft of the manuscript was written by S.C., H.L., and S.Z. and they put forward the main revision suggestion to the article, and all authors commented on previous versions of the manuscript and have read and approved the final manuscript.

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Supplementary Materials

Supplementary Material: primer sequence of TGF-β1, Smad3, Smad7, and collagens I and III. (*Supplementary Materials*)

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