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Research Article
Modified Taohong Siwu Decoction Promotes Repair of Damaged Endometrium in a Rat Model of Intrauterine Adhesion

[1]Hongping Niu, [2]Na Liu, [1]Caiyan Zhang, [3]Yonghui Zhang, [3]Xingxiu Zhan, [1]Xingyan Li, [3]Yanping Qian and [4]Lijuan Jiang

[1]Department of Gynecology, The First Clinical Medical College, Yunnan University of Chinese Medicine, Kunming, Yunnan 650500, China
[2]Department of Pharmacy, Yunnan University of Traditional Chinese Medicine, College of Traditional Chinese Medicine, Kunming, Yunnan 650500, China
[3]Department of Gynecology, The First Affiliated Hospital of Yunnan University of Chinese Medicine, Kunming, Yunnan 650021, China
[4]Department of Medical Ethics Committee, The First Affiliated Hospital of Yunnan University of Chinese Medicine, Kunming, Yunnan 650021, China

Abstract

Background and Objective: Modified Taohong Siwu Decoction (MTSD), a traditional Chinese medicine, consists of components that are known to enhance blood circulation and reduce fibrosis. In this study, the effects of MTSD on endometrial fibrosis and endometrial receptivity were investigated in a rat model of IUA and in Transforming Growth Factor-β1 (TGF-β1)-stimulated endometrial stromal cells (ESCs). **Materials and Methods:** Female Sprague Dawley rats were subjected to a dual-injury model of IUA. Fibrotic characteristics were induced in ESCs by exogenous supplementation of TGF-β1. The effect of MTSD on endometrial fibrosis was assessed using histochemical methods. Real-time PCR, immunohistochemical labeling and western blotting were used to determine the relative expression of genes and proteins involved in the TGF-β1/Smad pathway and endometrial receptivity. **Results:** The MTSD treatment group had a significantly higher number of endometrial glands but less endometrial fibrosis than the IUA treatment group. In response to IUA, TGF-β1 and Smad3 expression were upregulated. Conversely, Smad7, integrin αvβ3 and Leukemia Inhibitory Factor (LIF) were downregulated. Following MTSD therapy, TGF-β1 and Smad3 levels were dramatically reduced, while Smad7, integrin αvβ3 and LIF levels were significantly elevated. The TGF-β1 induced Smad3 expression in ESCs but downregulated the Smad7, integrin αvβ3 and LIF expression. By promoting the expression of Smad7, integrin αvβ3 and LIF, MTSD administration inhibited the expression of Smad3 induced by TGF-β1. **Conclusion:** The MTSD plays a crucial role through the TGF-β1/Smad signaling pathway in the repair of injured endometrium after IUA.

Key words: Intrauterine adhesion, Modified Taohong Siwu Decoction, TGF-β1/SMAD pathway, endometrial receptivity, endometrial stromal cells

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Corresponding Author: Lijuan Jiang, Department of Medical Ethics Committee, The First Affiliated Hospital of Yunnan University of Chinese Medicine, Kunming, Yunnan 650021, China Tel: +86-0871-63631649

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Intrauterine adhesion (IUA) occurs when the endometrium's basal layer gets damaged following trauma and infection[1]. Typically, IUA manifests as fibrosis of the endometrium or scarring of the endometrium. The IUA is associated with various clinical symptoms, including menstruation abnormalities, infertility, miscarriages and abnormal pregnancy[2,3]. A hysteroscopic adhesiolysis combined with hormone therapy is the most common treatment for stimulating the regrowth of the endometrium from residual stem cells and progenitor cells[4]. However, in mild-to-moderate IUAs, recurrence and deadhesion rates are high leading to poor prognosis[5]. Therefore, therapeutic interventions with greater effectiveness should be explored to repair and improve endometrial function in IUA patients.

Several studies have shown increased fibrosis markers in endometrial tissue after IUA[6,7]. In addition, IUA is reported to correlate with Transforming Growth Factor-β1 (TGF-β1) and the Smad pathway proteins[8,9]. Niu *et al.*[10] have reported the Chinese Herbal Medicine Tiaoshen Tongluo Recipe reversed the elevated expression of TGF-β1, Smad mRNA and protein in endometrial stromal cells (ESCs) as well as in a rat model of IUA.

Endometrial receptivity (ER) is the state of acceptability of the endometrium to embryo implantation. The ER is highly correlated with the rate of embryo implantation and pregnancy likelihood[11]. The ER is a critical predictive fertility factor in assessing IUA therapy. Leukemia Inhibitory Factor (LIF) and integrin αvβ3 are recognized as important ER markers. Zhou *et al.*[12] found that the irisin-treated rats with poor implantation outcomes had improved blastocyst implantation by promoting both LIF production and integrin secretion.

Siwu Decoction is an ancient Chinese prescription for gynecological conditions. It enhances blood flow, regulates menstruation and enriches blood supply[13,14]. Additionally, the permutation and combinations of ingredients have led to many Siwu Decoctions for treating abdominal pain in women during the menstrual cycle[15-17]. The Taohong Siwu Decoction stands out among Siwu Decoctions for its nourishing and promoting blood circulation properties. Modified Taohong Siwu Decoction (MTSD) administration in ovarian reserve-depleted patients reduces symptoms such as anxiety, irritability, vaginal dryness and fertility problems[18,19]. Furthermore, Yuan *et al.*[20] have demonstrated that, MTSD can improve ovarian function in mice suffering from premature ovarian failure by upregulating TGF-β1, TGF-βRII and Smad2/3. However, as of now, MTSD's molecular mechanism for treating IUA remains unknown. Therefore, this study aimed to examine the effect of MTSD therapy in reducing adhesions, tissue fibrosis and improving ER in rats with dual injuries established by IUA.

MATERIALS AND METHODS

Study area: This investigation was carried out in May, 2023 at the Yunnan University of Chinese Medicine.

Chinese herbal medicine: The MTSD granules were supplied by the Yunnan Provincial Hospital of Traditional Chinese Medicine. Dispensing granules for the MTSD contained 10 g of Peach kernel, 10 g of *Carthamus tinctorius*, 20 g of cooked Rehmannia, 15 g of *Paeonia lactiflora* Pall, 20 g of *Angelica sinensis*, 15 g of *Ligusticum wallichii*, 20 g of *Semen cuscuta*, 20 g of *Cistanche salsa*, *Cistanche deserticola*, 30 g of *Rhizoma polygonati*, 6 g of *Aulastomum gulo*, 15 g of *Caulis spatholobi* and 6 g of *Glycyrrhiza uralensis*. The MTSD granules were diluted in boiling water to make up 2 g/mL before each gavage.

Animals: Sprague Dawley rats (female, 250-280 g) were purchased from the Shanghai Sip-Bikai Laboratory Animal Co. Ltd. (Shanghai, China). The animals were housed in 12 hrs light/dark cycles at room temperature at 45-55% relative humidity, with food and water *ad libitum*.

Ethical consideration: All the animals were treated according to the guidelines of the Yunnan University of Chinese Medicine Ethics Committee.

IUA model: As described by Niu *et al.*[10], an IUA model was created in rats by inflicting a dual injury. Twenty rats of 10 weeks of age with regular estrous cycles of four to five days were included in the study. First, the rats were anesthetized with the intraperitoneal injection of 1% pentobarbital sodium. Next, the uterine horns were exposed by making a 2 cm incision in the lower abdomen. Each procedure was conducted on the right uterine horn, whereas the left uterine horn served as a control. First, a small incision was made inside the fallopian tube and then a 16-gauge needle was rotated inside the tube to cause a mechanical injury. Upon the occurrence of bleeding in the uterus horn, a saline flush and suturing were performed. Within 48 hrs of mechanical injury,

a 5 cm cotton thread soaked in lipopolysaccharide (0.6 mg/L) was inserted into the uterus. Afterward, sutures were applied to the wounds and the animals were allowed to heal. Rats were divided into four groups after the IUA operation and given 0 and 8 g/kg (low dose), 16 g/kg (moderate dose) and 32 g/kg (high dose) MTSD once a day at 8:00 am via oral gavage over a period of eight weeks. After that, the rats were sacrificed and their uteri were removed.

Hematoxylin and Eosin (H&E) and Masson staining The H&E and Masson staining of paraffin sections of rat uteri were performed as described by Niu *et al.*[10]. Each H&E-stained section were analyzed in five high-magnification fields, counting and averaging the number of glands within each field. In addition, the percentage of fibrosis area was calculated in five high-magnification fields for each Masson-stained slice. The Image-pro Plus software automatically calculated the average rate (Media Cybernetics, Inc., Maryland, USA).

Immunohistochemistry: The Immunohistochemistry was performed on paraffin sections by deparaffinizing, rehydrating and incubating them at 37°C for 30 min. After that, the sections were blocked with 5% bovine serum albumin (BSA) for 30 min. Afterward, incubation was performed overnight at 4°C with primary antibodies against TGF-β1, p-Smad3, Smad3, Smad7 (Santa Cruz Biotechnology, Santa Cruz, California), integrin αvβ3 and LIF (Boster Biological Technology, Wuhan, China). After 2 hrs of incubation with goat anti-rabbit secondary antibody, the sections were observed under a microscope (Nikon, Tokyo, Japan). Image-Pro Plus software (Media Cybernetics) was used to measure average optical intensity (AOI).

Drug-containing serum preparation: The MTSD granules (32 g/kg) were administered by gavage to the drug serum group, while the normal serum group received the same volume of saline twice a day for three consecutive days. Rats (n = 6/group) were fasted for 12 hrs and then fed with MTSD granules for one day. The blood was collected from their hearts at 1 hr after they had fasted. A high-speed centrifuge was used to centrifuge the supernatant for 10 min and the serum resulting from this process was treated as drug-containing serum.

ESCs isolation and culture: To study IUA *in vitro*, we isolated ESCs from rats undergoing early pregnancy, which tends to cause endometrial trauma during pregnancy[9]. Briefly, rat uteri were obtained on day 4 of pregnancy and minced before being incubated in Dulbecco's Modified Eagle Medium (DMEM) containing 1 mg/mL collagenase for 1 hr at 37°C with shaking (110 rpm). The supernatant was centrifuged after allowing cell debris to settle. The resultant pellet contained endometrial epithelial and stromal cells, which were separated using a 40 μm cell strainer. Stromal cells that passed through the strainer were subjected to decidualization. Upon reaching 70% confluency in DMEM, cells were treated with 0.5 mM cAMP and 100 nM medroxyprogesterone acetate to induce decidualization. For ESC passage, cells were digested with 0.25% Trypsin-EDTA solution (Thermo Fisher Scientific, Waltham, Massachusetts) until most cells detached, followed by centrifugation at 2000 rpm for 5 min. The cell pellet was then resuspended in a complete medium.

Cells treatment: We treated the ESCs with 50 ng/mL TGF-β1 for 48 hrs and added normal rat and drug-containing serum. The drug-containing serum of MTSD with final concentrations of 5% (low-dose), 10% (moderate-dose) or 20% (high-dose), respectively, was continued for cultured cells for 72 hrs. Controls received 15% normal rat serum as a blank control.

Real-time PCR: A total RNA extract was prepared from ESCs and endometrial tissues using the Trizol reagent from Invitrogen (Carlsbad, California). A NanoDrop ND 1000 instrument was used to measure the quality and quantity of the RNA, followed by reverse transcription using M-MLV reverse transcriptase and RNase inhibitor (Promega, Madison, Wisconsin, USA). Following the manufacturer's instructions, SYB Green PCR Master Mix (Applied Biosystems, Foster City, California, USA) was used to amplify GAPDH, TGF-β1, Smad3, Smad7, integrin αvβ3 and LIF RNA using the primers specified in Table 1. The mRNA levels were measured using the $2^{-\Delta\Delta Ct}$ method[21] and normalized to GAPDH.

Western blot analysis: The ESCs were first lysed with lysis buffer and centrifuged at 12,000×g for 15 min at 4°C for 15 min. Next, the protein was quantified using a BCA protein assay kit (Beyotime). Next, a 50 μg sample of protein was separated using SDS-PAGE and transferred to a PVDF membrane. Next, 5% non-fat milk was added to TBST (10 mM Tris-HCl, 100 mM NaCl, 0.1% Tween-20, pH 7.4) for an hour at room temperature, followed by overnight incubation in the primary antibody. The primary antibodies used in this study were TGF-β1, p-Smad3, Smad3, Smad7 (Santa Cruz

Table 1: Primers used in the study

		Primer sequence 5'→3'
TGF-β1	Forward	ACGTCAGACATTCGGGAAGC
	Reverse	TTCCGTCTCCTTGGTTCAGC
LIF	Forward	CAACTGGCACAGCTCAATGG
	Reverse	CAGTGGGGTTCAGGACCTTC
Smad3	Forward	TTCCATCCCCGAGAACACTAAC
	Reverse	GTGACTGGCTGTAGGTCCAAG
Smad7	Forward	CTCCTGCTGTGCAAAGTGTTC
	Reverse	ACAGTCTGCAGTTGGTTTGA
Integrin αvβ3	Forward	GGACAACTCTGGGCCGCTC
	Reverse	TCCTTCAGGTTACATCGGGGT
GAPDH	Forward	TGCTGGTGCTGAGTATGTCG
	Reverse	TCATGAGCCCTTCCACGATG

Biotechnology), integrin αvβ3 and LIF (Boster Biological Technology). For the development of the membranes, we used ECL and incubated the membranes for an hour at room temperature with horseradish peroxidase-labeled goat anti-rabbit secondary antibody. Finally, we calculated the relative expression of protein bands against the housekeeping protein using ImageJ software.

Statistical analysis: Three independent experiments were performed to obtain the data. Means±Standard Deviations were expressed for each experiment. Statistical significance was determined using one-way variance analysis and Dunnett's *post hoc* multiple comparisons. The data were analyzed using GraphPad Prism 8 (GraphPad, California, USA). The p-value of <0.05 was considered to be significant.

RESULTS

MTSD increases glands number and alleviates endometrial fibrosis in rats with IUA: A histological examination was performed after injury induction using H&E staining (Fig. 1a). The epithelium of the sham uterus mainly consisted of monolayer columnar epithelium. The endometrium had tubular glands and regular interspersions of the stroma. In the IUA control group, the luminal surface was found to be discontinuous, the basal glands were reduced and the luminal cavity was lost. Compared to rats with sham surgery, the IUA model had a significant reduction in endometrial glands. A dose-dependent increase in gland number was observed in MTSD-treated rats (Fig. 1b). Using Masson's staining, the endometrium collagen fibers in the Sham operation group were stained in blue and neatly arranged. In contrast, the mucous layer, the submucosa, muscles and blood vessels were stained in red (Fig. 1c). There was a lack of uniform staining of collagen fibers within the connective tissue in the IUA model group. Blue stained collagen fibers were decreased dose-dependently in endometrial mesenchymal in the groups receiving MTSD. There were few evenly distributed blue collagen fibers in the interstitial region of the high-dose group (Fig. 1d). The results showed that MTSD treatment can increase endometrial gland number and reverse endometrial fibrosis.

TGF-β1/Smad pathway and uterine endometrium function-related markers are moderated by MTSD in IUA: The IUA model had an increased relative expression of TGF-β1 and Smad3 genes. In contrast, Smad7, integrin αvβ3 and LIF expression were downregulated (Fig. 2). The TGF-β1 and Smad3 levels were considerably reduced following MTSD treatment compared to the untreated IUA model (Fig. 2a-b). However, MTSD caused dose-dependent increases in Smad7, integrin αvβ3 and LIF expression. When treated with the highest dose of MTSD, no significant differences were observed between the rats treated with sham surgery and the IUA model (Fig. 2c-e). Immunohistochemistry showed similar results for protein levels and phosphorylation patterns (Fig. 3a). Untreated IUA models showed the highest levels of TGF-β1, phosphorylated Smad3 and Smad3 (Fig. 3b-d). In addition, a decline in levels was noted after MTSD treatment. The highest levels were observed in the sham-operated rats, while levels decreased after IUA (Fig. 3e-g). Based on these results, MTSD appears to inhibit TGF-β1 signaling and increase endometrium cytokines following injury in rats.

Effects of MTSD on smad pathways and endometrial cytokines: According to recent work, TGF-β1 can alter the expression of the Smad pathway in ESCs[10]. In this study, MTSD was given to ESCs for 48 hrs after they had been

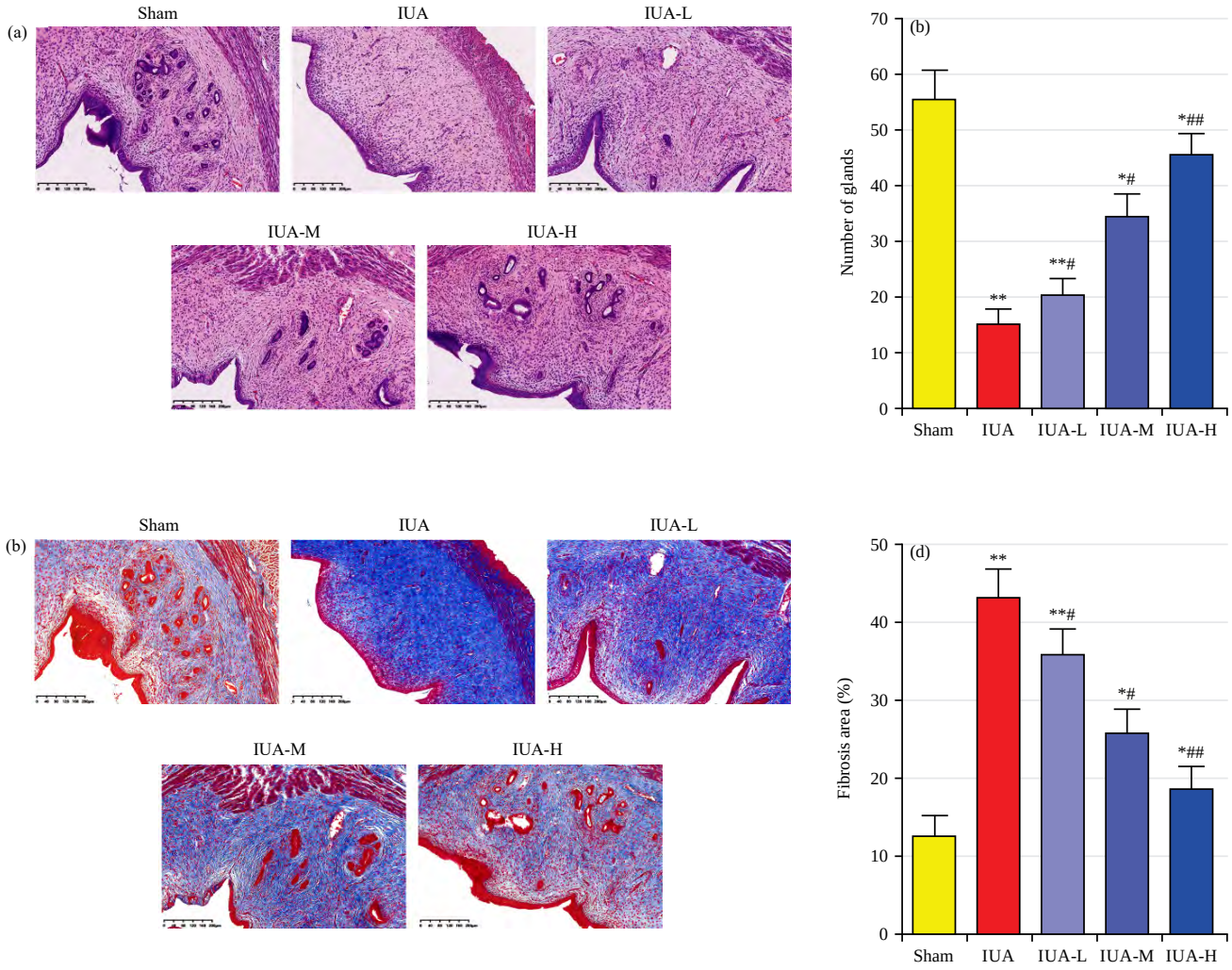

Fig. 1(a-d): Hematoxylin and Eosin (H&E) and Masson stains of rat uteri in each group, (a) Endometrial glands of each group were stained with H&E to detect any changes, (b) Statistical difference in the number of glands in the endometrium after endometrial damage and after treatment is shown, (c) Changes in intrauterine fibrosis in each group were measured with Masson staining and (d) After endometrial damage and treatment, statistical results of intrauterine fibrosis changes are presented

Following the intrauterine adhesion (IUA) procedure, the rats were treated with either 0 and 8 g/kg (IUA-L), 16 g/kg (IUA-M) or 32 g/kg (IUA-L) of Modified Taohong Siwu Decoction (MTSD) by oral gavage for 8 weeks. Rat uteri were collected from each group. Scale bar = 200 μm; *p<0.05, **p<0.01 compared to the Sham operation group and $^{\#}$p<0.05, $^{\#\#}$p<0.01 compared to the IUA control group

stimulated by 50 ng/mL TGF-β1. Then, a 5% (low dose), 10% (moderate dose) or 20% (high dose) concentration of rat serum containing MTSD was added to these ESCs, followed by another 72 hrs of culture. The real-time PCR was used to measure the mRNA expression levels of TGF-β1 (Fig. 4a), Smad3 (Fig. 4b), Smad7 (Fig. 4c), integrin αvβ3 (Fig. 4d) and LIF (Fig. 4e). In TGF-β1-stimulated ESCs, the addition of MTSD drastically enhanced the expression of Smad7, integrin αvβ3 and LIF while decreased the expression of TGF-β1 and Smad3. Furthermore, in protein levels (Fig. 5a), we observed that adding MTSD reduced Smad3 phosphorylation and Smad3 expression. Additionally, it increased the Smad7, integrin αvβ3 and LIF expression in TGF-β1-stimulated ESCs (Fig. 5b-f). These results indicated that MTSD modulates the TGF-β1/Smad pathway to moderate fibrotic tissue generation and endometrial dysfunction caused by injury repair processes in endometrial tissues.

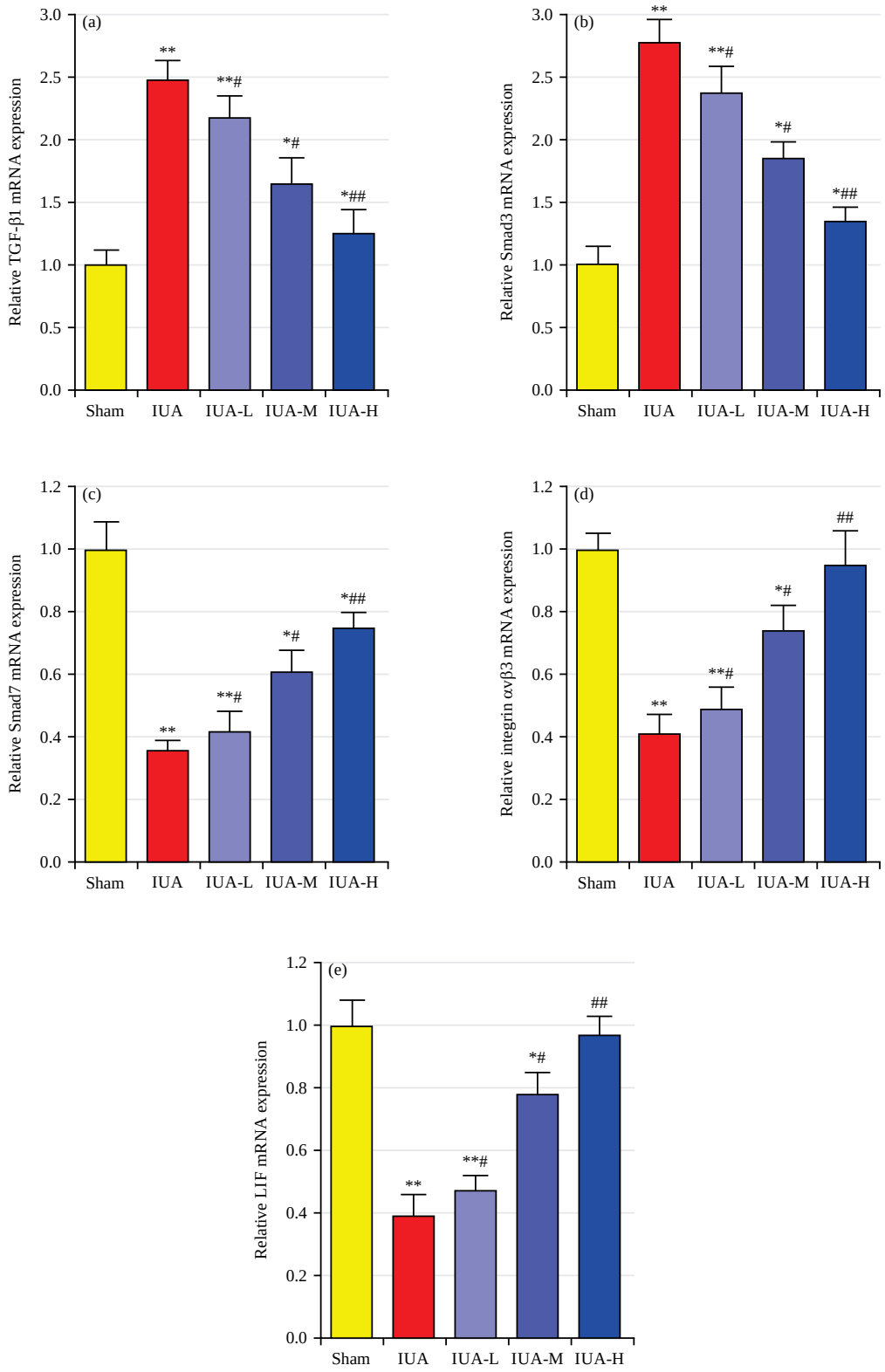

Fig. 2(a-e): Changes of TGF-β1/Smad and endometrium cytokines mRNA levels of rat uteri in each group, (a) Rat endometrium TGF-β1, (b) Smad3, (c) Smad7, (d) Integrin αvβ3 and (e) LIF mRNA expression were measured by real-time PCR

*p<0.05, **p<0.01 compared to the Sham operation group and #p<0.05, ##p<0.01 compared to the IUA control group

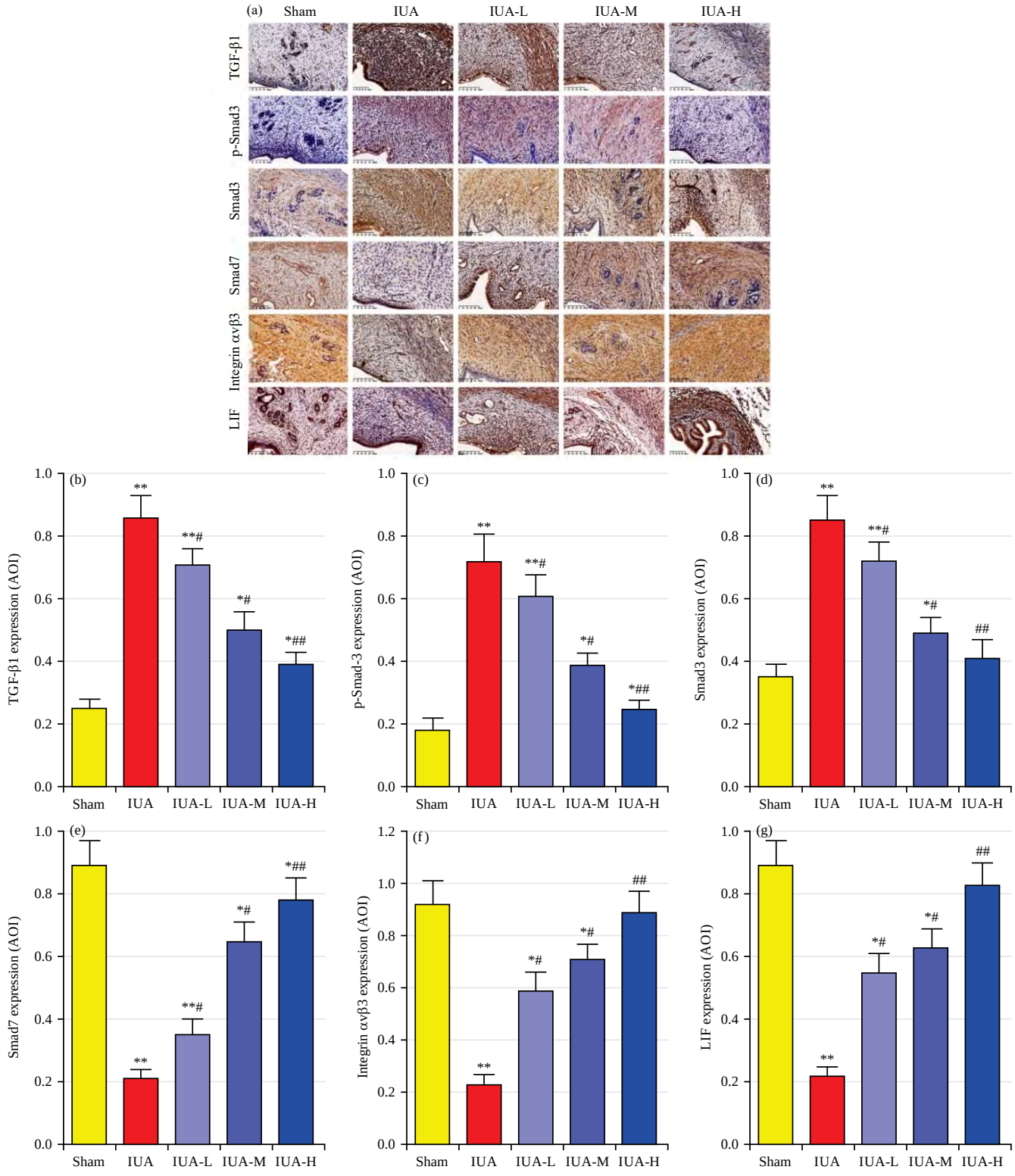

Fig. 3(a-g): Changes of TGF-β1/Smad and endometrium cytokines protein levels of rat uteri in each group, (a) Expression of TGF-β1/Smad and endometrium cytokines protein was detected by immunohistochemical (IHC) staining in the rat endometrium of each group, (b) Protein average optical intensity (AOI) of TGF-β1, (c) Phosphorylated-Smad3 (p-Smad3), (d) Smad3, (e) Smad7, (f) Integrin αvβ3 and (g) LIF was quantified

Scale bar = 100 μm; *p<0.05, **p<0.01 compared to the sham operation group and #p<0.05, ##p<0.01 compared to the IUA control group

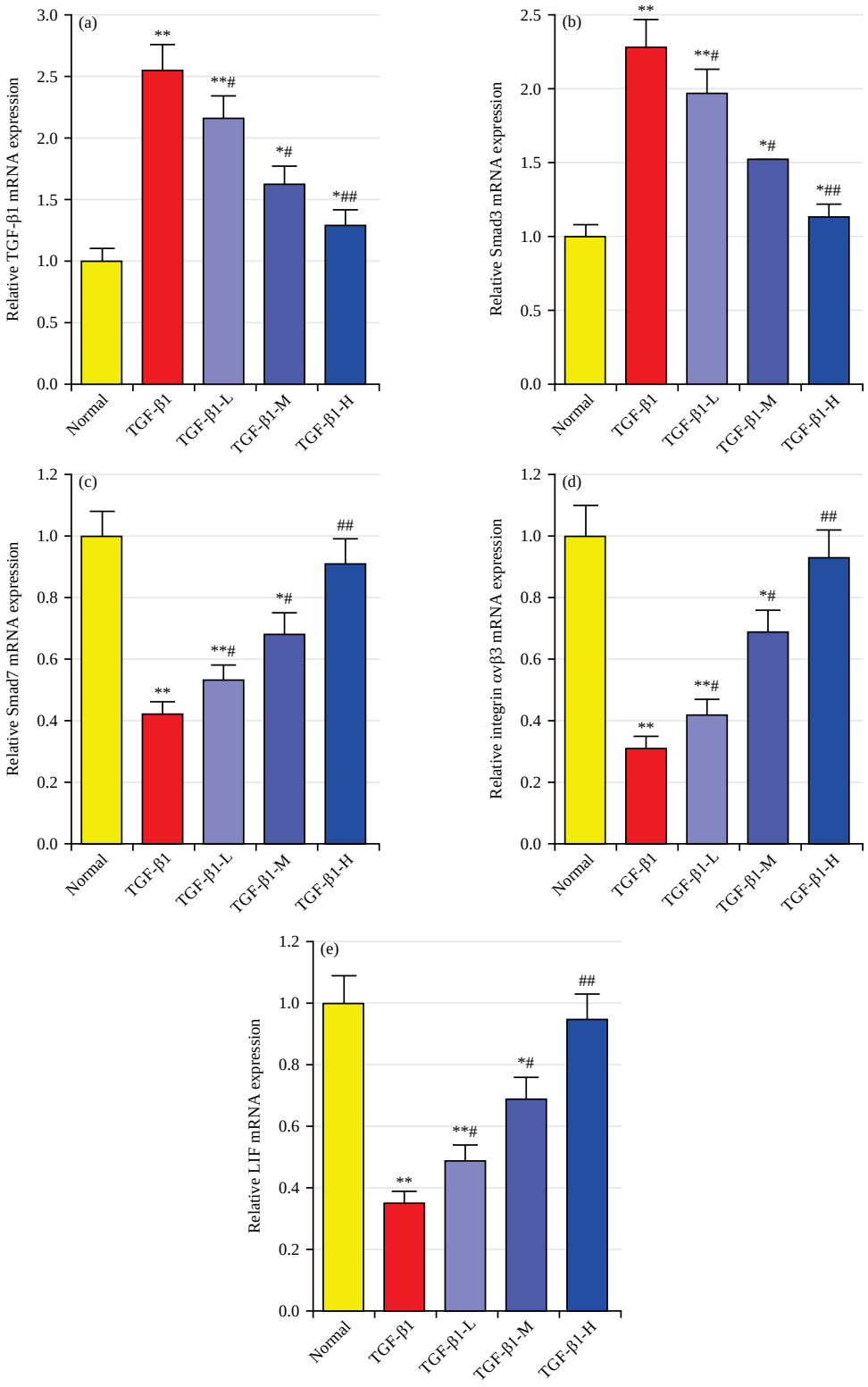

Fig. 4(a-e): Relative Smad and endometrium cytokines mRNA levels in ESCs. The relative mRNA expression of (a) TGF-β1, (b) Smad3, (c) Smad7, (d) Integrin αvβ3 and (e) LIF was detected by real-time PCR

After treating ESCs with 50 ng/mL TGF-β1 for 48 hrs, 15% normal rat serum (TGF-β1 group) or Modified Taohong Siwu Decoction (MTSD)-containing serum at final concentrations of 5% (TGF-β1-L), 10% (TGF-β1-M) or 20% (TGF-β1-H) were added to the culture for a further 72 hrs. The ESCs treated neither with TGF-β1 nor MTSD serum served as the normal control group. *p<0.05, **p<0.01 compared to the Normal control group and #p<0.05, ##p<0.01 compared to TGF-β1 control group

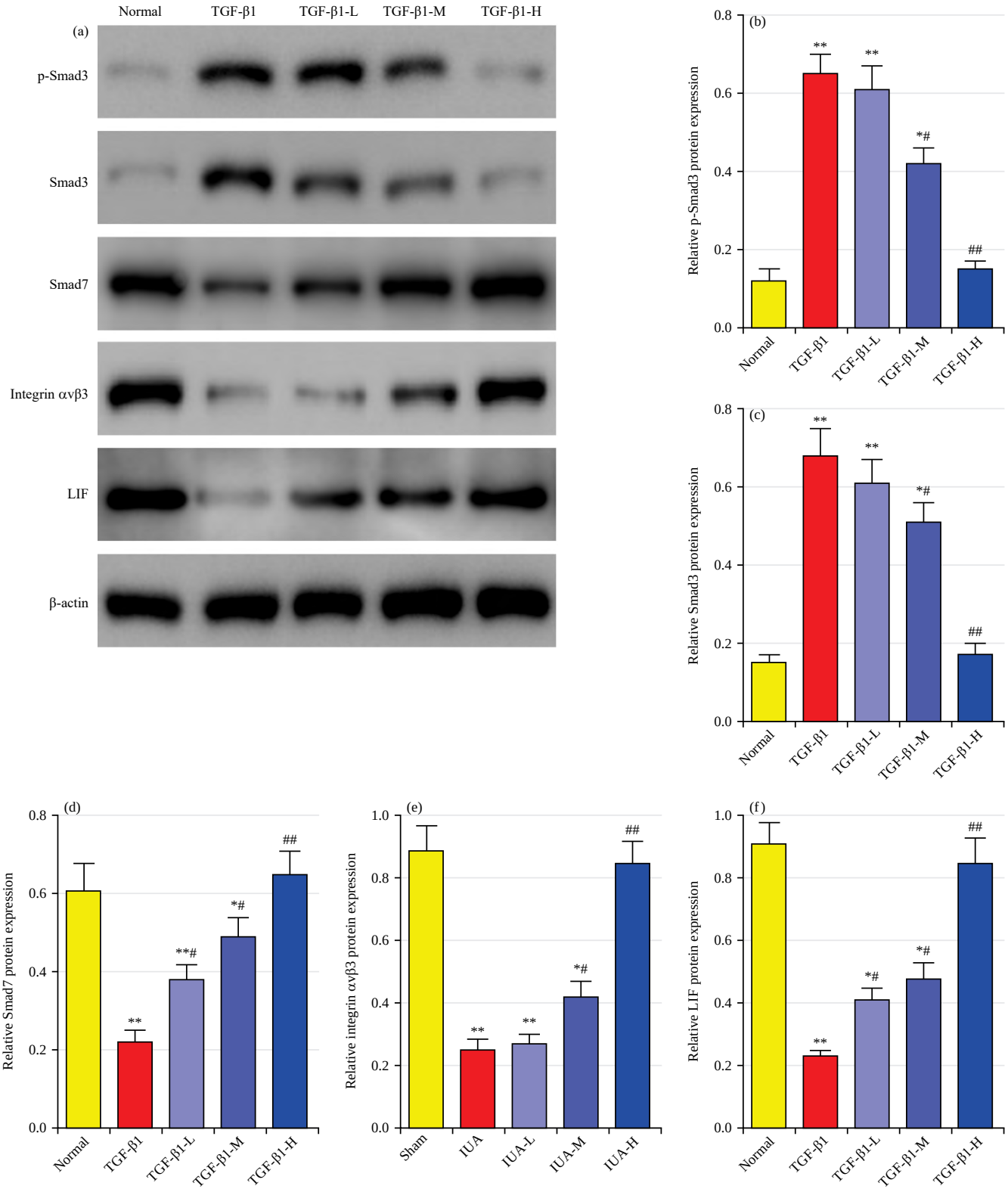

Fig. 5(a-f): Relative Smad and endometrium cytokines protein levels in ESCs, (a) Protein expression Smad and endometrium cytokines were assessed by western blotting, The protein expression of (b) p-Smad3, (c) Smad3, (d) Smad7, (e) Integrin αvβ3 and (f) LIF was quantified relative to β-actin

*p<0.05, **p<0.01 compared to the Normal control group and #p<0.05, ##p<0.01 compared to TGF-β1 control group

DISCUSSION

Fertility can be affected by IUA, a condition characterized by inadequate endometrial repair and fibrosis[22]. The TGF-β1/Smad is the vital fibrogenic pathway[23]. When trauma occurs, TGF-β1 is first released from platelets to the site of injury, followed by chemotaxis fibroblasts, monocytes and neutrophils in a dose-dependent manner. The TGF-β1 induces phosphorylation of SMAD2/3 by binding to TβR-I and TβR-II on fibroblast cell membranes and activating receptors, initiating transcription of target genes, promoting increased collagen synthesis, accumulation of extracellular matrix (ECM) and promoting the occurrence of tissue fiber fatigue. In contrast, Smad7 works by inhibiting the phosphorylation of Smad2 and/or Smad3, thereby blocking the signaling transduction[23-26]. Several studies have shown that inhibiting the TGF-β1/Smad pathway effectively alleviated the IUA[25,26]. According to Chen *et al.*[25], silencing kinase-insert domain-containing receptor (KDR) prevents the initiation and development of IUA by inhibiting the TGF-β1/Smads signaling pathway. According to Zhang *et al.*[26], aspirin inhibits endometrial fibrosis by suppressing the TGF-β1-Smad2/Smad3 pathway in IUA.

This study evaluated the potential of MTSD for alleviating IUA-induced fibrosis. We found that MTSD could increase the number of endometrial glands and reduce the area of endometrial fibrosis in IUA rats. This may be attributed to the inclusion of peach kernel, *Carthamus tinctorius* and *Caulis spatholobi* in the MTSD prescription, which is effective in promoting blood circulation. Moreover, IUA increased the expression level of fibrosis-associated genes, including TGF-β1 and Smad3. Besides, MTSD reduced the level of fibrosis in endometrial tissue in a double-injury model of IUA in rats. This study also found that the mRNA and protein expression levels of TGF-β1 and Smad3 in the uterine tissue of rats in the IUA model group were significantly higher than in the sham-operation group. Conversely, the mRNA and protein expression levels of Smad7 were significantly lower. At the same time, MTSD could reduce the mRNA and protein levels of TGF-β1 and Smad3 in the uterine tissue of rats in the IUA group, with an increase in the mRNA and protein expression of Smad7. These results suggested that MTSD can effectively delay the process of endometrial fibrosis by down-regulating the expression of Smad3 and hindering its phosphorylation, thereby blocking the pro-fibrotic process of TGF-β1, inhibiting the regulatory protein of Smad3 receptor and blocking the TGF-β1 signal transduction pathway by increasing the expression of Smad7.

To achieve a successful pregnancy, IUA treatment must result in a receptive endometrium. Endometrial receptivity is determined by various factors, including ovarian hormones, integrin αvβ3, LIF, etc.[27]. Integrin αvβ3 facilitates the transition of the endometrium from a non-adherent to an adherent state, which is crucial for the blastocyst adherent implantation[28,29]. In addition, a large amount of LIF is present in women's follicular fluid, endometrium and oviduct. The LIF is involved in many aspects of reproduction, including follicle maturation, implantation of embryos and maintaining pregnancy[28,30]. Xiao *et al.*[31] reported that infertile mice had lower integrin αvβ3 and LIF expression levels than normal mice. They further reported that Yichang decoction improves embryo implantation in mice by upregulating LIF and regulating integrin αvβ3 expression.

This study showed that the mRNA and protein levels of integrin αvβ3 and LIF in rat uterine tissues and ESCs of normal control groups were higher than those of IUA or TGF-β1 stimulated ESCs. This observation indicates that the endometrial injury and scar repair disrupted the endocrine environment of rats and had a negative impact on the expression of integrin αvβ3 and LIF. Overall, these events are not conducive to establishing good receptivity of the endometrium. However, the mRNA and protein expression levels of integrin αvβ3 and LIF in endometrium and ESCs in the MTSD treatment group were higher than in the model control group. This observation indicates that the MTSD can improve the endocrine environment of IUA rats by promoting the expression of integrin αvβ3 and LIF in the endometrium, making a conducive environment for the establishment of endometrial receptivity. The addition of Semen Cuscuta, *Cistanche* and *Rhizoma polygonati* in the MTSD prescription might be responsible for the aforementioned effect, as these components are known to have the effect of tonifying the kidney, nourishing essence and regulating sex hormone levels.

CONCLUSION

The present study demonstrated that MTSD modulated fibrosis and improved poor receptiveness in a rat model of IUA by inhibiting the TGF-β1/Smad pathway and promoting LIF and integrin αvβ3 expression. Therefore, it was proposed that the MTSD contributes to IUA prevention. The MTSD can be used as a therapeutic modality to moderate the accumulation of fibrotic tissue in endometrial tissue in order to improve IUA receptivity. These current results will be further validated in future exploratory studies.

SIGNIFICANCE STATEMENT

Conventional treatment of patients with intrauterine adhesion (IUA) often leads to high recurrence rate and poor prognosis and effective therapeutic interventions are urgently needed. This study aimed to investigate the molecular mechanism of Modified Taohong Siwu Decoction (MTSD), a traditional Chinese medicine known for promoting blood circulation and reducing fibrosis, in the treatment of IUA. We found that MTSD treatment significantly reduced endometrial fibrosis and increased the number of endometrial glands in a rat model of IUA. Additionally, MTSD downregulated the expression of TGF-β1 and Smad3, while upregulating Smad7, integrin αvβ3 and LIF, crucial markers of endometrial receptivity. Our findings suggest that MTSD modulates its effects through the TGF-β1/Smad signaling pathway, promoting the repair of injured endometrium and improving endometrial receptivity. These results provide valuable insights into potential therapeutic strategies for IUA patients, highlighting the promise of traditional Chinese medicine in addressing complex reproductive health issues.

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