J Surg Med. 2024;8(12):192-195.
https://doi.org/10.28982/josam.7862

JOSAM

Research article
https://jsurgmed.com/

Journal of Surgery and Medicine

e-ISSN: 2602-2079

The effect of fasudil on the uterine scar model created in rats

Filiz Yılmaz [1], Selma Aydemir [2], Serap Cılaker Mıcılı [2], Basak Baykara [2], Bayram Yılmaz [3]

[1] Hitit University, Erol Olcok Research and Training Hospital, IVF Center, Corum, Turkey
[2] Dokuz Eylül University, Faculty of Medicine, Department of Histology and Embryology, Izmir, Turkey
[3] Hitit University, Erol Olcok Research and Training Hospital, Department of Pathology, Corum, Turkey

ORCID of the author(s)

FY: https://orcid.org/0000-0003-0505-3905
SA: https://orcid.org/0000-0003-1263-9998
SCM: https://orcid.org/0000-0002-9830-0989
BB: https://orcid.org/0000-0002-4178-2235
BY: https://orcid.org/0000-0002-1737-9446

Corresponding Author
Filiz Yılmaz
Hitit University Erol Olcok Research and Training Hospital, IVF Center, Corum, Turkey
E-mail: drfilizyilmaz@gmail.com

Ethics Committee Approval
The experimental procedure was approved by the Dokuz Eylül University Local Ethics Committee (Protocol No. 34/2019).
This study was carried out in the Dokuz Eylül University Experimental Animals Laboratory in March 2020.

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
The authors declared that this study has received no financial support.

Previous Presentation
This research was presented as an oral presentation at the 4th International Scientific Research and Innovation Congress, 24–25 December 2022, Turkey.

Published
2024 December 3

Abstract

Background/Aim: Uterine healing post-obstetric surgery is crucial for maintaining fertility. This study investigates if fasudil has a therapeutic impact on scars formed by a full-thickness incision in a rat's uterus.
Methods: We divided 21 female Wistar Albino rats randomly into three groups: control, scar, and treatment. The control group underwent no surgical procedure. We created a uterine scar model in the scar and treatment groups. For 30 days, the treatment group was intraperitoneally administered a single dose of 20 mg/kg/day fasudil dissolved in saline, while the control and scar groups were given saline. After these 30 days, all rats were sacrificed. We removed the right and left uterine horns from all groups. The left side was set aside for histological analysis, while the right side was used for ELISA analysis (alpha-SMA and TGF-beta).
Results: The treatment group exhibited an increased uterine wall thickness compared to the scar group (P=0.03), although there was no discernible difference when compared with the control group. Both inflammation and fibrosis were notably higher in the scar group (P=0.01) but absent in both the treatment and control groups. The ELISA results, measuring alpha-SMA and TGF-beta, showed no statistically significant difference between the groups (P=0.321, P=0.375).
Conclusion: Fasudil effectively reduced inflammation and fibrosis in our experimental rat model, hence preventing scar formation. We believe our study adds significant value to the existing literature by potentially expediting tissue regeneration.

Keywords: uterine scar, fasudil, wound healing, alpha-SMA, TGF-beta

How to cite: Yılmaz F, Aydemir S, Mıcılı SC, Baykara B, Yılmaz B. The effect of fasudil on the uterine scar model created in rats. J Surg Med. 2024;8(12):192-195.

Introduction

Cesarean section is the most commonly performed obstetric surgery worldwide. It involves a full-thickness incision on the uterine wall, which is sutured by a specialist physician at the end of the delivery, leaving the primary wound to heal. Scar defects can develop if the cesarean-section wound does not heal properly, leading to gynecological issues such as abnormal uterine bleeding, postmenstrual spotting, painful menstrual periods, painful sexual intercourse, chronic pelvic pain, and secondary infertility. These complications can significantly affect the patient's quality of life [1-3]. Therefore, preventing the development of uterine scars can help avoid these many complications.

Fasudil, a potent and selective ROCK inhibitor, has been shown to suppress fibrosis in various diseases [4]. An *in vitro* study evaluated its effects on human urethral scar tissues, including changes to the cytoskeleton, collagen synthesis, and apoptosis of urethral fibroblasts. It demonstrated that fasudil could inhibit actin polymerization and collagen synthesis via the RhoA/ROCK pathway and induce apoptosis in scar fibroblasts [5]. In the treatment of cardiovascular diseases like cerebral and coronary vasospasm, angina, and hypertension, fasudil usage by patients did not result in serious side effects [6,7].

The RhoA/ROCK signaling pathway is linked to the expression of α-smooth muscle actin (α-SMA), which aids in the transformation of fibroblasts into myofibroblasts, facilitating wound healing and scar formation [8]. This expression of α-SMA signifies the activation of the RhoA/ROCK signaling pathway in fibroblasts [9]. α-SMA serves as a tool to differentiate myofibroblasts from fibroblasts, marking it as the go-to indicator for recognizing myofibroblasts. Myofibroblasts, present in both the development and modification phases of tissue damage, play a crucial role in generating extracellular matrix, including collagen [10]. They exist in all fibrotic diseases, such as scleroderma, as well as in liver, kidney, and lung fibrosis [11,12]. TGF-β stimulates myofibroblasts to overproduce extracellular matrix (ECM), leading to scar formation [13,14]. A separate study highlighted the role of RhoA in hypertrophic scar and keloid formation [15]. Furthermore, the RhoA/ROCK pathway is a key regulator in the contraction of smooth muscles within the myometrial layer of the uterus. This pathway operates through a distinct intermediary step in smooth muscle cells, enhancing myosin light chain (MLC) phosphorylation, thus triggering uterine contractions [16].

Existing literature indicates that the RhoA/ROCK pathway contributes to scar formation, including in the uterus. This led us to hypothesize that the RhoA/ROCK pathway could be involved in uterine scarring. We conducted a study to examine this concept, specifically investigating the impact of the ROCK inhibitor fasudil on scars produced by full-thickness incisions in rat uteruses.

Materials and methods

The Dokuz Eylül University Local Ethics Committee approved the experimental procedure (Protocol No. 34/2019). The study was conducted in the university's Experimental Animals Laboratory in March 2020.

The study involved 21 Wistar Albino female rats weighing between 180 and 220 g. Throughout the experiment, the rats were kept under normal environmental conditions and maintained a regular diet (21±2ºC, unlimited water and food). These rats (n=21) were randomly divided into three groups: Group 1, the control group (n=7), underwent no surgical procedures; Group 2 (n=7) received a uterine scar model; and Group 3 (n=7) received both a uterine scar model and a fasudil application.

Making uterine scar model with hysterotomy in rats

After administering 10 mg/kg xylazine hydrochloride and 70 mg/kg ketamine hydrochloride intramuscularly for anesthesia, the rats were placed in a dorsal horizontal position. The area set for the operation was cleaned with povidone-iodine. A transverse laparotomy incision of 2.5–3 cm was then made. We also made, on both horns of the uterus, a 1 cm vertical incision reaching up to the endometrial cavity [17,18]. This incision area was then sutured with 4.0 rapid vickryll, and the abdominal incision was also closed with the same material.

After 1 month, the same anesthesia and laparotomy method were performed through the same incision to surgically remove both horns. After removal, the left side was submerged in a 10% neutral formalin solution for histopathological assessment. After 3–5 days of fixation, it was embedded in paraffin blocks. The right side was preserved in dry ice for ELISA analysis. Subsequently, the rats were euthanized using high-dose anesthesia [17].

Following the hysterotomy, the rats were then treated with 80000 units/100mg of penicillin, administered intramuscularly for three consecutive days post-procedure [17].

Fasudil application

Fasudil (Santa Cruz, catalog number: sc-203418D), an anti-cicatrizant/anti-fibrotic agent, was dissolved in saline and administered as a single dose of 20 mg/kg/day intraperitoneally for 30 days [4]. The control and scar groups received saline during the experiment.

Histological and biochemical examination

The histological assessment was conducted using Hematoxylin-Eosin and Masson Trichrome stains. As previously described [19], the thickness of the uterine wall, as well as any inflammation and fibrosis, were evaluated microscopically. In the biochemical evaluation, levels of TGF-beta1 and alpha-SMA were determined. Adhering to the manufacturer's instructions, the levels of TGF-beta1 and alpha-SMA (catalog numbers E1688Ra and E2330Ra by BTLAB) were analyzed by ELISA.

Statistical analysis

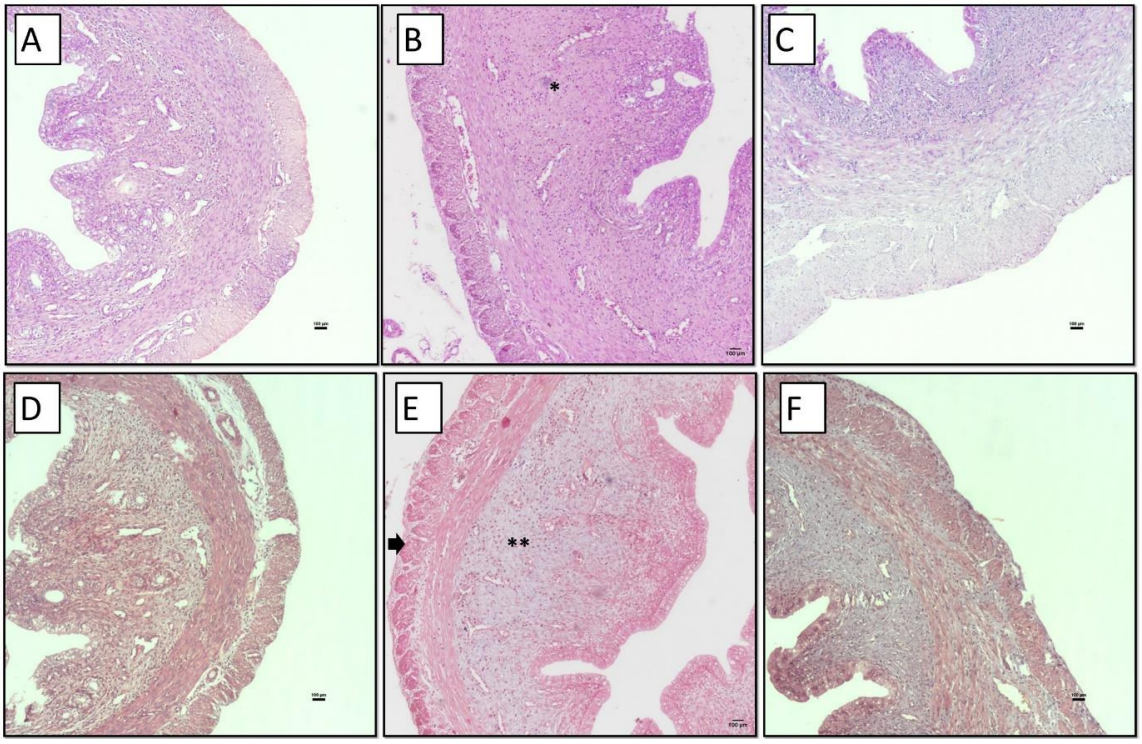
We performed a statistical analysis of the study data using the Statistical Package for Social Sciences (SPSS) 26.0 software. We calculated the mean and standard deviation of the data for the analysis. To determine the origin of the differences between the groups, we used the Kruskal Wallis and Mann-Whitney U tests.

Results

Histological Findings

We assessed whether fasudil treatment protected against scar formation by examining uterine healing post-injury. This was conducted using Hematoxylin-Eosin staining to identify general

Figure 1: Photomicrographs of the uterus tissue. Fibrosis (**), inflammation (*). Black arrows show thinning perimetrium. A, D: Control group, B, E: Scar group, C, F: Treatment group, Scale bar: 100 µm. A, B, C: Hematoxylin and Eosin Stain, D, E, F: Masson Trichrome stain.



morphological features in uterine histology. Masson Trichrome staining was used to assess the structure of the connective tissue and the degree of collagenization.

In the control group, the endometrium, myometrium, and perimetrium layers were identified as healthy. However, in the scar group, the perimetrium layer was thin, with noticeable increases in inflammation and fibrosis. Microscopic observation revealed fibrosis, augmented connective tissue, and angiogenesis. We saw inflammation characterized by PMNL cell presence, edema, and congestion.

Interestingly, the treatment group displayed histological findings similar to those of the control group (Figure 1). In the scar group, a reduction was observed in the thickness of the uterine wall. Contrastingly, a significant increase in wall thickness was noted in both the control and treatment groups (Table 1).

Biochemical Findings

Table 1 shows the levels of alpha-SMA and TGF-beta as determined by ELISA; the results are presented as mean (standard deviation). The alpha-SMA levels were 32.7 (5.6) ng/mL in the control group, 29.8 (3.8) ng/mL in the scar group, and 28.9 (4.2) ng/mL in the treatment group. There was no significant difference between the groups (P=0.375). Similarly, the levels of TGF-beta were 684.1 (180.1) ng/L in the control group, 597.4 (103.3) ng/L in the scar group, and 908.8 (222.3) ng/L in the treatment group. The difference between these groups was not statistically significant (P=0.321).

Table 1: Morphometric parameters and ELISA results (alpha-SMA and TGF-beta)

	Control group mean (SD)	Scar group mean (SD)	Treatment group mean (SD)	*P*-value
Uterine wall thickness (µm)	620.28 (45.56)	425.71 (55.76)*	596 (25.25)	0.01*
Inflammation	0.42 (0.34)	1.85 (0.69)*	0.42 (0.53)	0.03*
Fibrosis	0.14 (0.37)	2.42 (0.53)*	0.8 (0.69)	<0.001*
Alpha-SMA (ng/mL)	32.7 (5.6)	29.8 (3.8)	28.9 (4.2)	0.375
TGF-beta (ng/L)	684.1 (180.1)	597.4 (103.3)	908.8 (222.3)	0.321

Discussion

Over 50% of women with a history of cesarean section exhibit a uterine scar defect. This defect contributes to heightened maternal morbidity and longer hospital stays. The links between uterine scar defects, gynecological symptoms, obstetric complications, and possible subfertility makes it crucial to understand why scars form post-cesarean section and develop prevention strategies. Therefore, our study investigates whether fasudil has a therapeutic role in a scar model created by performing a full-thickness incision on a rat's uterus.

Previous studies on uterine scars have demonstrated the potential benefits of various antioxidants [17,20]. For example, Sayin et al. [20] developed a rat model of uterine scarring to assess the therapeutic effect of resveratrol. After 30 days of administering resveratrol, they observed a successful treatment outcome, as evidenced by increased uterine wall thickness and higher levels of VEGF, GPx, and SOD.

In a separate study, Mıcılı et al. [17] explored the effect of lipoic acid on uterine scarring, comparing results from 15 and 30-day treatments. They noted that the histological findings from the 30-day treatment group mirrored those of the control group, suggesting that lipoic acid could effectively treat uterine scars.

In our study, we similarly assessed the histological and biochemical effects of fasudil on uterine scar healing. Based on our findings, which included increased uterine wall thickness and reduced inflammation and fibrosis relative to the scar group, we propose that fasudil may be an effective treatment for uterine scars.

Previous studies have demonstrated the anti-scarring effects of fasudil on various organs [4,5]. Li et al. [5] evidenced the therapeutic impact of fasudil on human urethral scar tissue *in vitro*. Similarly, Qi et al. [4] presented findings suggesting fasudil effectively reduces inflammation and fibrosis in the treatment of hyperoxia-induced pulmonary fibrosis in neonatal rats. Histological evaluations often highlight inflammation and

increased fibrosis as indicators of scar development, which, if left unchecked, can lead to long-term complications in the uterus. Consequently, successful anti-scarring therapy is critical. In this study, we explored the influence of fasudil on the healing of uterine scar tissue and discovered that it exhibits therapeutic potential, aligning with earlier findings.

Upon reviewing the literature on studies examining the effects of fasudil, we found that it is most commonly administered intraperitoneally. Subcutaneous application was favored only in wound models created on the skin [21]. In all studies probing the therapeutic impact on internal organs like the heart, kidney, and lungs, fasudil was dispensed once daily and intraperitoneally [4,22,23]. Therefore, in our study, we opted to administer fasudil once daily and intraperitoneally.

The literature reveals that the function of the RhoA/ROCK pathway has been assessed using fasudil. This pathway's role in regulating glomerular adhesion and inflammation in diabetic nephropathy has been established through contrasting groups administered with and without fasudil [16,24]. The choice to use fasudil in our research was to investigate this pathway's effect on uterine scar tissue. Our results aligned with previous studies, suggesting the RhoA/ROCK pathway might influence uterine scar tissue. For our subsequent study, we plan to include molecular-level analysis to enhance our findings.

Limitations

We could not observe the initial effects because of the extended experimental duration. For our next study, we are planning to examine both short and long-term effects, allowing us to monitor immediate processes. Augmenting the study with groups having both short and long experimental durations, a larger sample size, and varied doses will enhance our research. Furthermore, we could measure antioxidant parameters biochemically in the tissue. Evaluations of RhoA/ROCK molecule expressions could indicate the pathway's presence.

Conclusion

Consequently, we believe that fasudil positively assists the uterine wound healing process. Further studies could potentially enable its adaptation for clinical use.

References

1. Bij de Vaate AJ, van der Voet LF, Naji O, Witmer M, Veersema S, Brölmann HA, et al. Prevalence, potential risk factors for development and symptoms related to the presence of uterine niches following Cesarean section: systematic review. Ultrasound Obstet Gynecol. 2014 Apr;43(4):372-82. doi: 10.1002/uog.13199. PMID: 23996650.
2. Florio P, Filippeschi M, Moncini I, Marra E, Franchini M, Gubbini G. Hysteroscopic treatment of the cesarean-induced isthmocele in restoring infertility. Curr Opin Obstet Gynecol. 2012 Jun;24(3):180-6. doi: 10.1097/GCO.0b013e3283521202. PMID: 22395067.
3. van der Voet LF, Bij de Vaate AM, Veersema S, Brölmann HA, Huirne JA. Long-term complications of caesarean section. The niche in the scar: a prospective cohort study on niche prevalence and its relation to abnormal uterine bleeding. BJOG. 2014 Jan;121(2):236-44. doi: 10.1111/1471-0528.12542. PMID: 24373597.
4. Qi XJ, Ning W, Xu F, Dang HX, Fang F, Li J. Fasudil, an inhibitor of Rho-associated coiled-coil kinase, attenuates hyperoxia-induced pulmonary fibrosis in neonatal rats. Int J Clin Exp Pathol. 2015 Oct 1;8(10):12140-50. PMID: 26722398; PMCID: PMC4680343.
5. Li XD, Wu YP, Chen SH, Liang YC, Lin TT, Lin T, et al. Fasudil inhibits actin polymerization and collagen synthesis and induces apoptosis in human urethral scar fibroblasts via the Rho/ROCK pathway. Drug Des Devel Ther. 2018 Sep 3;12:2707-13. doi: 10.2147/DDDT.S156095. PMID: 30214158; PMCID: PMC6126504.
6. Mohri M, Shimokawa H, Hirakawa Y, Masumoto A, Takeshita A. Rho-kinase inhibition with intracoronary fasudil prevents myocardial ischemia in patients with coronary microvascular spasm. J Am Coll Cardiol. 2003 Jan 1;41(1):15-9. doi: 10.1016/s0735-1097(02)02632-3. PMID: 12570938.
7. Shimokawa H, Rashid M. Development of Rho-kinase inhibitors for cardiovascular medicine. Trends Pharmacol Sci. 2007 Jun;28(6):296-302. doi: 10.1016/j.tips.2007.04.006. Epub 2007 May 7. PMID: 17482681.
8. Shimizu T, Liao JK. Rho Kinases and Cardiac Remodeling. Circ J. 2016 Jun 24;80(7):1491-8. doi: 10.1253/circj.CJ-16-0433. Epub 2016 Jun 1. PMID: 27251065; PMCID: PMC5563468.
9. Zhang XH, Sun NX, Feng ZH, Wang C, Zhang Y, Wang JM. Interference of Y-27632 on the signal transduction of transforming growth factor beta type 1 in ocular Tenon capsule fibroblasts. Int J Ophthalmol. 2012;5(5):576-81. doi: 10.3980/j.issn.2222-3959.2012.05.06. Epub 2012 Oct 18. PMID: 23166867; PMCID: PMC3484693.
10. Bochaton-Piallat ML, Gabbiani G, Hinz B. The myofibroblast in wound healing and fibrosis: answered and unanswered questions. F1000Res. 2016 Apr 26;5:F1000 Faculty Rev-752. doi: 10.12688/f1000research.8190.1. PMID: 27158462; PMCID: PMC4847562.
11. Zhou Y, Huang X, Hecker L, Kurundkar D, Kurundkar A, Liu H, et al. Inhibition of mechanosensitive signaling in myofibroblasts ameliorates experimental pulmonary fibrosis. J Clin Invest. 2013 Mar;123(3):1096-108. doi: 10.1172/JCI66700. Epub 2013 Feb 22. PMID: 23434591; PMCID: PMC3582144.
12. Zhao XH, Laschinger C, Arora P, Szászi K, Kapus A, McCulloch CA. Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. J Cell Sci. 2007 May 15;120(Pt 10):1801-9. doi: 10.1242/jcs.001586. Epub 2007 Apr 24. PMID: 17456553.
13. Putra A, Alif I, Hamra N, Santosa O, Kustiyah AR, Muhar AM, et al. MSC-released TGF-β regulate α-SMA expression of myofibroblast during wound healing. J Stem Cells Regen Med. 2020 Dec 11;16(2):73-9. doi: 10.46582/jsrm.1602011. PMID: 33414583; PMCID: PMC7772809.
14. Shinde AV, Humeres C, Frangogiannis NG. The role of α-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. Biochim Biophys Acta Mol Basis Dis. 2017 Jan;1863(1):298-309. doi: 10.1016/j.bbadis.2016.11.006. Epub 2016 Nov 4. PMID: 27825850; PMCID: PMC5163362.
15. Kiya K, Kubo T, Kawai K, Matsuzaki S, Maeda D, Fujiwara T, et al. Endothelial cell-derived endothelin-1 is involved in abnormal scar formation by dermal fibroblasts through RhoA/Rho-kinase pathway. Exp Dermatol. 2017 Aug;26(8):705-12. doi: 10.1111/exd.13264. Epub 2017 Mar 10. PMID: 27892645.
16. Aktas S, Un I, Omer Barlas I, Ozturk AB, Ilkay Karagul M. Evaluation of the Rho A/Rho-kinase pathway in the uterus of the rat model of polycystic ovary syndrome. Reprod Biol. 2019 Mar;19(1):45-54. doi: 10.1016/j.repbio.2019.01.005. Epub 2019 Jan 28. PMID: 30704840.
17. Micili SC, Goker A, Sayin O, Akokay P, Ergur BU. The effect of lipoic acid on wound healing in a full thickness uterine injury model in rats. J Mol Histol. 2013 Jun;44(3):339-45. doi: 10.1007/s10735-013-9485-8. Epub 2013 Feb 1. PMID: 23371355.
18. Lin N, Li X, Song T, Wang J, Meng K, Yang J,et al. The effect of collagen-binding vascular endothelial growth factor on the remodeling of scarred rat uterus following full-thickness injury. Biomaterials. 2012 Feb;33(6):1801-7. doi: 10.1016/j.biomaterials.2011.11.038. Epub 2011 Dec 1. PMID: 22136717.
19. Mohtasham Amiri Z, Tanideh N, Seddighi A, Mokhtari M, Amini M, Shakouri Partovi A, et al. The Effect of Lithospermum officinale, Silver Sulfadiazine and Alpha Ointments in Healing of Burn Wound Injuries in Rat. World J Plast Surg. 2017 Sep;6(3):313-8. PMID: 29218280; PMCID: PMC5714976.
20. Sayin O, Micili SC, Goker A, Kamaci G, Ergur BU, Yilmaz O, et al. The role of resveratrol on full-thickness uterine wound healing in rats. Taiwan J Obstet Gynecol. 2017 Oct;56(5):657-63. doi: 10.1016/j.tjog.2017.08.015. PMID: 29037554.
21. Bond JE, Kokosis G, Ren L, Selim MA, Bergeron A, Levinson H. Wound contraction is attenuated by fasudil inhibition of Rho-associated kinase. Plast Reconstr Surg. 2011 Nov;128(5):438e-450e. doi: 10.1097/PRS.0b013e31822b7352. PMID: 22030503; PMCID: PMC3205419.
22. Zhuang R, Wu J, Lin F, Han L, Liang X, Meng Q, et al. Fasudil preserves lung endothelial function and reduces pulmonary vascular remodeling in a rat model of end stage pulmonary hypertension with left heart disease. Int J Mol Med. 2018 Sep;42(3):1341-52. doi: 10.3892/ijmm.2018.3728. Epub 2018 Jun 13. PMID: 29901088; PMCID: PMC6089763.
23. Lai D, Gao J, Bi X, He H, Shi X, Weng S, et al. The Rho kinase inhibitor, fasudil, ameliorates diabetes-induced cardiac dysfunction by improving calcium clearance and actin remodeling. J Mol Med (Berl). 2017 Feb;95(2):155-65. doi: 10.1007/s00109-016-1469-1. Epub 2016 Aug 30. Erratum in: J Mol Med (Berl). 2017 Feb;95(2):167. PMID: 27576917.
24. Rao J, Ye Z, Tang H, Wang C, Peng H, Lai W, et al. The RhoA/ROCK Pathway Ameliorates Adhesion and Inflammatory Infiltration Induced by AGEs in Glomerular Endothelial Cells. Sci Rep. 2017 Jan 5;7:39727. doi: 10.1038/srep39727. PMID: 28054559; PMCID: PMC5215591.