Tropical Journal of Pharmaceutical Research March 2023; 22 (3): 517-523
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i3.8

Original Research Article

Glycyrrhizinate attenuates exosome-induced endothelial cell proliferation and permeability through p38 pathway

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Sent for review: 29 June 2022 *Revised accepted: 28 February 2023*

Abstract

Purpose: *To investigate the mechanism of action involved in the treatment of severe burn injury with glycyrrhizinate (DG).*
Methods: *Exosomes were purified from sera of patients with burn injury using an ultra-high-speed centrifuge, and verified by western blot. Cell proliferation and permeability were assessed using cell counting kit (CCK)-8, transepithelial-transendothelial electrical resistance (TEER), and Fluorescein Isothiocyanate (FITC) dextran assays, while immunoblotting was used for assay of the levels of p38, occluding, and zonula occludens 1 (ZO-1).*
Results: *Serum-derived exosomes (serum-exo) significantly suppressed cell proliferation while causing hyperpermeability in HUVECs ($p < 0.001$). Furthermore, DG alleviated the hyperpermeability and inhibition of cell proliferation caused by serum-exo ($p < 0.001$). In addition, the upregulation of p-P38 induced by serum-exo decreased upon DG treatment. Interestingly, the effect of DG was blocked by anisomycin, a specific p38 activator, indicating that p38 signaling pathway may contribute to the function of DG.*
Conclusion: *Glycyrrhizinate attenuates serum-exo-induced cell proliferation and permeability alteration via p38 signaling pathway, thereby making it a potential agent for the management of severe burn injury.*

Keywords: *Burn wound, Exosomes, Glycyrrhizinate, Permeability, Proliferation*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Microvascular hyperpermeability is present both at the local burn site and at distant tissues. Thus, alteration of permeability leads to aggravated tissue retention of proteinaceous fluids, which results in blockage of microcirculation and blood-tissue exchange, leading to hypoxia.

The S100A9-containing serum-derived exosomes (serum-exo) have been reported to induce alteration of permeability in HPMEC cells [1]. Exosomes are double-layer lipid vesicles carrying multiple bioactive substances such as microRNA, mRNA, mitochondrial DNA (mtDNA), proteins, and lipids which can be taken up by target cells. Therefore, exosomes play vital roles in conduction and signal transduction [2].

Exosomes are involved in endothelial cell regulation. For example, exosomes released from adipose-derived stem cells promote the proliferation, migration, and tube-like differentiation of human umbilical vein endothelial cells [3]. Endothelial cell dysfunction is manifested as changes in cell migration and permeability [4]. It has been suggested that serum-exo might play a critical role in burn injury by contributing to pulmonary microvascular hyperpermeability, which makes it a potential therapeutic target [1].

Licorice is a traditional Chinese medicine with anti-inflammatory, anti-tumor, antibacterial, anti-oxidant, and anti-arrhythmic properties [5]. Its main bioactive components are triterpene saponins and licorice flavonoids. A study has shown that glycyrrhizic acid, a member of the triterpene saponins, exerted a protective effect on mice infected with systemic *Candida albicans* [6]. However, the exact mechanism of glycyrrhizinate (DG) in burn treatment is not well understood.

The aim of this study was to investigate the impact of DG on changes in the proliferation and permeability of human umbilical vein endothelial cells (HUVECs) induced by serum exosomes.

EXPERIMENTAL

Cell culture

Human umbilical vein endothelial cells (HUVECs) were maintained on Dulbecco-modified Eagle medium (Hyclone, SH30243.01; Logan, UT, US) supplemented with 10 % FBS (GIBCO, 16000e044; Carlsbad, CA, USA). The culture medium also contained 1 % penicillin-streptomycin (Solarbio, P1400, Beijing, China) for selection. The cells were cultured at 37 °C in an atmosphere of 5 % CO_2.

Clinical samples and ethical issues

Blood samples (5 mL) were obtained from 3 patients with burn injuries. Then, the serum samples were separated by centrifuging the whole blood samples for 480 s at 3500 rpm at ambient temperature. The sera were then kept at -80 °C. Informed and written consent was obtained from each patient. This study received approval from the Ethics Committee of the Seventh People's Hospital Affiliated with Shanghai University of Traditional Chinese Medicine, Shanghai (approval no. 2017-IRBQYYS-028), and met the criteria stipulated in the guidelines of Helsinki Declaration [7].

Isolation and identification of serum exosomes

Exosomes were isolated using ultracentrifugation method. The serum samples were clarified by spinning at 10,000 g for 30 min at 4 °C. Then, the supernatants were taken up in 5-mL ultracentrifuge tubes which contained PBS. Following 3 sequential centrifugation steps, each at 17,000 g for 2 h, the pelleted exosomes were taken up in a small volume of PBS, filtered through a 0.22-µm PVDF filter, and preserved frozen at -80 °C until further analysis.

The exosomes were identified using immunoblot analysis of several known markers including CD9, CD81, and TSG101. The antibodies anti-CD9 (Ab92726), anti-CD81 (Ab109201), and anti-TSG101 (Ab125011) were purchased from Abcam.

PKH-67 tracer exosomes

The exosomes were stained using green fluorescent linker PKH67 (UR52303, Umibio, Shanghai, China). In this procedure, 1 mL of exosomes (1 µg/mL) was incubated with 2 µL PKH67 for 25 min at ambient temperature, followed by addition of 0.5 % BSA/PBS for uptake of unreacted dye. Then, the tagged exosomes were centrifuged at 100,000 g for 1 h and taken up in PBS for use in subsequent studies.

The HUVEC cells were plated at a level of 50,000 cells/well and incubated with or without PKH67-labeled exosomes for 24 h. The nuclei were stained with DAPI. The uptake of DAPI was monitored using fluorescent microscopy (Leica Microsystems, Wetzlar, Germany) 4 h later.

Cell Counting Kit (CCK)-8 experiment

This was performed with CCK-8 kit (SAB, USA). Approximately HUVEC cells were plated at a density of 2×10^3 cells/well, followed by 12-h incubation at 37 °C in a 5 % CO_2 incubator. Then, the cells were divided into two treatment groups which received either serum-exo or glycyrrhizinate (Aladdin, G111375, Beijing, China). At the end of treatment, 100 µL of CCK-8 reaction solution was added and incubated for 1 h. Finally, cell viability was evaluated by measuring absorbance at 450 nm.

Immunoblot assay

Protein extraction from cells was done with RIPA lysis buffer (JRDUN, Shanghai, P.R. China). The proteins were separated on SDS-PAGE and

electroblotted onto a PVDF membrane which was sealed with 5 % non-fat milk, followed by 12 h incubation of 1° antibodies at 4 °C, and rinsing with PBST. Then, the blots were incubated with secondary antibody for 60 min at 37 °C. The protein bands were visualized using ECL. The 1° immunoglobulins used were anti-CD9 (Ab92726), anti-CD81 (Ab109201), anti-TSG101 (Ab125011), anti-ZO-1 (Ab96587), anti-occludin (Ab167161), anti-p38 (Ab170099), and anti-p-p38 (Ab47363), all being products of Abcam, as well as 60004-1-1G (Proteintech).

Effects of different licorice monomers on cell permeability

The five major licorice monomers comprising glycyrrhizinate (DG), glycyrrhizin (PG), liquiritigenin (LN), iso-PG, and isoliquiritigenin (iso-LN) were applied to HUVECs together with serum-exo, and then the permeability of the cells was determined using Transepithelial-Transendothelial Electrical Resistance (TEER) and Fluorescein isothiocyanate (FITC)-dextran assays. Thus, 10^4 HUVEC cells in 100 µL of medium were plated in the top compartment of a Transwell plate, while 600 µL of medium was in the lower compartment. Then, the cells were maintained for 24 h at 37 °C in a 5 % CO_2 incubator, medium changed 24 h afterward. The TEER was determined every day and the values were computed using Eq 1.

TEER = dR-iR/A …… (1)

Where dR = daily resistance, iR = initial resistance, A = area of multicell base

For FITC-Dextran, 10^5 HUVEC cells were cultured to 100 % confluence, followed by addition of FITC-Dextran to the culture medium and incubation for 5 min at 37 °C in a 5 % CO_2 incubator. Then, 200 µL of the basic medium was set as base value, and the cells were cultured in a complete medium for one day, after which FITC was measured (excitation λ = 490 nm; emission λ = 520 nm).

Statistical analysis

All data analyses in the current study were conducted using GraphPad Prism 7.0 software (San Diego, CA, USA). At least three independent experimental repeats were performed for each data reported in this project. All results are presented as mean ± standard deviation (SD). One-way ANOVA and Tukey's post hoc tests were used for comparing data amongst groups. Values of $p < 0.05$ were taken as indicative of statistical significance.

RESULTS

Isolation and identification of serum-exo

In order to investigate the function of serum exosomes (serum-exo) in burn injury patients, exosomes from patients' sera were extracted using an ultracentrifuge. Their homogeneity was assessed with TEM imaging. The exosomes exhibited a spherical shape (Figure 1 A). In addition, the presence of exosomal biomarkers such as CD81, TSG101, and CD9 was validated using western blot (Figure 1 B). Together, these results indicated that the purified micro-vesicles from the serum of burn patients were exosomes. Then, the experiment was performed to determine whether the exosomes could be taken up by human umbilical vein endothelial cells (HUVEC), and PKH-67-labeled exosomes were co-cultured with HUVECs. As shown in Figure 1 C, the endocytosed exosomes were seen using laser-scanning microscope imaging, suggesting that the exosomes were successfully internalized by HUVEC.

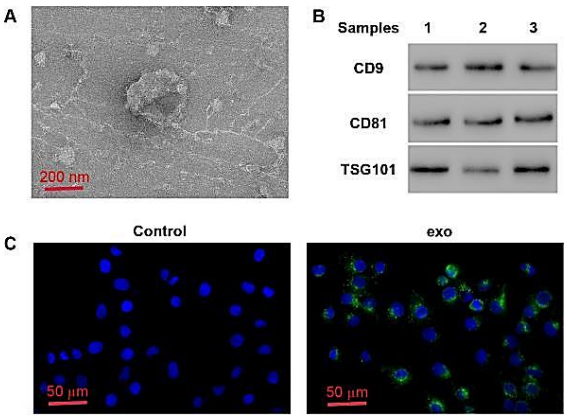

Figure 1: Extraction and confirmation of serum-exo from burn patients. (A) Exosome content and purity, as confirmed with TEM. (B) The expressions of exosomal markers CD9, CD81, and TSG101, as assayed using Western Blot. (C) PHK-67 staining assay indicated that the serum exosomes were absorbed by HUVECs cells through endocytosis

Serum-exo significantly inhibited cell proliferation and induced cell permeability

In order to investigate the influence of serum-exo on cell multiplication, CCK-8 assay was done on HUVECs. Interestingly, serum-exo significantly decreased the proliferation of HUVECs, when compared with vehicle control in a time-dependent manner (Figure 2 A). It is known that disruption of integrity of endothelial barriers such as burn injury results in microvascular hyperpermeability. To determine whether serum-

exo plays a role in the alteration of permeability, the permeability of serum-exo-treated HUVECs was examined using Transepithelial-Transendothelial Electrical Resistance (TEER) and FITC-Dextran assays. As shown in Figures 2 B and C, serum-exo significantly reduced the levels of TEER, whereas they increased the FITC-dextran in a time-dependent manner, which indicated elevation of transepithelial hyperpermeability.

It has been demonstrated that the integrity of tight junction (TJ) depends on integral membrane proteins such as claudins and occludin, and cytoplasmic scaffolding proteins such as ZO-1, ZO-2, and ZO-3 [8]. Likewise, Figure 2 D shows that ZO-1 and occludin protein concentrations were significantly decreased by treatment with serum-exo. In addition, serum-exo significantly enhanced p-38 phosphorylation in a time-based fashion in HUVEC without altering the total p38 protein abundance (Figure 2 D). Thus, the exosomes promoted the permeability of HUVECs by activating p38 MAPK signaling pathway, as well as disrupting the integrity of TJs and endothelial barrier.

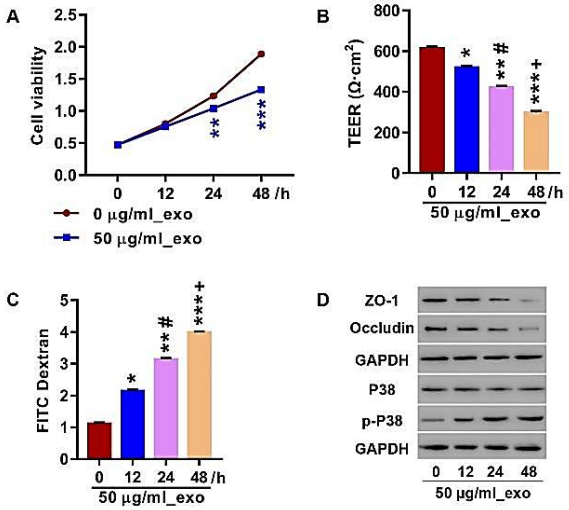

Figure 2: Serum exosomes significantly inhibited cell proliferation and induced cell permeability in HUVECs. The HUVECs were exposed to serum-exo at different times as indicated. (A) Cell proliferation, as determined using CCK8 assay. (B & C) The permeability of HUVECs, as determined using TEER (B) and FITC Dextran assays (C). (D) Expressions of relevant proteins, as determined using western blot. **P* < 0.05, ***p* < 0.01, ****p* < 0.001 vs. 0 h; $^{\#}p$ < 0.05 vs. 12 h; ^{+}p < 0.05 vs. 24 h

Cell permeability

As shown in Figures 3 A and B, the altered permeability induced by serum-exo was reversed by treatment with DG and LN, while no significant changes were observed in the treatment with the other monomers, suggesting the potential therapeutic effect of DG.

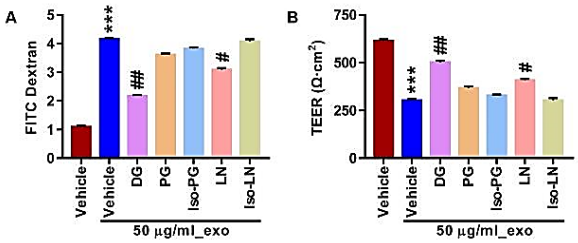

Figure 3: Effects of different licorice monomers on cell permeability of HUVECs. HUVECs were treated with five major licorice monomers comprising DG, PG, Iso-PG, LN, and Iso-LN (10 μmol/L each) alone, or in combination with exosomes (50 μg/mL). (A & B) The permeability of HUVECs, as determined using TEER (A) and FITC Dextran assays (B). ****P* < 0.001 vs. vehicle; $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01 vs. 50 μg/mL exo + vehicle

DG alleviated exo-induced inhibition of proliferation and permeability

To further evaluate the effect of DG on serum-exo-treated HUVECs, DG was applied to serum-exo-treated HUVECs. As shown in Figure 4 A, DG restored the proliferation profile of serum-exo-treated HUVECs in a dose-dependent manner. Furthermore, the TEER and FITC Dextran assays demonstrated that cell permeability was gradually restored as the dose of DG increased (Figures 4 B and C). Western blot data revealed that protein amounts of ZO-1, occluding, and phosphorylated p38 were restored as well (Figure 4 D). Thus, the effect of DG on cell permeability might be mediated through ZO-1 and occludin signaling, as well as p38 activity.

DG suppressed exo-induced inhibition of proliferation and permeability

In order to investigate the hypothesis that DG influenced cell permeability via the p38 pathway, anisomycin, a specific p38 activator, was used to treat the HUVECs that had been treated with serum-exo and DG. It was found that the rescue capability of DG toward serum-exo-induced inhibition of cell permeability was abolished by anisomycin, as exhibited by decreased TEER and increased FITC Dextran in the exo + DG + anisomycin treatment group (Figures 5 A and B). Furthermore, the alterations in the protein phosphorylation level of p38 induced by serum-exo and DG were reversed by anisomycin (Figure 5 C), suggesting that DG might be of benefit to patients with burn injury by specifically targeting serum-exo-induced hyperpermeability

via inhibition of p38 activity. Collectively, these data demonstrate that DG exhibited a potential therapeutic role in burn injury patients by reversing increased permeability through suppressing p38 activity.

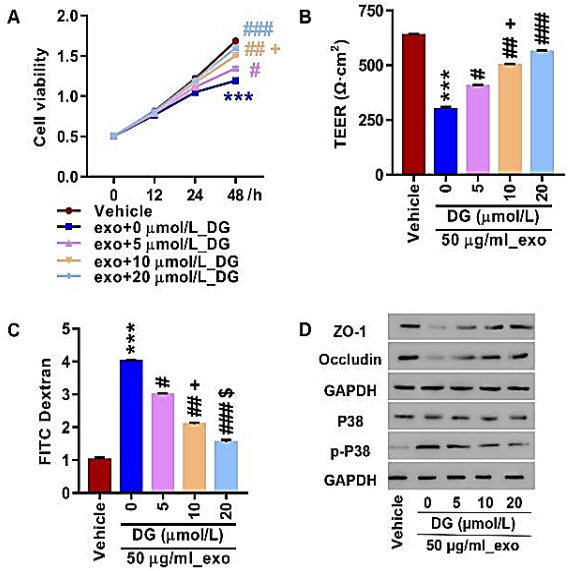

Figure 4: DG significantly reversed serum exosome-induced inhibition of proliferation and permeability in HUVECs. The HUVECs were treated with serum-exo (50 μg/mL) alone or in combination with DG at indicated concentrations (5, 10, and 20 μmol/L). (A) The proliferation of HUVECs at various time points for each treatment group, as determined with CCK8 assay. (B & C) Permeability of HUVECs, as determined with TEER (B) and FITC Dextran assays (C). (D) Expressions of occludin, p38, p-p38, and ZO-1. ***$P < 0.001$ vs. vehicle; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. exo+0 μmol/L DG; $^{+}p < 0.05$ vs. exo+5 μmol/L DG; $^{\$}p < 0.05$ vs. exo+10 μmol/L DG

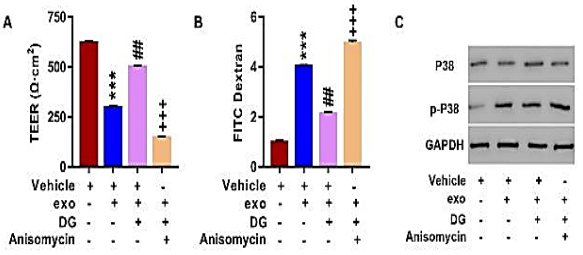

Figure 5: DG reversed serum exosome-induced inhibition of proliferation and cell permeability in HUVECs probably through the p38 pathway. After treatment with exosomes of HUVECs, DG (10 μmol/L) and p38 agonist anisomycin (10 μmol/L) were added to the cell culture separately or in combination. (A & B) The permeability of HUVECs, as determined using TEER (A) and FITC Dextran assays (B). (C) Expressions of p38 and p-p38. ***$P < 0.001$ vs. vehicle; $^{\#\#}p < 0.01$ vs. vehicle + exo; $^{+++}p < 0.001$ vs. vehicle + exo + DG

DISCUSSION

The treatment and management of severe burn injury take time during which emphasis is focused on both local burn lesions, internal impacts, and the psychological and social effects [10]. Despite the remarkable developments in these treatments, internal aggravations from burns are still the major causes of disability and mortality in serious burn injury patients, regardless of successful initial resuscitation [11]. It is known that microvascular hyperpermeability causes severe pathological changes that eventually lead to hypoxia-induced diseases. It has been reported that exosomes play critical roles in the diagnosis and treatment of hypoxia diseases [12].

Exosomes are double-layer lipid vesicles derived from the various types of cells, and they execute various roles. Numerous studies have elucidated the functions of exosomes in various biological and pathological processes [2]. For instance, S100A9-bearing serum-exo which was derived from subjects with severe burn wounds has been reported to induce permeability alteration in HPMECs via ZO-1 and occludin signaling as well as activation of p38 signaling, indicating their crucial function in impairment of tight joints and intactness of endothelial barricades [1].

An enhanced understanding of the mechanisms underlying the hyperpermeability caused by severe burn wounds will provide knowledge for the improvement of current therapy, or even for the discovery of novel therapy. It is known that licorice is of significant benefit to patients with burn wounds [13]. In addition, the effects of components of licorice on permeability have been reported in multiple publications.

For instance, glycyrrhizin was reported to increase the permeability and decrease elasticity modulus of cell membranes [14]. Similarly, glycyrrhizin was shown to induce mitochondrial permeability transition and trigger apoptosis [15]. On the other hand, glycyrrhizin has been reported to maintain normal retinal permeability and retinal capillary coverage through anti-inflammatory mechanisms [16].

Apparently, the effect of licorice on permeability is cell type-dependent. Furthermore, it has been reported that diammonium glycyrrhizinate exerted a beneficial effect on burn wounds by targeting post-burn hepatic dysfunction [17]. In this study, the potential therapeutic effect of glycyrrhizinate (DG), another bioactive integrant of licorice, on serum-exo-induced hyper-permeability in HUVECs was validated. However,

it has been suggested that licorice treatment suppresses cell proliferation and enhances the expressions of pro-apoptotic proteins [18]. Likewise, the data obtained in this study demonstrate that DG attenuated exo-induced endothelial cell proliferation and permeability. It was not surprising to observe its inhibitory effect on cell proliferation, given that licorice exhibits anti-cancer effect on multiple types of cancers [19].

However, the molecular mechanism associated with licorice or DG was not hitherto specifically identified. In this study, the results of biochemical analyses suggest that DG might affect permeability by restoring levels of occluding and ZO-1 as well as blocking p38 activity. It is known that ZO-1 and occludin control permeability by regulating endothelial adherens junction [8]. In addition, p38 MAPK is known to control endothelial cell permeability [9]. Given the functions of DG and serum-exo on ZO-1 and occludin through activation of p38, the data obtained in the current study provide insight into the reverse roles of DG and serum-exo.

CONCLUSION

The findings of this study provide direct experimental evidence for the influence of DG on cellular permeability and reveal the relationships amongst DG, ZO-1/occluding, and p38. In addition, this study validates the value of DG as a therapy for severe burn wounds, highlights the critical role of serum-exo in burn injury, and the importance of ZO-1, occluding, and p38 signaling.

DECLARATIONS

Acknowledgements

This study was funded by Shanghai Health and Family Planning Commission, Shanghai Chinese and Western Medicine Clinical Cooperation Pilot Construction Project-water burning injury (burn) {no. ZY(2018-2020)-FWTX-1106}, and Chinese Medicine Specialty Brand Building Multiplication Plan (Chinese Medicine Specialty of Burn Plastic Wound Repair) (no. PDZY-2021-0311).

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shun Xu conceived and designed the study. Shun Xu, Ai-Li Wang, Zao-Li Shen, Yu Chen and Qing Jia performed the experiments and collected and analyzed the data. Ai-Li Wang wrote the manuscript. All authors read and approved the final manuscript.

REFERENCES

1. *Qin D, Yang W, Pan Z, Zhang Y, Li X, Lakshmanan S. Differential proteomics analysis of serum exosomein burn patients. Saudi J Biol Sci 2020; 27: 2215-2220.*
2. *Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci 2019; 9: 19.*
3. *Zhang J, Yi Y, Yang S, Zhu Y, Hu X. Effects of adipose-derived stem cell released exosomes on proliferation, migration, and tube-like differentiation of human umbilical vein endothelial cells. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2018; 32: 1351-1357.*
4. *Ku YH, Cho BJ, Kim MJ, Lim S, Park YJ, Jang HC, Choi SH. Rosiglitazone increases endothelial cell migration and vascular permeability through Akt phosphorylation. BMC Pharmacol Toxicol 2017; 18: 62.*
5. *Yang R, Wang LQ, Yuan BC, Liu Y. The Pharmacological Activities of Licorice. Planta Med 2015; 81: 1654-1669.*
6. *Guo N. Protective effect of glycyrrhizine in mice with systemic Candida albicans infection and its mechanism.*

Zhongguo Yi Xue Ke Xue Yuan Xue Bao 1991; 13: 380-383.

7. *World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310: 2191-2194.*
8. *Tornavaca O, Chia M, Dufton N, Almagro LO, Conway DE, Randi AM, Schwartz MA, Matter K, Balda MS. ZO-1 controls endothelial adherens junctions, cell-cell tension, angiogenesis, and barrier formation. J Cell Biol 2015; 208: 821-838.*
9. *Wang W, Weng J, Yu L, Huang Q, Jiang Y, Guo X. Role of TLR4-p38 MAPK-Hsp27 signal pathway in LPS-induced pulmonary epithelial hyperpermeability. BMC Pulm Med 2018; 18: 178.*
10. *Öster C, Willebrand M, Ekselius L. Burn-specific health 2 years to 7 years after burn injury. J Trauma Acute Care Surg 2013; 74: 1119-1124.*
11. *Schaefer TJ, Nunez Lopez O. StatPearls. StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC., 2021.*
12. *Duan P, Tan J, Miao Y, Zhang Q. Potential role of exosomes in the pathophysiology, diagnosis, and treatment of hypoxic diseases. Am J Transl Res 2019; 11: 1184-1201.*
13. *Tanideh N, Rokhsari P, Mehrabani D, Mohammadi SS, Sabet SF, Ashraf MJ, Koohi HO, Shamsian S, Ahmadi N. The healing effect of licorice on pseudomonas aeruginosa infected burn wounds in experimental rat model. World J Plast Surg 2014; 3: 99-106.*
14. *Selyutina OY, Polyakov NE, Korneev DV, Zaitsev BN. Influence of glycyrrhizin on permeability and elasticity of cell membrane: perspectives for drugs delivery. Drug Deliv 2016; 23: 858-865.*
15. *Salvi M, Fiore C, Armanini D, Toninello A. Glycyrrhetinic acid-induced permeability transition in rat liver mitochondria. Biochem Pharmacol 2003; 66: 2375-2379.*
16. *Liu L, Jiang Y, Steinle JJ. Glycyrrhizin protects the diabetic retina against permeability, neuronal, and vascular damage through anti-inflammatory mechanisms. J Clin Med 2019; 8: 957.*
17. *Jin HH, Zheng GP, Chen WR, Liu D, Cheng X, Ren JB. The use of diammonium glycyrrhizinate in the treatment of hepatic dysfunction in burn patients. Zhonghua Shao Shang Za Zhi 2005; 21: 353-355.*
18. *Khan R, Khan AQ, Lateef A, Rehman MU, Tahir M, Ali F, Hamiza OO, Sultana S. Glycyrrhizic acid suppresses the development of precancerous lesions via regulating the hyperproliferation, inflammation, angiogenesis and apoptosis in the colon of Wistar rats. PLoS One 2013; 8: e56020.*
19. *Zhang Z, Xu M, Sun X, Guo X. Naturally occurring glycyrrhizin triterpene exerts anticancer effects on colorectal cancer cells via induction of apoptosis and autophagy and suppression of cell migration and invasion by targeting MMP-9 and MMP-2 expression. J BUON 2020; 25: 188-193.*