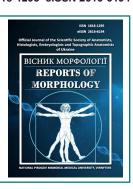
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Study of the effect of Saponaria officinalis herb extract on histological changes in the marginal vein of the ear under conditions of experimental thrombophlebitis

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Thrombophlebitis is recognized as one of the most common complications of chronic venous insufficiency (CVI). Its course is characterized by inflammation of the venous wall and the formation of blood clots in the lower extremities of varicose veins. Thrombophlebitis is diagnosed according to various data in 5-60 % of patients with CVI. The number of drugs with a phleboprotective effect and the ability to reduce the processes of thrombus formation in veins on the pharmaceutical market of Ukraine and the world is extremely limited. As a promising object, as a new phleboprotective drug, medicinal plant raw materials can be considered, namely the extract of the herb Saponaria officinalis, for which venotonic and antiexudative effects have been established in previous studies. The aim of this work was to study the effect of Saponaria officinalis herb extract on changes in the histological parameters of the ear vein in rabbits under conditions of experimental thrombophlebitis. Experimental thrombophlebitis was modeled on rabbits according to the standard method with applying a ligature to the marginal vein of the animal's ear and injecting Lugol's solution. Histological changes after phytocomposition administration (ETML) (20 mg/ kg intragastrically) were compared with the effectiveness of the reference drug Eskuvit (7.2 mg/kg for escin intragastrically). It was established that the therapeutic and prophylactic administration of ETML at a dose of 20 mg/kg significantly prevented thrombus formation in the marginal ear vein of rabbits, contributing to the softening of the thrombus material, thrombolysis, preventing the acquisition of an obliterating character for thrombotic masses. in all rabbits of the group. probably reduces the clinical indicators of transudative edema, which developed as a result of acute venostasis. ETML prevented the destruction of the vascular wall, reduced or eliminated degenerative changes and inflammatory reaction in the surrounding tissues. Significant advantages of ETML over the comparison drug Eskuvit have been established. Under the conditions of experimental thrombophlebitis, the therapeutic and prophylactic administration of ETML at a dose of 20 mg/kg has a powerful phleboprotective effect, reduces the processes of thrombus formation in the vein. The effect of ETML is statistically significantly higher than the effectiveness of the comparison drug Eskuvit. Keywords: Saponaria officinalis herb extract, experimental thrombophlebitis, histological changes, Escuvit, phleboprotective effect, anti-inflammatory effect, medicinal plants.

Introduction

The thrombotic and associated inflammatory process in the veins, leading to the development of thrombophlebitis, can affect both varicose-altered and non-varicose veins. In clinical practice, this distinction has led to the classification of thrombosis of varicose-altered superficial veins (varicothrombophlebitis) and thrombosis of non-varicose

superficial veins [41, 42, 43].

The prognosis of the disease and the treatment strategy for patients with varicothrombophlebitis and thrombosis of non-varicose superficial veins exhibit certain differences. However, therapeutic recommendations include both pharmacological agents with phleboprotective properties

and those capable of reducing thrombus size [33, 37, 38].

The most common risk factor for thrombophlebitis is the presence of varicose veins of the lower extremities. Transformation of superficial veins in patients with thrombophlebitis is detected in up to 80 % of cases. Conversely, thrombophlebitis develops in 5-60 % of patients with varicose vein disease of the lower extremities [21, 24].

The major etiological risk factors for thrombophlebitis include age, obesity, tobacco use, a history of deep vein thrombosis or thrombophlebitis, pregnancy and the postpartum period, oral hormonal contraceptive use, hormone replacement therapy, immobilization, recent surgeries and trauma, and oncological diseases. For instance, within the first month postpartum, the risk of developing de novo thrombophlebitis significantly increases. A positive correlation between thrombophlebitis and oncological diseases has been established in 10-18 % of patients [10].

Genetic factors play a significant role in the etiopathogenesis of thrombophlebitis. Hereditary thrombophilia, particularly Leiden factor V mutation, prothrombin gene mutation G20210A, and deficiencies of antithrombin III (AT III), protein C, and protein S, significantly increase the risk of thrombophlebitis [41].

In patients with thrombotic vein lesions, oxidative stress and dyslipidemia play a crucial role, increasing the risk of recurrent thrombophlebitis fivefold in cases of untimely or inadequate treatment [31, 34].

The most dangerous complications of thrombophlebitis include thrombus extension into the deep veins and the development of pulmonary embolism. According to various authors, the incidence of thrombus propagation into deep veins reaches 18-20 % (95 % CI 13.9-23.3), while the frequency of symptomatic pulmonary embolism in thrombophlebitis cases is reported at 6.5-7.5 % (95 % CI 3.9-11.8). It is crucial to consider the risk of thrombophlebitis extending into the deep venous system, which ranges from 7 % to 32 % [36, 38, 39, 41].

The pharmacotherapy of thrombophlebitis includes various drugs with different mechanisms of action, such as anticoagulants, anti-inflammatory agents, venotonic drugs (which stimulate venous blood flow), and phleboprotective agents (which restore venous wall integrity) [30, 32].

Pathogenetically justified pharmacotherapy of thrombophlebitis involves the use of drugs with membrane-protective, venotonic, antioxidant, and anti-inflammatory properties [23].

Given the chronic nature of venous insufficiency with periodic episodes of thrombophlebitis, an important task for pharmacology is the development of an effective phleboprotective agent suitable for long-term and safe use. Phytoniring-based drugs (agents derived from medicinal plant raw materials) meet these requirements. These preparations contain a complex of biologically active substances with multifunctional properties, membrane-stabilizing effects on affected veins, normalization of hemostatic processes (anticoagulant action), reduced thrombus formation risk, a

broad therapeutic dose range, and low toxicity.

A promising medicinal plant raw material for the development of a novel domestic phleboprotective agent is the thick extract of *Saponaria officinalis* (soapwort) herb (SOTE), developed by Doctor of Pharmaceutical Sciences, Professor Marchyshyn S. M. (I. Horbachevsky Ternopil National Medical University, Ukraine). Pharmacognostic studies have demonstrated that the investigated *Saponaria officinalis* extract contains a significant amount of triterpene saponins and other biologically active compounds, whose biological activity correlates with the pathogenesis of venous system disorders [5, 12].

The aim of this study was to evaluate the effect of Saponaria officinalis herb extract on changes in the histological parameters of the ear vein in rabbits under experimental thrombophlebitis conditions.

Materials and methods

To model experimental thrombophlebitis in rabbits, a comprehensive thrombophlebitis methodology in rabbits was selected [16]. The methodology includes ligation to induce blood stasis, as well as the administration of a chemical agent (Lugol's solution) to damage the vascular intima, given the proven greater effectiveness of using multiple thrombogenic factors in experiments.

The experiment was conducted on 24 rabbits weighing 2-2.5 kg. The animals were housed under standard vivarium conditions at the National Pirogov Memorial Medical University, Vinnytsia, with ad libitum access to food and water. The study was conducted in accordance with the National "General Ethical Principles of Animal Experimentation" (Ukraine, 2001), which comply with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [26]. The Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsia, found no violations of ethical standards in the conduct of the research (Protocol No. 11 dated November 18, 2024). The experiments were conducted with consideration of circadian and seasonal rhythms, and all procedures were performed under standard conditions from 9:00 to 10:00 a.m. A ligature was applied to the marginal ear vein of the rabbits after preliminary depilation and disinfection. Above the ligature, 0.1 mL of a 2 % Lugol's solution was introduced into the vein lumen. Following the administration of Lugol's solution, the ligatures were removed to restore blood circulation in the vessel. The laboratory animals were distributed as follows (six rabbits per group):

Group 1 – intact control;

Group 2 – pathological control (thrombophlebitis);

Group 3 – thrombophlebitis + therapeutic-prophylactic administration of SOTE at a dose of 20 mg/kg;

Group 4 – thrombophlebitis + therapeutic-prophylactic administration of Escuvit at a dose of 7.2 mg/kg based on escin content.

The tested SOTE extract and the reference drug Escuvit

were finely ground into powder, thoroughly mixed with distilled water to form a suspension, and administered intragastrically to the animals using a metal probe with an olive tip. The administration was performed once daily, starting seven days before pathology induction and continuing for ten days during its progression.

Rabbits from all groups were euthanized on the 10th day of the experiment via air embolism. The excised samples (approximately 3 cm from the ligation site) were fixed in a 10 % formalin solution, dehydrated in ethanol of increasing concentrations, and embedded in paraffin. The sections were stained with hematoxylin and eosin. Microscopic examination of the histological specimens was performed using a Granum microscope. Microphotography was conducted with a Granum DCM 310 digital video camera, and the images were processed on a Pentium 2.4 GHz computer using the Toup View software.

Results

The histological structure of the examined ear region in animals from the intact control group is characterized by a normally appearing epidermis lining both the outer and inner surfaces. The dermis of the skin proper contains hair follicles and sebaceous glands. Near the cartilage plate, transverse profiles of the external marginal ear vein are visible. The vessel lumen is narrow, and the venous wall is thin. The vessel wall consists of the intima (elongated endothelial cell nuclei), a very thin media (almost devoid of muscular components, with very fine and sparse collagen fibers), and an indistinct adventitia. The surrounding tissues of the vein showed no signs of inflammation or edema (Fig. 1).

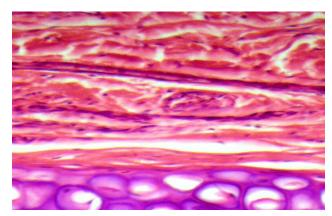


Fig. 1. External marginal ear vein of an intact rabbit. The condition of the vessel and surrounding tissues (indicated by arrows) corresponds to the norm. Hematoxylin-eosin. ×100.

In animals of the control pathology group, on the 10th day of experimental thrombophlebitis development, significant necrosis of the epidermis, dermis, and even the cartilage plate, as well as a diffuse inflammatory reaction, were observed in the injured area (Fig. 2a).

In areas adjacent to the necrosis, the venous lumen is markedly dilated and filled with a relatively dense red

thrombus. In certain regions, the venous wall is damaged, with visible adhesion of the thrombus to the destructed remnants of the vein. The surrounding dermis exhibits an inflammatory reaction of varying intensity and degenerative edema of collagen fibers (Fig. 2b).

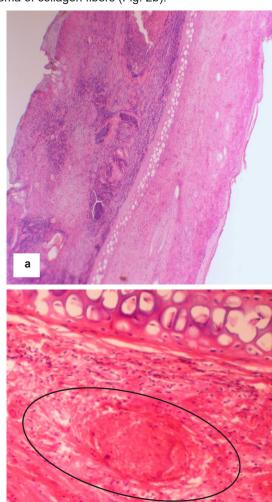


Fig. 2. Control pathology group. External marginal ear vein of the rabbit on the 10th day of thrombophlebitis development: a – extensive necrosis of all tissues in the injured area (×100); b – barrel-shaped dilation of the vascular lumen, occluded by a red thrombus (outlined), vascular wall partially destroyed, collagen fibers of the dermis in a state of degenerative edema, inflammatory reaction in the dermis (×200). Hematoxylin-eosin.

Additionally, in animals from the control pathology group, various stages of thrombus organization were observed along almost the entire examined segment of the ear vein. These stages ranged from the breakdown of erythrocytes and leukocytes and the appearance of connective tissue cells to partial or complete obliteration with lumen overgrowth by connective tissue. Moreover, perivascular tissue fibrosis and signs of inflammation in the dermis were detected (Fig. 3).

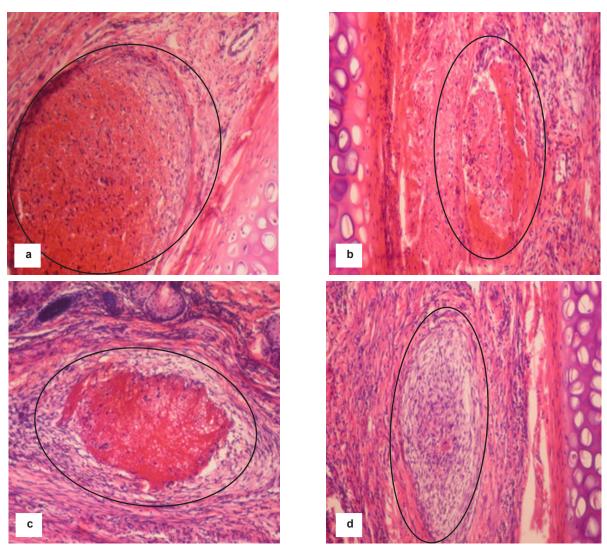


Fig. 3. Control pathology group. External marginal ear vein of a rabbit on day 10 of thrombo-phlebitis development. Lysis of thrombotic masses (outlined) (a), stages of thrombus organization (outlined) (b-c), complete obliteration of the vascular lumen (outlined) (d). Inflammatory reaction of varying intensity and perivascular fibrosis. Hematoxylin-eosin. ×250.

In rabbits that received SOTE at a dose of 20 mg/kg in a therapeutic-prophylactic regimen, no necrosis of the vein tissues was observed in the injury zone. The lumen of the external marginal ear vein was widened, but the thrombus did not occlude the vein and was parietal in nature. The thrombotic masses appeared significantly softened, and in some specimens, they were lysed.

The vein wall remained intact. The collagen stroma of the dermis was slightly edematous, but the inflammatory response was not pronounced (Fig. 4). Further along the vein, in areas more distant from the site of injury, no thrombus was observed in the vascular lumen. Various residual erythrocyte masses of different volumes and densities were present but did not obstruct blood flow. The condition of the vein wall was close to that of an intact vessel, and no fibrosis of the perivascular tissue was detected. Signs of inflammation in the ear tissues were either absent or significantly reduced (Fig. 5).

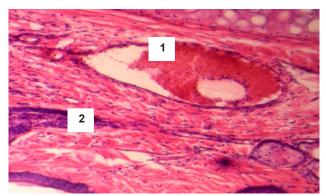


Fig. 4. External marginal ear vein of a rabbit that received SOTE at a dose of 20 mg/kg in a therapeutic-prophylactic regimen on day 10 of thrombophlebitis development. 1 – parietal thrombus in the widened vascular lumen, liquefaction of thrombotic masses; in some areas, 2 – initial infiltration of thrombotic masses by leukocytes. Hematoxylin-eosin. ×200.

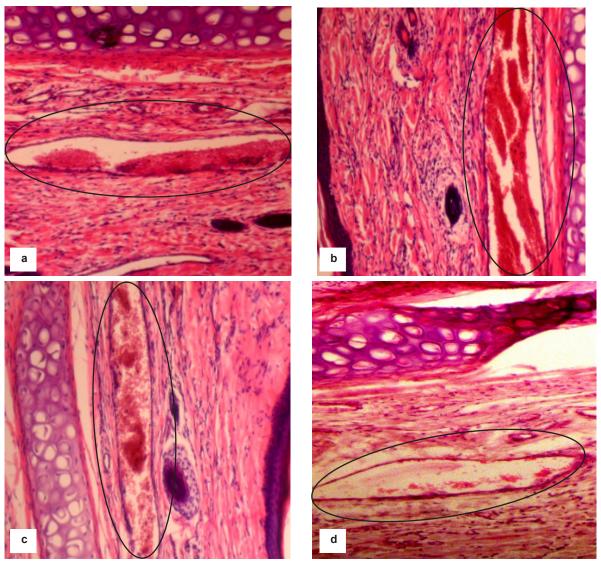


Fig. 5. External marginal ear vein of a rabbit that received SOTE at a dose of 20 mg/kg in a therapeutic-prophylactic regimen on day 10 of thrombophlebitis development. Vascular normalization: various volumes of liquefied erythrocyte masses (outlined) in the vascular lumen (a-d), vessel wall condition approaching normal, and a marked reduction in signs of inflammation in surrounding tissues. Hematoxylin-eosin. ×200.

The administration of the comparative drug Eskuvit in the same regimen prevented the formation of necrotic zones in the venous injury area. The expanded lumen of the marginal ear vein is filled with a thrombus of varying size. The thrombotic masses are not uniformly dense, with areas that are cracked and softened (which may allow blood flow in these regions), and some areas are infiltrated by leukocytes. The vessel wall is partially damaged, and the perivascular tissue is swollen. The tissues of the ear itself are infiltrated with inflammatory cells (Fig. 6).

Further along the vein, in some histological samples of the examined segment of the ear, the thrombus was still present. Processes of its organization were observed, and in parallel with the organization, distinct signs of canalization were found – the appearance of newly formed capillaries in the connective tissue that replaced the thrombus, which restored blood flow in this section of the vein. Closer to the edge of the ear, the state of the marginal vein in these rabbits was close to normal. In other cases, only erythrocyte masses of varying volumes were visible in the lumen of the vein. The condition of the collagen fibers in the dermis slightly normalized (Fig. 7).

Discussion

Chronic venous insufficiency (CVI) is one of the most prevalent diseases of modern times. The World Health Organization has included varicose veins in its list of "diseases of civilization": according to its data, this pathology affects 40 % of women and 32 % of men worldwide.

Varicose veins are the most common visible symptom of

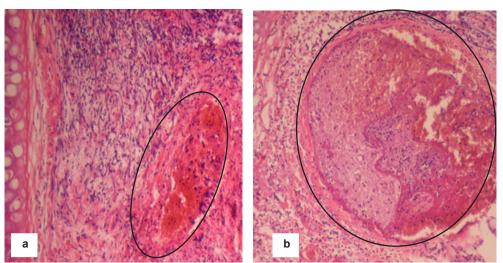


Fig. 6. The external marginal vein of the ear in the rabbit, which received Eskuvit at a dose of 7.2 mg/kg as escin in the therapeutic and prophylactic regimen, on the 10th day of experimental thrombophlebitis. Thrombotic masses of varying size (outlined by a line), which are not uniformly dense (a – b), fill the vessel lumen. The vessel wall is partially damaged. Hematoxylin-eosin. ×200.

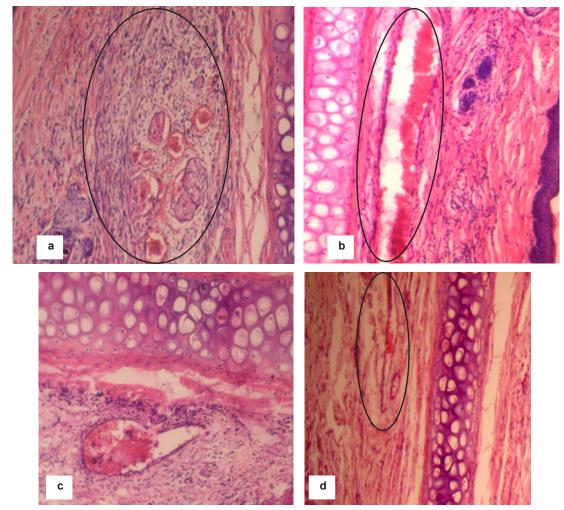


Fig. 7. External marginal vein of the rabbit's ear, which received Escuvit at a dose of 7.2 mg/kg as escin in a therapeutic-preventive regimen, on the 10th day of thrombo-phlebitis development. Organization of the thrombus (outlined with a line) and canalization of the thrombus (arrow) (a); remnants of erythrocytic masses (outlined with a line) in the lumen of the vessel (b); lysis of erythrocytes (c); vein with a small thrombus (outlined with a line) (d). Hematoxylin-eosin. ×200.

venous insufficiency, but they do not occur in every patient. Deep vein insufficiency is less common and is often caused by deep vein thrombosis. CVI imposes a significant economic burden on society in the form of medication and surgical treatment costs and, importantly, an immeasurable loss of work productivity due to pain and disability [18].

CVI is a multifactorial disease associated with endothelial dysfunction, inflammation, venous wall remodeling, valve incompetence, venous hypertension, and reflux [6]. Other causes include venous outflow obstruction and calf muscle pump failure due to obesity or leg immobility.

Pharmacological treatment is an essential component of conservative CVI management; therefore, the indications and benefits of this therapeutic approach in patients with venous system disorders are indisputable. The goals of pharmacological treatment include [13]: increasing venous emptying; affecting vascular walls and interstitial tissue; restoring metabolic balance (eliminating oxidative stress); reducing thrombotic predisposition in the lymphatic system; and minimizing cellular damage.

An important aspect of CVI treatment is the use of modern phlebotropic agents (venotonics, phleboprotectors) as first-line therapy. This is a substantial group of pharmacological agents, usually of natural origin, with the ability to enhance venous outflow from the lower limbs.

A crucial contribution to CVI therapy effectiveness is the use of modern venotonic agents (phleboprotectors). This is a large group of pharmacological agents, typically of natural origin, whose primary feature is their ability to enhance venous outflow from the lower limbs. This effect results from a synergistic influence on various components of the microcirculatory system and/or a direct impact on venous wall tone. Their mechanism of action is usually pleiotropic and includes stimulation of lymphatic flow, anti-inflammatory and antioxidant activity, improvement of hemorheology, and endothelial protection.

The most commonly used venotonics belong to four main groups of drugs, including flavonoids (hesperidin, diosmin, rutin, esculin), saponins (escin, horse chestnut extract, Ginkgo biloba), coumarins, synthetic venotonics, or combination drugs (calcium dobesilate, tribenoside, naftazone, benzarone).

However, the evidence base for the effectiveness of phleboprotective agents has been confirmed only for certain drugs from the extremely broad list of phleboprotectors. Therefore, despite extensive experience, comparisons of the clinical efficacy of pharmacotherapy for CVI symptoms using various venotonic drugs from different pharmacological groups do not allow for the definitive identification of the most effective drug. This is due to the insufficient number of randomized clinical trials and the significantly limited range of drugs available for comparison, which justifies the need for further research in this direction [14].

In our opinion, a promising addition to the arsenal of modern natural phleboprotective agents could be the use of an extract from the plant Saponaria officinalis L. (Caryophyllaceae), commonly known as common soapwort, which is widely distributed worldwide [19]. Phytopreparations derived from various parts of Saponaria officinalis are used in traditional medicine: the root as a blood-purifying, diaphoretic, and diuretic agent; the sap for liver diseases and to enhance bile outflow: and the leaves and roots for skin diseases [27, 28]. Literature data indicate that Saponaria officinalis contains a high level of saponins. The saponin fraction of common soapwort has demonstrated anti-inflammatory activity in vitro in a carrageenan-induced edema model in rats and has been shown to inhibit prostaglandin synthetase [17]. Purified saponins from the Saponaria officinalis fraction have demonstrated a hypocholesterolemic effect in vitro, which is believed to result from the saponins' ability to form an insoluble complex with cholesterol [4]. In addition to saponins, common soapwort also contains tannins, quillaic acid, flavonoids, sulfur-containing compounds, various phenolic compounds, and essential oils [20]. Experimental research data indicate that soapwort saponin extracts possess antibacterial and antifungal activity [1, 7].

Our results demonstrated the high phleboprotective efficacy of soapwort herb extract in an experimental model of thrombophlebitis. Microscopic findings confirmed the development of experimental thrombophlebitis, as evidenced by pathological changes both in the vessel itself and in the surrounding tissues, which occur due to temporary cessation of blood flow in a limited area of the ear, along with the simultaneous injection of Lugol's solution into the marginal vein cavity. By the 10th day of experimental thrombophlebitis development in animals from the control pathology group, thrombosis of the marginal vein was observed, characterized by lumen obstruction, vascular wall destruction, degenerative and inflammatory changes in the surrounding dermal tissues. Signs of different stages of thrombus organization were recorded - from erythrocyte and leukocyte breakdown, and the appearance of connective tissue cells, to complete occlusion of the lumen by connective tissue, cessation of blood flow in this area, and the development of vascular wall sclerosis of varying severity, as well as perivascular fibrosis.

The extract of Saponaria officinalis herb, when administered in a prophylactic-therapeutic regimen, significantly prevented thrombus formation in the marginal vein, promoting the softening of thrombotic material, thrombolysis, and preventing thrombotic masses from becoming obliterative in all rabbits in the group. As a result, the degradation products of the thrombus were "washed away" by the blood flow, and unlike in the control pathology group, hemocirculation was restored in the examined area of the ear. SOTE prevented vascular wall destruction, reduced or eliminated degenerative changes in the surrounding tissues, and exhibited anti-inflammatory effects.

The prophylactic effect of the reference drug Escuvit in this experimental model of thrombophlebitis varied among animals. In some cases, thrombosis in the marginal ear vein was prevented, while in others, active thrombus formation followed by its canalization was observed, facilitating the

restoration of blood flow in the thrombosed vessel. Under the influence of Escuvit, inflammatory and sclerotic changes in the ear tissues were reduced. Thus, in terms of the positive impact on the morphological manifestations of experimental thrombophlebitis, the reference drug Escuvit was significantly inferior to the studied SOTE.

The established pharmacological activity of SOTE in experimental thrombophlebitis, which significantly surpasses the reference drug Escuvit in restoring the histological parameters of the vein, is presumably realized through the additive synergy of the biologically active compounds (BAC) of Saponaria officinalis herb extract [5, 12]. These include triterpene saponins (4 % in the extract), hydroxycinnamic acids (7.36 % in the extract), and flavonoids (15.8 % in the extract).

The corticosteroid-like action of the saponins present in SOTE (saponaroside A, saponaroside D, saponrubin) suppresses the synthesis of pro-inflammatory intermediates (interleukins IL-1, IL-2, IL-6, IL-8, vascular endothelial growth factors, tumor necrosis factor- α (TNF- α), and interferon- γ), which promote inflammatory and phlogogenic responses [3, 11]. An important aspect of the phleboprotective action of saponins, including escin, is their ability to stimulate the synthesis of prostaglandin F_{2 α}, which exerts a venotonic effect and, according to Gallelli L. [8], prevents hypoxia-induced disruptions in the normal expression and distribution of endothelial cell platelet adhesion molecule-1 in the vein [3, 15].

The reduction of thrombus formation processes in the vein under the prophylactic-therapeutic administration of SOTE is likely also mediated by the normalizing effect of saponins on hemostatic processes in the damaged area. This includes inhibition of the coagulation component of hemostasis, direct inhibition of thrombin, and a reduction in thrombus formation in the vein [3].

The venotonic and antithrombotic effects of saponins are most likely the key mechanisms underlying the phleboprotective action of SOTE in experimental thrombophlebitis.

Hydroxycinnamic acids (chlorogenic acid, syringic acid, caffeic acid, quinic acid) and flavonoids (quercetin, isoquercetin, kaempferol), which are present in significant amounts in the Saponaria officinalis extract, as reported by Ullah R. et al. [29], Yang X. et al. [35], Shabbir U. et al. [25], and Zhao L. et al. [40], exhibit substantial anti-exudative and anti-inflammatory effects, contributing to the phleboprotective activity of the studied extract.

References

- [1] Abdolreza, N. (2013). Antibacterial effects of Saponaria officinalis extracts against avian pathogenic Escherichia coli (APEC). African Journal of Agricultural Research, 8(18), 2068-2071. doi: 10.5897/ajar11.1390
- [2] Bekendam, R. H., & Flaumenhaft, R. (2016). Inhibition of Protein Disulfide Isomerase in Thrombosis. *Basic Clin. Pharmacol. Toxicol.*, 119(3), 42-48. doi: 10.1111/bcpt.12573
- [3] Biswas, T., & Dwivedi, U. N. (2019). Plant triterpenoid saponins: biosynthesis, in vitro production, and pharmacological relevance. *Protoplasma*, 256(6), 1463-1486. doi: 10.1007/s00709-019-01411-0

The marked reduction in thrombus size under the prophylactic-therapeutic administration of SOTE in experimental thrombophlebitis can also be explained by the normalizing effect of SOTE flavonoids on the coagulation system. The anticoagulant mechanism of flavonoids involves their ability to inhibit platelet aggregation and prolong blood clotting time through the blockade of IIb/ IIIa platelet receptors and inhibition of the thromboxane A2 synthesis cascade. Additionally, the anticoagulant effect of flavonoids is associated with the inhibition of serine proteases [9, 22]. Quercetin, the primary flavonoid in Saponaria officinalis extract, has been shown by Bekendam R. H. and Flaumenhaft R. [2] to inhibit protein disulfide isomerase activity and block thrombus formation.

Thus, further in-depth preclinical and clinical studies of SOTE appear promising, as they may establish this phytopharmaceutical as an effective venotonic and phlebotropic drug with a pleiotropic mechanism of action.

Conclusions

- 1. Experimental thrombophlebitis in rabbits is characterized by the development of pathological changes both in the vein and in the surrounding tissues. Histologically, a picture of thrombophlebitis of the marginal vein was established, with occlusion of the vein lumen by a thrombus, destruction of the vascular wall, degenerative and inflammatory changes in the tissues surrounding the vessel, and the presence of varying degrees of vascular wall sclerosis and perivascular fibrosis.
- 2. The administration of SOTE in a therapeutic-preventive regimen at a dose of 20 mg/kg significantly prevented thrombus formation in the marginal vein, promoted softening of thrombotic material, activated thrombolysis, and prevented the formation of obliterating thrombi in all rabbits of the group. The extract of Saponaria officinalis herb prevented the destruction of the vascular wall, reduced or eliminated degenerative changes and inflammatory reactions in the surrounding tissues. The potent phleboprotective effect of the studied extract of Saponaria officinalis herb, according to histological examination, exceeded the effectiveness of Escuvit (at a dose of 7.2 mg/kg as escin).
- 3. The phleboprotective effect of SOTE is likely realized due to the additive synergy of its main groups of biologically active substances (triterpenoid saponins, hydroxycinnamic acids, flavonoids).
- [4] Böttger, S., & Melzig, M. F. (2011). Triterpenoid saponins of the Caryophyllaceae and Illecebraceae family. *Phytochemistry Letters*, 4(2), 59-68. doi: 10.1016/j.phytol.2010.08.003
- [5] Budniak, L., Slobodianiuk, L., Marchyshyn, S., Kostyshyn, L. & Horoshko, O. (2021) Determination of composition of fatty acids in saponaria officinalis L. ScienceRise: Pharmaceutical Science, 29(1), 25-30. doi: 10.15587/2519-4852.2021
- [6] Castro-Ferreira, R., Cardoso, R., Leite-Moreira, A., & Mansilha, A. (2018). The role of endothelial dysfunction and inflammation in chronic venous disease. Ann. Vasc. Surg., 46, 380-393. doi: 10.1016/j.avsg.2017.06.131

- [7] Czaban, J., Mołdoch, J., Wróblewska, B., Szumacher-Strabel, M., Cieślak, A., & Oleszek, W. (2013). Effect of triterpenoid saponins of field scabious, alfalfa, red clover and common soapwort on growth of Gaeumannomyces graminis var. tritici and Fusarium culmorum. Allelopathy Journal, 32, 79-90. do i: 10.1080/14786419.2017.1350668
- [8] Gallelli, L. (2019). Escin: a review of its anti-edematous, anti-inflammatory, and venotonic properties. Drug Des. Devel. Ther,. (13), 3425-3437. doi: 10.2147/DDDT.S207720
- [9] Guglemone, H. A., Agnese, A. M., & Montya, S. C. (2002). Anticoagulant effect and action mechanism of sulphated flavonoids from Flaveria bidentis. Thrombosis Researsh, 105(2), 183-188. doi: 10.1016/s0049-3848(01)00419-4
- [10] Heit, J. A., Spencer, F. A., & White, R. H. (2016). The epidemiology of venous thromboembolism. J. Thromb. Thrombolysis, 41(1), 3-14. doi: 10.1007/s11239-015-1311-6
- [11] Ishaq, M., DeGray, G., Mou, K., Aguilera, A., Yang, J., Lempicki, R. A., ... & Natarajan, V. (2007). Zap70 signaling pathway mediates glucocorticoid receptor-dependent transcriptional activation: role in the regulation of annexin 1 expression in T cells. *J. Immunol.*, 179(6), 3851-3858. doi: 10.4049/jimmunol.179.6.3851
- [12] Kostyshyn, L. V., Slobodianiuk, L. V., Marchyshyn, S. M., Demydiak, O. L. & Liashenko, L. Yu. (2020). Дослідження органічних кислот у траві та підземних органах Saponaria officinalis L. [Study of organic acids in herb and underground organs of saponaria officinalis L.]. Медична та клінічна хімія=Medical and Clinical Chemistry, 22(4), 77-82. doi: 10.11603/mcch.2410-681X.2020.i4.11743
- [13] Krasinski, Z., Krasińska, A., Markiewicz, S., & Zieliński, M. (2021). Patients with chronic venous insufficiency in the times of COVID-19 and the risk of thrombus formation suggestions on conservative treatment of such patients based on the principles of pathophysiology. Pol. Przegl. Chir., 93(2), 43-52. doi: 10.5604/01.3001.0014.8500
- [14] Lindner, I., Meier, C., Url, A., Unger, H., Grassauer, A., Prieschl-Grassauer, E., & Doerfle, P. (2010). Beta-escin has potent anti-allergic efficacy and reduces allergic airway inflammation. BMC Immunol., 11(1), 24. doi: 10.1186/1471-2172-11-24
- [15] Lorent, J. H., Quetin-Leclercq, J., & Mingeot-Leclercq, M. P. (2014). The amphiphilic nature of saponins and their effects on artificial and biological membranes and potential consequences for red blood and cancer cells. Org. Biomol. Chem., 44(12), 8803-8822. doi: 10.1039/c4ob01652a
- [16] Maloshtan L. M., Artemova K. O., Borodina N. V., & Kukht-enko A. S. (2023). Study of pharmacological activity of dry extract of Sakhalin willow shoots against the background of experimental thrombophlebitis. ScienceRise: Pharmaceutical Science, 4(44), 97-103. doi: 10.15587/2519-4852.2023.286723
- [17] Moniuszko-Szajwaj, B., Pecio, L., Kowalczyk, M., Simonet, A. M., Macias, F. A., Szumacher-Strabel, M., ... & Stochmal, A. (2013). New Triterpenoid Saponins from the Roots of Saponaria officinalis. Natural Product Communications, 12(8), 1687-1690. doi: 10.1177/1934578x1300801207
- [18] Ouriel, K. (2018). Central Venous Pathologies: Treatments and Economic Impact. Methodist Debakey Cardiovasc J., 14(3), 166-172. doi: 10.14797/mdcj-14-3-166
- [19] Pavela, R. (2016). Extract from the roots of Saponaria officinalis as a potential acaricide against Tetranychus urticae. Journal of Pest Science, 90(2), 683-692. doi: 10.1007/ s10340-016-0828-6

- [20] Petrović, G. M., Ilić, M. D., Stankov-Jovanović, V. P., Stojanović, G. S., & Jovanović, S. Č. (2017). Phytochemical analysis of Saponaria officinalis L. shoots and flowers essential oils. Natural Product Research, 32(3), 331-334. doi: 10.1080/14786419.2017.1350668
- [21] Pihlaja, T., Romsi, P., Ohtonen, P., Jounila, J., & Pokela, M. (2020). Post-procedural Compression vs. No Compression After Radiofrequency Ablation and Concomitant Foam Sclerotherapy of Varicose Veins: A Randomised Controlled Non-inferiority Trial. Eur. J. Vasc. Endovasc. Surg., 59(1), 73-80. doi: 10.1016/j.ejvs.2019.08.020
- [22] Rakhmatullina, A. A., Turaev, R. G., & Kiselev, L. V. (2015). The influence of camphene derivatives on coagulation processes blood. Medical Journal, 96(3), 455-458.
- [23] Robertson, N. U., Schoonees, A., Brand, A., & Visser, J. (2020). Pine bark (Pinus spp.) extract for treating chronic disorders. Cochrane Database Syst. Rev., 9(9), CD008294-CD008298. doi: 10.1002/14651858
- [24] Saberianpour, S., Modaghegh, M. H. S., Rahimi, H., & Kamyar, M. M. (2021). Role of mechanosignaling on pathology of varicose vein. Biophys. Rev., 13(1), 139-145. doi: 10.1007/s12551-021-00783-z
- [25] Shabbir, U., Rubab, M., & Daliri, E. B. (2021). Curcumin, Quercetin, Catechins and Metabolic Diseases: The Role of Gut Microbiota. Nutrients, 13(1), 206-212. doi: 10.3390/ nu13010206
- [26] Sneddon, L. U., Halsey, L. G., & Bury, N. R. (2017). Considering aspects of the 3Rs principles within experimental animal biology. J. Exp. Biol., (220), 3007-3016. doi: 10.1242/jeb.147058
- [27] Subbarayappa, B. V. (2001). The roots of ancient medicine: an historical outline. Journal of Biosciences, 26(2), 135-143. doi: 10.1007/bf02703637
- [28] Talluri, M. R., Gummadi, V. P., & Battu, G. R. (2018). Chemical Composition and Hepatoprotective Activity of Saponaria officinalis on Paracetamol-Induced Liver Toxicity in Rats. Pharmacognosy Journal, 10(6s), s129-s134. doi: 10.5530/pj.2018.6s.24
- [29] Ullah, R., Nadeem, M., Khalique A., & Imran, M. (2016). Nutritional and therapeutic perspectives of Chia (Salvia hispanica L.): a review. J. Food Sci. Technol., (4),1750-1758. doi: 10.1007/s13197-015-1967-0
- [30] Vazquez-Padron, R. I., & Allon, M. (2016). New Insights into Dialysis Vascular Access: Impact of Preexisting Arterial and Venous Pathology on AVF and AVG Outcomes. Clin. J. Am. Soc. Nephrol., 11(8), 1495-1503. doi: 10.2215/ CJN.01860216
- [31] Weaver, J. C., Krilis, S. A., & Giannakopoulos, B. (2018). Oxidative post-translational modification of βeta 2-glycoprotein I in the pathophysiology of the anti-phospholipid syndrome. Free Radic Biol. Med., 125, 98-103. doi: 10.1016/j. freeradbiomed.2018.03.048
- [32] Winter, M. P., Schernthaner, G. H., & Lang, I. M. (2017.). Chronic complications of venous thromboembolism. J. Thromb. Haemost., 15(8), 1531-1540. doi: 10.1111/jth.13741
- [33] Woller, S. C., Stevens, S. M., Kaplan, D., Wang, T. F., Branch, D. W., Groat, D., ... & Elliott, C. G. (2022). Apixaban compared with warfarin to prevent thrombosis in thrombotic antiphospholipid syndrome: a randomized trial. Blood Adv., 6(6),1661-1670. doi: 10.1182/bloodadvances.2021005808
- [34] Yang, X., Feng, Y., & Liu, Y. (2021). Fuzheng Jiedu Xiaoji formulation inhibits hepatocellular carcinoma progression in patients by targeting the AKT/CyclinD1/p21/p27 path-

- way. Phytomedicine, (87), 153575-153579. doi: 10.1016/j. phymed.2021.153575
- [35] Yang, X., Li, Y., Li, Y., Ren, X., Zhang, X., Hu, D., ... & Shang, H. (2017). Oxidative Stress-Mediated Atherosclerosis: Mechanisms and Therapies. Front. Physiol., 8, 600. doi: 10.3389/fphys.2017.00600
- [36] Ząbczyk, M., Natorska, J., & Undas, A. (2021). Factor XIII and Fibrin Clot Properties in Acute Venous Thromboembolism. Int. J. Mol. Sci., 22(4),1607. doi: 10.3390/ ijms22041607
- [37] Zanza, C., Racca, F., Longhitano, Y., Piccioni, A., Franceschi, F., & Artico, M. (2021). Risk Management and Treatment of Coagulation Disorders Related to COVID-19 Infection Risk Management and Treatment of Coagulation Disorders Related to COVID-19 Infection. Int. J. Environ. Res. Public Health, 18(3), 1268-1274. doi: 10.3390/ijerph18031268
- [38] Zhang, L., Kong, Y. H., Wang, D. W., Li, K. T., & Yu, H. P. (2021). Anticoagulant management by low-dose of low molecular weight heparin in patients with nonvalvular atrial fibrillation following hemorrhagic transformation and complicated with venous thrombosis: Five case reports and literature review. Medicine, 100(7), e24189 doi: 10.1097/MD.0000000000024189

- [39] Zhang, R., Ni, L., Di, X., Wang, X., & Ma, B. (2021). Systematic review and meta-analysis of the prevalence of venous thromboembolic events in novel coronavirus disease-2019 patients. J. Vasc. Surg. Venous Lymphat. Disord., 9(2), 289-298. doi: 10.1016/j.jvsv.2020.11.023
- [40] Zhao, L., Wang, H., & Du, X. (2021). The therapeutic use of quercetin in ophthalmology: recent applications. Biomed. Pharmacother., (137), 111371-111378. doi: 10.3389/ fcvm.2021.596506
- [41] Zhu, V. W., Zhao J. J., Gao, Y., Syn, N. L., & Zhang, S. S. (2021). Thromboembolism in ALK+ and ROS1+ NSCLC patients: A systematic review and meta-analysis. Lung Cancer, (157), 147-155. doi: 10.1016/j.lungcan.2021.05.019
- [42] Zielsdorf, S., Narayanan, L., Kantymyr, S., Barbetta, A., & Kwon, Y. (2021). Surgical shunts for extrahepatic portal vein obstruction in pediatric patients: a systematic review. HPB (Oxford), 23(5), 656-665. doi: 10.1016/j.hpb.2020.11.1149
- [43] Zoccarato, M., Nardetto, L., Basile, A. M., Giometto, B., Zagonel, V., & Lombardi, G. (2021). Seizures, Edema, Thrombosis, and Hemorrhages: An Update Review on the Medical Management of Gliomas. Front. Oncol., 11, 617-966. doi: 10.3389/fonc.2021.617966

ДОСЛІДЖЕННЯ ВПЛИВУ ЕКСТРАКТУ ТРАВИ SAPONARIA OFFICINALIS НА ГІСТОЛОГІЧНІ ЗМІНИ КРАЙОВОЇ ВЕНИ ВУХА ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ТРОМБОФЛЕБІТУ Цубанова Н. А., Волощук Н. І., Застрижна М. Л.

Тромбофлебіт визнано одним із найбільш розповсюджених ускладнень хронічної венозної недостатності (ХВН). Його перебіг характеризує запалення венозної стінки з подальшим тромбоутворенням у варикозно розширених венах нижніх кінцівок. Тромбофлебіт діагностують за різним даними у 5-60 % пацієнтів із ХВН. Кількість лікарських засобів, які здатні зменшувати процеси тромбоутворення у венах (флебопротектори), недостатньо представлена як в Україні, так і на фармацевтичному ринку інших країн. Найбільш перспективними флебопротекторами вважають засоби рослинного походження, серед яких важливе місце займає екстракт трави Saponaria officinalis, для якого у попередніх дослідженнях встановлено венотонізуючу та антиексудативну дію. Метою даної роботи було вивчити вплив екстракту трави Saponaria officinalis на зміну гістологічних показників вени вуха у кролів за умов експериментального тромбофлебіту. Експериментальний тромбофлебіт моделювали на кролях за стандартною методикою з накладанням лігатури на крайову вену вуха тварини та введенням розчину Люголя. Гістологічні зміни на тлі досліджуваної фітокомпозиції (20 ма/кг в/шл) порівнювали з дієвістю референс-препарату Ескувіту (7,2 ма/ка за есцином в/шл). Показано, що екстракт трави Saponaria officinalis за умов лікувально-профілактичного введення в значній мірі попереджував тромбоутворення у крайовій вені вуха кролів, сприяючи розм'якшенню матеріалу тромбу, тромболізису, запобігаючи набуттю облітеруючого характеру для тромбічних мас у всіх кролів дослідної групи, зменшував клінічні показники транссудативного набряку, який розвинувся внаслідок гострого веностазу. Таким чином, екстракт трави Saponaria officinalis перешкоджав руйнуванню судинної стінки, викликав депресію або повне нівелювання дегенеративних та запальних змін в оточуючих тканинах. За умов експериментального тромбофлебіту лікувально-профілактичне введення екстракт трави Saponaria officinalis у дозі 20 мг/кг чинить потужну флебопротекторну дію, зменшує процеси тромбоутворення у вені. Дія екстракт трави Saponaria officinalis значуще перевищує ефективність препарату порівняння Ескувіту.

Ключові слова: екстракт трави Saponaria officinalis, експериментальний тромбофлебіт, гістологічні зміни, Ескувіт, флебопротекторна дія, протизапальна активність, лікарські рослини.

Author's contribution

Tsubanova N. A. - conceptualization, research, review writing and editing, formal analysis and validation.

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