DOI 10.26724/2079-8334-2024-2-88-207-210
UDC 615.322:615.014.2:616.14-002

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EVALUATION OF THE EFFECTIVENESS OF DRY EXTRACT OF SAKHALIN WILLOW SHOOTS ON IRON HEMOSTASIS IN EXPERIMENTAL THROMBOPHLEBITIS

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The dry extract of the shoots of the Sakhalin willow was taken as the object of the study, as a therapeutic and preventive agent. The experiment was conducted on rabbits. Experimental thrombophlebitis was induced by injecting an intravenous solution of 2 % Lugol in a dose of 0.1 ml into the marginal vein of the ear. To assess the state of antioxidant protection in blood serum and the therapeutic effect of the dry extract of Sakhalin willow shoots, the following indicators were selected: the level of transferrin saturation, the concentration of iron in the blood, the total iron-binding capacity of the blood serum, the unsaturated iron-binding capacity of the serum. In the group of animals that received dry extract of Sakhalin willow shoots in a prophylactic-therapeutic regimen, a decrease in the concentration of iron in the blood serum was observed, and at the same time, an increase in the total iron-binding capacity of the blood serum and the unsaturated iron-binding capacity of the serum. It was proved that the extract of Sakhalin willow shoots affected the indicators of antioxidant protection against the background of experimental thrombophlebitis in rabbits. This action is related to the metal-chelating properties of the flavonoids identified in the extract.

Key words: Sakhalin willow, thrombophlebitis, iron hemostasis, antioxidant protection, metal-chelating activity**.**

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ОЦІНКА ЕФЕКТИВНОСТІ СУХОГО ЕКСТРАКТУ ПАГОНІВ ВЕРБИ САХАЛІНСЬКОЇ НА ГЕМОСТАЗ ЗАЛІЗА НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО ТРОМБОФЛЕБІТУ

В якості об'єкта дослідження було взято сухий екстракт пагонів верби сахалінської, як лікувально-профілактичний засіб. Експеримент проводили на кролях. Експериментальний тромбофлебіт викликали введенням у ділянку крайової вени вуха внутрішньовенного розчину 2% люголю в дозі 0,1 мл. Для оцінки стану антиоксидантного захисту в сироватці крові та терапевтичного ефекту сухого екстракту пагонів верби сахалінської були обрані показники: рівень трансферинового насичення, концентрація заліза в крові, загальна залізозв'язуюча здатність сироватки крові, ненасичена залізозв'язуюча здатність сироватки. В групі тварин, які отримували сухий екстракт пагонів верби сахалінської в профілактично-лікувальному режимі, спостерігали зниження концентрації заліза в сироватці крові та водночас збільшення загальної залізозв'язуючої здатності сироватки крові та ненасиченої залізозв'язуючої здатності сироватки. Доведено, що екстракт пагонів верби сахалінської впливав на показники антиоксидантного захисту на фоні експериментального тромбофлебіту у кролів. Ця дія пов'язана з метал-хелатуючими властивостями флаваноїдів, що були ідентифіковані в екстракті.

Ключові слова: верба сахалінська, тромбофлебіт, гемостаз заліза, антиоксидантний захист, метал-хелатуюча активність.

The work is a fragment of the research project "Development of the composition and production technology of a medicinal product prescribed for patients who have suffered from COVID-19 based on a vitamin complex", state registration No. 0122U002011.

Varicose thrombophlebitis (VT) is the most common complication of varicose veins (VV) of the superficial veins of the lower extremities that represents an actual and social problem. It is known that the frequency of development of this complication is up to 90 % of all thrombotic lesions, and the recurrence rate of VT of superficial veins is almost 20 % [9].

Thrombophlebitis is a disease of the circulatory system, which is manifested by inflammatory processes in the walls of blood vessels (veins) and subsequent thrombus formation and impaired blood flow. In the pathogenesis of thrombotic complications, an important role is played by intravascular hemolysis, during which free hemoglobin comes from erythrocytes the circulatory circle of vessels. Iron (ferrum), contained in hemoglobin, in the free state has a toxic effect on the endothelium of blood vessels and damages the membranes of blood cells. Also, this excess of ferrum is due to the ability to start chain free radical reactions that lead to LP (lipid peroxidation) in biological membranes, damage to proteins and nucleic acids. According to many researchers, iron ions capable of catalyzing free radical oxidation (FRO) are present in all biochemical fluids, signs of their appearance in an amount exceeding the transferrin capacity lead to the development of pathological orientation in FRO reactions [1, 4].

In the contemporary scientific literature, ideas about the mechanisms of control of iron hemostasis are rapidly changing, and data on changes in the balance in the "oxidant-antioxidant" system in pathologies of the venous system burdened by thrombophlebitis are not presented. Therefore, the study of this problem is relevant.

The dry extract of the shoots of the Sakhalin willow (DEoSWS) was taken as the object of the study, as a therapeutic and preventive agent, which is standardized by the number of polyphenolic compounds [10].

The purpose of the study was to evaluate the effectiveness of the developed therapeutic and prophylactic agent based on dry extract of the shoots of the Sakhalin willow on the processes of free radical oxidation of varicose disease complicated by thrombophlebitis.

Materials and methods. The experiment was conducted on 18 sexually mature rabbits, weighing 2.5±2.6 kg. All animals were kept in the same conditions on a balanced diet at the Educational and Scientific Institute of Applied Pharmacy in the vivarium of the Educational and Scientific Center for Medical and Biological Research of the National Academy of Sciences. The animals were kept in a separate room with adjustable microclimate parameters: air temperature 18–22 °C, relative humidity 50–65 %, light mode "12 hours day/night". The laboratory was sterilized daily using UV irradiation. Animals had free access to water (pre-settled tap water from drinking bowls). Animals were cared for according to standard operating procedures. All stages of the research were carried out in accordance with Directive 2010/63/EU of the European Parliament and the Council of the EU dated September 22, 2010 "On the protection of animals used for scientific purposes".

The animals were divided into three groups: Group I – the control group with experimental thrombophlebitis; Group II – animals that were previously injected intragastrically with DEoSWS at a dose of 30 mg/kg before modeling thrombophlebitis; Group III – similarly received the reference drug "Eskuvit" in a dose of 20 mg/kg – tablets (AG "Halychpharm" Ukraine). The dose for animals was calculated taking into account body weight according to Rybolovlev's method (Rybolovlev YuR, 1979).

Experimental thrombophlebitis was induced by injecting an intravenous solution of 2 % Lugol in a dose of 0.1 ml into the marginal vein of the ear [6].

To evaluate the state of antioxidant protection in blood serum and the therapeutic effect of DEoSWS, the following parameters were chosen: the level of transferrin saturation, the concentration of iron in the blood (CIB), the total iron-binding capacity of the blood serum (TIBCBS), the unsaturated iron-binding capacity of the serum (UIBCBS).

Measurements of these indicators were carried out with the help of a test set LLC NVP (Filicit-Diagnostika, Ukraine). Blood for the experiment was taken from the marginal vein of the ear of the rabbit before modeling thrombophlebitis, at the peak of development (1 day) of thrombophlebitis and on the 10th day of the experiment in all experimental groups.

The obtained results were processed using the Statistica 11 program on the basis of variance analysis of data using the Mann-Whitney test, with a probability level of $p<0.05$.

Results of the study and their discussion. Previously, the pharmacological activity of DEoSWS was studied and anti-inflammatory, anticoagulant, membrane-stabilizing and disaggregation activities were established. Therefore, it was appropriate to study the effect of the plant extract on iron hemostasis in the conditions of experimental thrombophlebitis.

Based on the obtained data, an increase in the concentration of iron in the blood serum was observed in the control pathology group (CP) on the first day of the experiment by 1.76 times compared to the initial data, and at the same time a decrease of 1.44-fold and 3.5-fold in TIBCBS and UIBCBS (Figs. 1, 2, 3).

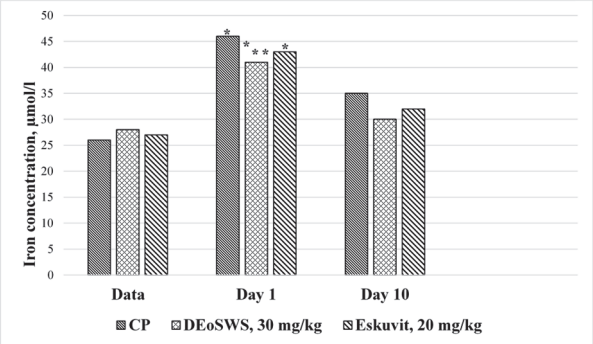

Fig. 1. Iron concentration in serum in conditions of thrombophlebitis. Notes: 1. * – deviations are reliable with respect to the original data ($p<0.05$); 2. ** – deviations are significant in relation to the control pathology ($p<0.05$)

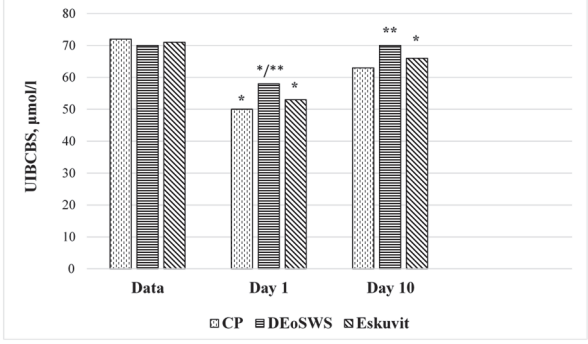

Fig. 2. Fluctuations in the blood serum level of TIBCBS in conditions of thrombophlebitis.

Notes: 1. * – deviation are reliable with respect to the original data ($p<0.05$); 2. ** – deviations are significant in relation to the control pathology ($p<0.05$)

In the group of animals that received DEoSWS in the prophylactic-therapeutic regimen, on the first day, a decrease in the concentration of iron in blood serum by 11 % was observed compared to the group of CP, and at the same time, an increase of TIBCBS by 14 % and UIBCBS by 52 %. Under the influence of "Eskuvit" there was also a decrease in the concentration of iron by 7 % and an increase of TIBCBS and UIBCBS by 6 % and 29 %, respectively, in the blood serum, however, no statistically significant changes were noted, except for UIBCBS in relation to CP.

Suppression of the activity of the antioxidant system (AOS) in blood serum was evidenced by an increase in the level of transferrin saturation (TS) in the CP group up to 85 % on the first day of the experiment (Fig. 4).

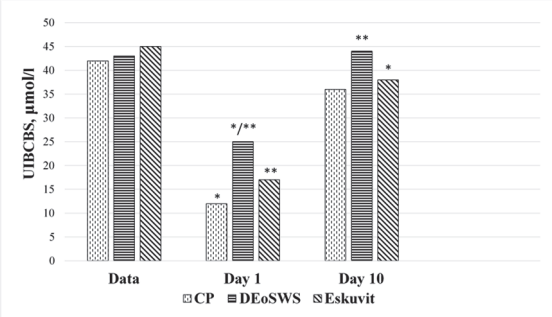

Fig. 3. Fluctuations in the level of UIBCBS in blood serum in conditions of thrombophlebitis. Notes: 1. * – deviations are reliable with respect to the original data ($p<0.05$); 2. ** – deviations are significant in relation to the control pathology ($p<0.05$)

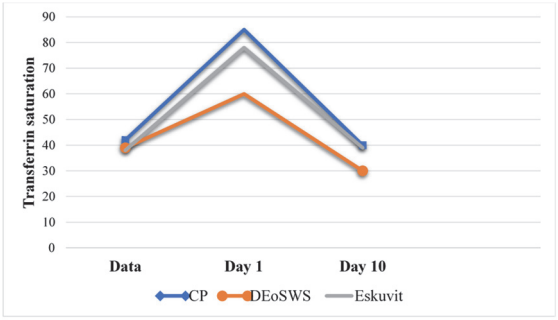

Fig. 4. Dynamics of saturation of transferrin with iron ions in conditions of thrombophlebitis

The obtained data are confirmed by literature data on the reduction of AOS as transferrin is saturated with iron ions by more than 50 %, which indicates a violation of the binding of iron to transferrins and the appearance of free (unbound) iron, which can cause a cytotoxic effect [8].

This indicates a violation of the "oxidant-antioxidant" balance at the peak of the development of experimental thrombophlebitis towards the predominance of oxidants and exhaustion of the body's antioxidant defense mechanisms. The results are also confirmed by literature data regarding the feedback principle between the concentration of iron and transferrin in the blood plasma. An increased level of iron has an inhibitory effect on the synthesis of transferrin in inflammatory diseases of various genesis [3].

In the group treated with DEoSWS for 1 day, the level of TS significantly decreased and was 30 % less than the control pathology. In the group treated with the reference drug, the TS indicator for 1 day was almost at the same level as the CP indicator and reduced TS by 9 %. At the end of the experiment, a decrease in TS was observed almost to the initial data in all experimental groups. This can be considered as an adaptive reaction of the body in response to a pathological process, which allows increasing the activity of antioxidant protection enzymes.

Analyzing the positive effect of the extract of Sakhalin willow shoots on the indicators of antioxidant protection against the background of experimental thrombophlebitis in rabbits, it can be argued that this effect is related to the metal-chelating properties of the flavonoids that were identified in the extract. It is known that all phenols, which include flavonoids, are able to form phenoxyl radicals and thus stop oxidative reactions in cells. Also, flavonoids are able to chelate metal ions, which play an important role in the initiation of free radical reactions [9, 10]. Flavonoids act as hydrogen atom donors, binding metals to free radicals. Chelation of metal ions of free radicals is an important mechanism of antioxidant action of natural flavonoids [7].

Flavonoids can act as structural antioxidants. Penetrating into the hydrophobic part of the membrane, flavonoid molecules reduce lipid mobility, which reduces the effectiveness of the interaction of free radicals with new lipid molecules [5].

The reason for the high antioxidant activity of flavonoids may be the inhibitory effect of the agents on enzymes and on the binding of hormones to cytoplasmic receptors in cells [5].

It is known that flavonoids form stable chelating complexes capable of binding metal ions with variable valence. The formation of such complexes leads to the inhibition of free radical processes. Due to their chelating properties, flavonoids, after ingestion with food, can affect the ionic balance and oxidative status of cells and tissues. In this state, phyto-complexes of metals are much more effective interceptors of oxygen anion radicals than the original components.

Among the poly phenols in the willow shoot extract are tannins, which can also participate in oxidative reactions for oxygen transfer. Their ability to easily donate electrons and protons makes them

more suitable as free radical scavengers. Tannins prevent the development of uncontrolled lipid self-oxidation and the accumulation of toxic lipid products [10, 11].

In this way, the dry extract from the shoots of the Sakhalin willow realized antioxidant-chelating and anti-inflammatory activity and showed a therapeutic-preventive effect against the background of experimental thrombophlebitis.

Conclusions

1. The proposed dry extract of the shoots of Sakhalin willow, which includes polyphenolic compounds (including flavonoids), used as a therapeutic and preventive agent against the background of experimental thrombophlebitis, significantly improved the negative effects on iron metabolism: it reduced the level of iron, increased TIBCBS and UIBCBS and reduced transferrin saturation in blood serum of rabbits.

2. Therapeutic and prophylactic administration of “Eskuvit” tablets against the background of experimental thrombophlebitis reduced the negative effect of free iron, had a positive effect on iron metabolism indicators and showed an antioxidant effect, however, according to some indicators (transferrin saturation) it was inferior to DEoSWS.

3. Medicinal and prophylactic extract of Sakhalin willow shoots, based on polyphenols, improved the condition of animals when modeling thrombophlebitis, showed anti-inflammatory and antioxidant metal-chelating properties, which allows it to be offered in complex therapy as a prophylactic agent for venous disease of the lower extremities.

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Стаття надійшла 6.04.2023 р.