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Artículos

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Efectos farmacológicos comparativos de Venarus Plus, Venarus y Detralex en la disfunción endotelial inducida por L-NAME, el tono venoso y la agregación plaquetaria

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Abstract:

In the present study we compared the pharmacological activity of Venarus Plus, Venarus and Detralex 1000 mg on the reversion of endothelial dysfunction (ED), and on the effect on venous tone, vascular permeability, and platelet aggregation. We used 150 Wistar male rats, weighing 180-220 g, and 80 adult albino rabbits weighing 2800 - 3200 g. Endothelial dysfunction (ED) was induced with the non-selective inhibitor of NO synthase, N-nitro-L-arginine-methyl ether (L-NAME). Functional vascular tests and biochemical markers were used to determine the reversion of the functional disorders. The anti-inflammatory effects of the drugs was evaluated in rabbits using o-xylene. The venotonic effects of the compounds was carried out on an isolated segment of the rat portal vein with Ca^{2+} solutions at a concentration of 0.08-1.75 mM. Our results show that the maximum daily therapeutic dose of Venarus Plus, produces a significant decrease in the ED coefficient (CED), an increase in NO synthesis, and an extended ADP-induced platelet aggregation time. The studied drugs dose-dependently reduce vascular permeability disorders caused by the application of o-xylene, which was manifested in a profound decrease the size of spots and the extension of the time interval before their onset. To study the Ca^{2+}-mediated smooth muscle response, showed that the maximum force of vein contraction occurs with a higher dosage of drugs in the presence of a lower concentration of Ca^{2+}, the effects of the drugs are comparable.
Keywords: endothelial dysfunction, venous tone, diosmin, chronic venous insufficiency.

Resumen:

Las enfermedades venosas crónicas son uno de los problemas urgentes de la medicina moderna. Estudios recientes han demostrado una gran importancia de la disfunción endotelial (DE) y el estrés oxidativo en su patogénesis. Para la reversión de los cambios que ocurren, actualmente se usan medicamentos del grupo flavonoide, particularmente diosmina y hesperidina. Utilizamos 150 ratas macho Wistar, con un peso de 180-220 g y 80 conejos albinos adultos con un peso de 2800 a 3200 g. La disfunción endotelial (DE) se indujo con el inhibidor no selectivo de NO sintasa, N-nitro-L-arginina-metil éter (L-NAME). Se utilizaron pruebas vasculares funcionales y marcadores bioquímicos para determinar la reversión de los trastornos funcionales. Los efectos antiinflamatorios de las drogas se evaluaron en conejos usando o-xileno. Los efectos venotónicos de los compuestos se llevaron a cabo en un segmento aislado de la vena porta de la rata con soluciones de Ca^{2+}, a una concentración de 0.08-1.75 mM. Nuestros resultados muestran que la dosis terapéutica diaria máxima de Venarus Plus produce una disminución significativa en el coeficiente de DE (DE), un aumento en la síntesis de NO y un tiempo extendido de agregación plaquetaria inducida por ADP. Los fármacos estudiados reducen de forma dependiente de la dosis los trastornos de permeabilidad vascular causados por la aplicación de o-xileno, que se manifestó en una disminución profunda del tamaño de las manchas y la extensión del intervalo de tiempo antes de su aparición. Al estudiar la respuesta del músculo liso mediada por Ca^{2+}, se demostró que la fuerza máxima de contracción venosa se produce con una dosis más alta de medicamentos en presencia de una concentración más baja de Ca^{2+}, los efectos de los medicamentos son comparables.
Palabras clave: disfunción endotelial, tono venoso, diosmina, insuficiencia venosa crónica.

Introduction

Chronic venous diseases (CVDs) and the search for effective methods of their treatment are one of the urgent problems of modern medicine. According to generalized data from epidemiological studies, 35-60% of the working-age population in different countries suffers from CVDs [1]. In Russia, it has been shown that 67% of women and 50% of men have lower extremity chronic venous disorders [2].

The basic pharmacotherapy of CVD includes pleiotropic medications (venoactive drugs, phleboprotectors, venotonics). This is a large, varied group of biologically active substances obtained by processing plant raw materials or chemical synthesis, combined with pharmacological and clinical effects [3]. G-benzopyronsare is the most studied from the main venotonics. They are flavonoids, medications based

on diosmin and hesperidin, wich are actively used nowadays for the correction of venous tone and reduction of the core symptoms of chronic venous insufficiency. Numerous studies have confirmed a wide range of biological effects of diosmin, including its anti-ulcer, anti-mutagenic, antioxidant, and anti-inflammatory effects [4].

Diosmin medications are produced both in granules and in film-coated tablets, but ultrasound micronization is important to increase the bioavailability of the drug. This was demonstrated in a study in healthy volunteers when they received labeled forms of micronized and non-micronized diosmin [5.]

The drug Detralex 1000 mg, registered in France, consisting of naturally obtained diosmin 900 mg (90%) and 100 mg (10%) of flavonoids in terms of hesperidin is a representative of the micronized purified flavonoid fraction (MPFF). The Russian analog is the drug Venarus, which has a similar quality and percentage composition but is completely synthetic in nature.

It is planned to develop and register the drug Venarus Plus, containing the active components hesperidin 100 mg (in terms of 100% substance), diosmin 900 mg (in terms of 100% substance); oligomeric procyanidol of Vitis vinifera seeds-300.0. The combination of synthetic components with natural flavonoids obtained from the grape seeds is due to the results of modern studies, which have shown that these substances can reduce the symptoms of chronic venous insufficiency, helping to strengthen the walls of varicose veins and restore their elasticity, having a powerful anti-inflammatory effect, removing edema and reducing the risk of thrombosis [6-10].

The results of numerous clinical trials indicate that in the early stages of the disease (C0S—C2S), all pleiotropic medications have a good therapeutic effect on subjective symptoms, but not external manifestations (telangiectasia, varicose reticular and subcutaneous veins) of CVD. However, for the pharmacotherapy of early stages of CVD, preference should be given to pleiotropic drugs and their combinations, the effectiveness, and safety of which have been proven in randomized controlled clinical trials.

In the present study we compared the pharmacological activity of Venarus Plus, Venarus and Detralex 1000 mg on the reversion of endothelial dysfunction (ED), and on the effect on venous tone, vascular permeability, and platelet aggregation.

Material and Methods

We assessed the comparative effects of three drugs: Venarus Plus, Detralex and Venarus. Venarus Plus contains the active components hesperidin 100 mg + diosmin 900 mg + oligomeric procyanidol of vitisvinifera seeds 300.0, produced by JSC "PE "Obolenskoe", Russia. Detralex 1000 mg (Les Laboratoires Servier, Russia), which is a micronized purified fraction of flavonoids containing 90% diosmin (900 mg) and 10% (100 mg) flavonoids in terms of hesperidin; and Venarus (JSC "PE "Obolenskoe"), consisting of hesperidin 100 mg and diosmin 900 mg in terms of 100% of the substance.

Eighty white male Wistar rats weighing 180-220 g, were used to induce ED. For that the non-selective NO-synthase inhibitor, N-nitro-L-arginine-methyl ether (L-NAME) was intraperitoneally administered, at a dose of 25 mg/kg/day for 7 days. On the 7th day, the animals were anesthetized with 300 mg/kg chloral hydrate, the left carotid artery was catheterized to register hemodynamic parameters. The hemodynamic parameters: heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP) were continuously measured using a sensor and hardware complex for invasive assessment of hemodynamic parameters BIOPAC MP-150 (USA), with TSD-104A module and the computer program Asq Knowledge 4.2. Acetylcholine solution (40 μ/kg) and sodium nitroprusside (30 μ/kg) were intravenously administered as functional tests.

All animals were divided into 8 groups: group 1: intact rats administered with saline solution in equivolume doses (Control), group 2-rats administered with L-NAME intraperitoneally for 7 days, group 3 – rats administered with L-NAME intraperitoneally + Venarus Plus at a minimum therapeutic dose of 86 mg/kg/day, group 4-rats administered with L-NAME intraperitoneally + Detralex 1000 mg at a minimum therapeutic dose of 86 mg/kg/day, group 5-rats administered with L-NAME intraperitoneally + Venarus in the minimum therapeutic dose of 86 mg/kg/day, group 6 – rats administered with L-NAME intraperitoneally + Venarus Plus at a maximum therapeutic dose of 260 mg/kg/day, group 7-rats administered with L-NAME intraperitoneally + Detralex 1000 mg at a maximum therapeutic dose of 260 mg/kg/day, group 8-rats administered with L-NAME intraperitoneally + Venarus at a maximum therapeutic dose of 260 mg/kg/day. The medications were administered once a day, for 7 days.

The development and degree of ED reversion in experimental animals were evaluated calculating the coefficient of ED (CED) [11-15]. Colorimetric evaluation of the level of NO metabolites (i.e. the total concentration of nitrates and nitrites, NOx) was based on the development of color in the diazotization reaction with sulfonamide nitrite, which is part of the Griss reagent [16-20]. Platelet aggregation was studied by a visual microtechnique using adenosine diphosphate (ADP), collagen, thrombin, ristomycin, and epinephrine as inducers [21-26].

The anti-inflammatory activity of the drug was studied using Oyvin and Monakova method [27-32,37]. For this purpose, experiments were conducted in mature albino rabbits weighing 2800-3200 g. The rabbits had a 13 cm fur-trimmed area on their abdomen. Then the animals were fixed and singly administered with the test drug and comparison drugs at the maximum (100 mg/kg/day) and minimum (34 mg/kg/day) therapeutic dose 9 hours before the injection of Evans blue solution, which was used as a permeability indicator. The indicator of capillary permeability was the time when blue-colored spots appeared on the skin and their diameter.

To evaluate the Ca^{2+}-dependent smooth muscle response, 70 male Wistar rats were divided into 7 groups. Group 1 –control, rats administered with saline solution in equivolume doses, group 2 –rats administered with Venarus Plus at the minimum therapeutic dose of 86 mg/kg/day, group 3 –rats administered with Detralex 1000 mg at the minimum therapeutic dose of 86 mg/kg/day, group 4 –rats administered with Venarus at the minimum therapeutic dose of 86 mg/kg/day, group 5 –rats administered with Venarus Plus at the maximum therapeutic dose of 260 mg/kg day, group 6-rats administered with Detralex 1000 mg at the maximum therapeutic dose of 260 mg/kg/day, group 7 –rats administered with Venarus at the maximum therapeutic dose of 260 mg/kg/day. The medications were administered per os, once a day for 7 days.

After anesthesia (chloral hydrate, 300 mg/kg), a portal vein section of 25±4 mm was dissected from each animal. Data on the pacemaker activity of the interstitial cells of Cajal in the portal vein were used for selecting a section of the venous bed. The specimen was placed vertically in the box of the BIOPAC tissue testing station (initial tension 0.5 g) with the STM-200 electrodes of the electro stimulator. The lumen of the isolated vein was ligated, which excluded the contact of solutions with the endothelium. The test was performed with Ca^{2+} solutions at a concentration of 0.08-1.75 mmol [33,34,36]. The solutions were added to the perfusate sequentially from the minimum to the maximum concentration of Ca^{2+}. A modified Krebs-Henseleit solution was used as the base solution, in which the concentration of Ca^{2+} was changed; isotonicity was achieved by changing the content of sodium chloride (all reagents - Reakhim, Russia). The solutions were oxygenated with a mixture of 95% O_2 and 5% CO_2. The temperature of all solutions was 30°C. Data were registered and processed using the Biopac AsqKnowledge 4.2 software.

Statistical processing of the data was done by assessing for data distribution. The distribution type was determined by the Shapiro-Wilk test. In a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. Intergroup differences were analyzed using parametric (Student's t-test) or

nonparametric (Mann-Whitney test) methods, depending on the type of distribution. The calculations were made using the statistical software package Microsoft Excel 7.0.

Results

L-NAME administration to male rats, during 7 days, induced on the 8th day a statistically significant increase in systolic and diastolic blood pressure from 135.7±4.1 and 99.9±3.3 to 188.3±6.1 and 143.0±2.9 mm Hg, respectively, an increase in CED from 1.2±0.1 to 5.0±0.6 ($p<0.05$) and a decrease in the terminal NO metabolites from 45.19±2.89 to 22.69±1.50 (Table 1).

TABLE 1
The effect of Venarus Plus Detralex 1000 mg and Venarus on the arterial blood pressure and CED

Indicator Group	SBP, mm Hg	DBP, mm Hg	CED, relative units	NO, µmol/ml
Control	135.7 ±4.1^{y}	99.9±3.3^{y}	1.2±0.1^{y}	45.19±2.89^{y}
L-NAME	188.3 ±6.1*	143.0±2.9*	5.0±0.6*y	22.69±1.50*^{y}
L-NAME + Venarus Plus (86 mg/kg/day)	179.0 ±5.4*	143.6±5.7*	2.3±0.2*^{y}	32.66±1.60*^{y}
L-NAME + Detralex 1000 мг (86 mg/kg/day)	185.3 ±4.6*	134.1±3.4*	2.4±0.4*^{y}	31.34±1.64*^{y}
L-NAME + Venarus (86 mg/kg/day)	186.8 ±4.1*	136.2±3.2*	2.6±0.4*y	30.08±1.62*y
L-NAME + Venarus Plus(260 mg/kg/day)	174.9 ±4.9*	134.1±4.2*	1.6±0.1*^{y}	42.68±1.68*^{y}
L-NAME +Detralex 1000 mg (260 mg/kg/day)	174.0 ±4.0*	135.3±3.2*	2.0±0.1*^{y}	34.42±2.20*^{y}
L-NAME + Venarus (260 mg/kg/day)	174.3 ±4.1*	135.8±3.4*	2.2±0.1*y	31.12±2.10*y

* p005 compared with control group yp005 in comparison with LNAME Data are expressed as M±m n10

Intraperitoneally administration of the studied drugs did not induce a decrease in blood pressure. However, there was a dose-dependent significant decrease in CED. It should be noted that in the maximum therapeutic dose, the effects of Venarus Plus produced a more pronounced effects when compared with the other two drugs.

The administration of a non-selective NO-synthase inhibitor altered platelet aggregation, which is expressed in its acceleration. Venarus Plus, Detralex 1000 mg, and Venarus administration resulted in a dose-dependent reversion of the altered platelet aggregation, which is manifested in the elongation of platelet aggregation. It was found that in the maximum therapeutic dose, the effectiveness of Venarus Plus was higher than that of comparison drugs when ADP was used as inducer (Table 2).

TABLE 2
The effect of Venarus Plus Detralex and Venarus on the platelet aggregation

Inducer Group	ADP, sec	Collagen, sec	Ristomycin, sec	Adrenalin, sec
Control	43.6±1.5^{y}	33.0±0.6^{y}	41.5±1.9^{y}	102.4±3.8^{y}
L-NAME	30.2±1.3*	27.1±1.1*	31.6±1.2*	79.4±2.7*
L-NAME + Venarus Plus (86 mg/kg/day)	34.1±1.3*^{y}	32.5±0.8*^{y}	35.5±1.2*^{y}	89.6±2.7*^{y}
L-NAME + Detralex 1000 мг (86 mg/kg/day)	34.6±1.5^{y}	31.5±1.0^{y}	36.2±1.5^{y}	92.3±3.9^{y}
L-NAME + Venarus (86 mg/kg/day)	34.2±1.4^{y}	31.0±1.0^{y}	35.4±1.5^{y}	89.5±3.7^{y}
L-NAME + Venarus Plus(260 mg/kg/day)	39.3±1.2^{y}	32.2±1.0^{y}	36.8±1.6^{y}	99.2±3.4^{y}
L-NAME +Detralex 1000 mg (260 mg/kg/day)	35.2±1.4^{y}	32.1±1.0^{y}	37.2±1.36^{y}	96.9±3.9^{y}
L-NAME + Venarus (260 mg/kg/day)	34.8±1.4^{y}	32.0±1.0^{y}	36.2±1.36^{y}	94.3±3.7^{y}

* p005 compared with control group yp005 in comparison with LNAME Data are expressed as M±m n10

In the study of Ca^{2+}-mediated smooth muscle response, it was found that in the control group there was an increase in the vein tone against the background of adding Ca^{2+} to the solution starting from a concentration of 0.76 mmol/l, whereas 7-day use of the study drug and comparison drugs caused a significant increase in the venous tone from a concentration of Ca^{2+} 0.25 mmol/l (Table 3). At the same time, the sensitivity of the smooth muscle vein wall to Ca^{2+} at a concentration of 0.76 mmol/l was significantly higher than in the control. The effect of drugs is dose-dependent, which is manifested in achieving the maximum contractile force with a higher dosage of drugs in the presence of a lower concentration of Ca^{2+}. However, there was no significant difference between the study drug and the comparison of drugs, as shown in figure 1.

TABLE 3
The effect of Venarus Plus, Detralex 1000 mg and
Venarus on the contractility of the isolated vein segment

Ca^{2+} concentration Medication, dose	Ca^{2+} 0.08 mmol/l	Ca^{2+} 0.15 mmol/l	Ca^{2+} 0.25 mmol/l	Ca^{2+} 0.76 mmol/l	Ca^{2+} 1.75 mmol/l
Control	0.55±0.01	0.55±0.01	0.55±0.01	0.69±0.03	0.79±0.03
Venarus Plus (86 mg/kg/day)	0.52±0.01	0.54±0.01	0.65±0.01*	0.81±0.02*	0.90±0.03*
Detralex 1000 mg (86 mg/kg/day)	0.55±0.01	0.53±0.01	0.59±0.02	0.80±0.02*	0.89±0.03*
Venarus (86 mg/kg/day)	0.55±0.01	0.53±0.01	0.57±0.02	0.78±0.02*	0.88±0.03*
Venarus Plus (260 mg/kg/day)	0.55±0.01	0.55±0.01	0.66±0.01*	0.87±0.02*	0.91±0.03*
Detralex (260 mg/kg/day)	0.55±0.01	0.55±0.01	0.67±0.01*	0.87±0.01*	0.92±0.02*
Venarus (260 mg/kg/day)	0.55±0.01	0.55±0.01	0.65±0.01*	0.86±0.01*	0.90±0.02*

* p005 compared with control group Data are expressed as M±m n10

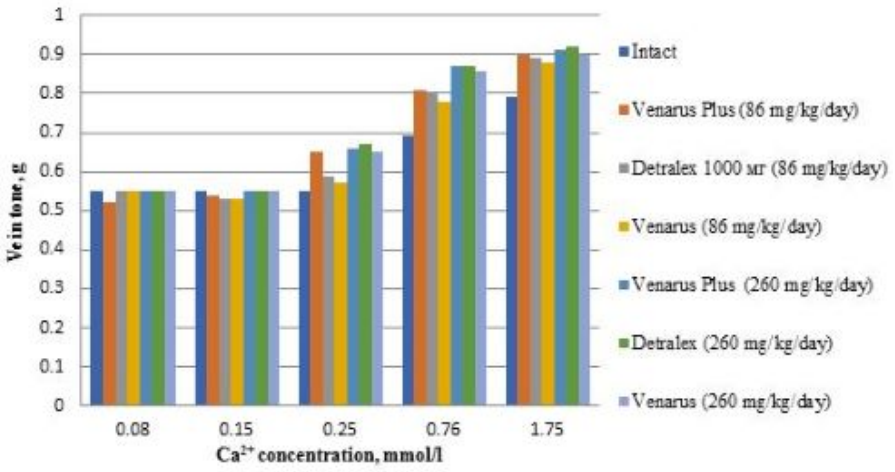

FIGURE 1
The effect of Venarus Plus, Detralex 1000 mg, and
Venarus on the contractility of the isolated vein segment

The results of the study of the anti-inflammatory activity of drugs using the Oyvin and Monakova [27] method is presented in Table 4, were it is shown a decrease in vascular permeability when the drugs are administered, as evidenced by a decrease in the size of spots and an extension of the latency time of their manifestation. It should be noted that Venarus Plus, Detralex 1000 mg, and Venarus, at the dose of 100 mg/kg/day, were better to reduce vascular permeability. Thus, it can be stated that the studied drugs, with dose-dependent dynamic reduce vascular permeability disorders caused by the application of 0-xylene.

TABLE 4
The effect of Venarus Plus, Detralex 1000 mg and Venarus on the vascular permeability

Medications	The mean area of the spots, cm^2	latency time of spots manifestation, sec
Control	6.58±0.08	202±6.11
Venarus Plus (34 mg/kg/day)	5.17±0.06*	255±5.43*
Detralex 1000 mg (34 mg/kg/day)	5.20±0.06*	243±7.30*
Venarus (34 mg/kg/day)	5.16±0.06*	240±7.30*
Venarus Plus (100 mg/kg/day)	4.39±0.05*	285±6.54*
Detralex (100 mg/kg/day)	4.38±0.05*	290±6.15*
Venarus (100 mg/kg/day)	4.38±0.05*	283±6.25*

* $p<0.05$ compared with control group. Data are expressed as (M±m; n=10)

Discussion

The greater effectiveness of Venarus Plus in the treatment of endothelial dysfunction is probably due to the additional pharmacological effects of pro-anthocyanidins from vitisvinifera seeds (VVS) – natural flavonoids. In experiments with the use of VVSfor rabbit aortic specimens, activation of the constitutive form of NO-synthase (cNOS) was detected, which is involved in the formation of endothelial nitric oxide, involved in the relaxation of blood vessels [6]. In vitro experiments have also shown that VVSproanthocyanidins modulate the inflammatory response by suppressing iNOS expression, increasing the synthesis of prostaglandin E_2 and nitric oxide, and inhibiting the translocation of the main complex of transcription proteins responsible for the expression of the immune response, cell cycle, and apoptosis genes - nuclear factor kappa B (NF-kappa B) [7]. A decrease in the release of superoxide and iNOS, determined using NO-sensitive electrodes, was also shown in another study. The experiment evaluated the effects of VVS on coagulation and platelet-dependent inflammatory response. The results showed a significant decrease in platelet aggregation when adding the substance to blood products [8]. A significant antithrombotic effect of proanthocyanidins was demonstrated by Sano et al. in rodents under physiological conditions and in an arterial thrombus model [9]. In a randomized study, Polagruto et al., which included smokers, found that ADP-stimulated platelet activity was significantly lower in the group receiving VVS compared to the placebo group [10,35].

It was found that VVS irreversibly inhibits the proteolytic enzymes collagenase and elastase, glycosidase hyaluronidase, and beta-glucuronidase, which destroy the components of the extracellular matrix-glucuronic acid, collagen, and elastin [7].

Conclusion

1. According to the study of endothelium protective action of Venarus Plus it was established that the test drug has pronounced endothelium protective effect, significantly reducing the CED and slowing platelet aggregation in induced ED. It should be noted that at the maximum daily therapeutic dose, the endothelium protective action of Venarus Plus exceeds the effectiveness of the comparison drugs.

2. Based on the results of the study of the effect of Venarus Plus on the vein contractile activity, it can be concluded that the study drug has a comparable ability to increase the contractile activity of an isolated segment of the portal vein in response to an increase in the concentration of Ca^{2+}.

3. Based on the results of the study of the effect of Venarus Plus on vascular permeability, it can be concluded that the study drug has a dose-dependent, comparable to comparison drugs, the ability to reduce vascular permeability disorders caused by the application of o-xylene.

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