

# Antioxidant Composition Causes Antiexsudorific Effect in the Model of Chronic Venous Insufficiency

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Venous hypertension and swelling of the hind limbs was demonstrated to develop in rat model of chronic venous insufficiency. It was found that 14-day course of dihydroquercetin (50 mg/kg) and lipoic acid (50 mg/kg) administered intragastrically prevented the increase in hind limb volume and reduced the severity of edema of rat muscle tissue in chronic venous insufficiency.

**Key Words:** *chronic venous insufficiency; edema; dihydroquercetin; lipoic acid*

Treatment of chronic venous insufficiency (CVI) of lower limbs is a pressing medical and socioeconomic problem [9] due to high prevalence of this disease (>20% population in economically developed countries) [2]. A variety of treatment options and development of new drugs do not reduce the number of patients with this pathology [9,11,13,14].

The use of drug therapy, including preventive measures, is a promising trend [1].

Elimination of limb edema caused by both venous and lymphatic components is essential in the treatment of CVI patients. In the venous stasis and hypertension, edema is related to imbalance between transcapillary filtration and reabsorption of the interstitial fluid and lymphatic drainage [3,7].

We previously showed that the composition of dihydroquercetin and lipoic acid exhibits marked lymphokinetic and capillary protective activity [4,11].

Here we studied the effects of composition of dihydroquercetin and lipoic acid on edema in rats with CVI.

## MATERIALS AND METHODS

Experiments were carried out on male Wistar rats ( $n=15$ ) weighing 250-350 g. CVI was modeled by

restriction of vena cava blood flow. To this end, laparotomy was performed under ether anesthesia, a fragment of caudal vena cava proximal to the right renal vein was isolated, and the ligature was applied. Then, heparin in a dose of 250 U/kg was injected into the femoral vein and the caudal vena cava was partially occluded (by  $\frac{2}{3}$ ). In sham-operated (SO) animals, the same surgery was performed, except vessel occlusion. The animals of experimental group intragastrically received (daily) the composition of dihydroquercetin (50 mg/kg) and lipoic acid (50 mg/kg) in 1% starch suspension. Controls and SO animals received an equivalent volume of 1% starch suspension. The first dose was given on day 1 after surgery and the last dose was administered 1 h before measurements.

On day 14, the volume of the hindlimb paws was assessed by oncometry, venous pressure measured, and a fragment of shin muscles was isolated for histological examination.

Before pressure measurements, the rats were narcotized with diethyl ether; heparin was injected intravenously in a dose of 250 IU/kg. The pressure in the caudal vena cava was measured by a direct method at the level of iliac vein inflow. Access was performed through the femoral vein.

After sacrifice (ether overdose) a fragment of calf muscle was isolated, fixed in 10% neutral formalin, dehydrated in ascending alcohol concentrations, em-

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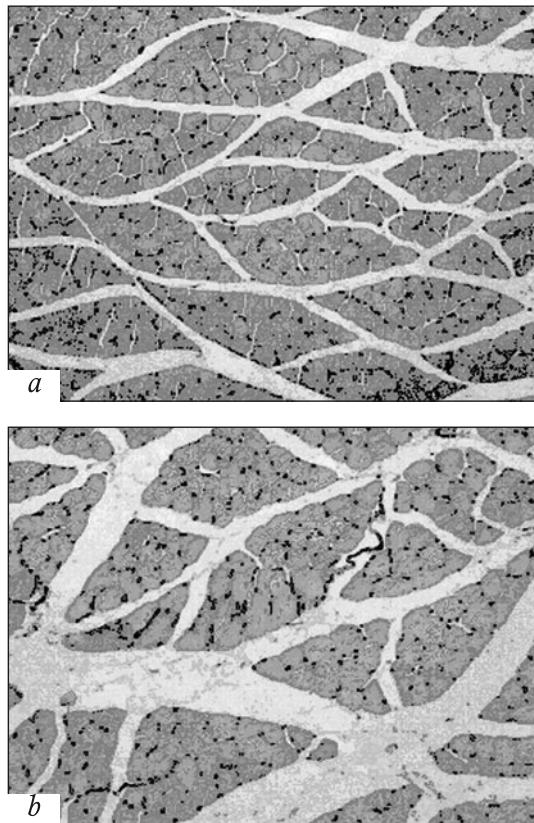
bedded in paraffin, and 5- $\mu$  sections were prepared. The deparaffinized sections were stained with hematoxylin and eosin. The area of interstitial space in relation to standard tissue area on histological sections of shin muscles (in %) was estimated using Adobe Photoshop CS2 at  $\times 10$ .

Statistical processing of the results was performed using Statistica 8.0 software. The data are presented as mean and standard error of the mean ( $M\pm m$ ). The significance of intergroup differences was evaluated using nonparametric Mann-Whitney *U* test.

## RESULTS

Significant increase in venous pressure (by 2.8 times,  $p<0.05$ ) was observed in animals of the control group on day 14 compared to SO animals (Table 1). Against the background of venous hypertension in this group, a significant increase (by 8%) in the volume of hindlimb paws was revealed compared with SO animals (Table 2). Histologically, the area of interstitial space increased by 1.2 times in comparison with SO animals ( $p<0.05$ ), which attested to muscle tissue swelling (Table 3, Fig. 1).

Thus, blood flow restriction in the caudal vena cava was accompanied by venous hypertension, increase in the limb volume, and edema of muscle tissue, *i.e.* the model reproduced the characteristic signs of



**TABLE 1.** Effects of the Dihydroquercetin (50 mg/kg) and Lipoic Acid (50 mg/kg) Composition Administered Intragastrically as a 14-Day Course on Venous Pressure in CVI Rats ( $M\pm m$ )

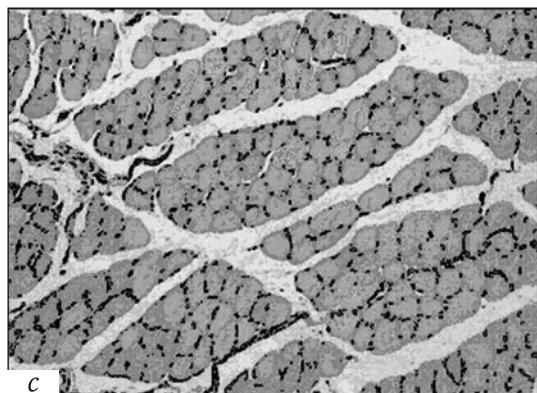
Group	Venous pressure, mm H <sub>2</sub> O
Control ( $n=5$ )	112.0 $\pm$ 3.7*
SO ( $n=5$ )	40.8 $\pm$ 2.9
Dihydroquercetin and lipoic acid ( $n=5$ )	103.2 $\pm$ 4.0*

**Note.** \* $p<0.05$  compared to SO group.

CVI in humans [6,8].

Administration of dihydroquercetin and lipoic acid composition in CVI model had no effect on venous pressure (Table 1). However, the composition significantly reduced the increase in limb volume and prevented the formation of tissue edema. Thus, the volume of hindlimbs paws in animals treated with dihydroquercetin and lipoic acid was significantly lower than in the control and did not significantly differ from that in SO rats. The area of interstitial space in the experimental group was lower than in the control by 4% ( $p<0.05$ ; Tables 2, 3, Fig. 1).

It is known that the increase in limb volume can be caused by plethora or edema [2,7,10]. Increased



**Fig. 1.** Shin muscles of SO animals (a), CVI animals (b), and CVI animals treated with dihydroquercetin and lipoic acid composition (c). Interstitial edema. Hematoxylin and eosin staining,  $\times 10$ .

**TABLE 2.** Effects of Dihydroquercetin (50 mg/kg) and Lipoic Acid (50 mg/kg) Composition Administered Intragastrically as a 14-Day Course on Hindlimb Paw Volume in CVI Rats ( $M\pm m$ )

Group	Initial, ml		On day 14, ml	
	left paw	right paw	left paw	right paw
Control ( $n=5$ )	1.86 $\pm$ 0.04	1.89 $\pm$ 0.04	2.01 $\pm$ 0.03 <sup>o</sup>	2.05 $\pm$ 0.04 <sup>o</sup>
SO ( $n=5$ )	1.90 $\pm$ 0.03	1.92 $\pm$ 0.02	1.87 $\pm$ 0.02	1.91 $\pm$ 0.01
Dihydroquercetin and lipoic acid ( $n=5$ )	1.85 $\pm$ 0.04	1.93 $\pm$ 0.02	1.90 $\pm$ 0.02 <sup>*</sup>	1.93 $\pm$ 0.03 <sup>*</sup>

**Note.**  $p<0.05$  compared to: <sup>\*</sup>control, <sup>o</sup>SO group.

**TABLE 3.** Effects of Dihydroquercetin (50 mg/kg) and Lipoic Acid (50 mg/kg) Composition Administered Intragastrically as a 14-Day Course on the Ratio of Interstitial Space Area (%) to Standard Tissue Area on Histological Sections of Shin Muscles in CVI Rats ( $M\pm m$ )

Group	Area of interstitial space/ standard tissue area, %
Control ( $n=5$ )	40.0 $\pm$ 1.2 <sup>o</sup>
SO ( $n=5$ )	32.2 $\pm$ 1.3
Dihydroquercetin and lipoic acid ( $n=5$ )	36.2 $\pm$ 1.3 <sup>o*</sup>

**Note.**  $p<0.05$  compared with <sup>\*</sup>control, <sup>o</sup>SO group.

venous pressure in CVI leads to hyperdistention of veins and loss of tone. Venous stasis inevitably impairs reabsorption and the volume of interstitial fluid increases. The resulting fluid excess is removed from the tissue via lymph capillaries. At a certain stage of the disease, this balance is disturbed, reserve capacity of the lymphatic system becomes insufficient, and edema develops [2,4,7,10].

In our study, the composition of dihydroquercetin and lipoic acid administered to rats with experimental CVI prevented the increase in hindlimb volume and decreased muscle edema.

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