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MECHANISM OF ENDOTHELIUM-DEPENDENT ACTION OF LEONURUS TURKESTANICUS EXTRACT

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ABSTRACT

This article investigates the endothelial-dependent mechanism of action of *Leonurus* extract. The research demonstrates that the extract effectively influences aortic preparations even in the absence of the endothelial layer. These findings are supported by experiments using the eNOS inhibitor L-NAME, which serve as crucial evidence in understanding the role of the endothelium. The results provide a scientific foundation for exploring endothelial-mediated physiological processes and assessing the vasodilatory effects of the extract. The efficiency of the extract in modulating endothelial responses is noted to be linked to its influence on nitric oxide production processes mediated by the eNOS enzyme.

KEYWORDS: *Leonurus*, eNOS, L-NAME.

INTRODUCTION

Literature indicates that the endothelial layer plays a crucial role in maintaining vascular functionality, specifically in regulating vascular tone. Endothelial cells, located in the inner layer of blood vessel walls, synthesize local mediators that regulate blood flow within organs. One key functional molecule synthesized by endothelial cells is nitric oxide (NO), an essential vasodilator.[1] Endothelial dysfunction and structural abnormalities are significant factors in the pathogenesis of arterial hypertension, atherosclerosis, and other cardiovascular diseases. In many cardiovascular disorders, initial damage to endothelial cells triggers a cascade of pathological morpho-functional changes, ultimately resulting in widespread dysfunction.[2]

The role of endothelial cells spans a broad range of functions, including regulating vascular tone, hemostasis, immune response, migration of blood cells along the vascular wall, synthesis of inflammatory mediators and inhibitors, and maintaining barrier functions.[3]

Endothelial Dysfunction (ED)

Endothelial dysfunction is characterized by an imbalance in the mediators that regulate vascular processes. It is often associated with structural changes in blood vessels, diminished responsiveness to external stimuli, and impaired production of vasoactive endothelial factors.[4] Various factors affecting NO metabolism contribute to endothelial dysfunction. Risk factors for ED, particularly in the context of cardiovascular disease, include physical inactivity, smoking, excessive salt consumption, exposure to toxins, estrogen deficiency, and disruptions in carbohydrate, lipid, or protein metabolism.

Nitric Oxide (NO) and Endothelial Function

The amino acid L-arginine serves as a substrate for NO synthesis via the enzyme NO synthase (eNOS) in endothelial cells. Activation of eNOS by calcium-calmodulin complexes leads to the oxidation of L-arginine, producing NO in picomolar concentrations.[5] In its inactive state, eNOS associates with caveolin within the plasma membrane, suppressing its activity. Stimuli such as acetylcholine, bradykinin, serotonin, thrombin, ADP, and glutamate can dissociate eNOS from this complex, thereby enhancing NO production.[3,6]

Statins, even at low concentrations, stimulate NO production by inhibiting the formation of eNOS-caveolin complexes, providing a therapeutic basis for correcting ED.[3,6]

NO-Mediated Vasodilation

In smooth muscle cells, NO activates guanylate cyclase (GC), increasing the concentration of cyclic guanosine monophosphate (cGMP), which in turn activates protein kinase G (PKG). This signaling pathway leads to the phosphorylation of the myosin light chain, resulting in relaxation.[8]

Endothelial cells also regulate Ca2+ homeostasis and modulate the functional state of smooth muscle cells (SMCs) through various vasoactive factors, ensuring vascular tone regulation.[9] By activating the NO/sGC/cGMP/PKG signaling pathway, NO decreases intracellular Ca2+ concentration ([Ca2+]i), facilitating relaxation through the activation of IP3R and Ca2+-ATPase in the sarcoplasmic reticulum (SR).[7]

Experimental Validation

To evaluate the role of the endothelium in the relaxant effects of the studied extract, experiments were conducted on isolated aortic preparations with and without the endothelial layer. This approach assessed the endothelial contribution to the observed vascular relaxation.

The research object included an extract from the local plant motherwort (Leonurus turkestanicus) and rat aorta preparations.

MATERIALS AND METHODS

Experiments were conducted on aorta preparations from male white non-bred rats (200–250 g). The rats were euthanized using cervical dislocation, and the aorta was surgically extracted, placed in a specialized chamber (5 ml), and perfused with Krebs-Henseleit physiological solution (mM): NaCl 120.4; KCl 5; NaHCO3 15.5; NaH2PO4 1.2;

MgCl2 1.2; CaCl2 2.5; C6H12O6 11.5, HEPES pH 7.4. For specific experiments, Ca2+-free Krebs solutions were used, with 1 mM EGTA added. The physiological solutions were oxygenated with carbogen (95% O2, 5% CO2) and maintained at +37°C with a U-8 ultrathermostat. After removing connective tissue and fat from the aorta, it was cut into 3–4 mm ring segments. The aorta rings were attached to a Radnoti (Isometric Transducer, USA) sensor using platinum wire hooks. The rings were allowed to equilibrate for 60 minutes before an initial preload of 1 g (10 mN) was applied. The contraction force was recorded with a mechanotron, and data was processed using OriginLab OriginPro v. 8.5 SR1 (EULA, Northampton, MA 01060–4401, USA). The isometric contraction force (mN) of the rat aorta in vitro was statistically recalculated as a percentage (%).[10]

RESULTS AND ANALYSIS

The experiments investigated the potential modulation of vascular endothelial layer functional activity by extracts under their relaxant effects. Standard isometric conditions were used to study rat aorta preparations with and without the endothelial layer.

Inducing Contraction: Contraction was induced in aorta preparations by 1 µM phenylephrine (PE), achieving a contraction amplitude of up to 10 mN, which was considered the baseline for experiments.

Verification of Endothelial Integrity: The presence or absence of the endothelial layer was confirmed using 1 µM acetylcholine.

In isometric conditions of rat aorta vascular preparations, 1 µM acetylcholine reduced the contraction amplitude induced by 1 µM phenylephrine by 57.8±4.3%. In preparations where the endothelial layer was mechanically removed with a cotton swab, acetylcholine had almost no effect on the contraction induced by phenylephrine. Effect of Leonurus Extract: Previous studies indicated that the Leonurus extract effectively influenced voltage-gated Ca^{2+} (L-type) channels[11], and receptor-operated Ca^{2+} channels In the current study[12], the relaxant effect of Leonurus extract significantly decreased under conditions where the endothelial layer was removed. Different concentrations of the extract (µg/mL) reduced the relaxant effect by 41.5±4% compared to controls. When compared to the intact endothelial condition, the relaxant effect decreased by 33.6±3.3% (Figure 1).

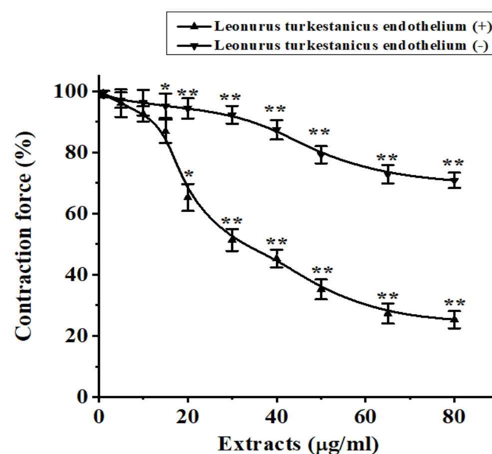

Figure 1: Concentration-dependent relaxant effect of *Leonurus* extract on contraction induced by 1 µM phenylephrine in rat aorta vascular preparations with (+) and without (–) endothelial layer. The contraction force induced by 1 µM phenylephrine was taken as 100% in the control. (In all cases, significance indicators: $p < 0.05$, $p < 0.01$; n=5).

The experimental results demonstrate that the tested extract caused significant changes in aortic preparations with the endothelial layer removed. These findings suggest that the examined substance may exert endothelial-dependent activity. To further clarify this hypothesis, subsequent experiments were conducted in the presence of the eNOS inhibitor L-NAME (100 μM). In these experiments, a noticeable reduction in the relaxant effect of the tested extract was observed in aortic preparations incubated with L-NAME. In the presence of 100 μM L-NAME, the *Leonurus* extract reduced the contraction force induced by phenylephrine in aortic preparations by 37.5 ± 4%. Compared to conditions with an intact endothelial layer, the reduction was measured at 37.6 ± 3% (Figure 3).

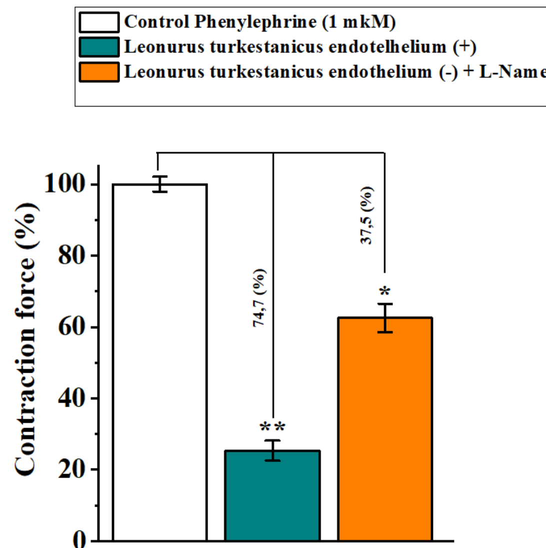

Figure 3: Concentration-dependent relaxant effect of Leonurus extract on rat aortic vessel preparations under incubation with the eNOS blocker L–NAME at 100 μM. The contractile force induced by 1 μM PE was considered 100% in the control. (In all cases, statistical significance was set at $p<0.05$, $p<0.01$; n=6).

The series of experimental results demonstrates that the studied *Leonurus cardiaca* extract exhibits a potent relaxant effect, primarily mediated through endothelium-dependent mechanisms. The attenuation of this relaxant effect in conditions where the endothelium is removed or in the presence of L-NAME highlights the critical role of NO-synthase. By activating NO-synthase and the sGC/cGMP/PKG signaling pathway, the extract facilitates the reduction of Ca^{2+} influx through Ca^{2+}L and Ca^{2+}R channels in the plasmalemma, while also inhibiting Ca^{2+} release from the sarcoplasmic reticulum (SR). These actions lead to a decrease in $[Ca^{2+}]_i$ levels in smooth muscle cells, resulting in relaxation of the smooth muscle.

REFERENCES

1. Покровский В.И., Виноградов Н.А. Оксид азота, его физиологические и патофизиологические свойства // Терапевтический архив, 2005; 1(С): 82-87.
2. Abdullaev A. A, Inamjanov D. R, Abduazimova D. S, Omonturdiyev S. Z, Gayibov U. G, Gayibova S. N, Aripov T. F. Sílybum Mariánum's Impact on Physiological Alterations and Oxidative Stress in Diabetic Rats. Biomed Pharmacol J, 2024; 17(2).
3. Петрищев Н.Н, Власов Т.Д. Физиология и патофизиология эндотелия Дисфункция эндотелия причины, механизмы, фармакологическая коррекция // СПб ГМУ, 2003; С. 4-38 б.

4. Оганов Р.Г., Масленникова Г.Я. Смертность от сердечно-сосудистых и других хронических неинфекционных заболеваний среди трудоспособного населения России // Кардиоваскулярная терапия и профилактика, 2002; №3 С. 4-8 б.
5. Loscalzo J., Nutr J. L-arginine and atherothrombosis // The Journal of Nutrition, 2004; 134: 2798-2800.
6. Бувальцев В.И. Роль коррекции метаболизма оксида азота в организме при профилактике гипертонического ремоделирования сердечно- сосудистой системы // Российский кардиологический журнал, 2002; №5 – С. 74-81.
7. Маслова М.Н., Сеченова И.М. Молекулярные механизмы стресса // Российский физиологический журнал, 2005; – № 11. – С. 1320-1328.
8. Ковалев И.В., Баскаков М.Б., Капилевич Л.В., Медведев М.А. Роль оксида азота в регуляции электрической и сократительной активности гладких мышц // Бюллетень сибирской медицины, 2012; – №1 – С. 7-25.
9. Бирулина Ю.Г., Гусакова С.В., Рязанцева Н.В., Ковалев И.В., Смаглий Л.В., Алейник А.Н. Влияние гипоксии и реоксигенации на механическое напряжение гладких мышц сосудов при фаолации α_1–адренорецепторов // Вестник науки Сибири, 2015. – Спецвып. 15. – С.390-394.
10. Vandier Ñ., Guennec Jean-Yves L., Bedfer G. What are the signaling pathways used by norepinephrine to contract the artery? A demonstration using guinea pig aortic ring segments // Adv. Physiol. Educ, 2002; 26(1-4): 195-203.
11. Ismailova Sh.N., Omonturdiev S.Z., Inomjanov D.R., Abdullaev .I.Z, Gayibov U.G., Mamajanov M.M. Influence of leonurus extract (leonurus turkestanicus) on the aortic contraction activity// NamDU Ilmiy Axborotnomasi 2024-6-son p-164-167.
12. Ismailova Sh.N, Inomjanov D.R, Abdullaev I.Z, Abdullaev A.A, Bogbekov A.I, Abduazimova D.Sh, Gayibov U.G and Aripov T.F. Determination of the role of Na+/Ca^{2+} exchanger system in the relaxant effect of motherwort (leonurus turkestanicus) extract // The Pharma Innovation Journal, 2024; 13(11): 47-49. DOI:ttps://dx.doi.org/10.22271/tpi.2024.v13.i11a.25911