# INVESTIGATION OF THE ENDOTHELIUM-DEPENDENT VASORELAXANT EFFECTS OF THE ITL-2 POLYPHENOL ISOLATED FROM ISATIS TINCTORIA L.

**G.M. Raimova[1], S.S. Khodjiyev[2], K.E. Nasirov[3]**
Institute of Biophysics and Biochemistry, National University of Uzbekistan, 100174, Tashkent, Uzbekistan[1,2,3]

***https://doi.org/10.5281/zenodo.17774275***

***Abstract***. *The article presents the results of the study of the effect of polyphenol ITL-2 on L-type calcium channels of the plasma membrane of rat aortic smooth muscle cells. L-type Ca2+ channels are Ca2+ carriers in smooth muscle cells and play an important role in changing vascular tone. The results showed that the relaxant effect of polyphenol ITL-2 extract is associated with a decrease in intracellular [Ca2+]in due to inhibition of L-type calcium channels.*

***Keywords:*** *polifenol aorta, polysaccharide, L-type Ca2+ channels, contraction, KCl and phenylephrine, relaxant.*

## INTRODUCTION

It is important to note that Isatis tinctoria L. has been used in folk medicine for centuries as an anti-inflammatory, antioxidant and tonic remedy [13,14]. Today, in the context of the widespread prevalence of diabetes mellitus, its complications and the growing need for safe natural sources for treatment, the scientific study of biologically active substances isolated from this plant is of particular importance [2,5]. From this point of view, this study is relevant, since it is aimed at assessing the possibilities of creating effective antidiabetic herbal remedies based on local raw materials. In recent years, special attention has been paid by researchers to the function of vascular endothelium, which is considered an important link in the pathogenesis of arterial hypertension, atherosclerosis, metabolic syndrome and type 2 diabetes mellitus [1,2]. Endothelial dysfunction is one of the main factors negatively affecting the functional stability of the cardiovascular system, which is manifested by impaired vascular tone, increased thrombogenicity and impaired microcirculation [3,11]. In diabetes mellitus, decreased nitric oxide (NO) synthesis or its bioavailability, increased oxidative stress, and peroxynitrite formation due to its interaction with superoxide radicals lead to profound impairment of endothelial function [4,5]. This leads to increased vasoconstriction, increased blood pressure, activation of platelet aggregation, and accelerated atherogenesis [6,7].

Insulin resistance and hyperinsulinemia are key factors in the development of endothelial dysfunction, promoting the formation of atheromas through activation of the sympathetic nervous system, impaired lipid metabolism, increased LDL levels, and increased expression of adhesion molecules [8,10]. At the same time, angiotensin II blocks insulin signaling, reduces glucose transport, and worsens hyperglycemia [10]. In the early stages of diabetes mellitus, hemorheological disturbances are observed - increased plasma viscosity, increased aggregation of erythrocytes and platelets, decreased fibrinolytic activity, increased von Willebrand factor, which predispose to vascular damage and the development of diabetic angiopathies [9]. In this regard, the search for new agents aimed at restoring the functional activity of the endothelial layer and eliminating its damage is an important task. In particular, the study of the vasorelaxant and

endothelium-protective effects of plant extracts rich in natural polyphenols is of great scientific and practical interest [12-14]. In addition to this, the aim of this study was to investigate the endothelium-dependent vasorelaxant effects of biologically active substances isolated from the Isatis tinctoria L. plant and their potential role in the correction of vascular dysfunction in diabetes mellitus, which is of great importance for the field of medicine and pharmacology.

**Research methods and materials.** Diabetic type 2 diabetes was experimentally diagnosed in 30 non-sporadic white mice with massoy tela weighing 280–340 g. A model of type 2 diabetes mellitus (T2DM) was induced in white rats by feeding them for 60 days with a high-fat diet (55% of calories due to fat) on the 61st day of the experiment after a 12-hour fasting period, freshly prepared streptozotocin solution (Sigma, USA) was injected once intraperitoneally at a dose of (35 mg/kg in 0.1 M citrate buffer with pH 4.5). The control group of animals (five) received 0.3 ml of saline solution. The experimental model of type 2 diabetes mellitus was conducted in a natural light regimen on a complete, nutrient-balanced diet for laboratory animals.

It is worth mentioning that in the experiments, the model rats were opened by cervical dislocation and the aorta was surgically isolated. The prepared aortic preparations were placed in a special chamber (5 ml) 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; pH 7.4.) and held for ~60 min. until equilibrium was reached. Then, the aortic preparation was induced to contract with KCl (50 mM) and phenylephrine (1 μM), and the force of contraction was studied in a comparative manner. [12]. Physiological solutions were oxygenated with carbogen (95% O2, 5% CO2) and maintained at +37°C using a U-8 ultrathermostat. In the experiments, the endothelial layer was mechanically removed from aortic preparations using a cotton swab and checked using acetylcholine to ensure its removal [13]. Experiments were also conducted with the eNOS blocker L-NAME, the guanylate cyclase enzyme inhibitor methylene blue, and the cyclooxygenase enzyme inhibitor indomethacin. The contractile activity of aortic rings was transmitted to a signal amplifier via a Grass FT.03 (Grass-Telefactor, USA) mechanotransducer and recorded on a computer using the *Logger Lite* program.

Our experiments were initially carried out in rats with induced diabetes. In this study, aortic preparations isolated from these diseased rats were induced to contract with KCl and phenylephrine (PE) and compared with the results obtained in healthy rat aortic preparations. In particular, in aortic preparations isolated from diseased rats, the force of contraction induced by KCl (50 mM) and PE (1 μM) was reduced by 36.5±4.4% and 45.8±3.9%, respectively, compared to the control. According to these results, it was found that diabetes reduces the force of aortic smooth muscle cells (ASMCs) induced by PE (1 μM) to a greater extent than KCl (50 mM). This is explained by the dysfunction of the receptor-controlled Ca2+ transport systems and the inositol 1,4,5-triphosphate cascade in PE-induced contractions. It can be suggested that the decrease in the contractile activity of SMHs of the sick rat aorta is related to the dysfunction of the Sa2+-transport systems in the plasma membrane and sarcoplasmic reticulum (SR). The statistical processing of data and formulating illustration with auxiliary computer program Origin 6.1 (Microsoft, SShA).

**Results and their analysis.** In our experiments, changes in the ion transport systems in the blood vessel microvessels during diabetes induced by metformin and ITL-2 polyphenol were observed. It was found that in diabetic rats induced by metformin and ITL-2, the force of contraction of the aorta induced by KCl (50 mM) increased from 36.5±4.4% to 49.7±3.6% and 44.2±4.2%, respectively, compared to rats not treated with these compounds (Fig. 1). The most

positive effect was observed in the aorta of rats treated with ITL-2, i.e., an increase in the force of contraction was observed by 23.3±3.2%.

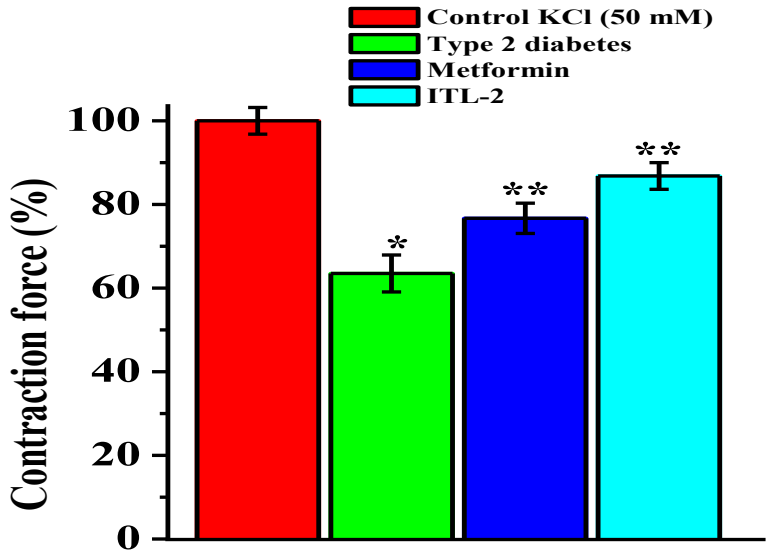



***Figure 1. Contractile force induced by KCl (50 mM) in rat aortic preparations treated with type 2 diabetes and metformin, ITL-2 compounds.***

The ordinate axis represents the contractile force of the aortic preparation in percent, with the contractile force induced by 50 mM KCl taken as 100% (*p<0.05; **p<0.01; n=3). It has been reported in the literature that the contraction of aortic vascular smooth muscle preparations induced by KCl occurs due to the activation of voltage-gated Ca2+ channels located in the cell plasmalemma, which leads to an increase in intracellular Ca2+ ions [14]. In view of this, the results obtained may be due to the normalization of the activity of voltage-gated Ca2+ channels, which leads to an increase in intracellular Ca2+ concentration.

The dynamics of Ca2+ in the cell is regulated by Ca2+ transport systems in the SR together with Ca2+L–, Ca2+R– channels in the membrane [15]. Usually, in vitro conditions, the α1-adrenoreceptor agonist - PE is used to determine and assess the participation of Ca2+ transport systems in the mechanisms of contraction of the SNS [16]. In subsequent experiments, it was found that the contraction force of rat aortic blood vessels treated with metformin, ITL-2 combination, induced by PE (1 μM) increased compared to diabetic. In this case, it was found that the contraction force of rat aorta treated with metformin, ITL-2 increased from 45.8±3.9% to 71.2±3.9%, and 64.9±3.6%, respectively (Fig. 2).

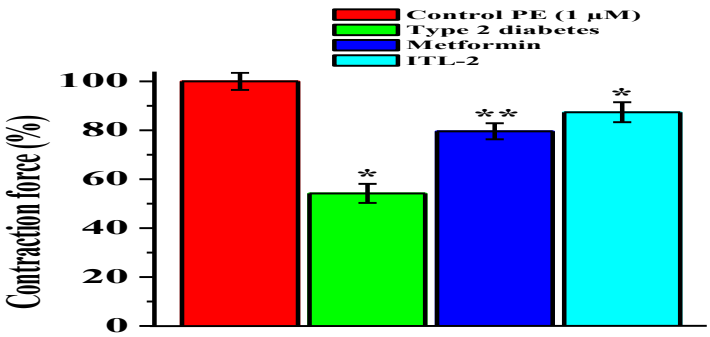


***Figure 2. Contractile force induced by PE (1 μM) in rat aortic preparations with type 2 diabetes and treated with a combination of metformin and ITL-2.***

The ordinate axis represents the percentage of the aortic preparation contractile force, with the force induced by 1 μM PE being taken as 100% (*p<0.05; **p<0.01; n=4). It is known that PE acts on α1-adrenoreceptors located in the plasmalemma of NMSs, leading to the activation of phospholipase C (PLC) through G-protein activation, which in turn leads to the formation of inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol 4,5-biphosphate (PIP2) [17]. The resulting IP3 activates the inositol 1,4,5-triphosphate receptor (IP3R) located in the SR membrane, which increases the release of [Ca2+]SR into the cytosol and stimulates an increase in the amount of [Ca2+]in in the SNS and the onset of contraction. DAG, in turn, activates receptor-gated Ca2+ channels in the plasmalemma through tyrosine kinase systems, which increases the entry of Ca2+ ions from outside the cell into the cell and causes muscle contraction [17].

It should be emphasized that the results obtained suggested that these compounds, together with the Ca2+L– and Ca2+R– channels of the plasmalemma of the SNS that are damaged by diabetes, can restore the functional activity of IP3R in the SR. Also, the literature provides information on the destruction of the vascular endothelial layer by diabetes mellitus. In this regard, metformin may affect the vascular function of ITL-2 through a number of endothelium-dependent mechanisms. In experiments, it was found that the removal of the endothelial layer from the aortic preparation significantly affected the vasorelaxant effect of the ITL-2 combination and metformin. In particular, in the aorta preparation with the removed endothelial layer, the relaxant effect of ITL-2 (800 μM) on the force of PE-induced contraction in the aorta preparation with the removed endothelial layer was reduced from 61.5 ± 3.6% to 23.7 ± 4.4% (Fig. 3, B). In similar experiments, it was observed that metformin (600 μM) reduced the relaxant effect on PE-induced contraction force in endothelium-free aortic preparations from 82.2±3.9% to 48.7±4.2% (Fig. 3, C).

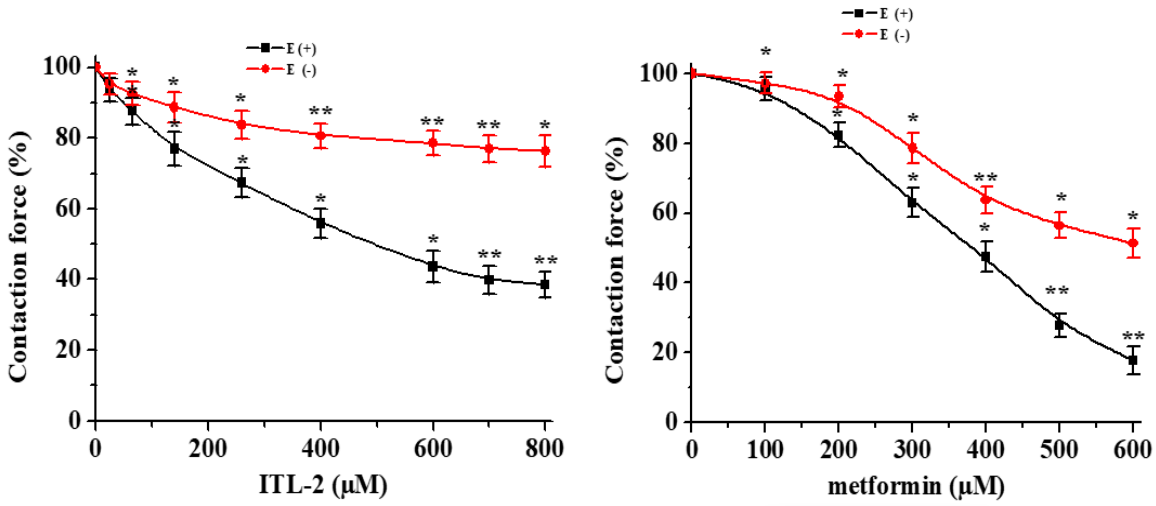



***Figure 3. Effects of ITL-2 (A) and metformin (C) on PE-induced contractile force in rat aortic preparations with and without endothelium (+) and without endothelium (–).***

The ordinate axis represents the contractile force of the aortic preparation, taken as 100%. The abscissa axis represents the concentration of polyphenols (μM). (*p<0.05; **p<0.01; n=5).

The results showed that polyphenols have a strong vasorelaxant effect, which is endothelium-dependent, and that activation of NO synthase may promote smooth muscle

relaxation by reducing the influx of Ca2+ ions through Ca2+L and Ca2+R channels in the plasma membrane.

It must be acknowledged that endothelial cells produce a number of vasoactive factors, among which nitric oxide (NO) plays a leading role in vascular smooth muscle relaxation [18, 19]. NO molecules are lipophilic, therefore they freely penetrate the CSF. Guanylate cyclase, activated by nitric oxide, synthesizes cyclic guanosine monophosphate (cGMP), which serves as an activator of another enzyme, protein kinase G. Protein kinase G activates some potassium channels, which causes hyperpolarization of the NMS (shifts the membrane potential to the negative side) [14]. In addition, it inhibits the entry of Ca2+ into the cell from the membrane and the exit of Ca2+ from the SR, ensuring the relaxation of NMS.

In this regard, the effect of the NO-synthase inhibitor L-NAME (ethyl ester of Nω-nitro-L-arginine), which is a structural analogue of the substrate of the NO-synthase enzyme, on the effect of the studied polyphenols was studied. In these experiments, it was found that the relaxant effect of ITL-2 and metformin polyphenol on the force of PE-induced aortic contractions was reduced to 23.7±3.2% and 20.9±4.4%, respectively, compared to the control, in the presence of 100 μM L-NAME (Table 1).

***Table 1. Effect of endothelium, L-NAME, methylene blue, and indomethacin on the vasorelaxant activity of ITL-2 and metformin polyphenol under PE-induced contraction conditions (*p□0.05; **p□0.01; n=4).***

| **Experimental conditions** | **Vasorelaxation %** | |
|---|---|---|
| | **ITL-2** | **Metformin** |
| Endothelium (+) | 61,5±3,6%** | 82,2±3,9% ** |
| Endothelium (-) | 23,7±4,4%* | 48,7±4,1%* |
| L-NAME (100 μM) | 29,2±3,7%** | 52,4±4,3%* |
| Methylene blue (10 μM) | 37,8±4,3%* | 61,3±4,0%* |
| Indomethacin (10 μM) | 58,6±3,8%** | 79,6±3,7%** |

In such experiments, it was observed that the effect of ITL-2 polyphenol on the force of contraction of aortic preparations induced by PE under the conditions of incubation with the guanylate cyclase enzyme inhibitor methylene blue (10 μM) decreased from 61.5±3.6% to 37.8±4.3%, and the effect of metformin polyphenol decreased from 82.2±3.9% to 61.3±4.0% (Table 1). Also, in experiments with the participation of the cyclooxygenase enzyme inhibitor - indomethacin (10 μM), no changes in the relaxant effect of polyphenols were observed.

**Conclusion.** It is necessary to highlight that the NO/sGC/cGMP/PKG signaling pathway plays an important role in the significant reduction of the vasorelaxant effect of ITL-2 and metformin polyphenols in the absence of the endothelial layer and under the influence of the NO synthase inhibitor L-NAME and the guanylate cyclase inhibitor methylene blue. The greatest reduction in the relaxant effect was observed in ITL-2 and metformin polyphenols. Activation of the NO/sGC/cGMP/PKG signaling pathway under the influence of these polyphenols reduces the entry of Ca2+ ions through Ca2+L and Ca2+R channels in the plasma membrane, as a result of which the amount of [Ca2+]in in smooth muscle cells decreases and smooth muscle relaxation is ensured.

**REFERENCES**

1. Rajendran P. et al. The vascular endothelium and human diseases. Int J Biol Sci. 2013;9(10):1057–1069.
2. Davignon J., Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004;109:III27–III32.
3. Tang S.C. et al. Diabetic nephropathy and endothelial dysfunction. J Nephrology. 2011;24(1):83–91.
4. Förstermann U., Sessa W.C. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;33:829–837.
5. Ceriello A., Motz E. Oxidative stress and diabetes. ATVB. 2004;24:816–823.
6. Libby P. et al. Inflammation in atherosclerosis. JACC. 2009;54:2129–2138.
7. Deanfield J.E. et al. Endothelial function and clinical relevance. Circulation. 2007;115:1285–1295.
8. Reaven G.M. Insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2003;88:3497–3509.
9. Ziegler D. et al. Endothelial dysfunction in prediabetes. Diabetology. 2008;51:1020–1029.
10. Muniyappa R., Quon M.J. Insulin action in vascular endothelium. Curr Opin Clin Nutr Metab Care. 2007;10:523–530.
11. Halcox J.P. et al. Prognostic value of endothelial function. Circulation. 2002;106:653–658.
12. Heinrich M. et al. Fundamentals of Pharmacognosy and Phytotherapy. Elsevier; 2012.
13. Wang Y. et al. Anti-inflammatory and antioxidant activities of Isatis tinctoria. J Ethnopharmacol. 2014;155:1405–1412.
14. Zhang X. et al. Pharmacological properties of Isatis tinctoria. Pharmacogn Rev. 2018;12:45–52.
15. M. Guli Raimova, S. Siroj Khodzhiev, E. Kabul Nasirov, K. Nozima Makhmudova, R. Rustam Makhmudov The Effect Of Polyphenols On Hemostasis System Disorders In Rats With Experimental Diabetes Texas Journal of Multidisciplinary Studies ISSN NO: 2770-0003
16. https://zienjournals.com June 2025.45-49.
17. Esimbetov A.T., Zaripov A.A., Usmanov P.B. Izuchenie endothelium - zavisimoe vazerolaksantnoe deystvie flavonoid chrysina // Universum: khimiya i biologiya. – 2019. – No. 11(65). - P.47-51.
18. Jackson W.F. Potassium Channels in Regulation of Vascular Smooth Muscle Contraction and Growth // Adv Pharmacol. - 2017. - V.78. - P.89-144.
19. Jackson W.F. Ion Channels and Vascular Tone // Hypertension – 2000. – V.35. - P.173-178.
20. Lee C.H., Poburko D., Sahota P., Sandhu J., Ruehlmann D.O., van Breemen C. The mechanism of phenylephrine-mediated [Ca2+]i oscillations underlying tonic contraction in the rabbit inferior vena cava // J Physiol. - 2001. - V.534. - P.641-650.
21. Grześk E., Malinowski B., Tejza B., Wiciński M., Kołtan S., Szadujkis-Szadurska K., Kulczyńska M., Kołtan A., Grześk G. Effect of phospholipase c inhibition on vascular smooth muscle contraction // Medical and Biological Sciences - 2015. - V.29. - P.33-39.
22. Kovalev I.V. Mechanistic regulation of oxidizing nitrogenous electrical and secretory functions of smooth muscles // Abstract of the medical abstract. (03.00.13 – physiology, 03.00.02 – biophysics). - Tomsk. – 2012. – S.35.
23. Tousoulis D., Kampoli A., Tentolouris C., Papageorgiou N., Stefanadis C. The role of nitric oxide on endothelial function // Curr. Wasc. Pharmacol. – 2012. – V.10(1). – P.4-18.