Central Asian Research Journal For Interdisciplinary Studies (CARJIS). ISSN (online): 2181-2454
Volume 4 | Special Issue | July, 2025 | SJIF: 5,965 | UIF: 7,6 | ISRA: JIF 1.947 | Google Scholar |
https://carjis.afu.uz/

UDK 577.352.4

THE INTERACTION OF LIVER MITOCHONDRIAL MEGAPORES WITH POLYPHENOL COMPOUNDS RELATED TO THE PYRUVATE-MALATE SUBSTRATE

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Annotatsiya

Ushbu tadqiqotda ba'zi polifenol birikmalarining kalamush jigar mitoxondriyalarida siklosporin A (Cs-A) ga sezgir megaporlar funktsiyasiga ta'siri o'rganildi. Piruvat-malat substratlari va 10 mkM Ca^{2+} tomonidan qo'zg'atilgan mitoxondriyal megaporlardagi konformatsion o'zgarishlar tahlil qilindi. Natijalarga ko'ra, geksagidroksifenolil-1-(o-b-D-glyukopiranozid)-2-(o-4-D-galloyl-b-D-glyukopiranozid) 100 mkM konsentratsiyada nazoratga nisbatan megapor faolligini 70,5±4,8% ga inhibe qilgan. Bundan tashqari, geksagidroksifenol-1-(o-2-o-galloil-b-D-glukopiranozid)-1-(o-b-D-ksilopiranozid) 100 mkM konsentratsiyada 72,5±2,9% inhibitiv ta'sir ko'rsatdi.

Kalit so'zlar: jigar mitoxondriyalari, megaporlar, polifenol birikmalar.

Аннотация

В данном исследовании изучалось влияние некоторых полифенольных соединений на функцию циклоспорина А (Cs-A)-чувствительных мегапор в митохондриях печени крыс. Анализировались конформационные изменения митохондриальных мегапор, индуцированные пируват-малатными субстратами и 10 мкМ Ca^{2+}. Согласно результатам, гексагидроксифеноил-1-(о-β-D-глюкопиранозид)-2-(о-4-D-галлоил-β-D-глюкопиранозид) в концентрации 100 мкМ ингибировал активность мегапор на 70,5±4,8% по сравнению с контролем. Кроме того, гексагидроксифеноил-1-(о-2-о-галлоил-β-D-глюкопиранозид)-1-(о-β-D-ксилопиранозид) в концентрации 100 мкМ продемонстрировал ингибирующий эффект 72,5±2,9%.

Ключевые слова: митохондрии печени, мегапоры, полифенольные соединения.

Abstract

In this study, the effect of certain polyphenol compounds on the function of cyclosporin A (Cs-A) sensitive megapores in rat liver mitochondria was investigated. The conformational changes of mitochondrial megapores induced by pyruvate-malate substrates and 10 μM Ca^{2+} were analyzed. According to the results, hexahydroxyphenoyl-1-(o-β-D-glucopyranoside)-2-(o-4-D-galloyl-β-D-glucopyranoside) at a concentration of 100 μM inhibited megapores activity by 70.5±4.8% compared to the control. Additionally, hexahydroxyphenoyl-1-(o-2-o-galloyl-β-D-glucopyranoside)-1-(o-β-D-xylopyranoside) at 100 μM showed an inhibitory effect of 72.5±2.9%.

Key words: Liver mitochondria, megapore, polyphenolic compounds.

Introduction.The mitochondrial permeability transition pore (mPTP) is a protein complex located between the inner and outer mitochondrial membranes, through which molecules up to 1500 Da can pass freely. The opening of the mPTP is triggered by several factors, including Ca^{2+} ions, various reactive compounds, and oxidative stress. This pore can be inhibited by cyclosporin A (Cs-A) or bongkrekic acid. When the pore opens, the mitochondrial membrane potential ($\Delta\Psi m$) is lost. As a result, the mitochondrial matrix swells due to the influx of water and other substances, leading to the disruption of the

outer membrane's integrity. Pro-apoptotic proteins are released from the intermembrane space into the cytosol, ultimately causing cell death [1,2]. One of the key organelles involved in regulating cellular Ca^{2+} homeostasis is the mitochondrion [3]. Mitochondria are among the first elements to respond to oxidative stress, which can contribute to the development of various pathological processes [4]. Under oxidative stress conditions, the conformational state of the mitochondrial permeability transition pore (mPTP) located in the inner mitochondrial membrane shifts to an open state, allowing small molecules to non-specifically enter the mitochondrial matrix [4, 5]. This is due to the activity of the Ca^{2+}-dependent cyclosporin A (Cs-A)-sensitive permeability transition pore (PTP), which is directly linked to apoptosis and necrosis processes [6, 7]. The opening of the Cs-A-sensitive pore leads to mitochondrial swelling, outer membrane rupture, and the release of intermembrane components into the cytosol, which ultimately triggers apoptosis [8]. Additionally, oxidative stress disrupts the antioxidant-prooxidant system in tissues, leading to enhanced lipid peroxidation. Lipid peroxidation exacerbates many pathological processes [9, 10].

In this context, it is crucial to study the cellular and subcellular mechanisms by which biologically active compounds extracted from plants affect the organism, as this helps in identifying potential pharmacological compounds. Among biologically active substances, polyphenolic compounds hold a special place. Polyphenols are a broad spectrum of biologically active compounds that exhibit various properties, such as antioxidant, hepatoprotective, antihypoxic, and membraneotropic activities [11, 12, 13]. To better understand the mechanisms of action of polyphenolic compounds extracted from plants, and to develop new therapeutic agents based on these compounds, it is essential to investigate their effects on cells and mitochondria.

The aim of this study is to explore the impact of polyphenolic compounds extracted from Plantago major L— specifically hexahydroxyphenyl-1-(O-β-D-glucopyranoside)-2-(O-4-D-galloyl-β-D-glucopyranoside) and hexahydroxyphenyl-1-(O-2-O-galloyl-β-D-glucopyranoside)-1-(O-β-D-xylopyranoside) — on the mitochondrial permeability transition pores (mPTP) of liver mitochondria.

Materials and methods: The studies were conducted on albino rats weighing 180-200g. Liver mitochondria were isolated from the rats using the Schneider method [14] by differential centrifugation. The composition of the isolation medium was as follows: 250 mM sucrose, 10 mM tris-HCl, 1 mM EDTA, pH 7.4. The centrifugation process was carried out in two stages. In the first stage, centrifugation was performed at 1500 rpm for 7-8 minutes. In the second stage, centrifugation was carried out at 6000 rpm for 15 minutes. The purified mitochondria were transferred to a special container using a pipette. For the experiments, mitochondria were diluted in an EDTA-free isolation medium at a 10:1 volume ratio and stored in a container with ice.

To study the effect of the polyphenolic compounds on the mitochondrial PTP, an incubation medium with pyruvate-malate oxidation substrates was used (mM): sucrose – 200, pyruvate – 5, malate – 5, KH_2PO_4 – 1, Ca^{2+} – 10 μM, EGTA buffer – 0.02, HEPES – 20, tris-HCl – 20, rotenone – 0.002, oligomycin – 1 μg/ml, pH 7.2; mitochondrial protein – 0.4 mg/ml) [15].To study the Ca2+-dependent PTP state of the mitochondrial membrane, the kinetics of mitochondrial swelling were assessed at an optical density of 540 nm wavelength, with a protein concentration in the medium of 0.3-0.4 mg/ml at a temperature of 26°C.The obtained results were statistically analyzed using the Origin 6.1 computer software. Results were considered significant at $P<0.05$.

Results and discussion: According to the results of the experiments, the swelling of liver mitochondria induced by 10 μM Ca^{2+} was

evaluated as the opening of the PTP. (Figure 1) The 25 µM concentration of the polyphenolic compound, isolated from Plantago major L, consisting of hexahydroxy-diphenyl-1-(O-2-O-galloyl-β-D-glucopyranoside)-1-(O-β-D-xylopyranoside), was found to reduce the swelling of liver mitochondria from rats induced by 10 µM Ca^{2+} by 15.9 ± 2.7% compared to the control group. This result demonstrates a significant inhibitory effect compared to the control.

The 50 µM concentration of this polyphenolic compound inhibited mitochondrial swelling by 33.1 ± 6.5% compared to the control. Moreover, the 100 µM concentration of hexahydroxy-diphenyl-1-(O-2-O-galloyl-β-D-glucopyranoside)-1-(O-β-D-xylopyranoside) was found to reduce liver mitochondrial swelling by 70.5 ± 4.8% relative to the control. These results indicate that the polyphenolic compound inhibits the opening of the mitochondrial PTP induced by Ca^{2+}. The findings are significant as they suggest that the mitochondrial PTP serves as a "target" for pharmacological compounds, and the inhibition of this structure by hexahydroxy-diphenyl-1-(O-2-O-galloyl-β-D-glucopyranoside)-1-(O-β-D-xylopyranoside) leads to the stabilization of the mitochondrial membrane (Figure 1).

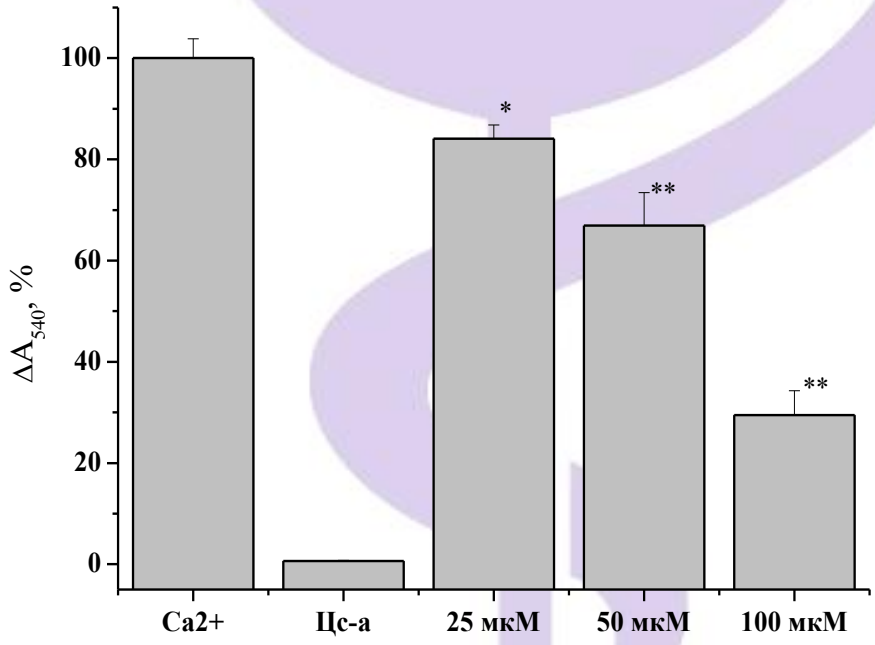

Figure 1. The effect of Xylopir-T polyphenol on Ca^{2+}-dependent megaporin in rat liver mitochondria. IM (mM): sucrose – 200, KH_2PO_4 – 1, pyruvate – 5, malate – 5, Ca^{2+}-EGTA buffer – 0.02, HEPES – 20, tris-HCl – 20, pH 7.2 (* - P<0.05, ** - P<0.01; n=5).

According to the experimental results, the 50 µM concentration of hexahydroxy-diphenyl-1-(O-β-D-glucopyranoside)-2-(O-4-D-galloyl-β-D-glucopyranoside), isolated from *Plantago majorL*, inhibited the swelling of liver mitochondria induced by 10 µM Ca^{2+} by 24.2±2.2%. Furthermore, the 100 and 150 µM concentrations of this polyphenolic compound inhibited the swelling of liver mitochondria induced by 10 µM Ca^{2+} by 65.2±3.5% and 83.7±2.6%, respectively (Figure 3).

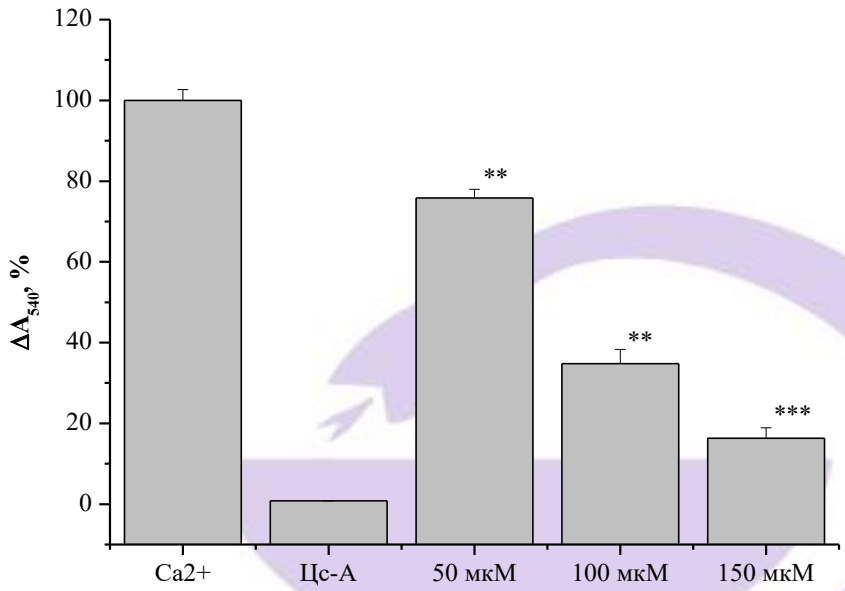

Figure 2. The effect of Glucopir-T polyphenol on Ca^{2+}-dependent megaporin in rat liver mitochondria. IM (mM): sucrose – 200, KH_2PO_4 – 1, pyruvate – 5, malate – 5, Ca^{2+}-EGTA buffer – 0.02, HEPES – 20, tris-HCl – 20, pH 7.2 (** - P<0.01, *** - P<0.001; n=5).

The obtained results indicate that the studied polyphenolic compounds cause dose-dependent inhibition of Ca^{2+}-dependent mitochondrial megaporin in rat liver mitochondria. Among these polyphenolic compounds, hexahydroxy-diphenyl-1-(O-β-D-glucopyranoside)-2-(O-4-D-galloyl-β-D-glucopyranoside) was found to inhibit mitochondrial megaporin more strongly compared to the second polyphenolic compound. Like other polyphenolic compounds [16], these polyphenolic compounds were also shown to inhibit mitochondrial megaporin.

Conclusion: The studied polyphenolic compounds, exhibiting high antioxidant properties, were shown to inhibit mitochondrial megaporin in rat liver mitochondria. The hexahydroxy-diphenyl-1-(O-2-O-galloyl-β-D-glucopyranoside)-1-(O-β-D-xylopyranoside) polyphenolic compound at a 100 μM concentration inhibited mitochondrial megaporin by 70.5±4.8%, while the hexahydroxy-diphenyl-1-(O-β-D-glucopyranoside)-2-(O-4-D-galloyl-β-D-glucopyranoside) polyphenolic compound at a 150 μM concentration inhibited it by 83.7±2.6%.

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