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Tannins as Modulators in the Prevention of Mitochondrial Dysfunction

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Abstract

In this article, the effects of octagalloyl-glucose and nonagalloyl-glucose hydrolyzable tannins isolated from the leaves of *Pistacia vera L.*, a plant of the *Anacardiaceae* family, on lipid peroxidation (LPO), mitochondrial permeability transition pore (mPTP) status, and ATP-dependent K^+ (mitoKATP) channel activity in mitochondria, which are directly involved in various pathologies, as well as their antiradical properties were studied *in vitro*. *In vitro* experiments showed that octagalloyl-glucose and nonagalloyl-glucose tannins inhibited the Fe^{2+}/ascorbate-induced LPO process in mitochondria by 86.7 ± 4.4% and 85.5 ± 4%, respectively, at a concentration of 10 μM, while they blocked mitochondrial mPTP by 88.2 ± 4.5% and 90.3 ± 5.1%, respectively, at a concentration of 15 μM in succinate + rotenone medium. MitoKATP-channel of rat liver mitochondria was activated by octagalloyl-glucose at 10 μM concentration by 90.9 ± 2.6%, nonagalloyl-glucose at 5 μM concentration by 84.2 ± 1.8%, but at 10 μM concentration, it was able to activate by 50.5 ± 5.3%. Octagalloyl-glucose and nonagalloyl-glucose tannins at a concentration of 15 μM neutralized DPPH radical by 91.7 ± 1.83% and 85.9 ± 3.5%, respectively. According to the obtained results, taking into account that the high antiradical and antioxidant activity of the studied hydrolyzable tannins (octagalloyl-glucose and nonagalloyl-glucose) at low concentrations have a stabilizing effect on the mitochondrial membrane, it is possible to conduct additional experiments that allow creating membrane-active preparations from them.

Keywords: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Hydrolysable tannins, Lipid peroxidation (LPO), Mitochondrial ATP dependent potassium (mitoKATP) channel, Mitochondrial membrane, Mitochondrial permeability transition pore (MPTP), Nonagalloyl glucose, Octagalloyl glucose, *Pistacia vera L.*

Introduction

These findings suggest that some hydrolysable tannins, such as octagalloyl glucose and nonagalloyl glucose from *Pistacia vera L.*, are potent modulators of mitochondrial bioenergetics and redox homeostasis. Through their interaction with mitochondrial ion channels, these substances not only reduce oxidative damage but also participate in the regulation of calcium homeostasis and membrane potential. The ability of such tannins to influence the functional status of the MPTP and mitoKATP channels may be able to play a protective role by decelerating or inhibiting the onset of mitochondrial permeability transition, a key step towards cell death pathway activation [1,2].

Furthermore, antiradical activity in such tests as 2,2-diphenyl-1-picrylhydrazyl (DPPH) also attests to their capacity to scavenge ROS and thereby limit the initiation of cascades of lipid peroxidation. By preservation of structural and functional integrity of the mitochondrial membrane, these phytocompounds ensure preservation of ATP synthesis and cellular energy metabolism as a whole under stressful conditions [3].

Herein, natural antioxidant exploitation like tannins from plant origin has a potential therapeutic strategy against mitochondrial dysfunction-related disorders. These are cardiovascular diseases, neurodegenerative disorders, and metabolic syndromes where oxidative stress and mitochondrial dysfunction are the overweighing pathological features. Recent research is aimed at obtaining more insight into the molecular mechanism of tannin and mitochondrial channels interaction and optimization of their use in particular therapies [4,5].

Material and methods

Chemicals

Octagalloyl glucose and nonagalloyl glucose, belonging to the group of hydrolyzable tannins, were isolated from the leaves of *Pistacia vera L.* [6]. The purity of these compounds ranged between 86 – 90%. Octagalloyl glucose and nonagalloyl glucose tannins were obtained from the Proteins and Peptides Laboratory of the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan. All reagents which are used in the experiments purchased by Sigma Aldrich company.

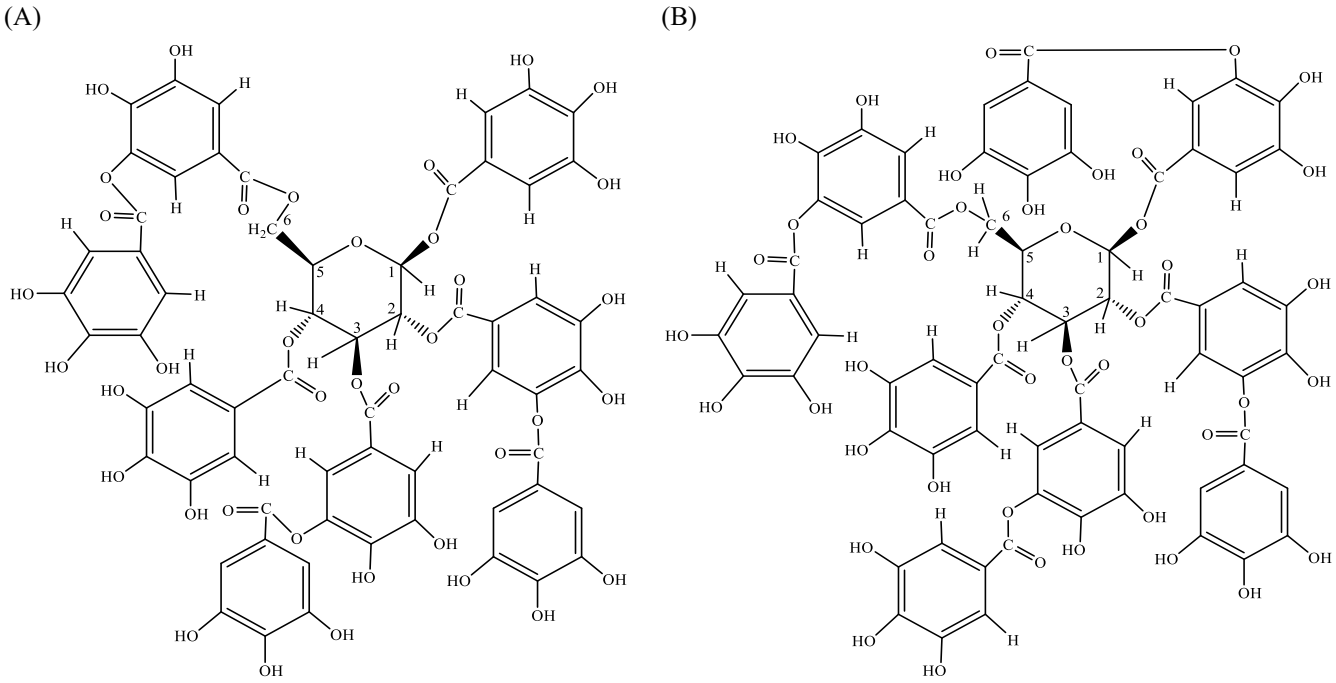


Figure 1 Chemical structure of octagalloyl-glucose (A) and nonagalloyl-glucose (B) hydrolyzable tannins.

Isolation of liver mitochondria

Liver mitochondria were isolated by differential centrifugation [7,8]. The isolation medium consisted of 250 mM sucrose, 10 mM Tris-HCl, and 1 mM EDTA (pH 7.4). After decapitation, the rat's abdominal cavity was opened, and the liver was excised and placed in a beaker containing ice-cold isolation medium. The liver mass was measured, then mechanically homogenized. The tissue was further homogenized in a Teflon homogenizer at a 1:6 g/mL ratio with the isolation medium. The homogenate was subjected to the first centrifugation at 1500 rotation/min (400× g) for 7 min at 0 - 2 °C using an RS-6MC centrifuge with an angled rotor. This step removed large tissue fragments and cell debris. The resulting supernatant was centrifuged again at 6,000 rpm for 15 min at 0 - 2 °C. The mitochondria, which had settled at the bottom of the tube, were separated from the supernatant. Any remaining liquid and lipid droplets on the tube walls were removed using filter paper.

The purified mitochondria were resuspended in isolation medium without EDTA at a 10:1 g/mL dilution

to prepare a mitochondrial suspension, which was stored in a special ice-cooled container for experiments. Protein concentration in mitochondria was determined using the Biuret method [9].

Determination of lipid peroxidation

Lipid peroxidation (LPO) in mitochondria was assessed using the Fe^{2+}/ascorbate system. This process leads to mitochondrial swelling due to the loss of membrane barrier function, which was measured spectrophotometrically. The incubation medium contained 125 mM KCl, 10 mM Tris-HCl (pH 7.4). LPO was induced by adding 10 μM $FeSO_4$ and 600 μM ascorbate, with a mitochondrial protein concentration of 0.3 - 0.4 mg/mL in the cuvette [10].

Determination of antiradical activity

Antiradical activity was evaluated by measuring the kinetics of stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction using octagalloyl glucose [11].

Determination of mitochondrial megapore (mPTP) activity

The state of the Ca^{2+}-dependent cyclosporin A-sensitive mitochondrial permeability transition pore (MPTP) was assessed spectrophotometrically at 540 nm by monitoring changes in mitochondrial swelling kinetics [12]. The incubation medium contained 200 mM sucrose, 20 μM EGTA, 20 mM Tris, 20 mM HEPES, 1 mM KH_2PO_4, 5 mM succinate, and 2 μM rotenone (pH 7.4). Mitochondrial protein concentration was 0.3 - 0.4 mg/mL, and the temperature was maintained at 26 °C.

Determination of ATP-sensitive potassium channel activity in mitochondria

The activity of mitochondrial ATP-sensitive potassium channels (mitoK_ATP) was determined spectrophotometrically at 540 nm by analyzing changes in mitochondrial swelling kinetics [13]. The incubation medium contained 125 mM KCl, 10 mM HEPES, 5 mM succinate, 1 mM $MgCl_2$, 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, and 2 μM rotenone (pH 7.4). The mitochondrial protein concentration in the cuvette was 0.3 - 0.4 mg/mL, and the temperature was maintained at 26 °C.

For statistical analysis of mitoK_{ATP} activity, the results were compared between the −ATP and +ATP conditions. The effects of tannins were evaluated by comparing results with the +ATP condition.

Animal ethics

The Animal Use Committee of our institution approved all experimental procedures and preoperative care guidelines. Animals were housed in standard vivarium conditions (humidity: 55 - 65%, temperature: 22 ± 2 °C) with free access to drinking water and laboratory food. All manipulations with animals were carried out in accordance with the European Convention for the Protection of Animals Used for Scientific Purposes (1998) and the International Bioethical Guidelines of the Institute of Biophysics and Biochemistry of the National University of Uzbekistan (BRC/IBB-N44/2024/75-1). All operations were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering. Albino Wistar rats weighing 180 - 220 g were used in the experiments.

Statistics

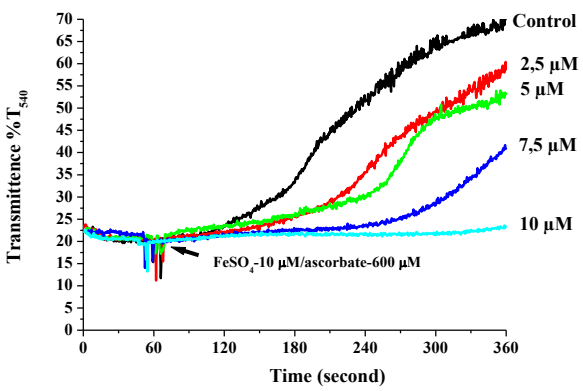
Statistical analysis and graphical illustrations were performed using OriginPro 8.5 (USA). Data were obtained from 5 to 8 independent experiments (n = 5 - 8). A paired t-test was used for analyzing combined data, while an unpaired t-test was applied for comparisons between individual groups. Statistical significance was set at 2 levels: $p < 0.05$ and $p < 0.01$.

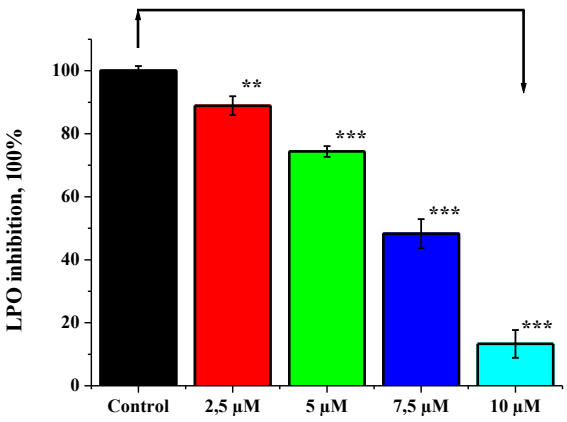
Results and discussion

The shape and number of mitochondria depend on the metabolic activity of the cell. They are plastic and dynamic, participating in the processes occurring in the cell. Therefore, mitochondrial dysfunction is studied separately in the development of various diseases. Most chronic liver diseases are associated with the degree of damage to mitochondria. Liver mitochondria have unique properties compared to mitochondria of other organs, as they are necessary for the metabolism of carbohydrates, lipids and proteins in the liver, and play a role in apoptosis and necrosis to ensure the viability of hepatocytes [14]. Mitochondria participate in metabolic (glucose and fatty acid metabolism) and bioenergetic processes, including ATP synthesis, membrane potential maintenance, and the generation of reactive oxygen

species (ROS) [15]. Mitochondrial dysfunction is associated with increased ROS production and oxidative phosphorylation impairment [16]. More than 50 mitochondrial transporters, belonging to a superfamily of nuclear-encoded proteins, have been identified in the inner mitochondrial membrane [17]. The transporters catalyze the transport of a range of metabolites, nucleotides, cofactors, amino acids, carboxylic acids, fatty acids, inorganic ions, and vitamins across the inner mitochondrial membrane [18]. Mitochondrial transporters play essential roles in a variety of cellular processes, including oxidative phosphorylation of lipids and carbohydrates, amino acid metabolism, macromolecule synthesis, ion homeostasis, cellular regulation, and differentiation [19]. These complex physiological processes depend upon the structural organization of mitochondrial membranes and their lipid composition [20]. Lipids provide membrane integrity and participate in ATP synthesis, cellular proliferation, metabolism, inflammation, and apoptosis as signaling molecules. Thus, structural alterations in the inner mitochondrial membrane and transport system dysfunction create the development of pathological conditions. For example, mPTP-dependent cell death is a consequence of mPTP in the open configuration, leading to necrotic cell death due to increased Ca^{2+} accumulation and ROS generation in the mitochondrial matrix [21]. ROS production, mitochondrial membrane potential, and inner membrane integrity—more precisely, the flow of various molecules across it—are part of mitochondrial biochemical and biophysical processes. More specifically, potassium ion entry into mitochondria is facilitated by a number of potassium channels in the inner mitochondrial membrane [22]. Pharmacologically, potassium ion entry into mitochondria influences membrane potential, mitochondrial volume, respiration, and ROS regulation [23]. Transition metals such as iron and copper play a key role in the production of reactive oxygen species (ROS) in the body. During the process of lipid peroxidation (LPO), free radicals not only damage proteins and DNA but also alter the function of cell membranes. As a result, various diseases can develop in the body, and their correction using plant-derived antioxidants and other phytochemical compounds has been demonstrated [24]. Based on this, the study investigated the effects of octagalloyl-glucose and nonagalloyl-glucose tannins, extracted from *Pistacia vera L.* leaves, on Fe^{2+}/ascorbate-induced LPO in liver mitochondria (**Figure 2**). The results showed that octagalloyl-glucose (**Figure 2(A)**) and nonagalloyl-glucose (**Figure 2(B)**), which are hydrolyzable tannins, exhibited inhibitory properties against Fe^{2+}/ascorbate-induced LPO at concentrations up to 10 µM, relative to the control. At a concentration of 10 µM, they inhibited LPO by 86.7 ± 4.4% and 85.5 ± 4.1%, respectively. Their half-maximal inhibitory concentrations (IC_{50}) were determined to be 7.3 ± 0.65 and 7.6 ± 0.41 µM, respectively. Similarly, other studied polyphenolic compounds also demonstrated high antioxidant properties at low concentrations [25,26]. However, it was shown that biologically active plant compounds that do not contain tannins did not exhibit high antioxidant activity [27,28]. Additionally, it has been suggested that the number, position, and substitution of functional hydroxyl groups in polyphenolic compounds may influence their antioxidant activity [29].

(A)





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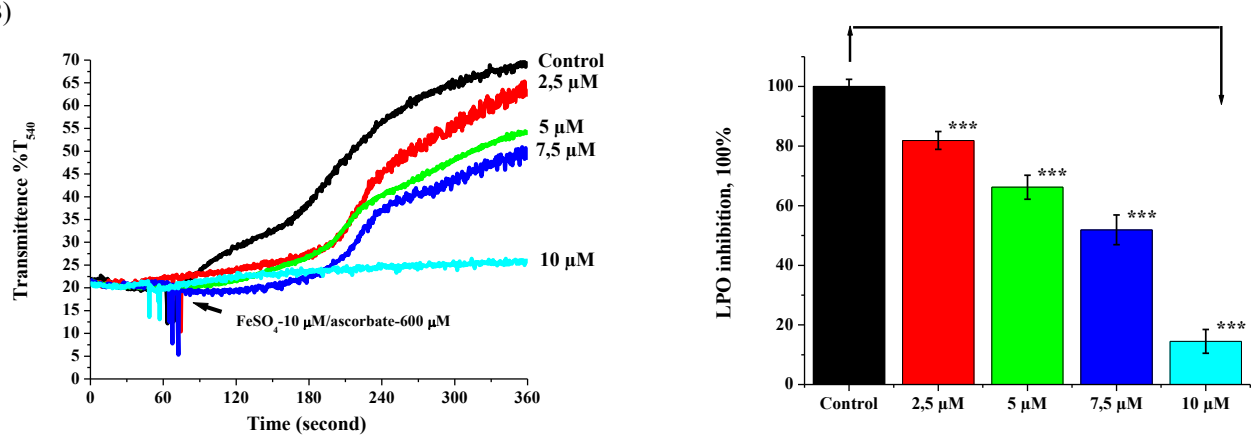
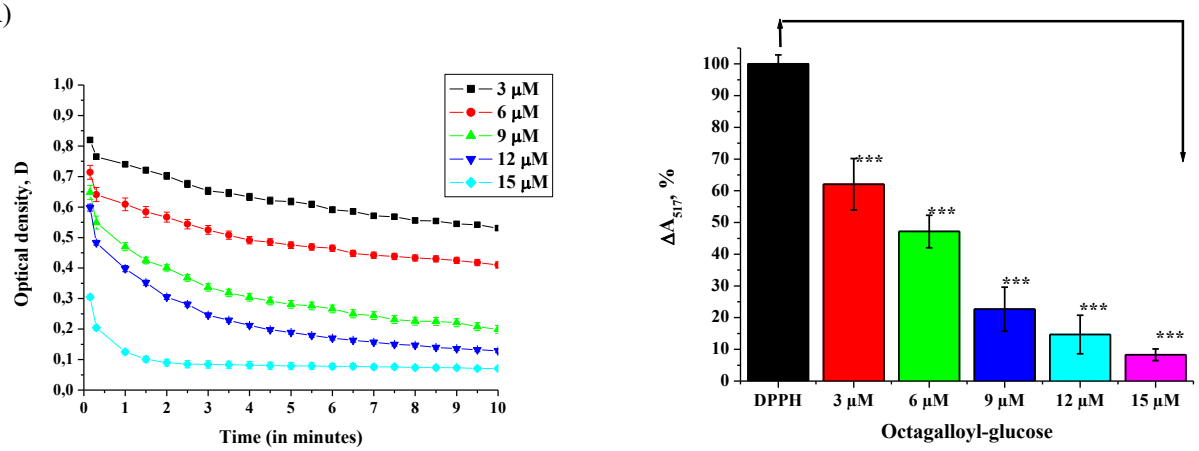


Figure 2 Effects of octagalloyl-glucose (A) and nonagalloyl-glucose (B) tannins on Fe^{2+}/ascorbate-stimulated mitochondrial LPO process (*–$p < 0.05$; **–$p < 0.01$, ***–$p < 0.001$; n = 5). (Left hand-side is original recording and right hand-side is graphic).

Thus, the studied tannins—octagalloyl-glucose and nonagalloyl-glucose—demonstrated high antioxidant properties *in vitro*. According to the literature, they may prevent the depletion of glutathione (GSH) caused by pro-oxidants and contribute to its restoration [30]. A decrease in mitochondrial GSH levels enhances the opening of mitochondrial megapore channels in a Ca^{2+}-dependent manner, leading to mitochondrial depolarization. This results in the release of pro-apoptotic proteins such as cytochrome *c*, apoptosis-inducing factor (AIF), and endonuclease G (EndoG) from mitochondria. Furthermore, excessive Ca^{2+} influx into mitochondria is observed, reducing electron flow and pyridine nucleotide oxidation while increasing reactive oxygen species (ROS) production [31]. Additionally, oxidized glutathione (GSSG) in mitochondria has been shown to block these effects without influencing the LPO process. Moreover, compounds with antioxidant properties that inhibit LPO have been shown to prevent mitochondrial membrane potential collapse [32]. The effect of tannins on the lipid peroxidation (LPO) process alone is not sufficient to fully assess their antioxidant properties. Therefore, in this study, the interaction of octagalloyl-glucose and nonagalloyl-glucose tannins with the DPPH radical was investigated to evaluate their radical-scavenging and/or inhibitory properties (**Figure 3**). The obtained results indicate that octagalloyl-glucose, at concentrations of 3 - 15 µM, interacted with the DPPH radical and inhibited it in a dose-dependent manner. At a concentration of 15 µM, the polyphenol neutralized 91.7 ± 1.83% of the DPPH radical (**Figure 3(A)**). Similarly, when studying the inhibitory properties of nonagalloyl-glucose at concentrations of 3 - 15 µM, its maximum inhibition of the DPPH radical at 15 µM was found to be 85.9 ± 3.5% (**Figure 3(B)**).

(A)



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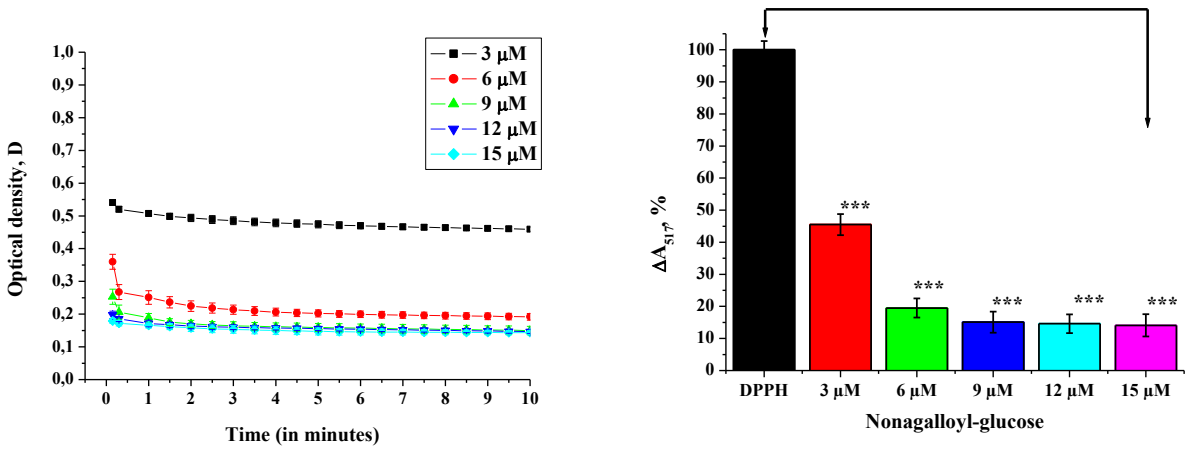


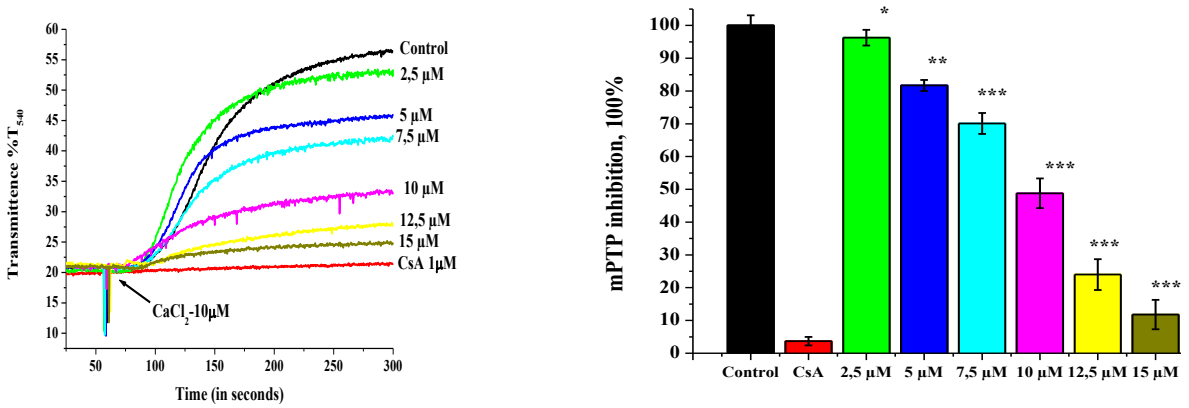
Figure 3 Octagalloyl-glucose (A) and nonagalloyl-glucose (B) tannins show antiradical property on DPPH (*–$p < 0.05$; **–$p < 0.01$, ***–$p < 0.001$; n = 5). (Left hand-side is original recording and right hand-side is graphic).

The half-maximal inhibitory concentrations (IC_{50}) of the hydrolyzable tannins, octagalloyl-glucose and nonagalloyl-glucose, were determined to be IC_{50} = 5.4 ± 0.6 µM and IC_{50} = 4.2 ± 0.5 µM, respectively. These results demonstrate that these tannins exhibit high antiradical activity at low micromolar concentrations, inhibiting the DPPH radical at small doses, similar to other studied hydrolyzable tannins [33,34].

The antiradical activity of hydrolyzable tannins has been reported to depend on their molecular weight, size, and the presence of galloyl and *ortho*-hydroxyl groups [35]. Furthermore, it has been shown that hydrolyzable tannins neutralize the DPPH radical through a 1:1 stoichiometric reaction [36]. This suggests that the presence of galloyl and *ortho*-hydroxyl groups in tannins is a key factor in their biological activity.

Based on these findings, we continued our study by investigating the effect of octagalloyl-glucose on the Ca^{2+}-dependent mitochondrial permeability transition pore (mPTP) (**Figure 4**).

(A)



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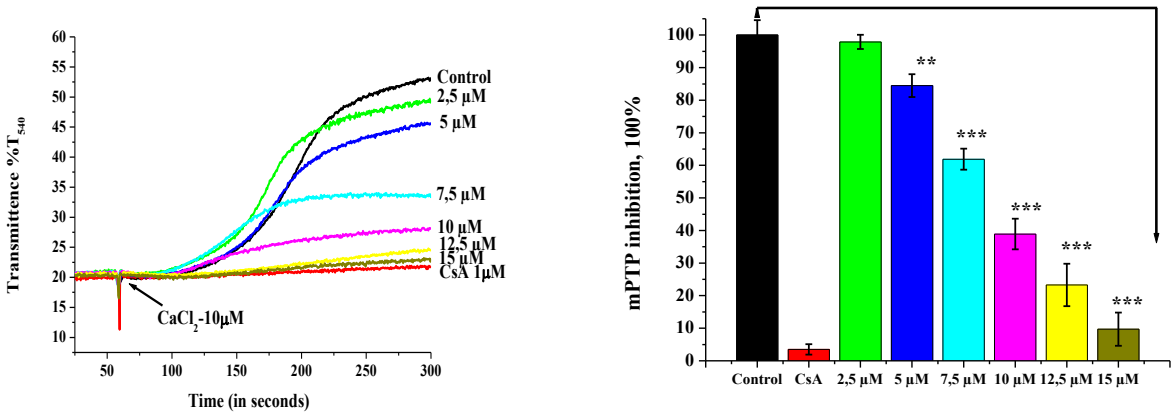


Figure 4 Effects of different concentrations of octagalloyl-glucose (A) and nonagalloyl-glucose (B) tannins on rat liver mitochondria on Ca^{2+}-dependent mPTP. Ca^{2+}-dependent high-amplitude contraction of mitochondria and complete inhibition of mPTP by CsA (5 µM) (* $-p < 0.05$, ** $-p < 0.01$, *** $-p < 0.001$; n = 5). (Left hand-side is original recording and right hand-side is graphic).

Initially, no mitochondrial swelling was observed in the incubation medium. However, after the addition of mitochondria, introducing 10 µM Ca^{2+} ions induced high-amplitude mitochondrial swelling. The presence of 5 µM CsA completely blocked this Ca^{2+}-induced swelling. It was found that the tannins octagalloyl-glucose and nonagalloyl-glucose inhibited Ca^{2+}-dependent mPTP in a concentration-dependent manner. Specifically, at 2.5 µM, both tannins exhibited only weak inhibition of Ca^{2+}-induced mPTP compared to the control. At 5 µM, octagalloyl-glucose and nonagalloyl-glucose inhibited mitochondrial permeability transition pores by 18.3 ± 1.7% and 15.5 ± 3.5%, respectively, compared to the control. At 15 µM, inhibition increased to 88.2 ± 4.5% and 90.3 ± 5.1%, respectively. However, increasing the polyphenol concentration beyond this point did not lead to further enhancement of inhibition. Therefore, the maximum effective inhibitory concentration of the tannins was determined to be 15 µM.

The results indicate that hydrolyzable tannins, such as octagalloyl-glucose and nonagalloyl-glucose, inhibit Ca^{2+}-induced mitochondrial permeability transition pore opening at low concentrations, thereby exerting a stabilizing effect on mitochondria. The half-maximal inhibitory concentrations (IC_{50}) for the inhibition of mitochondrial permeability transition pores in rat liver mitochondria were determined to be 9.8 ± 0.7 µM and 8.7 ± 0.3 µM, respectively. Previous studies have reported that hydrolyzable tannins exhibit inhibitory effects on mitochondrial permeability transition pores at concentrations of 50 - 200 µM [37]. However, in this study, octagalloyl-glucose and nonagalloyl-glucose significantly inhibited mitochondrial permeability transition at a concentration of just 15 µM.

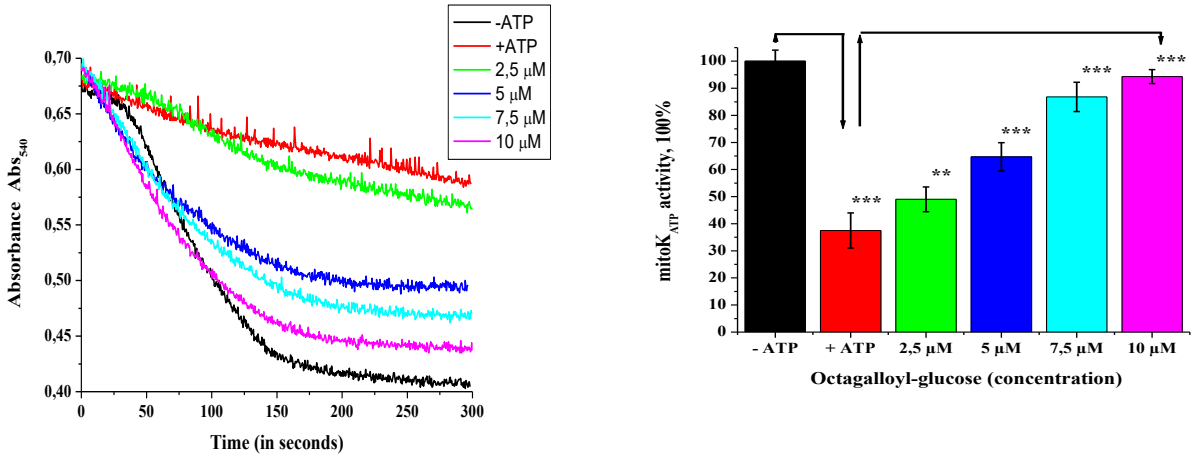
It has been shown that programmed cell death in fungal mitochondria resembles similar processes in mammals. Factors such as increased cytosolic Ca^{2+} levels and excessive ROS production can induce mitochondrial permeabilization, leading to the release of pro-apoptotic factors. In mammals, these changes are primarily driven by increased mitochondrial Ca^{2+} levels and oxidative stress [38]. Studies indicate that in fungal cell death, the regulation of mPTP involves the peptidylprolyl cis-trans isomerase gene (Cpr3) and the participation of endogenous mitochondrial Ca^{2+} and ROS. These findings suggest that tannins may prevent ROS generation and oxidative stress progression, thereby mitigating mitochondrial permeabilization.

It is well known that the activation of the ATP-sensitive K^+ channel in mitochondria (mitoKATP channel) plays a key role in cytoprotection across various cell types. In this study, the effects of octagalloyl-glucose and nonagalloyl-glucose on mitoK_{ATP} channel activity were investigated (**Figure 5**).

The experimental conditions for studying mitoK$_{ATP}$ channel activity in rat liver mitochondria were based on the incubation medium proposed by Vadziuk and Kosterin [39]. The addition of mitochondria to this medium led to a certain degree of swelling, indicating the opening of mitoK$_{ATP}$ channels. The inclusion of ATP at a physiological concentration (200 µM) partially inhibited mitochondrial swelling. This supports the characterization of the channel as an ATP-sensitive K^+ channel in mitochondria [40]. Under ATP-inhibited conditions, the addition of different compounds to the incubation medium allows for the modulation of mitoK$_{ATP}$ channel activity.

Some compounds activate the mitoK$_{ATP}$ channel, exhibiting activator properties (e.g., diazoxide, nicorandil), while others inhibit channel activity, displaying blocker properties (e.g., 5-hydroxydecanoate, MCC-134). The effects of octagalloyl-glucose (**Figure 5(A)**) and nonagalloyl-glucose (**Figure 5(B)**) tannins at concentrations of 2.5 – 10.0 µM on mitoK$_{ATP}$ channel activity under ATP-inhibited conditions were studied.

(A)



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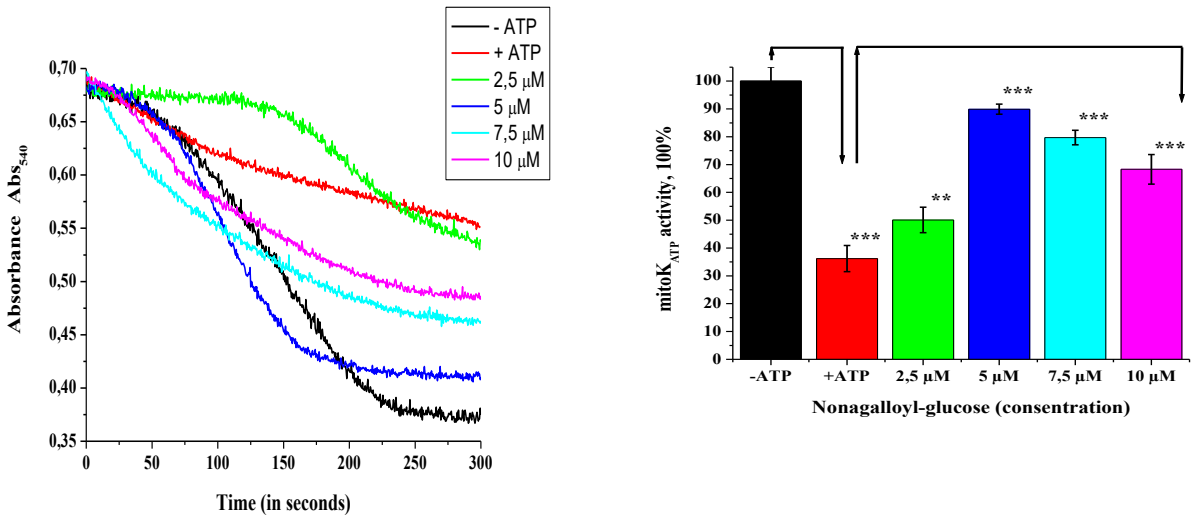

Figure 5 Effect of octagalloyl-glucose (A) and nonagalloyl-glucose (B) tannins on mitoK$_{ATP}$-channel activity of rat liver mitochondria. Effects on ATP-dependent K^+ channel. Addition of ATP at a physiological concentration of 200 µM to the incubation medium caused channel inhibition, and under these conditions the effect of the polyphenol was studied. (** $-p < 0.01$, *** $-p < 0.001$; n = 5). (Left hand-side is original recording and right hand-side is graphic).

Octagalloyl-glucose was found to have an activating effect on the mitoK$_{ATP}$ channel, increasing its activity up to 94.7 ± 2.5% at a concentration of 10 μM compared to the control (−ATP). Nonagalloyl-glucose exhibited a dome-shaped activation pattern within the 2.5 - 10.0 μM concentration range, reaching 84.2 ± 1.8% activation at 5 μM compared to the ATP-inhibited state. However, at 10 μM, the activation decreased to 50.5 ± 5.3%.

While both tannins exhibited similar effects in inhibiting lipid peroxidation (LPO) and mPTP, as well as demonstrating antiradical activity based on DPPH, their effects on mitoK$_{ATP}$ channel activation differed. Specifically, octagalloyl-glucose consistently activated the channel in a concentration-dependent manner, whereas nonagalloyl-glucose showed a biphasic effect—initially activating the channel but later leading to inhibition at higher concentrations. Similar biphasic responses have been observed in mitoK$_{ATP}$ channel activity when treated with polyphenol-rich plant extracts [41].

In this study, we investigated the effects of octagalloyl-glucose and nonagalloyl-glucose tannins on the mitoK$_{ATP}$ channel activity under ATP-inhibited conditions. Our findings demonstrate that these tannins exert differential regulatory effects on mitoK$_{ATP}$ channel activity, potentially contributing to their protective roles in mitochondrial function.

Octagalloyl-glucose exhibited a concentration-dependent activation of the mitoK$_{ATP}$ channel, reaching a maximum activation of 94.7 ± 2.5% at 10 μM. This suggests that octagalloyl-glucose may serve as a potent mitoK$_{ATP}$ channel opener, which could enhance mitochondrial protection by preventing calcium overload and oxidative stress. The activation of mitoK$_{ATP}$ channels is known to be linked to cardioprotective mechanisms, including reduced mitochondrial permeability transition pore (mPTP) opening and improved mitochondrial membrane potential regulation [42,43].

In contrast, nonagalloyl-glucose displayed a biphasic effect, initially activating the channel at lower concentrations (5 μM, 84.2 ± 1.8%) but inhibiting it at higher concentrations (10 μM, 50.5 ± 5.3%). This biphasic response suggests a dose-dependent modulation, where lower concentrations may enhance mitochondrial function, while higher concentrations could potentially lead to channel desensitization or secondary inhibitory effects. Similar biphasic responses have been observed with polyphenol-rich plant extracts, indicating the complexity of polyphenol interactions with ion channels.

Both tannins showed comparable effects in inhibiting lipid peroxidation (LPO) and mPTP opening while exhibiting antiradical activity based on DPPH assays. This suggests that, in addition to their effects on mitoK$_{ATP}$ channels, these tannins contribute to oxidative stress reduction, which is crucial for mitochondrial and cellular protection [44,45].

The observed differential effects between octagalloyl-glucose and nonagalloyl-glucose highlight the importance of structural variations in polyphenol activity on ion channels. The concentration-dependent activation of octagalloyl-glucose suggests its potential therapeutic application as a mitoK$_{ATP}$ opener, whereas the biphasic response of nonagalloyl-glucose underscores the necessity of precise dose optimization for effective use. Further studies are required to elucidate the exact mechanisms underlying these polyphenol-channel interactions and to explore their implications in mitochondrial protection and disease prevention.

Conclusions

In conclusion, the hydrolyzable tannins octagalloyl-glucose and nonagalloyl-glucose, isolated from the leaves of *Pistacia vera* L., exhibited high antiradical activity according to the DPPH assay in *in vitro* conditions. Additionally, they inhibited lipid peroxidation (LPO) processes and mitochondrial permeability transition pore (mPTP) opening in rat liver mitochondria. Octagalloyl-glucose demonstrated exclusively activating effects on ATP-sensitive potassium (mitoKATP) channels at the tested concentrations, whereas nonagalloyl-glucose exhibited both activating and inhibitory effects on these channels. These findings may hold significant theoretical and practical importance from a pharmacological perspective.

Declaration of generative AI in scientific writing

Only minimal assistance was used from QuillBot for paraphrasing selected sentences. All scientific content, interpretation, and conclusions were developed independently by the authors.

CRediT author statement

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References

[1] OS Ademowo, HKI Dias, DGA Burton and HR Griffiths. Lipid (per) oxidation in mitochondria: An emerging target in the ageing process? *Biogerontology*. 2017; **18(6)**, 859-879.

[2] M Xiao, H Zhong, L Xia, Y Tao and H Yin. Pathophysiology of mitochondrial lipid oxidation: Role of 4-hydroxynonenal (4-HNE) and other bioactive lipids in mitochondria. *Free Radical Biology and Medicine* 2017; **111**, 316-327.

[3] PR Angelova, N Esteras and AY Abramov. Mitochondria and lipid peroxidation in the mechanism of neurodegeneration: Finding ways for prevention. *Medicinal Research Reviews* 2021; **41(2)**, 770-784.

[4] R Endlicher, Z Drahota, K Stefkova, Z Cervinkova and O Kucera. The mitochondrial permeability transition pore—current knowledge of its structure, function, and regulation, and optimized methods for evaluating its functional state. *Cells* 2023; **12(9)**, 1273.

[5] Nozim N. Khoshimov, Alisher A. Mukhtorov, Kabil E. Nasirov, Rakhmatilla N. Rakhimov, Rahmatjon R. Mamadaminov. Effects of Polyphenols on changes in the transport of Ca2+ NMDA-receptors under the influence of L-glutamate. Research Journal of Pharmacy and Technology 2023; **16(3)**:1205-3.

[6] MR Rasuljonovich, SG Gaybullaevna, AN Tillakhodjaevna and DM Ravshanbekovna. Isolation of tannins from *Pistacia vera*. *Science and Innovation International Scientific Journal* 2023; **2(10)**, 80-88.

[7] FAO. *Directive 2010/63/EU on the protection of animals used for scientific purposes.* Official Journal of the European Union, Luxembourg, Luxembourg, 2010.

[8] WC Schneider and GH Hogeboom. Cytochemical studies of mammalian tissues: The isolation of cell components by differential centrifugation. *Cancer Research* 1951; **11(1)**, 1-22.

[9] Y Umidakhon, B Erkin, G Ulugbek, N Bahadir and A Karim. Correction of the mitochondrial NADH oxidase activity, peroxidation and phospholipid metabolism by haplogenin-7-glucoside in hypoxia and ischemia. *Trends in Sciences* 2022; **19(21)**, 6260.

[10] MI Asrarov, EJ Komilov and Ergashev. The mechanism of action of flavone luteolin on the function of rat liver mitochondria. *Problems of Biological Medical and Pharmaceutical Chemistry* 2015; **12**, 38-43.

[11] TV Pochinok, ML Tarakhovsky and VA Portnyagina. A rapid method for determination of antioxidative activity of drugs. *Pharmaceutical Chemistry Journal* 1985; **5**, 565-569.

[12] UG Gayibov, SN Gayibova, MK Pozilov, FS Tuxtaeva, UR Yusupova, GMK Djabbarova, ZA Mamatova, NA Ergashev and TF Aripov. Influence of quercetin and dihydroquercetin on some functional parameters of rat liver mitochondria. *Journal of Microbiology Biotechnology and Food Sciences* 2021; **11(1)**, e2924.

[13] OB Vadziuk and SA Kosterin. Diazoxide-induced mitochondrial swelling in the rat myometrium as a consequence of the activation of the mitochondrial ATP-sensitive (K^+)-channel. *Ukrainskii Biokhimicheskii Zhurnal* 2008; **80(5)**, 45-51.

[14] DD Esposti, J Hamelin, N Bosselut, R Saffroy, M Sebagh, A Pommier, C Martel and A Lemoine. Mitochondrial roles and cytoprotection in chronic liver injury. *Biochemistry Research International* 2012; **2012(1)**, 387626.

[15] NK Nozim, BK Islom, UDJ Ismoil, SK Siroj, UMA Abdugafor, OMM Ugli, EN Kabil, MZ Amindjanovna, SG Abdullayevna and NR Rakhmatilla. Effect of Polyphenols on Changes in

the Hemostatic System of Blood Plasma in Healthy and Model Rats with Alzheimer's Disease. *Trends in Sciences* 2024; **21(9)**, 8081.

[16] IZOGL Abdullaev, UG Gayibov, SZ Omonturdiev, SF Azamjonovna, SN Gayibova and TF Aripov. Molecular pathways in cardiovascular disease under hypoxia: Mechanisms, biomarkers, and therapeutic targets. *Journal of Biomedical Research* 2025; **39**, 1-16.

[17] F Palmieri and M Monne. Discoveries, metabolic roles and diseases of mitochondrial carriers: A review. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 2016; **1863(10)**, 2362-2378.

[18] JJ Ruprecht and ERS Kunji. Structural mechanism of transport of mitochondrial carriers. *Annual Review of Biochemistry* 2021; **90**, 535-558.

[19] UG Gayibov, SN Gayibova, HM Karimjonov, AA Abdullaev, DS Abduazimova, RN Rakhimov, HS Ruziboev, MA Xolmirzayeva, AE Zaynabiddinov and TFA Aripov. Antioxidant and cardioprotective properties of polyphenolic plant extract of Rhus glabra L. *Plant Science Today*. 2024; **11(3)**, 1-8.

[20] M Zaripova, SN Gayibova, RR Makhmudov, AA Mamadrahimov, NL Vypova, UG Gayibov, SM Miralimova and TF Aripov. Characterization of *rhodiola heterodonta* (crassulaceae): Phytocomposition, antioxidant and antihyperglycemic activities. *Preventive Nutrition and Food Science* 2024; **29(2)**, 135-145.

[21] DJ Robichaux, M Harata, E Murphy and J Karch. Mitochondrial permeability transition pore-dependent necrosis. *Journal of Molecular and Cellular Cardiology* 2023; **174**, 47-55.

[22] Z Shakiryanova, R Khegay, U Gayibov, A Saparbekova, Z Konarbayeva, A Latif and O Smirnova. Isolation and study of a bioactive extract enriched with anthocyanin from red grape pomace (Cabernet Sauvignon). *Agronomy Research* 2023; **21(3)**, 1293-1303.

[23] TF Aripov, UG Gayibov, SN Gayibova, AA Abdullaev, DSK Abduazimova, YI Oshchepkova and SI Salikhov. *In vitro* antioxidant and antiradical activity of the total polyphenols (substances of the antiviral drug rutan) of the leaves of tannic sumach rhus coriaria L. *Chemistry of Plant Raw Material* 2024; **4**, 138-147.

[24] IA Najar, GD Singh, S Javed and RK Johri. Effect of some phytoconstituents on Fe2+/ascorbate-induced lipid peroxidation. *Indian Journal of Experimental Biology* 2016; **54(12)**, 851-855.

[25] UG Gayibov, EJ Komilov, RN Rakhimov, NA Ergashev, NG Abdullajanova, MI Asrorov and TF Aripov. Influence of new polyphenol compound from *Euphorbia* plant on mitochondrial function. *Journal of Microbiology Biotechnology and Food Sciences* 2019; **8(4)**, 1021.

[26] N Ergashev, K Sayfieva, R Makhmudov and M Asrarov. Effect of tannins isolated from *Plantago major L.* and *Plantago lanceolata L.* on mitochondrial permeability transition pore in rat liver. *Trends in Sciences* 2024; **21(7)**, 7661.

[27] DR Isamukhamedova, NA Ergashev, RN Rakhimov and MI Asrarov. Effect of hydrolyzable tannins of *Euphorbia* plants on the rat antioxidant system under oxidative stress. *International Journal of Biochemistry Research & Review* 2024; **33(2)**, 40-46.

[28] DK Muratova, NA Ergashev, JJ Sobirov, UK Kurbanov and MI Asrarov. Effect of diterpene alkaloids on lipid peroxidation in mitochondria. *Nova Biotechnological et Chimica* 2021; **20(2)**, e850.

[29] BG Yuldoshev, NA Ergashev, EJ Komilov, DR Siddiqov and KS Ruziboyev. The effect of kaempferol and its glycosides on the lipid peroxidation of mitochondria. *Proceedings of the Russian Academy of Sciences* 2022; **5**, 78-82.

[30] Guli M. Raimova, Nozim N. Khoshimov, Kabil E. Nasirov, Abbaskhan S. Turaev, Malokhat E. Savutova. Anti-thrombotic action of sulfated polysaccharides on thrombosis caused by thromboplastin. Research Journal of Pharmacy and Technology. 2021; **14(11)**:6085-8.

[31] AV Mahmudov, OS Abduraimov, SB Erdonov, UG Gayibov and LY Izotova. Bioecological features of Nigella sativa L. in different conditions of Uzbekistan. *Plant Science Today* 2022; **9(2)**, 421-426.

[32] AV Mahmudov, OS Abduraimov, SB Erdonov, AL Allamurotov, OT Mamatqosimov, UG Gayibov and L Izotova. Seed productivity of Linum

usitatissimum L. in different ecological conditions of Uzbekistan. *Plant Science Today* 2022; **9(4)**, 1090-1101.

[33] N Ergashev, D Isamukhamedova, K Sayfieva, U Gayibov, R Rakhimov, R Makhmudov and M Asrarov. Anti-radical activity of some hydrolyzable tannins. *Universum Chemistry & Biology* 2023; **7**, 109.

[34] A Abdullaev, I Abdullaev, A Bogbekov, U Gayibov, S Omonturdiev, S Gayibova, M Turahodjayev, K Ruziboev and T Aripov. Antioxidant potential of rhodiola heterodonta extract: Activation of Nrf2 pathway via integrative *in vivo* and *in silico* studies. *Trends in Sciences* 2025; **22(5)**, 9521.

[35] S Sekowski, A Veiko, E Olchowik-Grabarek, A Dubis, AZ Wilczewska, KH Markiewicz, IB Zavodnik, E Lapshina, I Dobrzynska, N Abdulladjanova and M Zamaraeva. Hydrolysable tannins change physicochemical parameters of lipid nano-vesicles and reduce DPPH radical - Experimental studies and quantum chemical analysis. *Biochimica et Biophysica Acta - Biomembranes* 2022; **1864(1)**, 183778.

[36] M Carraro and P Bernardi. Calcium and reactive oxygen species in regulation of the mitochondrial permeability transition and of programmed cell death in yeast. *Cell Calcium* 2016; **60(2)**, 102-107.

[37] Y Kamei, M Koushi, Y Aoyama and R Asakai. The yeast mitochondrial permeability transition is regulated by reactive oxygen species, endogenous Ca^{2+}, and Cpr3, mediating cell death. *Biochimica et Biophysica Acta - Bioenergetics* 2018; **1859(12)**, 1313-1326.

[38] R Toczylowska-Maminska, A Olszewska, M Laskowski, P Bendnarczyk, K Skowronek and A Szewczyk. Potassium channel in the mitochondria of human keratinocytes. *Journal of Investigative Dermatology* 2014; **134(3)**, 764-772.

[39] Nozim N. Khoshimov, Guli M. Raimova, Kabul E. Nasirov, Zulayho A. Mamatova, Nodira I. Mamadaliyeva, Abbaskhan S. Turaev. The effect of Sulphated cellulose on System of Haemostasis. Research Journal of Pharmacy and Technology. 2021; **14(6)**:3283-9.

[40] GD Mironova, EV Kachaeva and AT Kopylov. Mitochondrial ATP-dependent potassium channel. I. The structure of the channel, the mechanisms of its functioning and regulation. *Vestnik Rossiiskoi akademii meditsinskikh nauk* 2007; **2**, 34-43.

[41] GD Mironova, MI Shigaeva, NV Belosludtseva, EN Gritsenko, KN Belosludtsev, EL Germanova and LD Lukyanova. Effect of several flavonoid-containing plant preparations on activity of mitochondrial ATP-dependent potassium channel. *Bulletin of Experimental Biology and Medicine* 2008; **146(2)**, 229-233.

[42] D Inomjonov, I Abdullaev, S Omonturdiev, A Abdullaev, L Maxmudov, M Zaripova, M Abdullayeva, M Abduazimova, D Menglieva, S Gayibova, M Sadbarxon, U Gayibov and T Aripov. *In vitro* and *in vivo* studies of crategus and inula helenium extracts: Their effects on rat blood pressure. *Trends in Sciences* 2025; **22(3)**, 9158.

[43] O Gaibullayeva, A Islomov, D Abdugafurova, B Elmurodov, B Mirsalixov, L Mahmudov, I Adullaev, K Baratov, S Omonturdiev and S Sa'dullayeva. *Inula helenium* L. root extract in sunflower oil: Determination of its content of water-soluble vitamins and immunity-promoting effect. *Biomedical and Pharmacology Journal* 2024; **17(4)**, 2729-2737.

[44] AQQ Azimova, AX Islomov, SA Maulyanov, DG Abdugafurova, LU Mahmudov, IZO Abdullaev, AS Ishmuratova, SQQ Siddikova and IP Askarov. Determination of vitamins and pharmacological properties of *Vitis vinifera* L. plant fruit part (mixed varieties) syrup-honey. *Biomedical and Pharmacology Journal* 2024; **17(4)**, 2779-2786.

[45] OS Zoirovich, AIZ Ugli, ID Raxmatillayevich, ML Umarjonovich, ZM Ravshanovna, GS Narimanovna, GU Gapparjanovich and AT Fatikhovich. The effect of ajuga turkestanica on the rat aortic smooth muscle ion channels. *Biomedical and Pharmacology Journal* 2024; **17(2)**, 1213-1222.