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RESEARCH ARTICLE

Comparative Analysis of the Effects of the Diterpene Alkaloid Napelline and its Derivative on the MitoK$^+_{ATP}$ Channel and mPTP in Rat Liver and Heart Mitochondria

Dilnoza Khasanovna Muratova[1,2,*], Nurali Azamovich Ergashev[2], Muzaffar Islamovich Asrarov[2], Mamurjon Komiljonovich Pozilov[1,2] and Xabiba Yakubovna Berdiyeva[1]

[1]Department of Biophysics, National University of Uzbekistan named after Mirzo Ulugbek, Tashkent 100174, Uzbekistan
[2]Institute of Biophysics and Biochemistry, National University of Uzbekistan named after Mirzo Ulugbek, Tashkent 100174, Uzbekistan

([*]Corresponding author's e-mail: dilnozaxasanovnam@gmail.com)

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Abstract

Mitochondria play crucial role in cellular energy metabolism and ion homeostasis, and their dysfunction is associated with various pathological conditions. The mitochondrial ATP-sensitive potassium channel (mitoK$^+_{ATP}$ channel) and the mitochondrial permeability transition pore (mPTP) are considered key regulators of mitochondrial function and potential targets for cytoprotective therapies. This study examined the effects of the diterpene alkaloid napelline and its derivative, 1-O-benzoylnapelline hydrochloride, on mitoK$^+_{ATP}$ channel activity and mPTP opening in liver and heart mitochondria isolated from male Wistar rats. Mitochondrial swelling assays were used to assess mitoK$^+_{ATP}$ channel activation in the presence of ATP (as an inhibitor), and calcium-induced swelling was used to evaluate mPTP opening. Napelline (50 μM) activated the activity of the liver mitoK$^+_{ATP}$ channel by 191.0 ± 2.3% compared to the control, and 1-O-benzoylnapelline (10 μM) - by 208%. In heart mitochondria, these values were 127.6 ± 3.1% and 156.5 ± 2.5%, respectively. Both compounds inhibited mPTP opening, with 1-O-benzoylnapelline showing greater efficacy: At 10 μM, it suppressed swelling by 92.3 ± 2.5% in liver and 78.6 ± 2.4% in heart mitochondria. Liver mitochondria exhibited a more pronounced response, indicating tissue-specific sensitivity. The increased activity of 1-O-benzoylnapelline may be attributed to the acylation of the C-1 hydroxyl group, which likely enhances its interaction with mitochondrial membranes. These results indicate that 1-O-benzoylnapelline is a more effective modulator of mitochondrial ion channels than napelline and may serve as a promising lead compound for the development of novel cytoprotective agents, warranting further investigation.

Keywords: Napelline, 1-O-benzoylnapelline, MitoK$^+_{ATP}$-channel, mPTP, Mitochondria, Liver, Heart

Introduction

Mitochondria serve as essential energy generators for cellular processes and play a crucial role in maintaining cell metabolism and structural integrity. Additionally, mitochondria regulate reactive oxygen species [ROS] production, mediate energy transduction processes, and contribute to cellular ion homeostasis. Dysregulation of mitochondrial ion channels has been linked to various pathological conditions [1].

Several studies have shown that ion channels located in the inner mitochondrial membrane possess essential functional properties. For instance, mitoK$^+_{ATP}$ channels play a role not only under physiological conditions but also in pathological states such as

ischemia, hypoxia, necrosis, and apoptosis. These channels are involved in the regulation of mitochondrial volume and the maintenance of mitochondrial integrity [2,3]. Biologically active compounds modulate mitochondrial volume by activating the mitoK$^+_{ATP}$ channel. Additionally, they contribute to the acceleration of ATP synthesis, stimulation of respiration, and myocardial protection during ischemia, as well as the prevention of apoptosis under oxidative stress [4]. According to A. Szewczyk and L. Wojtczak, the mitoK$^+_{ATP}$ channel, when open, facilitates the transport of K^+ ions into the mitochondrial matrix. The classical activator of this channel, diazoxide, can induce activation either by inhibiting the enzyme succinate dehydrogenase or by directly interacting with the protein subunit. As a result, K^+ ions are transported from the cytosol into the mitochondrial matrix. The classical channel blocker, glibenclamide, inhibits SUR receptors, while 5-hydroxydecanoate (5-HD) blocks protein components, thereby preventing receptor activity in the cytosolic region of the inner mitochondrial membrane and ultimately leading to channel inhibition [1]. It has been demonstrated that the mitoK$^+_{ATP}$ channel can be reactivated by specific compounds following ATP-mediated inhibition. These compounds include diazoxide, cromakalim, and pinacidil [3], as well as hypoxene, flocalin [5] and others. Agents that activate the mitoK$^+_{ATP}$ channel have been shown to induce cardioprotection by opening the channel and regulating reactive oxygen species (ROS) synthesis during ischemia-reperfusion [6]. Additionally, the mitoK$^+_{ATP}$ channel plays a crucial role in the long-term adaptation of animals to hypoxia [7]. Given the significant functional role of the mitoK$^+_{ATP}$ channel in the inner mitochondrial membrane under ischemic and hypoxic conditions, cardiomyocyte membranes and mitochondria are primarily chosen as the main objects of study [8]. At the cellular level, disturbances in oxygen homeostasis and the onset of hypoxia contribute to pathological conditions such as myocardial infarction and ischemia. The activation of the mitoK$^+_{ATP}$ channel triggers an adaptive protective mechanism in cells under hypoxic conditions, enhancing cellular resistance to stress [9,10]. Activation of the mitoK$^+_{ATP}$ channel plays a crucial role in protecting pancreatic and liver cells from the detrimental effects of hyperglycemia in experimental diabetes, as well as safeguarding the mitochondria of cardiac muscle cells during ischemia [11]. Consequently, significant attention has been directed toward understanding the regulatory mechanisms of the K^+_{ATP} channel, which is essential for inhibiting mitochondrial permeability transition pore (mPTP) opening and modulating mitochondrial volume. The mitoK$^+_{ATP}$-channel is critical for the functional activity of the cardiovascular system, and pharmacological regulation of its activity has been proposed as a potential therapeutic strategy for cardiovascular pathologies [12]. Most modulators targeting the mitoK$^+_{ATP}$-channel are synthetic compounds that either activate or inhibit the channel, often leading to dysregulation of cellular ion transport systems compared to naturally derived bioactive substances. Given that plant-derived bioactive compounds do not exert harmful effects on mitochondrial function, identifying new and effective mitoK$^+_{ATP}$-channel modulators remains a key focus in biophysics and pharmacology. Under physiological conditions, Ca^{2+} ions regulate normal energy metabolism; however, at elevated concentrations, they induce cell death via mitochondrial pathways. Excessive Ca^{2+} accumulation, accompanied by increased reactive oxygen species (ROS) production and inorganic phosphate accumulation, disrupts mitochondrial membrane permeability, leading to the opening of a non-selective, high-conductance pore in the inner membrane [13,14]. A crucial mitochondrial channel, the mitochondrial permeability transition pore (mPTP), is composed of proteins embedded within the mitochondrial membrane. This pore plays a key role in maintaining cellular homeostasis under normal conditions and contributes to cell death in pathological states such as ischemia, oxidative stress, and calcium overload. he passage of solutes with a molecular weight of up to 1.5 kDa through the mPTP leads to mitochondrial matrix swelling, loss of membrane potential, and ultimately the release of pro-apoptotic factors into the cytosol. The extent and duration of pore opening determine whether the cell undergoes apoptosis or necrosis [15-17]. Currently, several pharmacological agents that modulate mPTP activity have been identified. These modulators either induce or inhibit pore opening. Among the known inducers are doxorubicin,

progesterone, estradiol, and dehydroepiandrosterone, whereas inhibitors include cyclosporine A (CsA), buterol, bongkrekic acid, and ubiquinone [14,18-20]. It has been established that Ca^{2+}-induced mitochondrial collapse is prevented by the classical mPTP inhibitor, CsA, which stabilizes the pore in a closed conformation. CsA-mediated mPTP inhibition protects cardiomyocytes from ischemia-reperfusion injury and suppresses palmitate-induced apoptosis. Furthermore, CsA has been suggested to prevent necrotic cell death by mitigating pH-induced damage during ischemia-reperfusion, counteracting the toxic effects of Ca^{2+}-ionophores, and reducing oxidative stress. The pore-inhibitory properties of CsA have potential applications in preserving cells during normothermic transplantation [21]. In addition to its role in mPTP regulation, CsA possesses immunosuppressive properties, blocking cytokine gene transcription in activated T cells. Mechanistically, CsA inhibits the peptidyl-prolyl cis-trans isomerase activity of cyclophilin D (CypD) by forming a stable complex with it [22]. While mitochondria serve as the central organelles regulating apoptosis, the mPTP plays a particularly crucial role due to its regulatory function in cellular homeostasis. Consequently, extensive research has been dedicated to the pharmacological modulation of the mPTP as a potential therapeutic strategy for mitigating mitochondrial dysfunction [1,23]. The ability of certain compounds to inhibit mPTP opening and thereby enhance cell survival under stress conditions highlights the channel as a promising target for the development of novel cytoprotective agents.

The effects of several bioactive compounds, including diterpene alkaloids, polyphenols, and flavonoids, on the functional parameters of mitochondria under various pathological conditions have also been studied by researchers at the Molecular Biophysics Laboratory of the Institute of Biophysics and Biochemistry, National University of Uzbekistan [24-28]. Currently, alkaloids are widely used in various fields of medicine. Their biological activity and therapeutic potential largely depend on their physicochemical properties and physiological effects. Alkaloids are well-characterized secondary metabolites, and numerous alkaloids have been isolated, their derivatives synthesized, and their mechanisms of action extensively investigated in scientific laboratories. While several mechanisms of action of diterpene alkaloids have been elucidated, their influence on the ion permeability of the mitochondrial membrane in rat liver and heart remains unexplored.

Based on the above findings, the effect of the diterpene alkaloid napelline and its derivative on the mitoK^+_{ATP} channel in rat liver and heart mitochondria was investigated *in vitro*.

Materials and methods

Ethical statement

All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Local Ethics Committee of the Institute of Biophysics and Biochemistry, National University of Uzbekistan named after Mirzo Ulugbek [Protocol No. BEC/IBB-NUU/2019/02/22].

Animal handling and experimental conditions

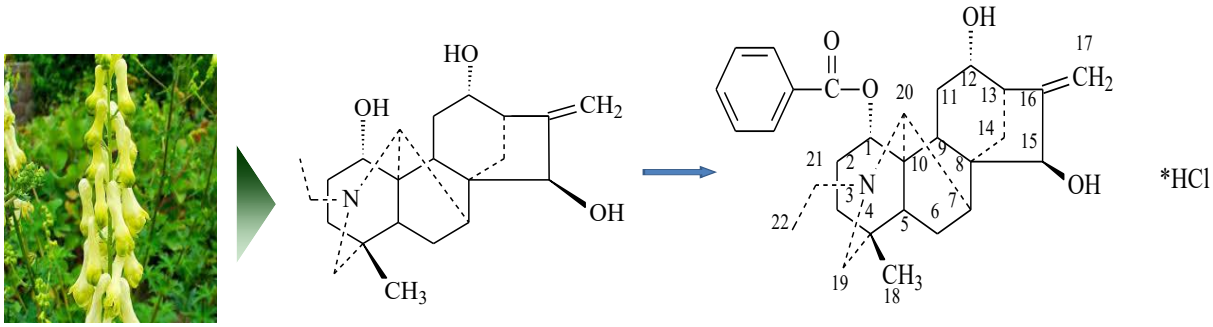
Male white outbred rats weighing 180 - 220 g were housed under standard laboratory conditions (temperature 20 - 24 °C, 65% humidity, natural light/dark cycle) with free access to food and water (ad libitum).

Extraction and isolation of alkaloids

Alkaloids were extracted from air-dried aerial parts of *Aconitum karakolicum* using 80% ethanol, followed by alkalization and chloroform extraction. The total alkaloid fraction was subjected to column chromatography on aluminum oxide and silica gel using gradient elution systems. Individual compounds, including napelline and songorine, were isolated based on their polarity and solubility. Structural identification was performed using TLC, IR, and NMR spectroscopy (^1H and ^{13}C NMR, JEOL 400/600 MHz, $CDCl_3$). Detailed procedures are provided in the Supplementary Materials.

The alkaloid napelline was isolated from plants of the genus *Aconitum karakolicum Rapcs* ($C_{22}H_{31}O_3$) growing on the territory of the Republic of Uzbekistan [29], and 1-O-benzoylnapelline ($C_{29}H_{37}O_4$) [30,31] was synthesized by introducing a benzoyl group into the napelline structure at positions C-1. **Figure 1** shows the structural formulas of napelline and its derivative. The

team of the "Alkaloidal Chemistry" laboratory of the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan identified and delivered the diterpene alkaloids employed in the research work.



Aconitum karakolicum Rapcs **Napelline** **1-O-benzoylnapelline**

Figure 1 Structural formulas of diterpene alkaloids studied in this work.

Mitochondria isolation

Mitochondria were isolated from the rat liver by differential centrifugation [32]. Under a mild ether anesthetic, the rats were immobilized, decapitated, the abdominal cavity was opened, and the liver was isolated. Liver from one rat was homogenized using Teflon-glass homogenizer and resuspended in isolation buffer, which contained 250 mM sucrose, 1mM ethylenediamine tetraacetic acid Na_2-salt (EDTA), and 10 mM Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), pH 7.4. Nuclei and whole cells were centrifuged at 1,500×g for 7 min. Mitochondria were sedimented by centrifugation of supernatant at 6,000×g for 15 min. The final mitochondrial pellet was suspended in a small volume of medium containing 250 mM sucrose, 10 mM Tris-HCl, was kept on ice prior to experiments.

Rat heart mitochondria were also isolated using differential centrifugation. Rat heart was homogenized in a medium [in mM]: Sucrose - 300, Tris-HCl - 10, EDTA - 2, albumin 0.2%, pH 7.4. To isolate a sufficient amount of mitochondria, 5 or 6 rat hearts were isolated and placed in chilled isolation medium. Laboratory equipment and petri dishes were stored in a freezer, and the procedure was carried out on ice. Rat hearts were cleaned of adipose tissue, blood and other large blood vessels in chilled 0.9% KCl solution. The cleaned heart was washed again, dried using filter paper, heart mass was determined and cut into small pieces using scissors. After that, the minced heart tissue was placed in a homogenizer and homogenized using a teflon pestle, poured with a 10:1 ratio of separation medium. Because the heart tissue is composed of transverse muscles, it was more homogenized than the liver tissue. The homogenate was poured into a centrifuge tube, placed in the rotor and centrifuged at 1,500 g for 7 min. At this stage, heavy aggregates settle. In the next step, centrifugation was performed at 6,000 g for 20 min. Isolated mitochondria were washed in medium without EDTA and albumin. Mitochondria isolation procedures were carried out under cold conditions. Mitochondria were kept on ice during the experiments. The mitochondrial protein content was determined by the Lowry method in modification of Peterson [33].

Method for assessing mitochondrial ATP-dependent K^+ channel conductance

The conductance of the ATP-dependent K^+ channel in mitochondria was assessed through optical density measurements at a wavelength of 540 nm. The incubation medium (IM) comprised the following components (mM): KCl (125), HEPES (10), succinate (5), $MgCl_2$ (1), K_2HPO_4 (2.5), KH_2PO_4 (2.5), rotenone (0.005), and oligomycin (0.001), adjusted to pH 7.4 [34]. The protein concentration in the medium ranged from 0.3 to 0.4 mg/mL.

Method for evaluating the condition of mitochondrial permeable pore

The kinetics of mitochondrial unfolding were examined during the assessment of the state of the Ca^{2+}-dependent permeable pore of the mitochondrial membrane. Optical density was recorded at a wavelength of 540 nm. The incubation medium was maintained at 26 °C, with a protein concentration of 0.3 - 0.4 mg/mL. The following incubation medium (IM) composition was employed to evaluate the permeability of the permeable transition pore (PTP) in mitochondria (mM): sucrose (200), EGTA (0.02), succinate (5), Tris (20), HEPES (20), KH_2PO4 (1), rotenone (0.002), oligomycin (1 μg/mL), adjusted to pH 7.2 [35].

Drugs and chemicals

The following chemical reagents were used: EDTA ("Sandoz", Switzerland), Tris-HCl ("Serva", Germany), KH_2PO_4, $MgCl_2$, K_2HPO_4, succinate, sucrose, KCl, ("Chemreaktivsnab", Russia), EGTA, HEPES, diazoxide, *olygomycin*, rotenone, ATP, $CaCl_2$ (Sigma", USA); Cyclosporine A ("Wako", China). All reagents are chemically pure.

Data analysis

The results were analysed statistically using the Origin Pro 7.5 (Microsoft, USA). The data were evaluated using parametric Student's t-test and are expressed as M ± m. The results that were deemed significant are indicated as follows: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Results and discussion

Our experiments were conducted *in vitro* to investigate the effect of the diterpene alkaloid napelline on the mitoK$^+_{ATP}$ channel at different concentrations. In the absence of ATP in the incubation medium (IM), the permeability of the mitoK$^+_{ATP}$ channel was considered 100%. The addition of 200 μM ATP to the IM inhibited the liver mitoK$^+_{ATP}$ channel. Under these conditions, different concentrations of napelline were tested, and activation of the mitoK$^+_{ATP}$ channel was observed starting from 10 μM, with the effect increasing in a concentration-dependent manner. The mitoK$^+_{ATP}$ channel was significantly activated by 191.0 ± 2.3% compared to the control at a 50 μM concentration of napelline (**Figure 2(a)**).

Several scientific studies have demonstrated that the mitoK$^+_{ATP}$-channel plays a crucial role in protecting cardiac tissues from ischemia-reperfusion injury [4,36]. Activation of K^+ channels in tissues prior to or during ischemia is directly linked to cytoprotection [37]. Given the involvement of mitoK$^+_{ATP}$ channel activation in cardioprotection, we investigated the effect of the diterpene alkaloid napelline on the cardiac mitoK$^+_{ATP}$ channel in our subsequent studies. In the absence of ATP in the incubation medium (IM), heart mitoK$^+_{ATP}$ channel blockade was considered as the control (100%). Upon the addition of 200 μM ATP, the channel was inhibited. However, in the presence of 50 μM napelline, the activity of the heart mitoK$^+_{ATP}$ channel was significantly enhanced to 127.6 ± 3.1% compared to the control (**Figure 2(b)**). Thus, the effect of the alkaloid napelline on the mitoK$^+_{ATP}$ channel in the liver and heart is more pronounced than that of the diterpene alkaloid songorine [25], which may be attributed to the presence of a hydroxyl group in its structure.

In subsequent experiments, the effect of 1-O-benzoylnapelline hydrochloride, a derivative of the alkaloid napelline, on the activity of the liver mitoK$^+_{ATP}$ channel was investigated. At a concentration of 10 μM, this compound increased mitoK$^+_{ATP}$ channel activity by 208% compared to the control (**Figure 2(c)**). In the next stage of our study, we investigated the effect of 1-O-benzoylnapelline at different concentrations on the activity of the heart mitoK$^+_{ATP}$ channel. In the presence of 200 μM ATP in the medium, a 0.01 μM concentration of 1-O-benzoylnapelline reactivated mitoK$^+_{ATP}$ channel activity by 20.0 ± 2.9%. Under these conditions, the activity of the heart mitoK$^+_{ATP}$ channel was significantly modulated by increasing concentrations of 1-O-benzoylnapelline (0.05, 0.1, 1 and 10 μM), enhancing channel activity by 47.7 ± 3.4%, 98.5 ± 2.8%, 134.5 ± 3.1%, and 156.5 ± 2.5%, respectively, compared to the control (**Figure 2(d)**).

Thus, the obtained results showed that 1-O-benzoylnapelline at low concentrations has a more active effect on the liver and heart mitoK$^+_{ATP}$ channel than the napelline. 1-O-benzoylnapelline was synthesized by acylating the hydroxyl group at the C-1

position of the napelline alkaloid using benzoyl chloride. The introduction of a benzoyl group is known to significantly enhance the antiarrhythmic properties of the alkaloid [38]. In addition, the increased lipophilicity contributes to enhanced activation properties. As a result, 1-O-benzoylnapelline acts as an activator of the mitoK$^+_{ATP}$ channel at lower concentrations compared to the parent napelline alkaloid. To evaluate this effect, channel activity was compared with that induced by diazoxide, a classic mitoK$^+_{ATP}$ channel activator (**Figure 3(a)**). Diazoxide was used as a control to assess the activation of the liver mitoK$^+_{ATP}$ channel.

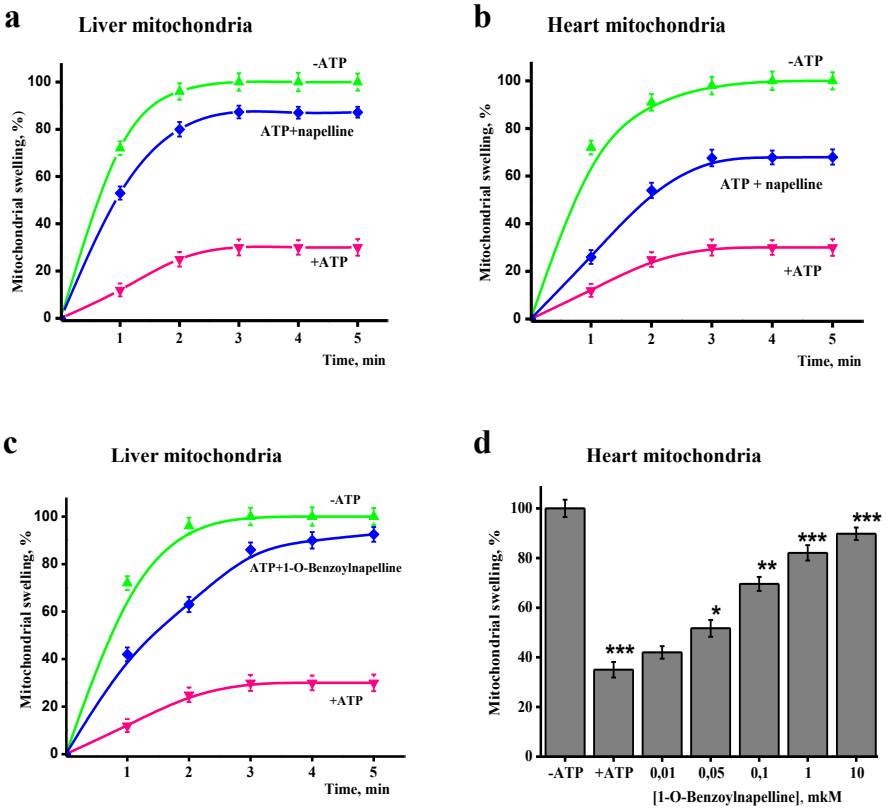


Figure 2 The effect of the alkaloids napelline and 1-O-benzoylnapelline on the swelling of rat liver and heart mitochondria. (IM: (in mM) KCl - 125, $MgSO_4$ - 1, K_2HPO_4 - 2.5, HEPES - 10, succinate - 5, rotenone - 5 μg/mL, oligomycin - 1 μg/mL, pH 7.4. ATP - 200 μM, napelline - 50 μM, 1-O-benzoylnapelline - 10 μM, n = 6).

These findings indicate that 1-O-benzoylnapelline functions as a mitoK$^+_{ATP}$ channel activator at a lower concentration than napelline. To compare these results, we examined the effect of diazoxide, a well-established activator of the mitoK$^+_{ATP}$ channel (**Figure 3(a)**). Diazoxide significantly increased liver mitoK$^+_{ATP}$ channel activity by 223% compared to the control.

Thus, both napelline and 1-O-benzoylnapelline exhibit an activating effect on the mitoK$^+_{ATP}$ channel in the liver and heart, similar to diazoxide. To further elucidate this mechanism, additional studies were conducted on the activated state of the mitoK$^+_{ATP}$ channel in the presence of 1-O-benzoylnapelline (**Figure 3(b)**), using glibenclamide - a classical inhibitor known for its specificity toward the mitoK$^+_{ATP}$ channel [36]. The addition of glibenclamide at a concentration of 10 μM to the incubation medium reduced the activating effect of 1-O-benzoylnapelline, resulting in re-inhibition of the mitoK$^+_{ATP}$ channel (**Figure 3(b)**).

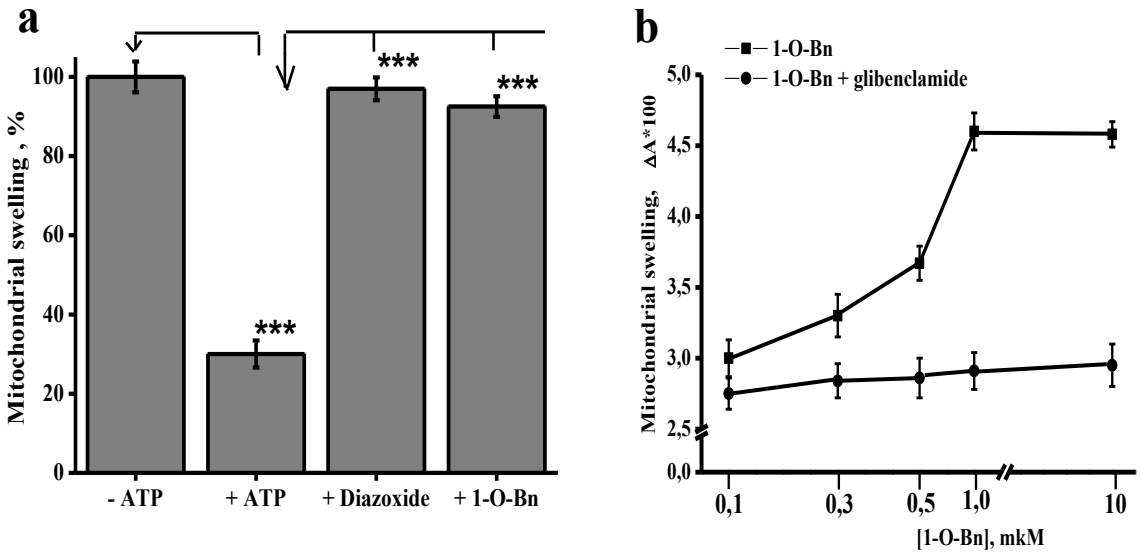


Figure 3 The effect of 1-O-benzoylnapelline and diazoxide on liver mitoK$^+_{ATP}$ channel activity (a) and the inhibition of liver mitochondrial swelling induced by 1-O-benzoylnapelline by glibenclamide (b). (The IM is presented in **Figure 2**. Concentrations of substances: ATP - 200 μM, diazoxide - 10 μM, 1-O-benzoylnapelline - 10 μM, glibenclamide - 10 μM. *** $p < 0.001$; n = 5 - 8).

The analysis of the obtained results suggests that the inhibition of the mitoK$^+_{ATP}$ channel by glibenclamide confirms that 1-O-benzoylnapelline specifically targets this channel. Both napelline and its derivative, 1-O-benzoylnapelline, exhibited an activating effect on the mitoK$^+_{ATP}$ channels of liver and heart mitochondria. However, 1-O-benzoylnapelline demonstrated its activating properties at relatively lower concentrations compared to napelline. This suggests that 1-O-benzoylnapelline may exert its effects with minimal side effects, as many alkaloids are known to exhibit pharmacological activity at low concentrations [39].

The synthesis of 1-O-benzoylnapelline was achieved through the acylation of napelline with benzoyl chloride, resulting in the substitution of the hydroxyl group at the C-1 position with a benzoyl residue. This structural modification significantly enhanced the biological activity of the alkaloid, potentially contributing to increased antiarrhythmic properties [38]. Additionally, the increase in lipophilicity due to benzoyl substitution may have played a key role in enhancing its activating effect on mitoK$^+_{ATP}$ channels.

The pronounced activity of 1-O-benzoylnapelline on liver and heart mitoK$^+_{ATP}$ channels can be attributed to the presence of the benzoyl group at the C-1 position. The findings indicate that 1-O-benzoylnapelline exerts a more potent effect on these channels at lower concentrations than napelline, suggesting its potential as a therapeutic agent for ischemic heart diseases. However, further in-depth investigations are required to confirm its clinical applicability.

The mitoK$^+_{ATP}$ channel plays a crucial role in both normal and pathological cellular processes by regulating mitochondrial volume and function [1]. The pharmacological activation or inhibition of mitochondrial potassium channels has been widely utilized to modulate cytoprotection and cell death [40]. Numerous studies have focused on identifying compounds that activate the mitoK$^+_{ATP}$ channel in cardiomyocytes [41]. It has been demonstrated that such activators depolarize heart mitochondria [42].

Given these findings, diterpene alkaloid derivatives warrant extensive investigation as potential cytoprotective and cardioprotective agents, particularly for mitigating ischemic damage by activating mitoK$^+_{ATP}$ channels in liver and heart mitochondria.

The activation of mitoK^+_{ATP} channels leads to mitochondrial membrane potential (ΔΨm) depolarization, thereby reducing the driving force for Ca^{2+} influx and preventing excessive mitochondrial Ca^{2+} accumulation. Since mitoK^+_{ATP} channel activation slows the rate of Ca^{2+} accumulation and promotes Ca^{2+} efflux from the mitochondrial matrix, the present study aims to examine the effects of diterpene alkaloids on the mPTP.

In various pathological conditions, the transition of the mPTP to an open state has been observed, and prolonged activation of this process leads to structural and functional disruption of cells. This necessitates the development of biologically active compounds capable of correcting such pathological states. Therefore, the search for local bioactive substances that can inhibit mitochondrial mPTP while simultaneously activating protective cellular mechanisms-such as defense against ischemia, hypoxia, Ca^{2+} overload, and oxidative stress-is of critical importance.

To investigate the membrane-active properties of the studied alkaloids, the effects of napelline and 1-O-benzoylnapelline on the mPTP state of rat liver and heart mitochondria were examined *in vitro*. A concentration of 10 μM Ca^{2+} was used as an inducer of mitochondrial swelling. The addition of Ca^{2+} to the incubation medium triggered mitochondrial swelling and induced the transition of the mPTP to a highly conductive state, which was taken as the control condition (100%) (**Figure 4**). Initial experiments demonstrated that the napelline inhibited Ca^{2+}-induced mitochondrial swelling in rat liver mitochondria in a concentration-dependent manner. Specifically, at a concentration of 50 μM, napelline reduced mitochondrial swelling by 18.0 ± 3.1% compared to the control, while at 100 μM, the inhibition reached 46.0 ± 2.9%. When evaluating the effect of napelline on heart mPTP, inhibition levels of 9.0 ± 3.0% and 38.0 ± 2.9% were observed at 50 and 100 μM, respectively (**Figure 4(a)**).

Under these conditions, the effect of 1-O-benzoylnapelline on the mitochondrial permeability transition pore (mPTP) in heart mitochondria was evaluated at concentrations of 0.5, 1, 5, and 10 μM. The results demonstrated that 1-O-benzoylnapelline inhibited mPTP opening by 18.0 ± 3.2%, 45.0 ± 3.7%, 67.2 ± 2.9%, and 78.6 ± 2.4%, respectively, compared to the control (**Figure 4(b)**). Experimental findings indicated that the inhibitory effect of 1-O-benzoylnapelline was most pronounced at a concentration of 10 μM. The half-maximal inhibitory concentration (IC_{50}) values for 1-O-benzoylnapelline in liver and heart mitochondria were determined to be 0.66 ± 0.02 and 1.5 ± 0.19 μM, respectively (**Figure 4(b)**).

In the subsequent experiments, the effect of the alkaloid 1-O-benzoylnapelline on mPTP activity in liver and heart mitochondria was investigated in the presence of cyclosporin A (CsA), a classical inhibitor of this pore. Under these conditions, the addition of 0.1 μM 1-O-benzoylnapelline to the incubation medium inhibited mitochondrial swelling in liver mitochondria by 28.0 ± 3.3% compared to the control. A concentration-dependent increase in the inhibitory effect was observed, with inhibition levels reaching 69.0 ± 3.1%, 88.0 ± 2.8%, 95.0 ± 3.2%, and 100.0 ± 2.9% at 0.5, 1, 5, and 10 μM concentrations, respectively (**Figure 4(c)**).

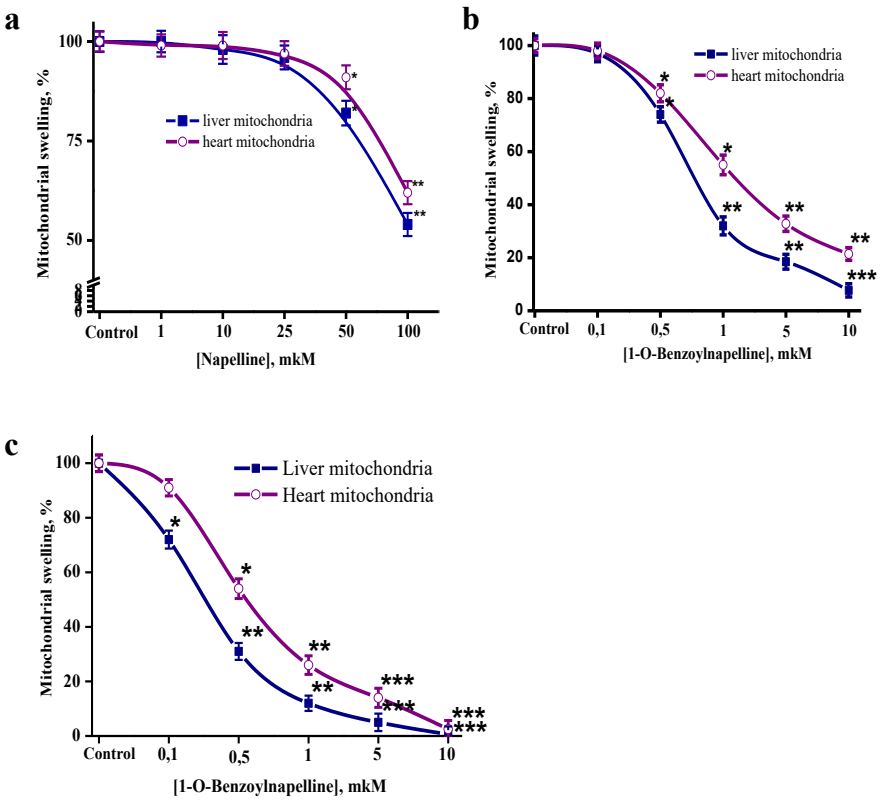


Figure 4 The effect of the alkaloids napelline and 1-O-benzoylnapelline on the Ca^{2+}-dependent swelling of rat liver and heart mitochondria. Incubation Medium [IM]: (in mM) sucrose - 200, EGTA - 0.02, succinate - 5, Tris - 20, HEPES - 20, KH_2PO_4 - 1, rotenone - 0.002, oligomycin - 1 μg/mL; pH 7.2. Ca^{2+} - 10 μM, CsA - 0.2 μM (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n = 4 - 6).

To assess the effect of 1-O-benzoylnapelline on mPTP activity in heart mitochondria in the presence of CsA, the same concentrations were applied to the IM. Under these conditions, significant inhibition of heart mPTP activity was observed, with inhibition levels of 9.0 ± 3.0%, 46.0 ± 3.6%, 74.0 ± 3.4%, 86.0 ± 3.5% and 97.5 ± 3.2% at 0.1, 0.5, 1, 5, and 10 μM concentrations, respectively. The half-maximal inhibitory concentration (IC_{50}) of 1-O-benzoylnapelline was determined to be 0.32 μM for liver mitochondria and 0.6 μM for heart mitochondria (**Figure 4(c)**).

At these concentrations, 1-O-benzoylnapelline induces the closure of the mPTP in both liver and heart mitochondria. The findings demonstrate that 1-O-benzoylnapelline effectively inhibits mPTP opening, aligning with previously reported data in the literature [21,43]. The inhibitory effect of 1-O-benzoylnapelline on mPTP in rat liver and heart mitochondria, particularly in the presence of CsA, may be attributed to its influence on cyclophilin D (CyP-D) activity. This suggests that the incorporation of a benzoyl group at the C-1 position enhances its inhibitory effect on CyP-D, thereby contributing to mPTP inhibition. Notably, napelline derivatives bearing a monobenzene substitution at C-1 have been reported to exhibit significant antiarrhythmic activity [38].

Among the investigated alkaloids, 1-O-benzoylnapelline demonstrated the most potent inhibitory effect on mPTP at relatively low concentrations *in vitro*, exerting a stabilizing influence on the mitochondrial membranes of rat liver and heart.

These findings suggest that 1-O-benzoylnapelline holds potential for the development of pharmacological agents aimed at treating pathological conditions associated with mitochondrial permeability transition.

The results of this *in vitro* study further indicate that 1-O-benzoylnapelline exhibits a more pronounced stabilizing effect on mPTP compared to the diterpene alkaloid napelline. The ability of biologically active compounds to inhibit mPTP may confer mitochondrial

protection against osmotic stress under pathological conditions, thereby preventing cell apoptosis and enhancing cellular resilience.

Our study revealed that the extent of mitochondrial collapse induced by Ca^{2+} is more pronounced in liver mitochondria compared to heart mitochondria. This observation suggests that liver tissues, despite their high regenerative capacity, exhibit lower resistance to Ca^{2+}-induced apoptosis. Consistent with existing literature, previous studies have demonstrated that, in comparison to liver mitochondria, brain mitochondria [44] and heart mitochondria [45] display greater resistance to Ca^{2+}-dependent permeability transition. This phenomenon is attributed to the tissue-specific nature of the process rather than differences in the absolute levels of Ca^{2+} exposure [45].

Thus, the findings further support the tissue-specific mechanisms underlying cellular protection against Ca^{2+}-induced apoptosis and necrosis. The half-maximal inhibitory concentrations (IC_{50}) of diterpene alkaloids on the permeability transition of liver and heart mitochondria are summarized in **Table 1**.

Table 1 The half-maximal inhibitory concentrations (IC_{50}) of diterpene alkaloids.

No.	Diterpene alkaloids	Liver mitochondria IC_{50} (μM)	Heart mitochondria IC_{50} (μM)
1	1-O-benzoylnapelline	0.66±0.02	1.5±0.19
2	Songorine	35.2±1.9	46.7±2.3
3	14-O-benzoyltalatisamine	49±3.2	95±3.5
4	Talatisamine	80±3.6	100±3.7

This table demonstrates that 1-O-benzoylnapelline effectively inhibits the Ca^{2+}-dependent high-conductance pore at low concentrations compared to other alkaloids [20,25]. Studies on the spasmolytic and spasmogenic effects of diterpene alkaloids indicate that among aconitine-type alkaloids, those containing aromatic ester groups at the C-1 and C-14 positions exhibit the highest activity. Notably, alkaloids with a benzoyl group at the C-1 position display stronger spasmolytic activity than those with an aromatic group at the C-14 position [30]. By blocking the mPTP at lower concentrations than other alkaloids, 1-O-benzoylnapelline plays a crucial role in promoting cell survival, likely through its regulatory effect on the balance between pro-apoptotic Bax and anti-apoptotic Bcl-2 proteins. Pharmacological modulators that inhibit mitochondrial permeability transition can be beneficial in conditions such as hypoxia and ischemia, particularly in the treatment of cardiovascular diseases. Given that MitoK^+_{ATP}-channel activators and mPTP inhibitors have demonstrated cardiocytoprotective properties [15,21], the pharmacological potential of 1-O-benzoylnapelline warrants further comprehensive investigation.

Conclusions

The structural modification of napelline by benzoylation at the C-1 position appears to enhance its activity, as evidenced by the superior effects of 1-O-benzoylnapelline on mitochondrial stability. This suggests that targeted chemical derivatization of natural compounds may offer a viable strategy for improving their bioactivity. The present study investigated the effects of diterpene alkaloids - specifically napelline and its derivative 1-O-benzoylnapelline - on the mitochondrial permeability transition and mitoK^+_{ATP} channel activity in rat liver and heart mitochondria. The findings demonstrated that 1-O-benzoylnapelline significantly inhibited Ca^{2+}-induced mitochondrial permeability transition and effectively activated the mitoK^+_{ATP} channel at low micromolar concentrations, suggesting its promising potential as a cardioprotective agent (**Figure 5**). These results align with earlier reports on the protective role of mitoK^+_{ATP} channel activation during ischemic and hypoxic stress, where controlled K^+ influx mitigates mitochondrial depolarization, limits ROS generation, and prevents apoptosis.

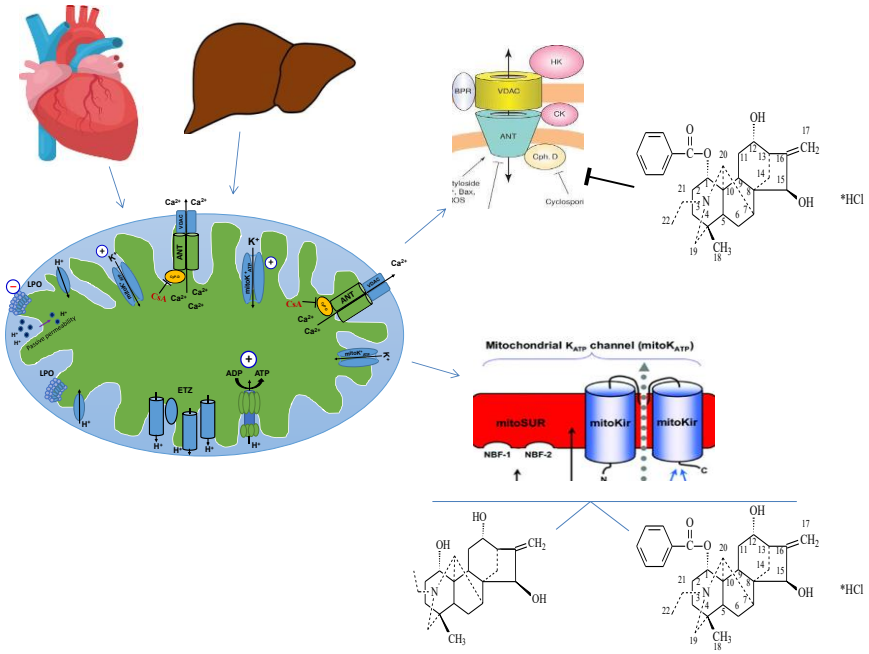


Figure 5 Mechanisms of action of diterpene alkaloids on mitochondrial membrane ion permeability *in vitro*.

Furthermore, although this study revealed tissue-specific differences in the sensitivity of liver and heart mitochondria to diterpene alkaloids, the underlying molecular mechanisms of this differential response remain unclear. These observations warrant further exploration to determine whether such effects are due to differences in channel subunit composition, mitochondrial membrane lipid environment, or other regulatory proteins. In conclusion, this work highlights the ability of 1-O-benzoylnapelline to inhibit mPTP and activate the mitoK$^+$ATP channel, both of which contribute to its significant cytoprotective properties. However, further extensive research is required to explore its full therapeutic potential.

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Declaration of Generative AI in Scientific Writing

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CRediT Author Statement

Dilnoza Khasanovna Muratova: Conceptualization, Methodology, Supervision, Validation, Funding acquisition, and Writing –original draft.

Nurali Azamovich Ergashev: Formal analysis, Methodology.

Muzaffar Islamovich Asrarov: Methodology, Formal analysis, Investigation, Validation.

Mamurjon Komiljonovich Pozilov: Data curation, Formal analysis.

Xabiba Yakubovna Berdiyeva: Data curation, Investigation.

References

[1] A Szewczyk and L Wojtczak. Mitochondria as a pharmacological target. *Pharmacological Reviews* 2002; **54(1)**, 101-127.

[2] M Akao, A Ohler, B O'Rourke and E Marbán. Mitochondrial ATP-sensitive potassium channels

inhibit apoptosis induced by oxidative stress in cardiac cells. *Circulation Research* 2001; **88(12)**, 1267-1275.

[3] ADT Costa, CL Quinlan, A Andrukhiv, IC West, M Jaburek and KD Garlid. The direct physiological effects of mitoK_{ATP} opening on heart mitochondria. *American Journal of Physiology-Heart and Circulatory Physiology* 2006; **290(1)**, H406-H415.

[4] DJ Hausenloy, R Schulz, H Girao, BR Kwak, D De Stefani, R Rizzuto, P Bernardi and F Di Lisa. Mitochondrial ion channels as targets for cardioprotection. *Journal of Cellular and Molecular Medicine* 2020; **24(13)**, 7102-7114.

[5] RB Strutynskyi, RA Rovenets and OOMoibenko. Mechanisms of cardiprotective activity of domestic activator of KATP channels flokali. *Tavricheskiy Medico-Biological Vestnik* 2012; **15(3)**, 226-229.

[6] H Ardehali and B O'Rourke. Mitochondrial K(ATP) channels in cell survival and death. *Journal of Molecular and Cellular Cardiology* 2005; **39(1)**, 7-16.

[7] GD Mironova, MI Shigaeva, EN Gritsenko, SV Murzaeva, EL Germanova, OS Gorbacheva and LLD Ukyanova. Activity of mitochondrial ATP-dependent potassium channel in animals with different resistance to hypoxia before and after the course of hypoxic training. *Bulletin of Experimental Biology and Medicine* 2011; **151(1)**, 25-29.

[8] AO Garlid, M Jaburek, JP Jacobs and KD Garlid. Mitochondrial reactive oxygen species: Which ROS signals cardioprotection? *American Journal of Physiology-Heart and Circulatory Physiology* 2013; **305**, 960-968.

[9] KD Garlid, P Paucek, V Yarov-Yarovoy, HN Murray, RB Darbenzio, AJ D'Alonzo, NJ Lodge, MASmith and GJ Grover. Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels: Possible mechanism of cardioprotection. *Circulation Research* 1997; **81(6)**, 1072-1082.

[10] OS Gorbacheva, NI Venediktova and GD Mironova. Study of the kinetics and regulation of the potassium cycle. *Pathogenesis* 2011; **9(3)**, 26-27.

[11] Y Nakai, H Horimoto, S Mieno and S Sasaki. Mitochondrial ATP-sensitive potassium channel plays a dominant role in ischemic preconditioning of rabbit heart. *European Surgical Research* 2001; **33(2)**, 57-63.

[12] GJ Grover and KD Garlid. ATP-sensitive potassium channels: A review of their cardioprotective pharmacology. *Journal of Molecular and Cellular Cardiology* 2000; **32(4)**, 677-695.

[13] P Bernardi, A Rasola, M Forte and G Lippe. The mitochondrial permeability transition pore: Channel formation by F-ATP synthase, integration in signal transduction, and role in pathophysiology. *Physiological Reviews* 2015; **95(4)**, 1111-1155.

[14] J Sileikyte and M Forte. The mitochondrial permeability transition in mitochondrial disorders. *Oxidative Medicine and Cellular Longevity* 2019; **2019(1)**, 3403075.

[15] JJ Lemasters, TP Theruvath, Z Zhong and AL Nieminen. Mitochondrial calcium and the permeability transition in cell death. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 2009; **1787(11)**, 1395-1401.

[16] A Rasola and P Bernardi. Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis. *Cell Calcium* 2011; **50(3**), 222-233.

[17] P Bernardi, C Gerle, AP Halestrap, EA Jonas, J Karch, N Mnatsakanyan, E Pavlov, SS Sheu and AA Soukas. Identity, structure, and function of the mitochondrial permeability transition pore: Controversies, consensus, recent advances, and future directions. *Cell Death & Differentiation* 2023; **30**, 1869-1885.

[18] M Crompton, H Ellinger and A Costi. Inhibition by cyclosporin A of a Ca^{2+}-dependent pore in heart mitochondria activated by inorganic phosphate and oxidative stress. *Biochemical Journal* 1988; **255(1)**, 357-360.

[19] C Piot, P Croisille, P Staat, H Thibault, G Rioufol, N Mewton, R Elbelghiti, TT Cung, E Bonnefoy, D Angoulvant, C Macia, F Raczka, C Sportouch, G Gahide, G Finet, X André-Fouët, D Revel, G Kirkorian, JP Monassier, G Derumeaux and M Ovize. Effect of cyclosporine on

reperfusion injury in acute myocardial infarction. *New England Journal of Medicine* 2008; **359(5)**, 473-481.

[20] DK Muratova, NA Ergashev and MI Asrarov. Effect of talatisamine and its derivate 14-O-benzoyltalatisamine on functional state of rat liver and heart mitochondria. *Biomedical & Pharmacology Journal* 2023; **16(4)**, 2333-2343.

[21] P Bernardi and FD Lisa. The mitochondrial permeability transition pore: Molecular nature and role as a target in cardioprotection. *Journal of Molecular and Cellular Cardiology* 2015; **78**, 100-106.

[22] A Nicolli, E Basso, V Petronilli, RM Wenger and P Bernardi. Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel. *Journal of Biological Chemistry* 1996; **271(4)**, 2185-2192.

[23] AP Halestrap. What is the mitochondrial permeability transition pore? *Journal of Molecular and Cellular Cardiology* 2009; **46(6)**, 821-831.

[24] UG Gayibov, EJ Komilov, RN Rakhimov, NA Ergashev, NG Abdullajanova, MI Asrarov and TF Aripov. Influence of new polyphenol compound from Euphorbia plant on mitochondrial function. *The Journal of Microbiology, Biotechnology and Food Sciences* 2019; **8(4)**, 1021-1025.

[25] MD Kh, NA Ergashev, AV Shkinev, MI Asrarov and UK Kurbanov. The effects of songorine on the activity of the ATP-dependent K^+-channel and the state of the megapore of rat liver mitochondria Exp. *Clinical Pharmacology* 2021; **84**, 12-15.

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

[27] MK Pozilov, U Gayibov, MI Asrarov, NG Abdulladjanova, HS Ruziboev and TF Aripov. Physiological alterations of mitochondria under diabetes condition and its correction by polyphenol gossitan. *Journal of Microbiology, Biotechnology and Food Sciences* 2022; **12(2)**, e2224.

[28] Z Ernazarov, MK Pozilov, MI Asrarov and SN Zhurakulov. Effects of dihydroquercetin, 1-aryltetrahydroisoquinoline, and conjugate on the functional condition mitochondrial membrane of the rat liver. *Nova Biotechnologica et Chimica* 2023; **22(1)**, e1414.

[29] MMN Sultankhodzhaev, LV Beshitaishvili, MS Yunusov and SY Yunusov. Alkaloids of Aconitum karakolicum. Structure of acetylnapelline. *Chemistry of Natural Compounds* 1978; **4**, 407-409.

[30] FN Dzhakhangirov, FM Tursunkhodzhaeva, MN Sultankhodzhaev and BT Salimov. Spasmolytic activity of diterpenoid alkaloids and their derivatives. *Chemistry of Natural Compounds* 2013; **49**, 702-706.

[31] MN Sultankhodzhaev, B Tashkhodzhaev, KK Turgunov, UK Kurbanov and NI Mukarramov. Structure and conformational analysis of napelline-type diterpenoid alkaloids. *Chemistry of Natural Compounds* 2017; **53(1)**, 99-104.

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

[33] GL Peterson. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry* 1977; **83**, 346-356.

[34] OB Vadzyuk and SA Kosterin. Diazoxide-induced swelling of rat myometrial mitochondria as evidence of activation of the ATP-sensitive K^+-channel. *Ukrainian Biochemical Journal* 2008; **80(5)**, 45-51.

[35] L He and JJ Lemasters. Heat shock suppresses the permeability transition in rat liver mitochondria. *Journal of Biological Chemistry* 2003; **278**, 16755-16760.

[36] A Paggio, V Checchetto, A Campo, R Menabò, G Di Marco, F Di Lisa, I Szabo, R Rizzuto and DD Stefani. Identification of an ATP-sensitive potassium channel in mitochondria. *Nature* 2019; **72(7771)**, 609-613.

[37] D Garlid, P Dos Santos, ZJ Xie, ADT Costa and P aucek. Mitochondrial potassium transport:

The role of the mitochondrial ATP-sensitive K^+ channel in cardiac function and cardioprotection. *Biochimica et Biophysica Acta-Bioenergetics* 2003; **1606(1-3)**, 1-21.

[38] FN Dzhakhangirov, MN Sultankhodzhaev, B Tashkhodzhaev and BT Salimov. Diterpenoid alkaloids as a new class of antiarrhythmic agents. Structure-activity relationship. *Chemistry of Natural Compounds* 1997; **33**, 190-202.

[39] L Zhang, M Siyiti, J Zhang, M Yao and F Zhao. Anti-inflammatory and anti-rheumatic activities *in vitro* of alkaloids separated from *Aconitum soongoricum Stapf. Experimental and Therapeutic Medicine* 2021; **21(5)**, 493.

[40] A Wrzosek, B Augustynek, M Żochowska and A Szewczyk. Mitochondrial potassium channels as druggable targets. *Biomolecules* 2020; **10(8)**, 1200.

[41] EL Holmuhamedov, A Jahangir, A Oberlin, A Komarov, M Colombini and A Terzic. Potassium channel openers are uncoupling protonophores: implication in cardioprotection. *FEBS Letters* 2004; **568(1-3)**, 167-170.

[42] H Zhu, J Dong, W Zhu, H Ding and Z Zhou. ATP-dependent potassium channels involved in cardiac protection induced by intermittent hypoxia against ischemia-reperfusion injury. *Life Sciences* 2003; **73(10)**, 1275-1287.

[43] MK Paul, K Rajinder and AK Mukhopadhyay. Characterization of rat liver mitochondrial permeability transition pore by using mitochondrial swelling assay. *African Journal of Pharmacy and Pharmacology* 2008; **2(2)**, 14-21.

[44] A Panov, S Dikalov, N Shalbuyeva, R Hemendinger, JT Greenamyre and J Rosenfeld. Species- and tissue-specific relationships between mitochondrial permeability transition and generation of ROS in brain and liver mitochondria of rats and mice. *American Journal of Physiology-Cell Physiology* 2007; **292(2)**, 708-718.

[45] R Endlicher, P Krivakova, H Lotkova, M Milerova, Z Drahota and Z Červinkova. Tissue specific sensitivity of mitochondrial permeability transition pore to Ca^{2+} ions. *Acta medica (Hradec Králové)* 2009; **52(2)**, 69-72.