

ANTI-RADICAL AND MEMBRANE-ACTIVE PROPERTIES OF GERANIIN POLYPHENOL COMPOUND

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Dildora Rahmatullaevna Isamukhamedova

Institute of Biophysics and Biochemistry under the National University of Uzbekistan, Tashkent, Uzbekistan

Nurali Azamovich Ergashev

Institute of Biophysics and Biochemistry under the National University of Uzbekistan, Tashkent, Uzbekistan

Rahmatilla Nurullaevich Rakhimov

Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

Muzaffar Islamovich Asrarov

Institute of Biophysics and Biochemistry under the National University of Uzbekistan, Tashkent, Uzbekistan

ABSTRACT

This research paper explores the anti-radical and membrane-active properties of geraniin polyphenol compound. Through experimentation, it was found that geraniin polyphenol exhibits a stabilizing effect on mitochondrial functional parameters and inhibits TssA-sensitive Sa2+-dependent mitochondrial megapore. Furthermore, the compound activates the permeability of the liver mitoK ATF channel and demonstrates membranotropic properties by regulating the size of the matrix. Notably, geraniin polyphenol showcases robust antiradical properties, indicating potent antioxidant activity. These findings shed light on the multifaceted pharmacological potential of geraniin polyphenol and its prospective applications in mitigating oxidative stress-related disorders.

KEYWORDS

Geraniin polyphenol, anti-radical properties, membrane-active, mitochondrial function, megapore inhibition, mitoK ATF channel, antioxidant activity.

INTRODUCTION

Studying the molecular mechanisms of pathological conditions at the level of subcells, including mitochondria, is one of the most pressing issues today. More than 90% of ATF needed for cell metabolism is synthesized by mitochondria. Mitochondria are therefore a convenient target for several molecular groups that protect against cellular damage and play a key role in the cytoprotective process [3]. In the literature, there is a lot of information about the functional parameters of cell components, in which the ion transport system, antioxidant system, formation of free radicals, oxidation-reduction reactions and membrane activity features are widely analyzed in the cell and mitochondrial membrane. Among them, basic functional parameters such as ion transport systems in mitochondria, i.e. mPTP, mitoK ATF channel, and formation of free radicals are of particular importance. Several mitochondrial proteins, including cyclophilin D (CypD), adenine nucleotide translocase (ANT), ATP-synthase, outer membrane potential-dependent anion channel (VDAC), phosphate transporter (PiC), and SPG-7, have been shown to be involved in channel activity. . Because CypD is not a transmembrane protein, it cannot form a channel by itself, but from the inhibitory effect of its ligand cyclosporine A, it is known to bind to channel-forming protein(s) and regulate channel activity. [5.15]. The unique regulatory feature of ATF-dependent K⁺ channel (mitoK ATF channel) in mitochondria includes functions of membrane potential, permeability of Na²⁺ ions, breakdown of free fatty acids and maintenance of ATF content in cells at a physiological level [7].

In fact, there are many signaling pathways in mitochondria, including cell proliferation and mitochondrial biogenesis. It also provides monitoring of cell death through oxidation-reduction reactions [16

]. In addition, the increase of free radicals in the cell, the appearance of reactive oxygen species (ROS) in the cell, and the rapid increase in the amount of lipid peroxides (LPO) and malondialdehyde (MDA) cause a change in the conformational state of ion channels in the membrane. The increased generation of these free radicals disrupts the function of passive and active transport of substances through ion transport systems. The activation of the potassium cycle during stress adaptation and the subsequent decrease in ROS formation may explain the known protective role of the mitoK ATF channel during ischemia-reperfusion. In recent years, the biophysical properties of the mitochondrial potassium channel and its physiological role have been well studied. Mitochondrial potassium channel is located in its inner membrane, and in laboratory conditions, a protein with the properties of this channel was isolated [14]. The participation of the mitoK ATF channel in the formation of the body's resistance to oxygen deficiency has been shown. MitoK ATF channel activation plays an important role in cardiomyocyte protection during ischemia. A number of mitoK ATF channel-activating synthetic substances (diazoxide and nicorandil) with potential cardioprotective properties have been discovered, which not only activate the mitoK ATF channel, but also activate cytoplasmic membrane potassium channels at high concentrations. However, there are no data on the effect of bioactive substances isolated from plants on the mitoK ATF channel in the literature. One of the most urgent tasks is to identify natural compounds that effectively affect the antioxidant system of liver mitochondria, inhibiting the formation of free radicals and mitoK ATF channel function under the influence of various stress factors, and studying their mechanisms of action.

Recent studies have shown that the mitochondrial megapore (mPTP) plays an important role in cell

signaling, apoptosis, and homeostasis of calcium ions. MPTP is activated by Ca^{2+} ions in the presence of inorganic phosphate and respiratory chain substrates [9]. In this case, the membrane of mitochondria is depolarized, their swelling is observed, the amount of ATP decreases and Ca^{2+} ions are released from the matrix, and the sensitivity of mPTP to cyclosporin A increases [2. 9]. At the same time, as a result of mitochondria bursting, its outer membrane breaks down, cytochrome *c* [8] and proapoptotic proteins are released from the intermembrane space into the cytosol [10]. The transition of MPTP to the open conformational state is fatal for the cell, causing disruption of bioenergetic processes and the development of various pathophysiological processes

[2]. Apparently, MPTP is involved in the development of various pathological processes. Hence, mPTP can be a pharmacological target in the treatment of various pathologies [2.8.10]. Based on this situation, it can be said that the study of the effect of various compounds, especially polyphenols, on the state of mPTP is one of the current topics.

In order to fulfill these tasks, in our research, we aimed to study the effect of geraniin (Fig. 1) polyphenol isolated from *Euphorbia franchetii* plant and its antiradical activity on mitoK_{ATP} channel activity of rat liver mitochondria and TssA-sensitive Ca^{2+} -dependent megapore.

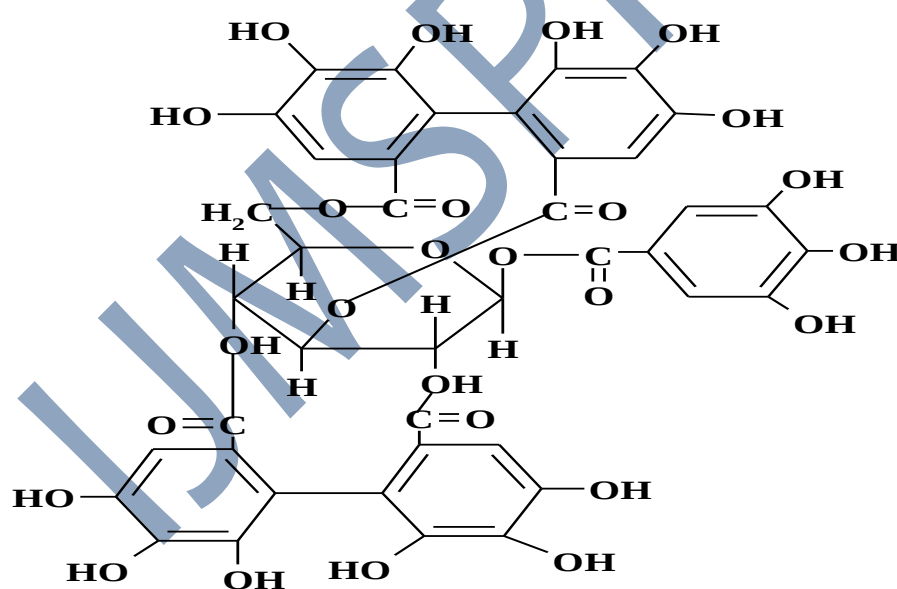


Fig. 1. Structural formula of geraniin polyphenol

RESEARCH MATERIALS AND METHODS

Rat liver mitochondria were isolated by differential centrifugation. The composition of the separation medium: 250 mM sucrose, 10 mM tris-HCl, 1 mM EDTA, [12] pH-7.4.

Mitochondrial K_{ATP}-channel permeability (0.3-0.4 mg/ml) was determined spectrophotometrically based on the change in optical density at a wavelength of 540 nm in 3 ml cells. The composition of the incubation medium was as follows: 125 mM KCl, 10 mM Hepes, 5

mM succinate, 1 mM magnesium chloride, 2.5 mM potassium hydrogen phosphate, 2.5 mM potassium dihydrogen phosphate, 5 μ M rotenone, and 1 μ g/ml mM oligomycin (rN-7.4). The volume of the incubation medium was 3 ml [13].

In the spectrophotometric method of determining the antiradical activity of bioactive substances, 2,2-diphenyl-1-picrylhydrazyl (DFPG) standard alcohol working solution 1:10 was determined [7].

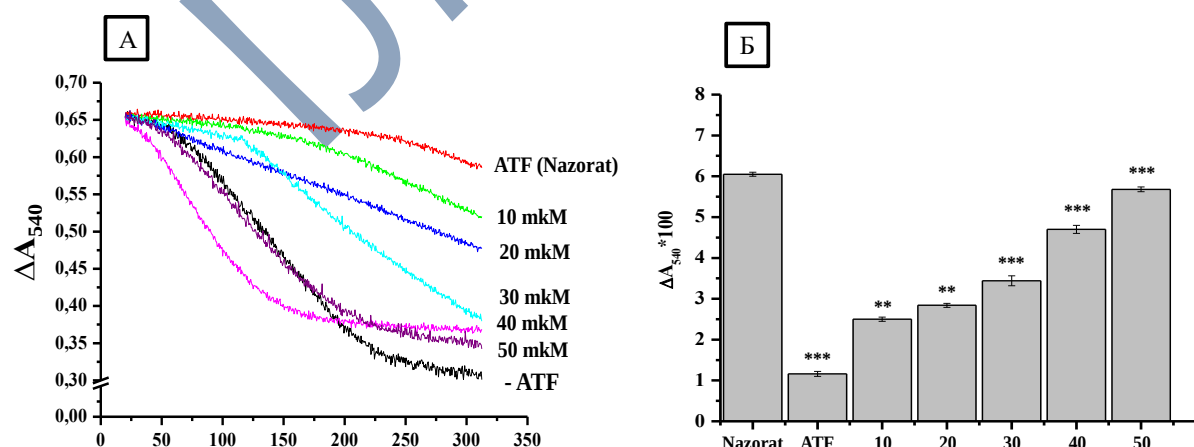
Also, in the study of TssA-sensitive Ca^{2+} -dependent megapore, from the incubation medium: sucrose - 200 mM, KN_2RO_4 - 1 mM, succinate - 5 mM, Ca^{2+} -EGTA-buffer - 20 μ M, Hepes - 20 mM, tris-HCl - 20 mM, rotenone - 2 μ M, rN 7.2 was used [12].

The amount of protein in mitochondria was determined by the biuret method. BSA was used as standard protein. The amount of protein in the cuvette was 0.3-0.4 mg/ml.

The obtained results and their analysis. Changes in the activity of potassium channels, which play an important role in the regulation of mitochondrial size and potential formation, can be noted. In particular, mitoKATF-channel activity is involved in the cycle of

potassium ions between mitochondria and cytosol. MitoKATF-channel inhibitors and activators exist. Bioactive substances extracted from plants can have different effects on mitoKATF-channel activity. In order to determine these, in our next experiment, the effect of geraniin polyphenol on liver mitoKATF-channel activity was studied.

In our studies, rat liver mitochondria geraniin on mitoKATF-channel activity the effect of polyphenol concentrations of 10, 20, 30, 40 and 50 μ M was studied (Fig. 2, A). In this case, the effect of the polyphenol substance was studied when mitoKATF-channel activity was inhibited by 200 μ M ATF. $41.32 \pm 0.95\%$ under the condition that ATF inhibits the mitoKATF-channel under the influence of 10 μ M concentration of polyphenol compound was found to activate. At the same time, $46.94 \pm 0.84\%$ activation of the polyphenol substance under the influence of 20 μ M concentration was shown. In our further studies, when the effect of 30, 40 and 50 μ M concentrations of geraniin on mitoKATF-channel activity of rat liver mitochondria was studied, these concentrations of polyphenol were $56.85 \pm 0.91\%$, respectively, compared to the condition when the channel was inhibited by ATF; It was found to activate up to $77.68 \pm 0.72\%$ and $93.88 \pm 0.54\%$ (Fig. 2, B).



**Figure 2. Geraniin effect of polyphenol on rat liver mitoK_{ATP} -channel. The original diagram of the effect of polyphenol on the liver mitoK_{ATP} channel , B - a histogram representing the activity of the mitoK_{ATP} channel .
n=5-6; **- R>0.01; ***-R>0.001.**

Geraniin polyphenol on the state of the Ca^{2+} -dependent megapore of rat liver mitochondria was investigated (Fig. 3, A,B). In our experiments, in the study of the rat liver mitochondria megapore, by adding 10 μM Ca^{2+} to the incubation medium, the mitochondrial megapore was shifted to the open conformational state, and this index was 6.88 ± 0.08 , which was considered as 100% control. At the same time, TssA, a special inhibitor of the channel, was used to determine whether the studied channel is really a megapore. When the experiment was continued by adding 5 μM TssA to the incubation medium with Ca^{2+} ions, indeed TssA inhibited the megapore up to 96.1% compared to the control, which was 0.27 ± 0.05 . From this then with polyphenol studies continue was made . Initially geranium 50 μM of polyphenol

concentration under the influence of liver Mitochondria depend on Ca^{2+} + TssA-sensitive Megapore status to control up to 9.01% gingered 6.26 ± 0.1 indicators were determined organize did _100 and 150 μM of polyphenol concentrations under the influence of while Ca^{2+} ions of the megapore in the presence of open conformational to the situation pass to control relatively suitable by 16.3 % (5.76 ± 0.06) and 42.7% (3.94 ± 0.16) was determined of polyphenol upper 200 μM concentration under the influence of while liver mitochondria Ca^{2+} ions of megapore in the presence of open conformational to the situation pass to control the indicator of 1.96 ± 0.08 organize up to 71.5% whining was determined of Geraniin a rat liver mitochondria megapore half maximum restraining concentration is $\text{IC}_{50} = 160.7 \pm 2.67 \mu\text{M}$.

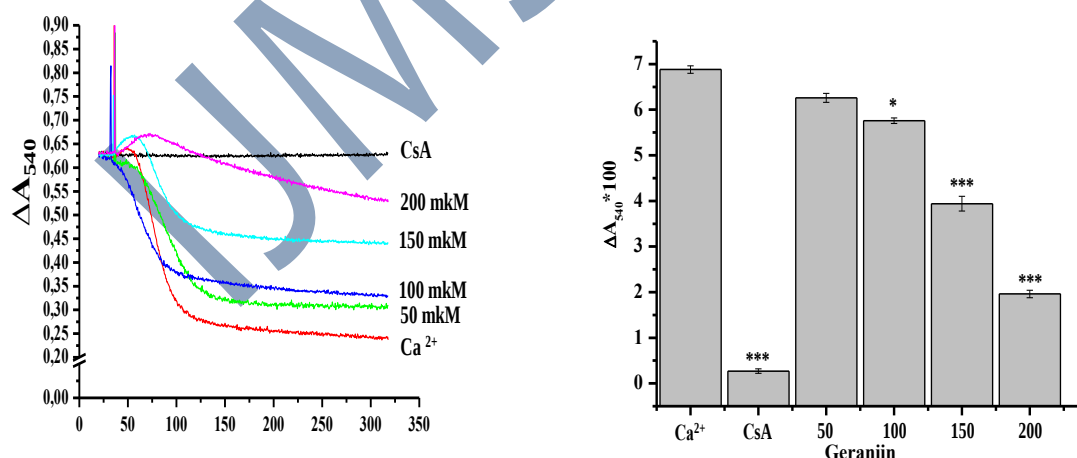


Fig. 3. Geraniin in rat liver mitochondria suppression of polyphenol effect. A - the original record taken with the V-5000 spectrophotometer ; B- geraniin is a graphical representation of the effect of polyphenols on mitochondrial function. (*P<0.05; **P<0.01; *P<0.001; n=5).**

Geraniin polyphenol substance using 2,2-diphenyl-1-picrylhydrazyl (DFPG) reagent at a wavelength of 517 nm. 2,2-diphenyl-1-picrylhydrazyl (DFPG) standard alcohol working solution 1:10 was prepared. The ratio of DFPG/polyphenol is 1:10. The optical density of the mixed solution is recorded in a spectrophotometer at a wavelength of 517 nm for 25 minutes (Fig. 4, A). Geraniin to the DFPG working solution in the

experiments 1.0 μM of polyphenol substance compared to the control of free radicals $23.08 \pm 0.22\%$, $39.05 \pm 0.17\%$ at 2.5 μM , and $69.05 \pm 0.15\%$ at 5 μM . 7.5 μM of this concentration neutralized free radicals by $90.20 \pm 0.27\%$ compared to the control (Fig. 3, B). It can be seen from the obtained results polyphenol fully demonstrated its antiradical properties.

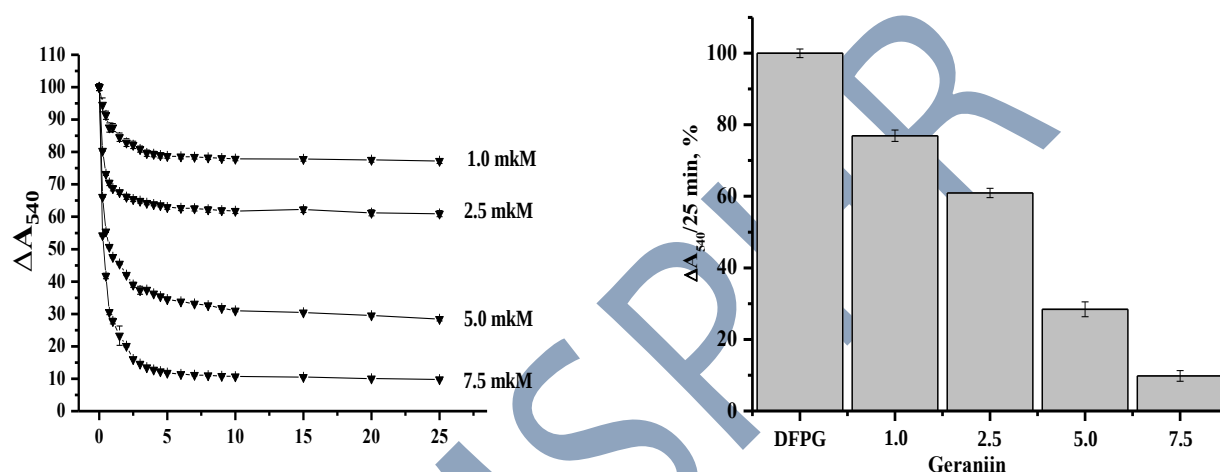


Fig. 4. The spectrophotometric method was used to determine the antiradical activity. Original image (A) 2,2-diphenyl-1-picrylhydrazyl (DFPG) standard alcohol working solution 1:10 was determined. histogram representing the antiradical activity of geraniin polyphenol (B).

CONCLUSION

The obtained results show that geraniin polyphenol has a stabilizing effect on mitochondrial functional parameters and an inhibitory effect on TssA-sensitive Sa^{2+} -dependent mitochondrial megapore. Also, the polyphenolic compound activates the permeability of the liver mitoK_{ATP} channel and has shown its membranotropic properties by regulating the size of the matrix. Geraniin polyphenol showed a strong antiradical property, which indicates that this compound has antioxidant activity.

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