

ISSN 0042-1685

**O'ZBEKISTON
BIOLOGIYA
JURNALI**

**Узбекский
Биологический
Журнал**



**Uzbek
Biological
Journal**

4-2023

ЎЗБЕКИСТОН RESPUBLIKASI FANLAR АКАДЕМИЯСИ
АКАДЕМИЯ НАУК РЕСПУБЛИКИ УЗБЕКИСТАН

**ЎЗБЕКИСТОН
BIOLOGIYA
JURNALI**

4

2023

**УЗБЕКСКИЙ
БИОЛОГИЧЕСКИЙ
ЖУРНАЛ**

Издается с января 1957 г. по 6 номеров в год

ТАШКЕНТ - 2022

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100047, Ташкент, ул. Я. Гулямова, 70.

Телефон 232-11-81

Технический редактор: М. Сагатов

На обложке: *Балобан. Итоги.*
Falco cherrug J.E. Gray, 1834
ssp. coatsi Dementtiev, 1945(1)
ssp. cherrug J.E. Gray, 1834 (2)
ssp. milvi pes Jerdon, 1871 (3)

Журнал зарегистрирован Агентством по печати и информации Республики Узбекистан 22.12.2006
Регистрационный номер 0052.

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EFFECT OF GOSSYPOL AND ITS DERIVATIVES ON FUNCTIONAL PARAMETERS OF
RAT LIVER MITOCHONDRIA

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У.Г. Гайибов, Э.Дж. Комилов, А.Т. Эргашев, М.И. Асраров, Т.Ф. Арипов
ГОССИПОЛ ВА УНИНГ ҲОСИЛАЛАРИНИ МИТОХОНДРИЯ ФУНКЦИОНАЛ ПАРАМЕТРЛАРИГА
ТАЪСИРИ

Госсипол полифенол бирикма бўлиб, кенг биологик хусусиятларга эга модда. Унинг турли моно- ва дихосилалари антиоксидант, шамоллашга қарши, антиаллерген, иммуномодулятор, вирусларга қарши ва ўсма касалликларига қарши кенг қўлланилади. Бундан ташқари госсипол ва унинг ҳосилалари асосида таёрланган дори воситалари гепатит вирусига қарши гепатопротектор сифатида кенг қўлланилмоқда.

Шунинг учун, патологик ҳолатлардаги митохондрия функцияларини коррекция қилувчи объект сифатида госсипол ва унинг ҳосилаларини циклоспоринсезгир порага таъсири ўрганилган. Бундан ташқари, юқорида келтирилган моддаларни митохондрия мембраналаридан бир ва икки валентли катионларни пассив транспортга таъсири ҳам ўрганилган.

У.Г. Гайибов, Э.Дж. Комилов, А.Т. Эргашев, М.И. Асраров, Т.Ф. Арипов
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МИТОХОНДРИЙ

Госсипол хорошо известное полифенольное соединение с широким спектром биологических активностей, также его различные моно- и дипроизводные используются в качестве антиоксидантных, противовоспалительных, антиаллергенных, иммуномодулирующих, противовирусных и противоопухолевых препаратов. Кроме того, в последние годы препараты на основе госсипола и его производных широко используются как гепатопротекторы при лечении вирусного гепатита.

Таким образом, для изучения действия госсипола и его некоторых производных, как объект корректировки функций митохондрий в патологических состояниях, в данной работе изучено влияние вышеуказанных соединений циклоспоринчувствительную пору (mitochondrial permeability transition pore) в митохондриях из печени крыс. Также было изучено влияние госсипола и его производных на пассивный транспорт различных одно- и двухвалентных катионов через мембраны митохондрий.

U.G. Gayibov, E.J. Komilov, A. T. Ergashev, M.I. Asrorov, T.F. Aripov
EFFECT OF GOSSYPOL AND ITS DERIVATIVES ON FUNCTIONAL PARAMETERS OF RAT LIVER
MITOCHONDRIA

Effect of gossypol and its mono-derivatives (monoamigossypol, monoaminogossypol) on ion transport across mitochondria membrane was investigated. Comparison investigation of influence of gossypol and its derivatives to transfer of protons through membrane shows different activities. However, gossypol has the highest activity than its derivatives. Mitochondrial permeability transition pore mPTP in vitro activities of gossypol and its derivatives were investigated too. Comparison results were the same.

Introduction. Mitochondria are involved in a wide range of cellular processes of importance for cell regulation including the transport of different metabolites across inner mitochondrial membrane (Palmeri, 2010). In this case biologically active compounds demonstrating effect on mitochondrial processes are of a great interest and searching for a novel and effective safe drugs to prevent mitochondria dysfunction remains an area of intensive research. Natural products derived from plants such as polyphenols have received considerable attention in recent years due to diverse pharmacological properties, including anti-inflammatory, anti-parasitic, antioxidant, antiviral, anticancer and other activities (Asrarov et al., 2015; Daglia, 2012; Lipińska et al., 2014; Xia et al., 2010). One of such kind of polyphenols is a polyphenol gossypol isolated from the seed, roots, and stem of the cotton plant (*Gossypium* sp.). The substance, a yellow pigment similar to flavonoids, is present in cottonseed oil (Gayibov et al., 2010)

The variety of activities of gossypol and its derivatives is indicative of several mechanisms of action and they can be considered polyfunctional modulators of cell processes. Thus gossypol was shown to inhibit oxidation of NADH substrate on isolated mitochondria (Arinbasarova et al., 2012). Gossypol can also chelate iron and other metal ions and be both a pro-oxidant and antioxidant (Depeyster and Wang, 1993). Moreover, the aromatic rings in gossypol render it a fairly hydrophobic compound able to penetrate biological membranes more readily (Laughton, 1989). With the help of differential scanning calorimetry and fluorescent methods it was shown that gossypol and its derivatives have expressed membrane activities that in turn made it interesting object for pharmacological goals. Additional chemical modifications of these natural compounds can improve their biological and pharmacological properties (Kosymbetov, 2006).

One of the important functional parameters of the cell is functional activity of mitochondrial permeability transition pore (mPTP). The mPTP is a causative event, leading to necrosis and apoptosis in hepatocytes after oxidative stress, Ca(2+) toxicity, and ischemia/reperfusion (Kim et al., 2013).

Inhibitors of the MPT have been shown to reduce cardiac ischemia-reperfusion injury (Martel et al., 2012). Furthermore, most cardioprotective strategies appear to reduce ischemic cell death either by reducing the triggers for the opening of the MPT, such as reducing calcium overload or reactive oxygen species, or by more direct inhibition of the MPT.

This work focuses on the study of influence of gossypol and its mono-derivatives (monoaminogossypol, MAG; monoanilinogossypol, MANG) on proton transportation across mitochondrial membranes and their effect on Ca²⁺-dependent mitochondrial permeability transition pore in vitro.

Materials and methods. Mitochondria were isolated as described by Frezza et al. (2007). The measurements of the permeability transition pore was done as described Marcu et al. (2012).

Mitochondrial swelling was monitored by absorbance at 540 nm in incubation buffer containing 200 mM sucrose, 20 μM EGTA, 5 mM succinate, 2 μM rotenone, 1 μg/ml oligomycin, 20 mM Tris, 20 mM HEPES, and 1 mM KH₂PO₄, pH 7.2 as described previously (He and Lemasters, 2002).

Statistical analyses were performed using the statistical package Origin 8 (OriginLab Corporation, USA).

Results. The biological activity of gossypol is mainly due to a disturbance of mitochondrial functions such as modification of the mitochondrial Ca²⁺ concentration associated with membrane fluidity (Martinez et al., 1993), disruption of oxidative phosphorylation by inhibition of mitochondrial succinic dehydrogenase (Jiang et al., 2002), release of cytochrome C from mitochondria into the cytosol suggesting a mitochondrial-mediated apoptotic mechanism (Hou et al., 2004). It is the diversity of mitochondrial dysfunctions that remains the problem of gossypol toxicity opened. The understanding of gossypol's mechanism of action on eukaryotic cells would raise a possibility of clinical implications.

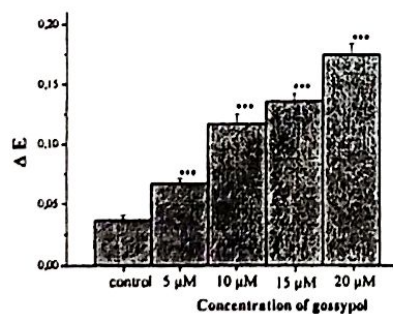


Figure 1. Influence of gossypol on passive transport of H⁺ across mitochondria membrane in isotonic NH₄NO₃-solution.

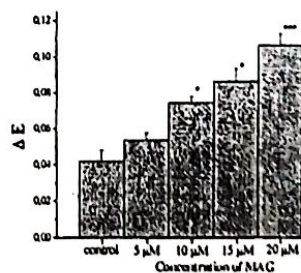


Figure 2. Influence of MAG on passive transport of H⁺ across mitochondria membrane in isotonic NH₄NO₃-solution.

The effect of gossypol on deenergized mitochondria from rat liver in isotonic NH₄NO₃-medium was dose-dependent and in concentrations 5 μM, 10 μM, 15 μM and 20 μM increased proton transportation 1.8, 3.2, 3.7 and 4.7 times respectively (Figure 1). Effect of MAG on passive transport of H⁺ in isotonic NH₄NO₃-solution shows that MAG increases the speed of mitochondria swelling in dose-dependent manner (Figure 2) and in the concentrations 5 μM, 10 μM, 15 μM and 20 μM the process sped up 1.3, 1.8, 2.1 and 2.5 times respectively. At the same time effect of MANG on proton transportation across mitochondria membranes was not observed.

Effect of gossypol and its derivatives on mitochondria permeability transition pore was also studied. Addition of $10\mu\text{M Ca}^{2+}$ to the incubation medium with induces intensive mitochondria swelling that indicates mPTP opening. Addition of gossypol in concentration of $1\mu\text{M}$ inhibits mPTP function to 6%. The raising of gossypol concentration to $3\mu\text{M}$, $5\mu\text{M}$ and $7\mu\text{M}$ inhibits mPTP activity to 34.4 %, 62.5 % and 86.1 %, respectively (Figure 3). Effect of MAG on the activity of mPTP was weaker in comparison with gossypol. At the same concentration of $5\mu\text{M}$, $10\mu\text{M}$, $15\mu\text{M}$ and $20\mu\text{M}$ and in presence of $20\mu\text{M Ca}^{2+}$ MAG inhibited mPTP to 9.1 %, 26.3 %, 52.4 % and 86.8 %, respectively (Figure 4). The second derivative of gossypol, MANG, showed even less activity comparing to gossypol and MAG. At a concentration of $15\mu\text{M}$, $30\mu\text{M}$, $50\mu\text{M}$ and $70\mu\text{M}$ MANG inhibited the activity of mPTP to 13 %, 25 %, 55 % and 91 %, respectively (Figure 5).

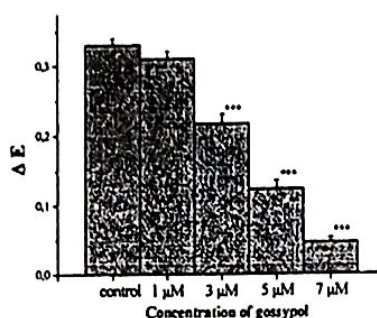


Figure 3. Influence of gossypol on mPTP.

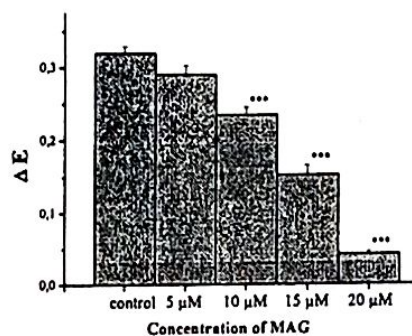


Figure 4. Influence of MAG on mPTP.

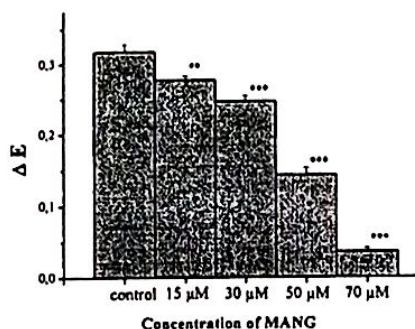


Figure 5. Influence of MANG on mPTP.

Discussion. Gossypol, a triterpenoid aldehyde, is a naturally occurring compound extracted from the cotton plant and other plants of the genus *Gossypium*. The compound has been shown to reveal bacteriostatic (Yildirim-Aksoy et al., 2004), antifungal (Puckhaber et al., 2002), antitumor (Moon et al., 2001), antifertility (Dodou et al., 2005) and anti-insect (Stipanovic et al., 2006) activities. Being biologically active, gossypol has received significant attention as a potential medicinal product and has been extensively studied over the past two decades.

Our results suggest that gossypol and MAG increase passive proton transport across mitochondria membrane thus showing protonophoric activity. At the same time gossypol possessed higher activity in comparison with MAG. MANG did show any protonophoric activity.

Experiments on mPTP showed that gossypol and its derivatives in dose-dependent manner can inhibit pore opening. Mitochondrial membrane is slightly permeable to some anions and cations, like NH_4^+ , but not permeable to chloride, sulphate, as well as to protons. Penetration of NH_4^+ does not cause mitochondria swelling as H^+ or OH^- are transported outside the cell thus protecting mitochondria from increased osmotic pressure. When both cation and anion ions penetrate into mitochondria the swelling process increases as increases osmotic pressure. NH_4^+ penetrates as neutral molecule of ammonia. We propose that in presence of gossypol and MAG cations and anions penetrate into mitochondria causing significant mitochondria swelling.

The higher protonophoric activity of gossypol in comparison to MAG and MANG is more likely because of its higher membrane active and antiradical activities. It was shown that antiradical and mem-

brane activity of gossypol derivatives were determined by the structure of the substituent and that gossypol and its derivatives were partially localized in the lipid bilayer and possibly induced formation of a new interdigitating phase (Tukfatullina et al. 2008).

Conclusion. Gossypol has been known for over a hundred years and the dichotomy between its toxicity and its unique biological activities places gossypol in an unusual position. And one of points for researchers is using gossypol's binaphthyl structure, functionality, and unique biological activities to make gossypol a potentially important value-added natural product. Our results suggest that the presence of carbonyl groups in the gossypol structure plays an important role in its protonophoric activity and ability to alter the function of mPTP that could be useful for further research efforts to obtain suitable gossypol derivatives for medical applications.

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Институт биоорганической химии АН РУз

Дата поступления
04.12.2017

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