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Research article / Оригинальная статья

Isolation of Formononetin-7-O-β-D-glucopyranoside from the Grass of *Ononis arvensis* L. and the Assessment of its Effect on Induced Platelet Activation

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Abstract

Introduction. Analysis of the clinical and laboratory picture of the SARS-CoV-2 infection suggests the presence of microcirculation and oxygen transport disorders, hemolysis of erythrocytes, intra-alveolar fibrin formation and microthrombus formation in the patient's pathogenesis. Accordingly, the search for potential anticoagulants, erythrocyte antiplatelet agents, membrane stabilizing drugs and mild thrombolytic drugs can prevent the development of life-threatening complications and reduce the mortality of COVID-19 patients.

Aim. Isolation of formononetin-7-O-β-D-glucopyranoside from the grass of *Ononis arvensis* L. and identification of the molecular mechanisms of its effect on platelet activation *in vitro*, induced by TRAP-6 (Thrombin receptor activated peptide) and ADP (adenosine diphosphate).

Materials and methods. Terrestrial parts of *Ononis arvensis* L. were collected in the SPCPU nursery of medicinal plants (Leningrad region, Vsevolozhsky district, Priozerskoe highway, 38 km). Isolation of formononetin-7-O-β-D-glucopyranoside was carried out by preparative high performance liquid chromatography on a Smartline device (Knauer, Germany) equipped with a spectrophotometric detector. The structure of formononetin-7-O-β-D-glucopyranoside was confirmed by one-dimensional and two-dimensional NMR spectroscopy (Bruker Avance III, 400 MHz, Germany), as well as high-resolution mass spectrometry (HR-ESI-MS) (Bruker Micromass Q-TOF, Germany). The study of the effect of formononetin-7-O-β-D-glucopyranoside on induced platelet activation was carried out on human platelets isolated from the blood of healthy volunteers. To research the effect of formononetin-7-O-β-D-glucopyranoside on platelet aggregation flow cytofluorometry with Cyto-FLEX (Beckman-Coulter, USA) was used.

Results and discussion. According to the method of fractionation and purification of the total extract of *O. arvensis* developed in previous studies, formononetin-7-O-β-D-glucopyranoside was isolated in an individual form for subsequent biological studies with a total yield of 30 % in comparison with its content in the original extract. In samples with formononetin-7-O-β-D-glucopyranoside and ADP, there is a pronounced inhibition of platelet activation – the percentage of active platelets ranges from 6.3–6.6 % at doses of formononetin-7-O-β-D-glucopyranoside 1 μM, 3 μM and 30 μM. The inhibitory effect of formononetin-7-O-β-D-glucopyranoside is not dose-dependent ($p \leq 0.05$). In samples with formononetin-7-O-β-D-glucopyranoside and TRAP, there is also a pronounced inhibition of platelet activation. The percentage of active platelets is 8 % at 1 μM formononetin-7-O-β-D-glucopyranoside doses, 15 % at 3 μM doses, and 16 % at 30 μM doses.

Conclusion. Administration of formononetin-7-O-β-D-glucopyranoside at doses of 1 μM, 3 μM, 30 μM strongly inhibits platelet activation induced by ADP and TRAP-6. For ADP, there is no dose-dependent effect, while for TRAP there is a weak dose-dependent effect, the greatest inhibition efficiency is achieved with the minimum investigated dose of 1 μM. In all cases, the results obtained are statistically significant.

Keywords: *Ononis arvensis*, platelets, formononetin-7-O-β-D-glucopyranosid, flavonoids, isoflavonoids, platelet activation, flow cytometry

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

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Выделения формононетин-7-О-β-D-глюкопиранозида из травы стальника полевого (*Ononis arvensis* L.) и оценка его влияния на индуцированную активацию тромбоцитов

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Резюме

Введение. Анализ клинической и лабораторной картины поражения вирусом SARS-CoV-2 позволяет говорить о наличии в патогенезе больных нарушений микроциркуляции и транспортировки кислорода, гемолиза эритроцитов, интраальвеолярного фибринообразования и микротромбообразования. Соответственно, поиск потенциальных антикоагулянтов, антиагрегантов эритроцитарного ряда, мембраностабилизирующих препаратов и мягких тромболитических препаратов способны предотвратить развитие угрожающих жизни осложнений и снизить смертность пациентов COVID-19.

Цель. Выделение формононетин-7-О-β-D-глюкопиранозида из травы стальника полевого (*Ononis arvensis* L.) и выявление молекулярных механизмов его влияния на активацию тромбоцитов *in vitro*, индуцированную TRAP-6 (Thrombin receptor activated peptide) и ADP (Аденозиндифосфат).

Материалы и методы. Надземные части стальника полевого (*Ononis arvensis* L.) были собраны в питомнике лекарственных растений СПХФУ (Ленинградская область, Всеволожский район, Приозерское шоссе, 38 км). Выделение формононетин-7-О-β-D-глюкопиранозида осуществляли методом препаративной высокоэффективной жидкостной хроматографии на приборе Smartline (Knauer, Германия), оснащенном спектрофотометрическим детектором. Структуру формононетин-7-О-β-D-глюкопиранозида подтверждали методом одномерной и двумерной ЯМР-спектроскопии (Bruker Avance III, 400 MHz, Германия), а также масс-спектрометрией высокого разрешения (HR-ESI-MS) (Bruker Micromass Q-TOF, Германия) в сравнении с литературными данными. Изучение влияния формононетин-7-О-β-D-глюкопиранозида на индуцированную активацию тромбоцитов проводили на тромбоцитах человека, выделенных из крови здоровых добровольцев. Для исследования влияния формононетин-7-О-β-D-глюкопиранозида на агрегацию тромбоцитов использовали метод проточной цитофлуорометрии на приборе Cyto-FLEX (Beckman-Coulter, США).

Результаты и обсуждение. Согласно разработанной в предыдущих исследованиях методики фракционирования и очистки суммарного экстракта травы *O. arvensis* был выделен формононетин-7-О-β-D-глюкопиранозид в индивидуальном виде для последующих биологических исследований со суммарным выходом 30 % в сравнении с его содержанием в изначальном экстрактом. В пробах с формононетин-7-О-β-D-глюкопиранозидом и ADP наблюдается выраженное ингибирование активации тромбоцитов – процент активных тромбоцитов колеблется в пределах 6,3–6,6 % при дозах формононетин-7-О-β-D-глюкопиранозида 1 μM, 3 μM и 30 μM. Ингибирующее действие формононетин-7-О-β-D-глюкопиранозида не носит дозозависимый характер ($p \leq 0,05$). В пробах с формононетин-7-О-β-D-глюкопиранозидом и TRAP также наблюдается выраженное ингибирование активации тромбоцитов. Процент активных тромбоцитов равен 8 % при дозах формононетин-7-О-β-D-глюкопиранозида 1 μM, 15 % при дозах 3 μM, и 16 % при дозе 30 μM. У ингибирующего эффекта формононетин-7-О-β-D-глюкопиранозида наблюдается слабая дозозависимость ($p \leq 0,05$).

Заключение. Введение формононетин-7-О-β-D-глюкопиранозида в дозах 1 μM, 3 μM, 30 μM выраженно ингибирует активацию тромбоцитов, индуцированную ADP и TRAP-6. Для ADP дозозависимого эффекта не возникает, в то время как для TRAP есть слабый дозозависимый эффект, наибольшая эффективность ингибирования достигается при минимальной исследованной дозе 1 μM. Во всех случаях полученные результаты являются статистически значимыми.

Ключевые слова: *Ononis arvensis*, тромбоциты, формононетин-7-О-β-D-глюкопиранозид, флавоноиды, изофлавоноиды, активация тромбоцитов, проточная цитофлуорометрия

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. А. М. Богоутдинова, А. К. Уэйли, А. О. Понкратова, А. А. Орлова, В. С. Шпакова, Н. Т. Фарманова, Д. Х. Нуруллаева, А. Т Шарипов – экспериментальная работа. М. Ю. Гончаров, С. П. Гамбарян, М. Н. Повыдыш – обсуждение результатов исследования, подготовка текста статьи.

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INTRODUCTION

According to the modern data, 20–25 % of all drugs mentioned in Pharmacopoeias come from natural sources, regardless whether they are used as unchanged individual natural compounds or with minor chemical modifications. In fact, about 50 % of drugs are produced on the basis of compounds first identified or isolated from plants [1].

Severe acute respiratory syndrome that develops with the new coronavirus infection (COVID-19) leads to a significant loss of life. Although the disease was initially primarily characterized with respiratory symptoms, it became clear that other systems were also involved, including the cardiovascular system. The analysis of the clinical and laboratory picture of SARS-CoV-2 virus infection suggests the presence disorders of microcirculation and oxygen transport, hemolysis of erythrocytes, intraalveolar fibrin formation and microthrombus formation in the pathogenesis. The prevention of these processes, preventive and early therapy, the administration of anticoagulants, antiplatelet agents, membrane stabilizing drugs and, possibly, mild thrombolytic OTC agents can prevent life-threatening complications and reduce the mortality of COVID-19 patients [2–4]. Thus, the studies aimed to the search for new anticoagulants, antiplatelet and antithrombotic agents are of great importance.

Currently, there are enough drugs on the pharmaceutical market from the group of antiplatelet agents with different mechanisms of action (clopidogrel, cyclopidine, dipyridamole, acetylsalicylic acid), however, in some cases, the risks of these drugs may outweigh their benefits. For example, in the presence of concomitant diseases associated with the risk of the increased bleeding, such drugs are contraindicated.

Numerous studies are devoted to investigation of the effect of drugs of natural origin on platelets and search of new natural molecules with cardiotropic activity [5–7]. However, in most studies, only the final result of the drugs action is noted without analyzing the molecular mechanisms responsible for inhibition or activatoin of platelets. It is known that oxidative stress, often associated with local or systemic inflammation underlies many cardiovascular diseases [8–10]. The antiplatelet and thrombolytic activity of natural compounds from the class of flavonoids has been reported. These compounds having a high antioxidant potential also increase the effectiveness of therapy for the disorders accompanied with oxidative stress.

Formononetin and its derivatives belong to the class of isoflavones. For a number of formononetin glycosides, including formononetin-7-O-β-D-glucopyranoside, antioxidant, cardioprotective, neuroprotective, anti-inflammatory and antiplatelet activity, etc. has been shown [11–16]. The study aim is the isolation of formononetin-7-O-β-D-glucopyranoside from the grass of *Ononis arvensis* L. and identification of the molecular mechanisms of its effect on platelet activation in vitro induced by TRAP-6 (Thrombin receptor activated peptide) and ADP (Adenosine diphosphate).

MATERIALS AND METHODS

The terrestrial parts of *Ononis arvensis* L. were collected in the nursery garden of medicinal plants of SPCPU (Leningrad region, Vsevolozhsky district, Priozerskoe highway, 38 km). The isolation of formononetin-7-O-β-D-glucopyranoside was performed with column chromatography (Dianion HP-20) and preparative high performance liquid chromatography on a Smartline device (Knauer, Germany) equipped with a spectrophotometric detector at a wavelength of 254 nm. A Kromasil chromatographic column 100-5C18, 250 × 30 mm in dimensions was used. The flow rate of mobile phase was 40 ml/min. The mobile phase composition: water (component A), acetonitrile (component B) with TFA content of 0.1 % (from H2O:CH3CN 5:95 to H2O:CH3CN 50:50, by volume). The structure of formononetin-7-O-β-D-glucopyranoside was confirmed with one-dimensional and two-dimensional NMR spectroscopy (Bruker Avance III, 400 MHz, Germany), as well as high-resolution mass spectrometry (HR-ESI-MS) (Bruker Micromass Q-TOF, Germany) (Luzhanin, V.G., 2021).

The study of the effect of formononetin-7-O-β-D-glucopyranoside on induced platelet activation was carried out on human platelets isolated from the blood of healthy volunteers. All experimental protocols were approved by the Ethics Committee of the Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences (Protocol No. 3-03 dated 02.03.20) and complied with the Declaration of Helsinki. The blood was collected into a tube with citrate buffer

supplemented with EGTA (2.5 μM), centrifuged for 7 minutes at 1400 g. Then, platelet-rich plasma (PRP) was collected.

To investigate the effect of formononetin-7-O-β-D-glucopyranoside on platelet activation induced by ADP or TRAP-6, PRP was diluted with HEPES buffer (150 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 m M D-glucose, 10 mM HEPES, pH = 7.4; dilution 1:13), then the samples were injected with the glucoside of formononetin at concentrations of 1 μM, 3 μM, 30 μM. Samples with the substance injected were incubated for 15 minutes at room temperature. Then, an activating factor, ADP 5 μM or TRAP 5 μM, and fibrinogen labeled with Alexa-Fluor 647 (Molecular Probes, Germany) were added to all samples with the exception of the control. After incubation with the activating factor for 5 minutes, the reaction was stopped by dilution in phosphate buffer (PBS) in a ratio of 10:1. For the analysis, we used the method of flow cytometry with Cyto-FLEX device (Beckman-Coulter, USA). Each sample was evaluated for 15,000 events.

RESULTS AND DISCUTION

Fractionation and purification of the total extract derived from 300 grams of O. arvensis for the isolation of formononetin-7-O-β-D-glucopyranoside as an individual compound for subsequent biological studies were performed according to the method developed in our previous study [17]. The raw materials were initially dried, milled and sieved through a sieve with a diameter of 1.0 mm. The plant material was repeatedly extracted with 96 % ethyl alcohol in a ratio of 1:6.

The first stage of exhaustive liquid-liquid extraction was carried out using equal volumes of hexane, then 50 ml of purified water was added to the alcohol extract, and exhaustive liquid-liquid extraction was similarly performed with an equal volume of dichloromethane. The content of formononetin-7-O-β-D-glucopyranoside in the residual aqueous-alcoholic solution after all stages of liquid-liquid extraction was 83 % in comparison with the original extract. Formononetin-7-O-β-D-glucopyranoside isolated as a result of previous studies, was used as a standard substance [18].

Losses of formononetin-7-O-β-D-glucopyranoside on the stage of liquid-liquid extraction can be explained with its partial solubility in dichloromethane and ethanol miscibility both with water and dichloromethane which can change the distribution coefficient of formononetin-7-O-β-D-glucopyranoside.

The residual water-alcohol solution was evaporated to an approximate volume of 75 ml and loaded into an open column with a reverse-phase Dianion HP 20 sorbent. The elution was performed like in our previous studies with a gradual decrease of the initial solvent polarity in steps of 10 % (from H2O:96 % EtOH 100:0 to H2O:96 % EtOH 0:100, v/v). The eluting fractions were collected to flasks of 100 ml. After HPLC analysis of the collected fractions, all fractions with a significant content of formononetin-7-O-β-D-glucopyranoside without interfering, co- or closely eluting peaks were combined and evaporated to the volume of 20 ml. The content of formononetin-7-O-β-D-glucopyranoside in the obtained fractions after purification with open column chromatography was 37 % in comparison with the initial extract.

Formononetin-7-O-β-D-glucopyranoside was then isolated as an individual compound with the preparative high performance liquid chromatography. The content of formononetin-7-O-β-D-glucopyranoside after purification on the preparative chromatograph in comparison with the initial extract was 30 %.

Formononetin, starting at a concentration of 1 μM, almost completely inhibits ADP-induced platelet activation (Figure 1).

Platelet activation caused by ADP was taken as 100 % and used as positive control. In the inactivated control

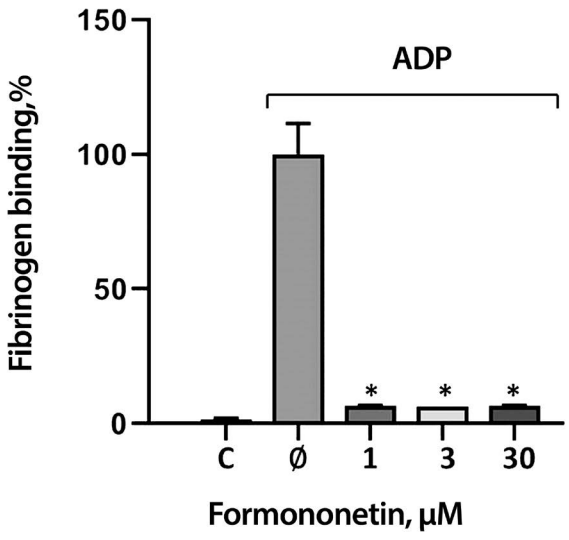

Figure 1. Formononetin inhibits ADP-induced platelet activation

sample 1.5 % of activated platelets were observed. In samples with formononetin-7-O-β-D-glucopyranoside and ADP, a pronounced inhibition of platelet activation is observed – the percentage of active platelets ranges from 6.3–6.6 % at doses of formononetin-7-O-β-D-glucopyranoside 1 μM, 3 μM and 30 μM. The data is presented as M ± SD, (N = 4, p ≤ 0.05).

Similar results were obtained with TRAP-6 platelet activation, the obtained results are presented in (Figure 2).

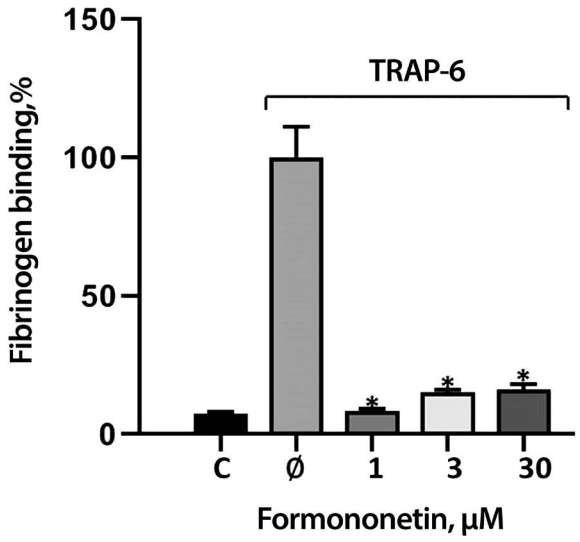

Figure 2. Formononetin inhibits TRAP-6-induced platelet activation

As in the first case, the platelet activation caused by TRAP-6 in the sample was taken as 100 %. The percentage of activated platelets in the control sample is 7.3 %. In samples with formononetin-7-O-β-D-glucopyranoside and TRAP, there is a pronounced inhibition of platelet activation. The percentage of active platelets is 8 % at 1 μM formononetin-7-O-β-D-glucopyranoside doses, 15 % at 3 μM doses, and 16 % at 30 μM doses. The data is presented as M ± SD, (N = 4, p ≤ 0.05).

CONCLUSION

Thus, we have supplemented the method for the isolation of formononetin-7-O-β-D-glucopyranoside from the grass of *Ononis arvensis* L., which made it possible to establish the yield percentage of formononetin-7-O-β-D-glucopyranoside in comparison to the total extract.

The obtained results showed that formononetin-7-O-β-D-glucopyranoside significantly inhibited platelet activation induced by ADP and TRAP-6. Further studies are needed to elucidate the molecular mechanisms of the inhibitory effect of formononetin on platelets.

REFERENCES

1. Newman D. J., Cragg G. M. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of natural products*. 2020;83(3):770–803. DOI: 10.1021/acs.jnatprod.9b01285.
2. Al-Ani F., Chehade S., Lazo-Langner A. Thrombosis risk associated with COVID-19 infection. A scoping review. *Thrombosis research*. 2020;1(192):152–160. DOI: 10.1016/j.thromres.2020.05.039.
3. Tsoupras A., Lordan R., Zabetakis I. Thrombosis and COVID-19: The Potential role of nutrition. *Frontiers in Nutrition*. 2020;7:177. DOI: 10.3389/fnut.2020.583080.
4. Gromov A. A., Kruchinina M. V., Rabko A. V. Coronavirus disease CIVID-19 - unused therapy opportunities. *Russkiy meditsinskiy zhurnal*. 2020;28(9):2–6. (In Russ.)
5. Nignpense E. B., Chinkwo K. A., Blanchard C. L., Santhakumar A. B. Polyphenols: modulators of platelet function and platelet microparticle generation? *International journal of molecular sciences*. 2020;21(1):146. DOI: 10.3390/ijms21010146.
6. Olas B. A review of in vitro studies of the anti-platelet potential of citrus fruit flavonoids. *Food and Chemical Toxicology*. 2021:112090. DOI: 10.1016/j.fct.2021.112090.
7. Ivkin D. Yu, Luzhanin V. G., Karpov A. A., Minasyan S. M., Poleshchenko Ya. I., Mamedov A. E., Povydysh M. N., Poroykov V. V., Narkevich I. A. Embinin is a perspective cardiotonic mean for natural origin. *Razrabotka i registratsiya lekarstvennykh sredstv = Drug development & registration*. 2018;3:166–170. (In Russ.)
8. Liguori I., Russo G., Curcio F., Bulli G., Aran L., Della-Morte D., Gargiulo G., Testa G., Cacciatore F., Bonaduce D., Abete P. Oxidative stress, aging, and diseases. *Clinical interventions in aging*. 2018;13:757. DOI: 10.2147/CIA.S158513.
9. Peoples J. N., Saraf A., Ghazal N., Pham T. T., Kwong J. Q. Mitochondrial dysfunction and oxidative stress in heart disease. *Experimental & molecular medicine*. 2019;51(12):1–3. DOI: 10.1038/s12276-019-0355-7.
10. Steven S., Frenis K., Oelze M., Kalinovic S., Kuntic M., Bayo Jimenez M. T., Vujacic-Mirski K., Helmstädter J., Kröller-Schön S., Münzel T., Daiber A. Vascular inflammation and oxidative stress: major triggers for cardiovascular disease. *Oxidative medicine and cellular longevity*. 2019:1–26. DOI: 10.1155/2019/7092151.
11. Panche A. N., Diwan A. D., Chandra S. R. Flavonoids: an overview. *Journal of Nutritional Science*. 2016:5. DOI: 10.1017/jns.2016.41.
12. Cheng Y., Xia Z., Han Y., Rong J. Plant natural product formononetin protects rat cardiomyocyte H9c2 cells against oxygen glucose deprivation and reoxygenation via inhibiting ROS formation and promoting GSK-3β phosphorylation. *Oxidative medicine and cellular longevity*. 2016:1–11. DOI: 10.1155/2016/2060874.

13. Huang Z., Liu Y., Huang X. Formononetin may protect aged hearts from ischemia/reperfusion damage by enhancing autophagic degradation. *Molecular Medicine Reports*. 2018;18(6):4821–4830. DOI: 10.3892/mmr.2018.9544.
14. Li T., Zhong Y., Tang T., Luo J., Cui H., Fan R., Wang Y., Wang D. Formononetin induces vasorelaxation in rat thoracic aorta via regulation of the PI3K/PTEN/Akt signaling pathway. *Drug design, development and therapy*. 2018;12:3675. DOI: 10.2147/DDDT.S180837.
15. Zhu H., Zou L., Tian J., Lin F., He J., Hou J. Protective effects of sulphonated formononetin in a rat model of cerebral ischemia and reperfusion injury. *Planta medica*. 2014;80(04):262–268. DOI: 10.1055/s-0033-1360340.
16. Zverev Ya. F. *Antitrombotsitarnaya aktivnost flavonoidov* [Antiplatelet activity of flavonoids]. *Voprosy pitaniya = Problems of Nutrition*. 2017;86(6):6–20. (In Russ.)
17. Luzhanin V. G., Whaley A. K., Ponkratova A. O., Grishukova E. A., Suloev I. S., Smirnov S. N., Serebryakov E. B. Isolation of individual compounds from the terrestrial parts of *Ononis arvensis* L. and *Solidago canadensis* L. *Razrabotka i registratsiya lekarst vennykh sredst v = Drug development & registration*. 2021;10(1):83–89. (In Russ.). https://doi.org/10.33380/2305-2066-2021-10-1-83-89.