

***Chelidonium majus* WATER EXTRACT INITIATES PLATELET AGGREGATION AND INHIBITS FIBRIN POLYMERIZATION IN BLOOD PLASMA**

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Chelidonium majus is a well-known source of biologically active compounds. Most of them are alkaloids, which are used in researches and for tradition medicine. In this study, we explored the influence of *C. majus* crude total extract onto blood coagulation system *in vitro*, primary and secondary hemostasis.

Aim. To study influence of *C. majus* extract onto blood coagulation process.

Methods. Turbidimetry of blood plasma, APTT test with chromogenic substrates, and platelet aggregation were used in the work.

Results. We demonstrated moderate stimulating effect of the extract on platelets (the rate of platelet aggregation increased up to 10%, followed by disaggregation). Extract also increased the rate of platelet aggregation stimulated by 12.5 mcM of ADP.

We observed the increase in the plasma clotting time in the presence of the extract, that corresponded to the 274, 411, 685, 1370 mcg of dry extract, from 70 s in control to 80, 90, 170 and 180 s, respectively, in turbidimetry test of plasma stimulated by APTT-reagent.

However, APTT test with the chromogenic substrate of thrombin (S2238) did not show significant influence of this plant's extract on thrombin activity.

Conclusions. Thus, we can conclude that anticoagulant activity of *Chelidonium majus* extract corresponded to the direct inhibition of fibrin polymerization.

Key words: *Chelidonium majus*, platelet activation, plasma coagulation, thrombin.

Chelidonium majus is known as celandine, common celandine, or greater celandine [1–3]. It belongs to the *Papaveraceae* family of dicotyledonous plants, and like its relative *Papaver somniferum*, comprises numerous biologically active compounds. This is a perennial herb, that grows in Europe and the Mediterranean, and is also common in America. Closely related species grow in Eurasia and Japan. Plants have a straight, branched stem 50–70 cm high.

Basal leaves are petiolate, deeply pinnate. They have three to five pairs of rounded or ovoid lobes. The upper leaves lobe is larger, usually, they are three-lobed.

The leaves are glaucous below and green above. The upper leaves are sessile. The flowers are actinomorphic, golden yellow, with a characteristic formula $*K_2C_4A_\infty G_{(2)}$ [4]. The fruit is a polyspermous pod-shaped capsule.

There are a lot of biologically active compounds, such as alkaloids, in *Chelidonium majus* herb. This plant, its juice, herb and roots are used for science and for traditional medicine. In traditional medicine, it was used to improve eyesight and as sedative, antispasmodic and for curing bronchitis, whooping cough, asthma, jaundice, gallstones, and gallbladder pain (celandine)

[1]. Juice with latex is used to treat skin diseases and problems such as warts, ringworm and corns [1].

Also isoquinolin extracted from *Chelidonium majus* was active against some pathogenic bacteria and *Candida* [5].

It consists of more than 35 compounds [2, 3]. It comprises a lot of alkaloids, derivatives of benzophenanthridine: homohelidonine, chelidonine, chelerythrine, chelidocystatin, coptisine, sanguinarine, berberine, protopine, sparteine and another [1, 6, 7]. It consists of non-alkaloid compounds, such as ascorbic acid, carotene, saponins, bitters, latex and protein [8, 9].

Also, it consists of agarose-like polysaccharides, yellow or orange milky juice. The experience of using this plant in traditional medicine was not so successful because of cytotoxicity [10], which was shown in clinical trials [11].

The aim of the present work was to study the influence of *Celidonium majus* extract onto hemostasis, blood coagulation process. This work highlights the influence of *C. majus* total water extract on protein and cellular hemostasis *in vitro*.

Material and Methods

We prepared *Chelidonium* herb in May during its flowering, dried in the shadow at 25 °C, and packed in cardboard boxes for the further use. The dried herb was cut into small pieces with scissors and steamed in a tris-HCl buffer, pH 7.4, and then was heated to 100 °C for 12 hours in a foam box. We routinely used 4 g of cut herb per 200 ml of buffer. Also, 1 ml of extract was dried on the small Petry dish with weight control before and after filling and drying, which helped to calculate the mass of dry extract per 1 ml. Blood plasma and platelet-rich plasma samples were obtained from blood of healthy donors. Volunteers signed informed consent prior to the blood sampling according to the Helsinki declaration. Platelet aggregation was studied using Solar AP2110 Aggregometer (Belarus). Blood plasma clotting was measured using Solar CGL-2410 Coagulometer (Belarus). Chromogenic substrate assay was performed using Multiscan EX (Thermo, USA).

Fibrin formation in blood plasma under the action of APTT-reagent was determined using POP spectrophotometer (Optizen, Korea). The scattering of light was measured at 350 nm. The following reagents were consecutively added to the 10 mm spectrophotometric

cuvette: blood plasma (100 mcl) was mixed with 100 mcl of APTT-reagent and incubated 3 minutes at 37 °C. Then 0.05 M tris-HCl buffer, pH 7.4, containing 0.15 M NaCl (400–600 mcl) and 100 mcl 0.025 M CaCl₂ were added to start the reaction. Analysis of the turbidity curves were performed using a specialized computer program.

Results and Discussion

In our study we used volumes of *Chelidonium majus* extract (0, 20, 30, 50, 100 mcl) that corresponded to the 0, 274, 370.5, 617.5, 1235 mcg of dry extract, that was characterized in [12].

For the determining of the effects of *Chelidonium majus* extract on platelets we performed aggregometry studies. Platelets in the platelet-rich blood plasma (250 mcl) were mixed with 25 mcl of 0.025 M of CaCl₂ at 37 °C and 20 mcl (274 mcg) of the extract or equivalent volume of 0.05 M tris-HCl buffer at 25 °C. We demonstrated moderate stimulating effect of the extract on platelets (the rate of platelet aggregation increased by up to 10%, followed by disaggregation). The extract also increased the rate of platelet aggregation stimulated by 12.5 mcM of ADP [12].

To study the effect of the extract of proteins on the blood coagulation system we applied activated partial thromboplastin time (APTT) test. It was performed in accordance with the following procedure. 0.1 ml of blood plasma was mixed with an equal volume of APTT-reagent (Siemens, Germany) and incubated for 3 min at 37 °C. Then the extract of *Chelidonium majus* or equal volume of 0.05 M tris-HCl buffer, pH 7.4, was added. After that the coagulation was initiated by adding of 0.1 ml of 0.025 M CaCl₂ solution. As a result, we observed the increase in the plasma clotting time in the presence of 0, 20, 30, 50, 100 mcl of the extract (that corresponded to the 0, 274, 370.5, 617.5, 1235 mcg of dry extract) from 70 s in control to 80, 90, 170 and 180 s, respectively (Fig. 1).

The inhibition of blood clotting could be explained by the direct action on the fibrin polymerization, or by the suppression of the clotting cascade factors activities. To clarify the reasons of the observed effect we measured the thrombin activity in the APTT-activated blood plasma using thrombin-specific chromogenic substrate S2238 H-D-Phe-Pip-Arg-pNA at 405 nm. The analysis was performed in 0.05 M Tris-HCl buffer, pH 7.4, at 37 °C. Chromogenic substrate was

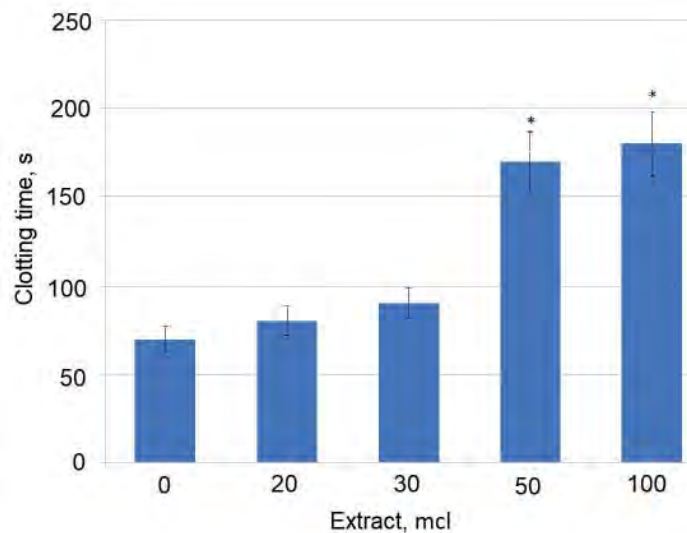


Fig. 1. Time of blood plasma clotting induced by APTT-reagent in the presence of *Chelidonium majus* extract: (0, 20, 30, 50, 100 mcl of extract corresponded to the 0, 274, 370.5, 617.5, 1235 mcg of dry extract). APTT — activated partial thromboplastin time. * $P \leq 0.05$.

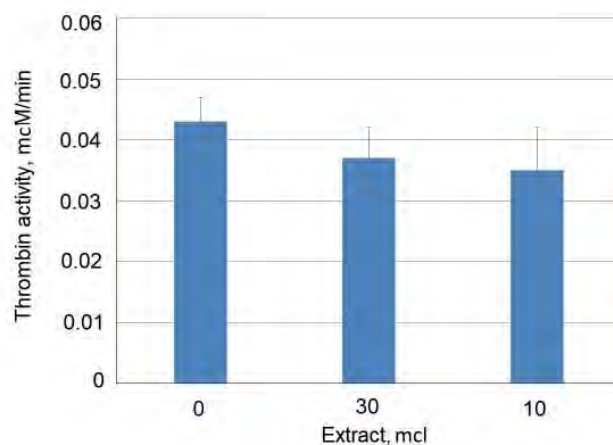


Fig. 2. Activity of thrombin generated in blood plasma under the action of APTT-reagent in the presence of *Chelidonium majus* extract: (10, 30 mcl corresponded to 123.5, 370.5 mcg of dry extract). APTT — activated partial thromboplastin time.

taken in the final concentration 30 mcM. The tested amount of *Chelidonium majus* extract ranged from 10 to 30 mcl that corresponded to the 123.5, 274, 370.5 mcg of dry extract. The experiment did not show any inhibition or facilitation of thrombin activity in the presence of studied extract (Fig. 2).

This finding allowed us to conclude that *Chelidonium majus* extract compounds did not act on the clotting cascade factors, and the overall anticoagulant effect of the extract can be connected to the direct inhibition of fibrin polymerization.

To prove this hypothesis, we performed a turbidity study of the fibrin formation in the

presence of *Chelidonium majus* extract. The tested amount of *Chelidonium majus* extract ranged from 30 to 100 mcl that corresponded to the 123.5 and 1235 mcg of dry extract. We demonstrated that fibrin polymerization was significantly inhibited by the studied extract, which was direct evidence of the action of *Chelidonium majus* extract on the fibrin polymerization (Fig. 3).

In this study, we showed the opposite influence of *Chelidonium majus* water extract on the factors of clotting cascade and on the platelets. The same result was obtained in the series of experiments: studied substance inhibited the clotting and slightly activated

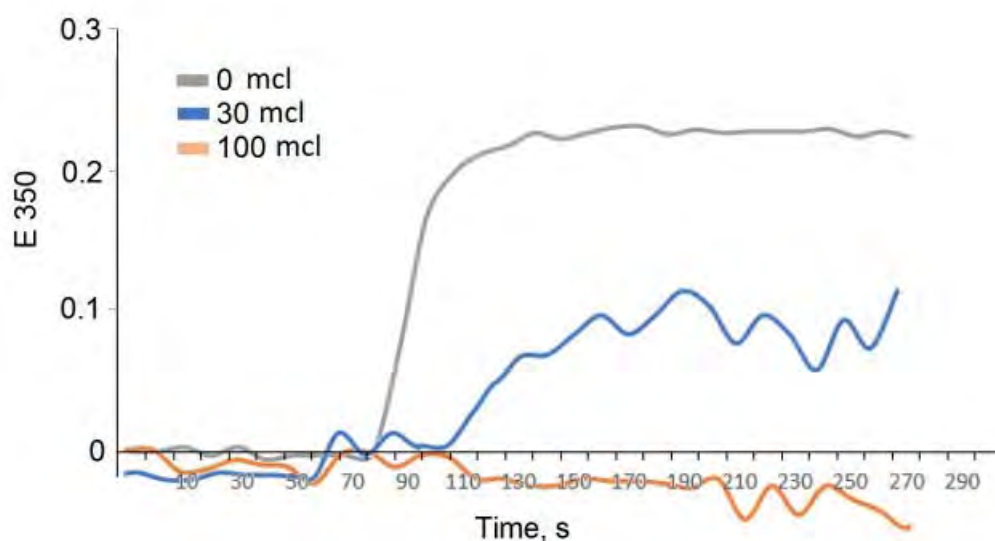


Fig. 3. Turbidity study of blood plasma activated by APTT-reagent in the presence of *Chelidonium majus* extract:

(10, 30 mcl corresponded to 123.5, 370.5 mcg of dry extract). APTT — activated partial thromboplastin time. Typical curves for 3 independent experiments are presented.

platelets. Platelets were moderately activated, but clot formation in the plasma was inhibited for a long period. We did not study these effects in intact fresh blood. There were difficulties in studying fibrin formation in turbidimetry methods in spectrophotometer cuvette, because of random oscillation of basal level of light scattering. This phenomenon may occur because of latex particles presented in the extract.

It is important to underline that the study was performed with *in vitro* isolated platelets or with platelet poor plasma components of the hemostasis system. This is a reduced model of the blood coagulation system, so the effects of pharmacokinetics and pharmacodynamics on the whole organism were not studied. Also, in this brief screening study, the goal of identification and isolation of active compounds was not performed. We suggest that some compounds of the extract may directly inhibit fibrin polymerization because there is no influence of the extract on thrombin in our experiments with a chromogenic substrate.

Also, it was shown that berberine, which is found in this plant, can activate platelets [12, 13] and cause their apoptosis [13]. It can also affect gene expression [14].

It is well-known that pure berberine is hydrophobic and did not form water solution. To make it soluble, the authors of [14] used a

carrier — fullerene C_{60} to immobilize berberine on its surface.

However, berberine from *Chelidonium majus* was shown to be water soluble. This alkaloid is carried by some proteins like CmMLP1 [6], which makes it well soluble in water. Also, it can react with HCl from the extraction buffer, forming more soluble salt. Hydrochloride of berberine, obtained by treating this alkaloid by HCl-containing buffer, is sparingly soluble in water and biochemically active compound [15, 16].

Conclusions

Thus, we can conclude that the anticoagulant activity of *Chelidonium majus* extract possibly corresponded to the direct inhibition of fibrin polymerization. The ability of the water extract of *Chelidonium majus* to activate platelets was demonstrated for the first time. We can suggest, that this effect may be caused by berberine. However, this effect was rather moderate. We presume that the studied effect on plasma was rather nonspecific, however this question needs further exploration.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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ВОДНИЙ ЕКСТРАКТ *Chelidonium majus* ІНІЦІЮЄ АГРЕГАЦІЮ ТРОМБОЦИТІВ І ПРИГНІЧУЄ ПОЛІМЕРИЗАЦІЮ ФІБРИНУ В ПЛАЗМІ КРОВІ

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Chelidonium majus (Чистотіл звичайний або великий) є добре відомим джерелом біологічно активних сполук, більшість з яких є алкалоїдами, що їх використовують у науці та традиційній медицині. Ми досліджували вплив водного екстракту *Chelidonium majus* на систему зсідання крові *in vitro*, первинний та вторинний гемостаз.

Мета. Вивчити вплив екстракту *Chelidonium majus* на процес згортання крові.

Методи. Турбідиметрія плазми крові, АЧТЧ-тест з використанням хромогенних субстратів та агрегація тромбоцитів.

Результати. Продемонстровано помірну стимулювальну дію екстракту на тромбоцити (швидкість агрегації тромбоцитів становила до 10 % з подальшою дезагрегацією). Екстракт також підвищував швидкість агрегації тромбоцитів, стимульовану 12,5 мкМ АDP.

В турбідиметричному тесті з використанням активації згортання АЧТЧ-реагентом спостерігали збільшення часу зсідання плазми крові за присутності екстракту в кількості, що відповідала 1370, 685, 411, 274 мкг сухого екстракту з 70 с в контролі до 170, 90, 80 і 180 с відповідно.

Водночас, тест АЧТЧ із хромогенним субстратом тромбіну (S2238) не показав істотного впливу екстракту цієї рослини на активність тромбіну.

Висновки. Таким чином, антикоагулянтна активність екстракту *Chelidonium majus* полягала у безпосередньому пригніченні полімеризації фібрину.

Ключові слова: *Chelidonium majus*, активація тромбоцитів, коагуляція плазми, тромбін.