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SHORT COMMUNICATION

Effect of the Water Extract of *Galega officinalis L.* on Human Platelet Aggregation *in vitro*

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***Galega officinalis L.* is a traditional medicinal plant from Bulgaria. It was found that the aqueous extract of *Herba Galegae* suppressed platelet aggregation *in vitro* induced by adenosine diphosphate, epinephrine, thrombin and collagen. The compounds with antiaggregating action have not as yet been isolated from *Galega officinalis*.**

Keywords: plant extract; *Herba Galegae*; *in vitro*; platelet aggregation

INTRODUCTION

The effect of various natural, synthetic and pharmaceutical compounds on platelet aggregation is of interest in the search for new medicinal substances and preparations (Mashkovski, 1988).

Galega officinalis is a plant used in traditional medicine for treatment of *diabetes mellitus*. Biologically active alkaloids, exhibiting a hypoglycaemic effect, were isolated from *Herba Galegae* (Hoppe, 1975; Petkov, 1982). There are data (Petkov, 1982) indicating that the plant extract has an anticoagulation effect. In connection with this, we studied the effect of water, ethanol and chloroform extracts of *Herba Galegae* on platelet aggregation *in vitro*. It was found that only the aqueous extract suppressed platelet aggregation.

MATERIAL AND METHODS

Plant material. The aerial parts of *Galega officinalis* at flowering stage were collected between May and August 1992 in different parts of Thrace, Bulgaria. The plant was verified by the Department of Botany, Faculty of Pharmacy (University of Sofia, Bulgaria).

Extraction. Aqueous extracts were obtained by maceration of 2 g dry matter in 20 mL distilled water for 20–24 h at 18°–20°C. The fresh extract was filtered twice and the effect on platelet aggregation studied immediately.

Isolation of human platelets. Blood was taken from volunteers who had received no medication for 15 days prior to blood collection. Blood was collected in disposable syringes at a ratio of 1 part 3.8% trisodium citrate and 9 parts venous blood (Zucker, 1989). Platelet-rich plasma (PRP) was prepared by centrifugation ($180 \times g$ for 10 min) and diluted to 300×10^6 platelets per mL with autologous platelet-poor plasma ($1800 \times g$ for 15 min).

Platelet aggregation. Aggregation was studied with a spectrophotometer set to operate at wavelength 600 nm and the results were recorded on an electronic recorder [XY-Recorder en dim 620. 02, VEB, Germany] (Born, 1962).

Reagents. Adenosine 5'-diphosphate (ADP) at a concentration of 1×10^{-3} M from Reanal (Hungary); epinephrine (1×10^{-4} M) and collagen (2 mg/mL) from Sigma Chemical Ltd (USA); human thrombin (8 units/mL) from the Research Institute of Haematology and Blood Transfusion (Sofia, Bulgaria) were used as aggregating agents.

Drugs. Sodium salicylate ($C_7H_5NaO_3$) at a concentration of 40 mg/mL in physiological saline, pH 7.4; verapamil (2.5 mg/mL); heparin (15 U/mL); dipyridamole

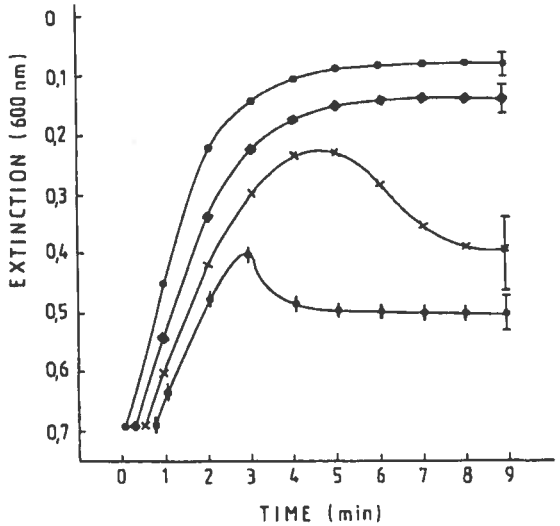

Figure 1. *In vitro* effect of 20 μL (×) and 40 μL (◆) aqueous extracts of *Herba Galegae*, containing 22.5 ± 2.6 mg/mL average dry matter and of a 20 μL aqueous solution of 40 mg/mL sodium salicylate (◆) as a basis for comparison of platelet aggregation of 400 μL platelet-rich plasma by adenosine diphosphate. For control (●) the aggregation of 400 μL PRP was used, after the addition of 20 μL ADP (1×10^{-3} M). Values are means of ± maximum standard errors for 10 independent experiments.

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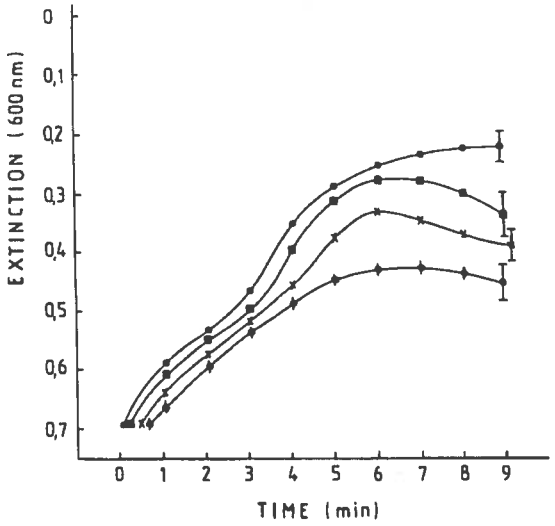

Figure 2. *In vitro* effect of 20 µL (■), 40 µL (×) and 60 µL (◆) aqueous extracts of *Herba Galegae* on platelet aggregation of 400 µL PRP by epinephrine. For control (●) the aggregation of 400 µL PRP was used, after addition of 50 µL epinephrine ($\cdot 10^{-4}$ M). Values are means of ± maximum standard errors for eight independent experiments.

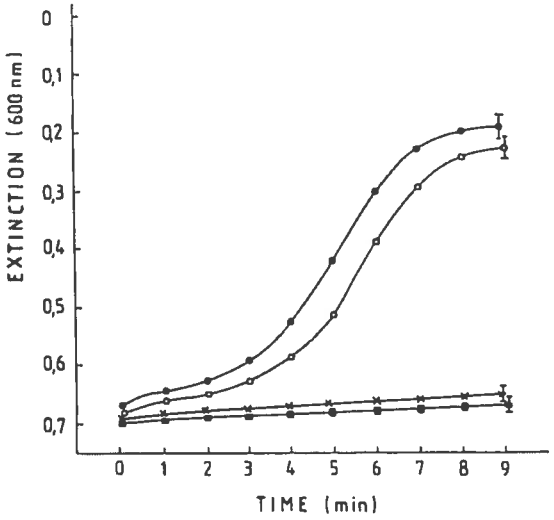

Figure 4. *In vitro* effect of 20 µL (○) and 40 µL (■) aqueous extracts of *Herba Galegae* on platelet aggregation of 400 µL PRP by collagen. The effect of 60 µL (2.5 mg/mL) verapamil (×) was used for comparison. For control (●) the aggregation of 400 µL PRP was used, after addition of 20 µL collagen (2 mg/mL). Values are means of ± maximum standard errors for eight independent experiments.

(5 mg/mL) from Pharmachim (Bulgaria) were used as a basis for comparison of the effect of the extract of *Herba Galegae*.

The extinction change that takes place during the aggregation of 400 µL platelet-rich plasma compared with platelet-poor plasma (whose extinction was taken as zero) after adding 20–50 µL aggregating agent at 37°C and a rate of stirring of 1000 rpm was the basis of measurement for aggregating effects. The effect of the water extract was studied after adding 20–60 µL fresh water extract to 400 µL PRP with stirring. The aggregating agents were added after 10–15 min and aggregation monitored for a further 9 min.

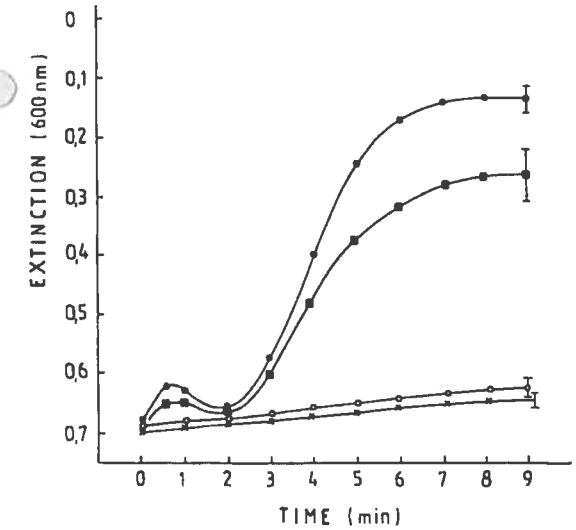

Figure 3. *In vitro* effect of 40 µL (■) and 60 µL (○) aqueous extracts of *Herba Galegae* on platelet aggregation of 400 µL PRP by thrombin. The effect of 20 µL (15 units/mL) heparin (×) was used for comparison. For control (●) the aggregation of 400 µL PRP was used, after addition of 50 µL thrombin (8 units/mL). Values are means of ± maximum standard errors for 10 independent experiments.

RESULTS AND DISCUSSION

A significant inhibitory action on platelet aggregation was found in the extract of *Galega officinalis L.* (*Herba Galegae*) which had on average dry matter content of 22.5 ± 2.6 mg/mL.

The anti-aggregating activity of the extract was verified for a number of known aggregating agents: ADP, epinephrine, thrombin and collagen. The effect of the extract on platelet aggregation with ADP is presented in Fig. 1 and as a basis for comparison it was similar to the effect of 20 µL aqueous solution of sodium salicylate at a concentration of 40 mg/mL. The inhibitory effect on platelet aggregation of 20–40 µL aqueous extract exceeded 50% [%

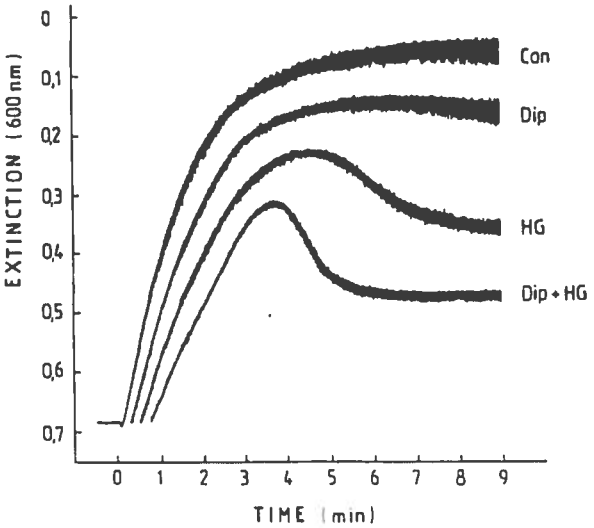

Figure 5. *In vitro* effect of separate application of 20 µL (5 mg/mL) dipyridamole (Dip) and 20 µL aqueous extract of *Herba Galegae* (HG), and combined application of 20 µL dipyridamole and 20 µL extract (Dip+HG) on platelet aggregation of 400 µL PRP, after addition of 20 µL ADP (1×10^{-3} M).

aggregation $= (E_0 - E) \times 100\% / (E_0 - E_f)$, where E_0 is the initial value of the extinction of PRP before the aggregating process, E_f is the final value of the extinction of PRP after finishing the aggregating process, E is the final value of the extinction of PRP with extracts or drugs after finishing the aggregating process]. The inhibitory effect of the extract was dose-dependent. Aggregation induced with different concentrations of ADP (from 1×10^{-3} M to 1×10^{-6} M) showed that the aqueous extract inhibited predominantly the second phase of aggregation with ADP. A similar inhibition of the second phase of aggregation was observed by aggregation with epinephrine (Fig. 2). The inhibitory effect depended on the volume of the extract used but did not affect the first phase of the aggregating process. The effect of the extract on platelet aggregation induced with thrombin is presented in Fig. 3. The aqueous extract in this case had a strong inhibitory effect on the initiation of the aggregating process (contrary to aggregation with ADP and epinephrine) and only weak effects on the latter phase of aggregation. Adding 60 μL of extract to 400 μL PRP totally inhibited aggregation, in a manner similar to that of 20 μL heparin (15 U). Adding 20–40 μL of extract to the same volume of PRP led to comparatively weak inhibitory effects in the later phase of the aggregating process. In this case a clearly expressed 'threshold volume' of the extract (about 50 μL) was observed, at which it fully inhibited platelet aggregation, but under the threshold volume the inhibitory effect was strongly reduced. In the case of aggregation with collagen (Fig. 4) the situation was similar. The aqueous extract with a threshold volume (~50 μL) totally inhibited platelet aggregation by inhibition of the initiation process, while under the threshold volume the extract had only a weak inhibitory effect on platelet aggregation.

A combined application of aqueous extracts of *Herba Galegae* with different drugs with anti-aggregating action (Mashkowski, 1988): dipyridamole, sodium salicylate, aspirin, verapamil, voltaren, heparin and others showed a common inhibitory effect on platelet aggregation of extract and drugs. For example, Fig. 5 shows an independent and a combined effect of dipyridamole and aqueous extract of *Herba Galegae* on platelet aggregation with ADP. 20 μL dipyridamole (5 mg/mL) added to 400 μL PRP produced a 15% inhibition of aggregation. 20 μL aqueous extract of *Herba Galegae* (22.5 ± 2.6 mg/mL) added to the same volume of PRP produced a 45% inhibition. A combined application of 20 μL dipyridamole and 20 μL extract inhibited 62% platelet aggregation. Therefore, the total effects of dipyridamole and the extract were equal and in other cases exceeded the sum of the independent inhibitory effects of dipyridamole and the extract applied separately. Several pathways of platelet activation are known (Kinlough-Rathbone *et al.*, 1977) for example, release of ADP; release of products of arachidonate metabolism and *via* platelet-activating factor (a pathway dependent on the other two). The aqueous extract of *Herba Galegae* inhibited platelet aggregation induced by ADP, epinephrine, thrombin and collagen. On the other hand, the aqueous extract inhibited the second phase of aggregation induced by ADP and epinephrine and the initiation of aggregation by thrombin and collagen (Zucker, 1989). This indicated that the extract acts through some common mechanism or pathway. Probably, it is through the prevention of the formation of interplatelet fibrinogen bridges, which are the support for irreversible aggregation. Ticlid (Bruno, 1983) has been shown to have a similar mechanism of action.

The study of the plant extract and its effect on platelet aggregation may be useful in the isolation of biologically active compounds from the extract. Such compounds, of anticoagulating and anti-aggregating action have not as yet been isolated from *Galega officinalis*.

REFERENCES

Born, G. V. R. (1962). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* **194**, 927–929.

Bruno, J.-J. (1983). The mechanism of action of ticlopidine. *Thromb. Res.* **4** (Suppl.), 59–67.

Hoppe, H. A. (1975). *Drogenkunde*, Vol. 1, p. 1311, 8 edn. Walter de Gruyter, Berlin.

Kinlough-Rathbone, R. L., Packham, M. A., Reimers, H.-J., Cazenave J.-P., and Mustard, J. F. (1977). Mechanism of platelet shape change, aggregation with release induced by collagen, thrombin or A23187. *J. Lab. Clin. Med.* **90**, 707–719.

Mashkovski, M. D. (1988). *Drugs* 11th edn, pp. 187–202. Medicina Publishing House, Moscow (in Russian).

Petkov, V. D. (1982). *Modern Phytotherapy*, pp. 307. Medicina i Fizkultura Publishing House, Sofia (in Bulgarian).

Zucker, M. B. (1989). Platelet aggregation measured by the photometric method. *Methods Enzymol.* **169**, 118–133.