Short Communication

Effects of the extract from *Conyza canadensis* on human blood platelet aggregation

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Abstract. The effects of different parts of extract from medicinal plant *Conyza canadensis,* used to control bleeding, on human blood platelet aggregation *in vitro* were investigated. Aqueous extract of *Conyza c.* from young or old plants, glycoconjugate part, polysaccharide part and aglycon part at the concentrations above 0.75 mg/ml strongly inhibited platelet aggregation induced by collagen (2 μg/ml) in dose-dependent manner. Polysaccharide part isolated from plant extract had the strongest inhibitory effect on aggregation stimulated by collagen and seems to be responsible for antiaggregatory properties.

Key words: Platelet aggregation — *Conyza canadensis* — Antiaggregatory properties

Blood platelets are multiresponding cells, both with respect to the different number of agonists and compounds. They can be activated by several physiologically important compounds including collagen and are involved in the blood clotting, inflammation process atherogenesis and cancers. In hemostasis, platelet plug formation dependent on platelet aggregation represents the primary response to vascular injury with the coagulation cascade and fibrin formation. Blood platelet aggregation under physiological conditions is an important process to stop bleeding, but it is considered that excessive platelet aggregation causes thrombosis and atherosclerosis (Wu 1996). Recently, various compounds, especially phenolics obtained from plants were tested for their ability to reduce platelet aggregation (Zbikowska et al. 1999; Rein et al. 2000; Olas et al. 2002).

Conyza canadensis (*Asteraceae*) is an annual plant that has spread from North America to Europe. *Conyza c.* is a useful remedy to control bleeding. This common weed is a source of volatile oil and is used even today in herbal medicine (Lasserre et al. 1983; Lenfeld et al. 1986), however phytochemically and pharmacologically is uninvestigated. Therefore, the aim of our study was to assess the effects of extract and its different parts isolated from *Conyza c.* on blood platelet aggregation induced by collagen. We also focus our attention on the action of extract of *Conyza c.* from young and old plants on blood platelet aggregation. The preliminary studies indicate that extract from *Conyza c.* possesses anticoagulant and antioxidant activities (Woźniak et al. 2004; Pawlaczyk et al. 2005; Olas et al. 2006).

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Plant material

Flowering plants of *Conyza canadensis* (L.) Cronq. were collected in Wroclaw, Poland, in August 2004. The identity of the plants was certified by Prof. Tomasz J. Nowak from Wroclaw University, and a voucher specimen (No. 019361) has been deposited in the Botanical Garden of Wroclaw University, Poland.

Extraction and purification of plant substances

Dried flowering parts of plants (200 g) were minced and mixed with 65% ethanol (2 l) and left to stand at room temperature for two days. Then the mixture was refluxed for 12 h. The extract was filtered and evaporated to dryness on a rotary evaporator to obtain a paste-like residue (56.1 g). The extract was dissolved in distilled water and filtered through a paper filter to give a clear liquid described as the crude plant extract. This extract was purified from ballast substances in

the extraction process using different organic solvents by the method described by Pawlaczyk et al. (2002) to obtain the plant fraction enriched in carbohydrate substances. The resulting precipitate was dialyzed against water for 120 h (cut-off of dialysis tubing membrane 12.5 kDa). The non-dialyzed portion was evaporated to dryness to obtain the macromolecular purified fraction (purified active fraction – glycoconjugate). After mild acidic hydrolysis (0.1 mol/l HCl in pH 2.40 at room temperature, 15 min), the aglycon part was removed as a precipitate by centrifugation (10.000 rpm, 15 min). The aglycon part was washed with water to remove HCl, dissolved in water by adding 1 mol/l NH_4OH to obtain pH 9.7. Next, the resulting solution of the aglycon was dialyzed against alkaline solution of pH 9.7 for 48 h. The retentat was neutralized by 0.1 mol/l HCl to obtain the precipitate, which was washed with water and evaporated to dryness (aglycon part). The soluble in water rest of the plant extract received after mild acid hydrolysis was neutralized to pH 7.0 by 0.1 mol/l NaOH and dialyzed against water for 48 h to remove NaCl. The residue in dialysis tubing membrane – the retentat was evaporated to dryness (polysaccharide part).

Platelet aggregation

Blood was collected into ACD solution (citric acid/citrate/dextrose; 5 : 1; v/v) and centrifuged for 10 min at 250 × *g* at room temperature. Platelet aggregation was investigated according to a method previously reported using a Chrono-log Lumi-aggregometer (Nowak and Wachowicz 2002). After incubation of platelet-rich plasma (3×10^8 platelets/ml) with extract or its parts at 37°C for 2 min with stirring, 20 μl of collagen (2 μg/ml) was added and aggregation was measured. The maximal aggregation (100%) was defined as the maximum change in light transmission observed over 5 min in the presence of DMSO without any extract nor its parts. Antiaggregatory activities were defined as the percentage inhibition of aggregation.

Cell viability

The activity of lactic dehydrogenase (marker of platelet lysis) in the extracellular medium after treatment of blood platelets

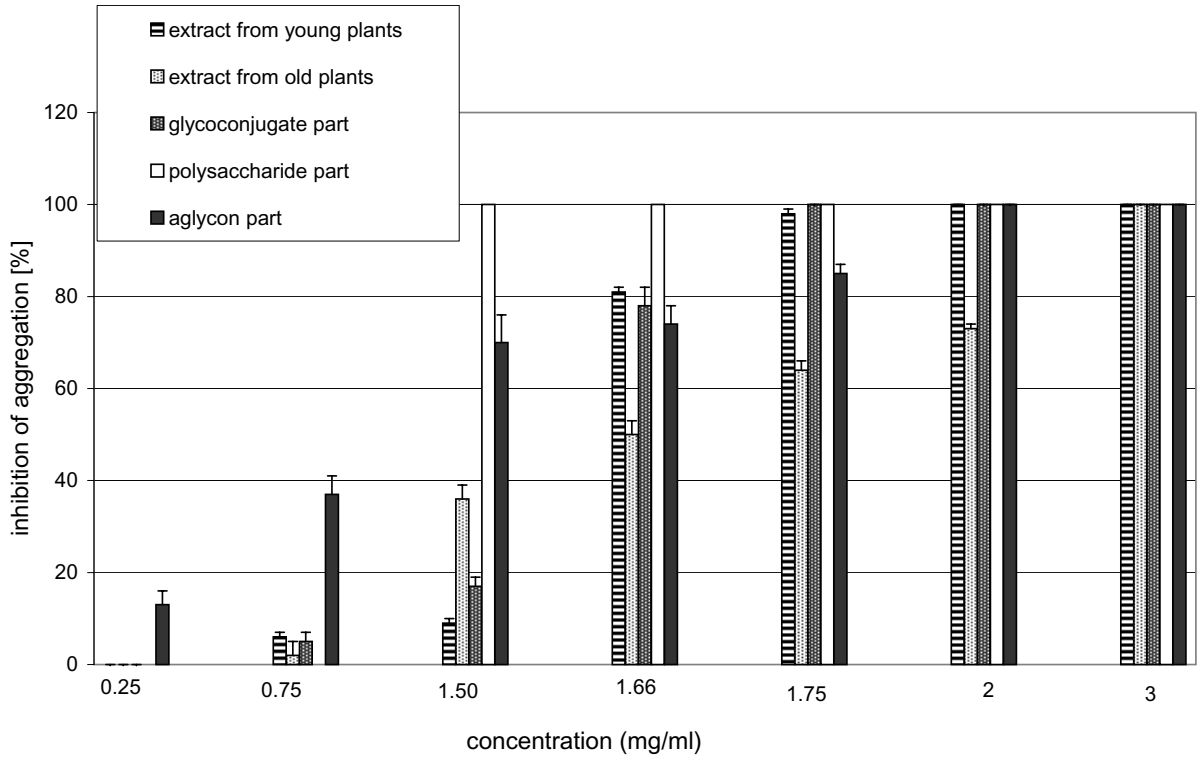

Figure 1. Effects of different concentrations of extract from *Conyza c.* and its parts on platelet aggregation induced by 2 μg/ml collagen. The maximal aggregation (100%) was defined as the maximum change in light transmission observed over 5 min in the presence of DMSO without any extract nor its parts. Antiaggregatory activities were defined as the percentage inhibition of aggregation. The results are representative of six independent experiments, and are expressed as means ± SEM ($n = 6$). The effects were statistically significant according to the Anova I test; $p < 0.05$ for samples with tested compounds (for all samples at the concentrations 1.5–3 mg/ml and for lower concentrations of aglycon part) compared with control platelets treated only with collagen.

Table 1. The effects of different concentrations of polysaccharide part of extract from *Conyza c.* on inhibition of platelet aggregation induced by collagen (2 μg/ml); $p < 0.05$ *versus* control (for concentrations: 0.85 mg/ml and higher)

Cconcentration (mg/ml)	% inhibition of aggregation *versus* control
0.025	0
0.050	0
0.250	0
0.750	0
0.850	27 ± 1.8
0.950	75 ± 2
1.200	100
1.500	100
2.000	100

with the highest concentration of tested extracts and their parts was measured spectrophotometrically according to Wroblewski and La Due (1955).

The effects of extract isolated from *Conyza c.* and its different parts on platelet aggregation induced by collagen were tested *in vitro* by turbidimetry method. We observed that extract of *Conyza c.* from young or old plants strongly inhibits platelet aggregation induced by collagen (2 μg/ml) and this effect was dose-dependent ($p < 0.05$) (Figure 1). Polysaccharide part showed the stronger inhibitory effect on platelet aggregation induced by collagen than extracts or other parts of extract at the same concentration (Figure 1).

The cytotoxicity of extracts from young or old plants and their different parts to human blood platelets was also evaluated. It was shown that the highest tested doses of young or old extracts and their different parts did not cause the lyses of platelets determined as a leakage of lactic dehydrogenase into the extracellular medium (data not shown).

Tested medicinal plant – *Conyza c.* contains a variety of flavonoids and tannins in addition to the essential oils. Aqueous extract contains the polysaccharides formed from mainly glucose, galactose, arabinose, glucuronic acid and has anticoagulant activities. However, the role of polysaccharides in medicinal properties of this plant is not clear. Here, we present evidences that polysaccharide aqueous extract obtained from *Conyza c.* possess not only antioxidative properties (Olas et al. 2006), but may inhibit blood platelet activation measured by aggregation. The obtained results indicate that polysaccharide part may also be responsible for antiaggregatory effect of extract from *Conyza c.* (Table 1). Moreover, the reduced platelet aggregation in the presence of extract may be partly a result of its antioxidative activities. The present study suggests that extract from *Conyza c.* is useful not only in control bleeding but also for protection against hyperactivity of platelets associated with cardiovascular diseases and atherosclerosis.

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