PHARMACOLOGY AND TOXICOLOGY

Effects of Water and Ethanol Extracts from *Paeonia lutea* Root Bark on Hemostatic Parameters

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Anticoagulant and fibrinolytic activities, as well as inhibitory effect on platelet aggregation of water (2%) and ethanol extracts from the peony root bark (*P. lutea*) are demonstrated *in vitro*. Chronic peroral administration of the ethanol extract activates anticoagulant and fibrinolytic processes in rat blood. Water extract apart from anticoagulant activity, strongly enhanced blood fibrinolytic potency and inhibited platelet aggregation, which implies its possible antithrombotic properties.

Key words: *plant extracts; hemostasis; fibrinolysis; platelet aggregation*

Plant extracts have a pronounced effect on the blood coagulation system, exhibiting a wide range of anticoagulant and hemostatic properties [3]. Many of them are widely used in clinical practice. Anticoagulant effect of chestnut and marigold water extracts is attributed to the presence of glycosaminoglycans [1]. Chronic 30-day peroral administration of dry extracts from sweet clover (*Melilotus officinalis*) exerted a profound anticoagulant effect in rats due to the presence of coumarin. Chronic peroral administration of water extracts from root bark of peony *P. suffruticosa* produced anticoagulant and fibrinolytic effects in rats [5]. Several active fractions were isolated from the extract after partial rectification. A protein-free fraction exhibited heparin-like anticoagulant activity. In a protein-containing fraction, a complex of a heparin-like anticoagulant with protein was found, which exhibited nonenzymatic fibrinolytic activity (NFA) [4]. Antithrombotic effect of some plant preparations, whose inhibitory action on platelet aggregation is associated with the presence of swietenines and swietenolides, has been reported [9].

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Our aim was to study the effects of water and ethanol extracts from peony *P. lutea* (ecologically pure phytomaterial) on fibrinolytic and anticoagulant activities, and on primary hemostasis *in vitro* and after chronic administration to rats.

MATERIALS AND METHODS

Water (2%) and ethanol (16% ethanol) extracts were obtained from ecologically pure phytomaterial of peony *P. lutea* grown in the Botanical Garden of M. V. Lomonosov Moscow State University. Anticoagulant activity of extracts was determined *in vitro* by measuring recalcification time of rat serum after addition of 0.1 ml of extract and activated partial thromboplastin time (APTT) [10]. NFA of the extract was determined by the ability of the extract to induce lysis of non-stabilized fibrin plates in the presence or absence (total fibrinolytic activity, TFA) of 3% ε-aminocaproic acid (ACA). [2]. In order to evaluate plasminogen activator activity, the extract was applied to heated and unheated stabilized fibrin plates [6]. Platelet aggregation was measured by turbidimetry [7] after extract addition to the platelet-rich rat plasma activated with ADP (2 μM). Light scattering was measured on a Russian-made aggregometer, and the difference be-

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TABLE 1. *In Vitro* Effects of Water and Ethanol Extracts from *P. lutea* on Anticoagulant and Fibrinolytic Indices and Platelet Aggregation in Rat Plasma (*M±m*)

Preparations	Anticoagulant activity, sec			TFA, mm^2	NFA, mm^2	Plasminogen activator activity, mm^2	Platelet aggregation, rel. units
	recalcification time	thrombin time	APTT				
0.85% NaCl	125±7.8	10±0.3	—	0	0	0	5.3±0.13 (100)
Water extract	>600**	14.3±0.2*	—	49±1.1**	20±0.7**	22.4±3.0**	4.3±0.3 (81)*
16% ethanol	136±4.8	12.2±0.3	40±2.3	0	0	0	8.3±0.75 (100)
Ethanol extract	552±13**	86.5±9.7**	480±11**	38±1.5**	29±1.0**	21.0±2.2**	4.4±0.8 (53.2)**

Note. Here and in Table 2: in parentheses: % of control; *$p<0.05$, **$p<0.01$ compared with the control.

tween maximum and minimum values observed after addition of ADP was expressed in relative units.

In vivo experiments were performed on random-bred albino rats weighing 180-200 g. The extracts were given *per os* in a daily dose of 0.5 ml/200 g for 7-8 days. Blood was drawn from *v. jugularis* 20-24 h after the last extract administration; 3.8% sodium citrate (9:1) was used as anticoagulant. After centrifugation at 1000*g* for 5.5 min, 0.2 ml platelet-rich plasma was taken from the samples, dissolved in isotonic NaCl (1:3) and used in platelet aggregation assay [7]. The residue was recentrifuged at 3000*g* for 10 min, anticoagulant [10] and fibrinolytic indices [2], and activity of plasma XIIIa factor [8] were measured.

RESULTS

Water and ethanol extracts exhibited *in vitro* high TFA, NFA, and plasminogen activator activity (Table 1). NFA of the ethanol extract was 30% higher than that of the water extract, while TFA of the water extract was 23% higher than that of the ethanol extract, probably due to enzymatic fibrinolysis. The ethanol extract considerably inhibited platelet aggregation *in vitro*. The water extract possessed less pronounced but statistically significant antiaggregation activity. Both water and ethanol extracts displayed potent anticoagulant activity *in vitro*, serum recalcification time being the most indicative test for the water extract and thrombin coagulation time and APTT for the ethanol extract.

Both extracts considerably increased TFA and NFA in the blood of experimental animals (Table 2). However, plasminogen activator activity was 1.7-fold higher in rats treated with ethanol extract. In this group, plasma factor XIIIa activity decreased to a greater extent (by 53.9%) than in rats treated with water extract (29%). Water and ethanol extracts increased anticoagulant activity assessed by APTT test 1.2-fold and 1.4-fold, respectively.

The antithrombocytic effect displayed by both extracts *in vitro*, after peroral chronic administration was observed only in rats treated with water extract. In rats treated with ethanol extract, the inhibition of aggregation in plasma was negligible and platelet aggregation practically did not differ from the control.

Thus, anticoagulant, fibrinolytic, and antithrombocytic effects *in vitro* are characteristic of both water and ethanol extracts. In chronic peroral administration, the most pronounced effect of these extracts is activation of enzymatic and non-enzymatic fibrinolysis. The positive effect of ethanol extract on plasminogen activation and its anticoagulant properties were more pronounced in comparison with water extract as demonstrated not only by prolongation of APTT, but also by a significantly lower activity of plasma factor XIIIa, indicating its anticoagulant-fibrinolytic effect.

TABLE 2. Effects of Chronic Peroral Administration of Water and Ethanol Extracts from *P. lutea* on Fibrinolytic Indices, APTT, Factor XIIIa, and Platelet Aggregation in Rat Plasma (*M±m*)

Groups	APTT, sec	TFA, mm^2	NFA, mm^2	Factor XIIIa, U/ml	Plasminogen activator activity, mm^2	Platelet aggregation, rel. units
0.85% NaCl	41±7.3	98±2.9	34±1.2	23.1±0.8	9.2±0.7	3.5±0.3 (100)
Water extract	58±6.3*	70±3.7**	54±2.1**	34.4±1.2**	15.2±3.0*	2.05±0.3 (58.5)*
16% ethanol	65±4.3	78±6	34±1.0	22.1±0.9	12±1.1	7.05±0.43 (100)
Ethanol extract	78±2.5**	36±8.0**	50.3±1.1**	32.7±1.5**	26±1.8**	6.3±0.43 (90.5)

Under the same conditions, water extract exhibits a wider spectrum of hemostatic activities: anticoagulant, fibrinolytic and inhibitory effects on platelet aggregation. Considerable inhibition of platelet aggregation and enhanced blood fibrinolytic potency after treatment with water extract from *P. lutea* root suggest its possible antithrombotic activity.

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