Original Article

Study of specific properties of Polygonum Aviculare L. dry extract as a hemostatic agent

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Abstract

Polygonum aviculare L., has been used in traditional medicine for many years. Hemostatic activity of the Polygonum aviculare L. dry extract (PADE) on the duration and amount of bleeding was studied in the current research. Moreover, the effect of the PADE was investigated on platelet count and capillary permeability. The results showed that the PADE administration increased platelet count by 212%, decreased retraction time by 50% and increased adhesive capacity by 225% and significantly reduced capillary permeability in the experimental animals. According to the results of this experiment, the Polygonum aviculare L. dry extract can be recommended to use in medical practice as a hemostatic agent in various hemorrhages.

Keywords: Polygonum aviculare L., thrombin, adhesion, retraction, hemostatic drugs.

INTRODUCTION

Hemostasis is the natural process that leads to cessation of bleeding/ hemorrhage from a blood vessel in the body. The problem of the hemorrhage stopping very often demands an urgent crucial medical action. There are effective hemostatic agents, such as vitamin K (vicasol), calcium-containing drugs (calcium chloride, calcium gluconate and animal-derived of blood clotting factors) in the management of bleeding (Fatemeh 2020).

Long-term use of chemical drugs such as hemostatic agents can cause adverse effects on the body. Herbs derived medicines from natural sources appear to be safe or no side effects. Investigations exploring the potential use of herbal medications should be encouraged and economically more favorable for patients. Hence, search for a medication with hemostatic and anti-inflammatory activities facilitating higher capillary permeability to be used in medical practice is of high value (Handan 2009, Ximena 2003).

Polygonum aviculare L., also called knotweed a member of the Polygonaceae family, has been used in traditional medicine for the treatment of hemorrhage, diarrhea, dysentery, atherosclerosis, hemoptysis, and hemorrhoids, and as a coagulant and sedative treatment for many symptoms associated with hypertension, helps for inducing apoptosis in the breast cancer cell line MCF-7 (Sun 2014, Chin-Yuan 2006, Habibi 2011, Yoon-Young 2013).

Shepherd's purse also called Capsella bursa-pastoris is a well-known medicinal plant that commonly used in the traditional medicine for many purposes. According to the previous studies, Shepherd's purse preparations have a hemostatic, anticancer, analgesic and anti-inflammatory effects. Moreover, it is also used to control different types of bleeding (Ali Esmail 2015, Atieh 2019, Sahar 2017).

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Nevertheless, it is unknown whether Polygonum aviculare L. has hemostatic activity on the hemorrhages. The content of total flavonoids of Polygonum aviculare L. has been described. As mentioned earlier, our aim was to evaluate the hemostatic effect of the Polygonum aviculare L. dry extract on the duration and amount of bleeding.

Review of Experimental Data

Reagents. All other organic and nonorganic reagents (of an analytical grade) were purchased from local commercial suppliers.

Preparation of Poligonium Aviculare L. extract

Aerial part of Poligonium Aviculare L. were purchased as a finished product (JC Asel, Uzbekistan). Maceration is an extractive technique that was carried out at room temperature. Aerial parts of the Polygonum aviculare L. were dried at room temperature for 2 weeks and subjected to extraction with 70% methanol at room temperature followed by evaporation of combined hydromethanolic solutions through rotatory evaporation at 50° C, The liquid extract was kept in refrigerator at 40°C under it was being used (Tukhtaev 2019).
Animals. The experiments on the duration of bleeding and amount of bleeding were performed on 24 rats of the both sexes, body mass 160-185 g, 6 animals in each group. The animals were maintained in standard cages at 22±1 °C in a 12:12 h light:dark cycle with free access to food and water. The experiments were conducted in accordance with Uzbekistan Law 11.794. The project was approved by the Tashkent pharmaceutical institute Animal Ethics Committee on October 17, 2011 (reference no. 43/11. The method of blood collection should be described in the protocol approved by the Institute animal ethics committee. All procedures in this study were approved by the Guide of Tashkent pharmaceutical institute for animal experimentation. All procedures were performed under isoflurane anesthesia.

Establishment of bleeding animal model

The experimental model was established by transecting animal's tail at 0.5 cm from the tip to standardize the degree of injury and the mean diameter of the tail tip was 2.5 mm, and the mean bleeding area was 4.9 mm^2. Therefore, animals placed in a prone position on a platform with their tails resting 10 cm below the surface as previously described. The procedure was performed under isoflurane anesthesia in this experiment.
The duration of bleeding is marked by a stopwatch from the moment the first drop of blood appears until the bleeding stops completely in seconds. In all groups after the treatment, the tail tip was blotted and measured by mean of a blotting paper. Animal's blood was collected on blotting paper and weighed before and after the procedure, that the difference in the weight of the blotting paper before and after the procedure indicated the amount of bleeding (Handan 2009, Ximena 2003).
The experimental animals were randomly assigned into four groups of six (6) rats each as follows:
Group I served as the control and received 1.5 ml of distilled water.
Groups II and III were treated with the PADE by orally at the doses of 75 mg/kg and 150 mg/kg, respectively 30 min prior to the beginning of the experiment.
Group IV were treated with the Shepherd's purse extract by orally at the dosage of 75 mg/kg and 150 mg/kg, respectively.
Shepherd's purse extract served as the control on the duration and amount of bleeding in this experiment.

Determination the effect of the PADE platelet count and its functional activity

The experiment was conducted for the determination the mechanism of action of the PADE on stopping of bleeding, such as to study its effect on platelet count and functional activity. The experiment was conducted in six rabbits weighing 2.6-3.3 kg. Animals received of medication at suspension state of 75 mg/kg by intravenously. Blood tests were performed at the 30th, 60th, and 120th minutes before and after medication injection. A platelet count was determined by the Mindray BC-5000 automated hematology analyzer (Mindray Bio-medical Electronics Co Ltd, China).
Thrombocyte adhesion was determined according to the method described by Chekalina S.I. (Ximena 2003). The platelet adhesion activity is characterized by the percentage of platelets adhering during the movement of stabilized blood on a magnetic stirrer, the number of platelets adhered is detected by the difference in their content before and after mixing.
Briefly: 0.2 ml of citrated blood is poured into a microtube, placed on a magnetic stirrer for 15 minutes. Blood for platelet counting is taken before and after mixing. The percentage of adhesion is calculated by the formula:
A-B / A * 100 = adhesion in%.
Spontaneous platelet aggregation was determined according to the method described by Wiu b Hoak method (Fatemeh 2020). It is an important phenomenon in many pathological states.
It is known from the literature that at the same time hemostatic agents are used to stop bleeding with medications for the strengthening capillaries also. To achieve our aim, the effect of the PADE was studied on capillary permeability in animals. The experiment was performed in eighteen mice weighing 18-3.22g by the method of K.N.Manakova (1984). The animals were divided into 3 groups in each of 6:
Group I served as the control and received 1ml of distilled water.
Groups 2 and 3 were administered orally of the PADE at the dosage of 75 mg/kg and 150 mg/kg, respectively. Thirty minutes after injection, the drug was administered by intraperitoneal at the dose of 50 mg/kg of trypan blue solution (0.3%). Then, 15 minutes after the trypan blue injection, 0.02 mL of p-xylene was injected under the foot's plantar aponeurosis and was determined the time of blue formation.
Investigations on thromboelastogram
The clotting process under the effect of preparations was estimated by thromboelastograms (TEG) recorded on «Tromb-2» thrombelastograph. The thromboelastograms allowed us to determine the following factors:
The following indicators were taken into account on thromboelastograms:
R - blood reaction time, which characterizes the first phase of the blood clotting process;
K-time of clot formation or thromboelastrographic constant of thrombin, which depends on the concentration of formed

thrombin and the amount of fibrinogen;
R/K is a constant use of prothrombin,
R-K is a coagulation constant that expresses the total duration of blood clotting;
MA is maximum amplitude and affected by the concentration of fibrinogen, the number and quality of platelets;
E is coefficient of clot elasticity, ITP-thrombohemorrhagic potential (MA/S)
Ci is hypercoagulation index.

Statistical analysis.

Statistical analysis was performed by GraphPad Prism 5.0 (CA, USA). Multiple comparisons of the means were carried out using ANOVA test. The differences were considered statistically significant when $p < 0.05$. All values were presented as mean ± SEM (stand error of mean).

RESULT AND DISCUSSION

Effect of Polygonum aviculare L. dry extract on the duration and amount of bleeding

The duration and amount of bleeding results of PADE are illustrated in Table 1.

Table 1. The effect of the PADE on the duration and amount of bleeding in animals after 60 min injection.

Groups	Dose, mg/kg	Weight of blotting paper, mg		
		Bleeding time, min	Before the experiment, mg	After the experiment, mg
Control	1,5	320±20	407±24	407±24
group				
PADE	75	177±14*	407±24	140±12*
PADE	150	143±11*	407±24	100±10*
Capsella extract	75	211,6±14*	407±24	170±14*

The values represent as Mean ± SEM for 6 rats each. * $P < 0.05$ as compared with control group value.

As shown in Table 1, the PADE shortened the duration of bleeding at 60 min by 320 ± 20 min (Group I), 177 ± 14 min (Group II) and 143 ± 11 (Group III) min, respectively. The Shepherd's purse extract shortened the duration of bleeding by 211.6 ± 13.94 min (Group IV).

The results showed that the PADE reduced the bleeding time when compared in both Group I and Group IV by 1.8-2.2 and 1.5 times.

A similar effect of the PADE was observed on the amount of bleeding; it reduced significantly the amount of bleeding to 140 ± 12 mg (Group II) and 100 ± 10 mg (Group III) respectively.

Study of the effect of the PADE on platelet count and its functional activity

The effect of the PADE on platelet count and functional activity was presented in Table 2. As shown in the table, it reduced the of platelets count by 30-120% at 38-112 minutes at dose of 75 mg/kg in peripheral blood of the experiment.

The maximum effect of dry extract occurred within 120 minutes of the experiment and increased platelet count from 400±12·109/l to 850±15·109/l.

Table 2. The effect of the PADE on the on the platelets count and its functional activity.

Studied parameters	At 30, 60 and 120 min after administration of PADE			
	result	at 30 min	at 60 min	at 120 min
Platelets, 10^9/l,%	400±12 / 100	550±20* / 138	800±16* / 200	850±15* / 212
Blood clot retraction, min.%	13±0,8 / 100	9±0,6* / 75	7±0,4* / 58	6±0,4* / 50
Platelets adhesion,%	20±1,0 / 100	28±3,0* / 140	38±4,0* / 190	45±2,4* / 225
Platelets spontaneous aggreation, %	25±1,4 / 100	36±2,6* / 144	44±2,4* / 176	35±2,4* / 140

The values represent as Mean ± SEM for 6 rats each. * = $P < 0.05$ as compared with control group value.

The PADE administration the functional activity of platelets was noted, as well as platelet adhesion increased from 20±2% to 38±4% at 60 minutes. Platelet adhesion occurred at the maximum level and increased from 20±2% to 45±4% or 123% at 120 min.

The PADE increased spontaneous platelet aggregation from 25 ± 1.4% to 36 ±2.6% at 30 min and it increased spontaneous adhesion of platelets from 35 ± 2 to 44 ± 2.4% at 60 to 120 min respectively.

Specifically, blood clotting under the influence of dry extract ranged from 13 ± 0.8% to 9 ± 0.6% at the 30th minute of the experiment, 7 ± 0.4% and 6 ± 0.4% at 60 and 120 min, respectively, the time of blood clot retraction decreased by 75%, 58% and 50%.

Table 3. Effect of PADE on thromboelastogram indicators of rabbits' at a single oral administration at dose of 5 mg/kg (M±м; п=6)

Indicators	The time from PADE administration, min			
	Outcome	30	60	120
Blood reaction time R, mm	20±2,0	8±0,5	7±0,5*	45±3,0

Clot formation time K, mm	12±1,0	10±2,6	10±1,0	25±2,0
Coagulation constant R+K, mm	32±2,2	18±6,0*	17±1,0*	70±5,2*
The constant of using prothrombin R/K	1,7±0,1	0,8±0,1	0,7±0,2	1,8±0,1
Maximal amplitude MA, mm	48±1,0	54±1,4	49±1,0*	43±1,0
Coagulation constant t, mm	110±10	110±10	110±10	120±11
The syneresis constant S, mm	122±11	120±11	110±11	145±13
Total clotting time T, mm	135±12	128±11	117±11	190±16
C_i	0,82±0,05	2,8±0,2*	1,8±0,1*	1,1±0,1*
E	82±6	89±6	92±8*	82±6

The values represent as Mean ± SEM for 6 rats each. * = P < 0.05 as compared with control group value.

As can be seen from the data in Table 1, we observed hypocoagulation, which is expressed and considerable changes of the basic indicators were noted after 120 minutes. Reaction time (R) has increased by 62 % from 20±2,0 to 45±3.0 mm, clot formation time has increased by 63 % from 12±1.0 to 25±2.0 mm. Parameter R+K was increased from 32±2.2 to 70±5.2 mm or by 62,7%. At that, maximum amplitude of MA has decreased by 18% from 48±1.0 to 43±1.0 mm while hypercoagulation index Ci has increased by 50% from 0,82±0,05 to 1,1±0,1*, and E was equal to 82±6 and has increased by 82±6.

The effect of PADE on capillary permeability

As shown in Table 4, the mean time of appearence of blue color was 11.7 ± 0.95 minutes in the control group leg.

Table 4. Influence of the PADE on capillary permeability.

Groups	Dose (mg/kg)	The time of appearance of blue color in animal leg, in min	Results, in %
Control group	1,5 ml H_2O	11,7±0,95	100
ADE	75	14,74±1,05*	126
ADE	150	16,38±1,1*	140

The values represent as Mean ± SEM for 6 rats each. * = P < 0.05 as compared with control group value.

The time of occurrence of the blue color of the animal legs which received the PADE was 14.74 ± 1.05 and 16.38 ± 1.1 minutes. Thus, the Polygonum aviculare L. dry extract increased the time of appearance of blue color on animal leg by 26% and 40%, compared to the control group, respectively. Hereby, the dry extract was having a strengthening effect on capillary permeability.

The present simple experiments confirmed the efficacy of Polygonum aviculare L. dry extract to shorten bleeding time in animals. The current research is the first in the medical literature about PADE as a natural potential medicine for the bleeding. In addition, the hemostatic plant extracts or their possible commercial derivatives could be more effective and cheaper than the topical hemostatic agents available at the present time.

Therefore, based on the experimental results, it can be concluded that under PADE was reduced the retraction time of blood coagulation and significantly increased the platelets count in the blood and their adhesion, aggregation and thromboelastogram.

Hence, the PADE has a significant coagulation effect at the studied doses, reducing the blood clotting time and the amount of blood released to a certain degree of mathematical accuracy. Its effect can be explained by contained vitamin K component.

Therefore, the new local PADE is not inferior to the well-known Shepherd's purse (Capsella bursa-pastoris) in terms of the effect of the blood clotting process on some of the studied indicators. This mechanism of action can be explained by the stimulating effect on platelet count and functional activity, thromboplastin, thrombin formation, as well as a decrease in the antithrombin III index, as in the Shepherd's purse.

An attempt was made to find a solution to another problem in the blood system by proving the blood-clotting effect of the remedy obtained based on PADE. In separate experiments, the acute toxicity, cumulative, local tickling, allergic, embryotoxic and chronic effects of the studied remedies were studied, and it was found that they are less toxic, free from side effects, allergic, cumulative and local tickling properties. In addition, the studied drugs do not adversely affect the cardiovascular, respiratory, central and peripheral nervous and immune systems of the body. When these drugs are administered to the body for a long time, it was found that they do not have any adverse effects on the histostructure of vital internal organs of the body and the biochemical parameters of peripheral blood.

Based on the results, it was proved for the first time that PADE is a new local hemostatic drug with high therapeutic activity, no side effects, economically viable for patients, as well as some adverse changes in blood clotting. PADE tincture and "hemostat" drugs have successfully passed clinical trials as hemostatic drugs and were introduced into medical practice. At present, PADE tincture and hemostat drugs are produced on an industrial scale. Documents of the pharmacological report of the remedy PADE were prepared for submission to the Pharmacological Committee of the Republic of Uzbekistan for consideration in order to obtain permission for clinical trials as a hemostatic drug.

CONCLUSION

Based on the results described above, PADE significantly shortened the duration and amount of bleeding in a rat model. PADE increased of the platelet count and its functional activity. Moreover, PADE increased of the strengthening of the capillary wall. Further research is needed to determine which active ingredients in the plant shorten bleeding time. This plant is a potential for use as a hemostatic agent in medical practice.

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