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Effect of Setulin Hemostatic Drug Excipient on the Hemostasis in Rabbits with Experimental Hypocoagulation.

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ABSTRACT

The current article presents the research outcomes on properties of Setulin hemostatic drug excipient produced on the basis of *Lagochilus setulosus* extracts. The authors investigate the effect of the given preparation on a blood hemostasis in rabbits with the hypocoagulation, caused by introduction of heparin. The conducted studies revealed that oral introduction of Setulin hemostatic drug excipient in a dose of 50 mg/kg causes the expressed hemostatic effect associated with the activation of thromboplastin formation and transformation of prothrombin into thrombin owing to acceleration of contact and phospholipid coagulation starting mechanisms (I and II phases of blood coagulation). In 60-90 minutes after introduction, Setulin completely removes hypocoagulative effect of heparin.

Keywords: *Lagochilus setulosus*, hemostatic drug excipient, Setulin, hemostasis, heparin, blood coagulation, thrombin, prothrombin.

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INTRODUCTION

Lagochilus genus is presented by set of species, which are extended in different geographical areas. It is known that the plants of this genus contain medicinal substance called lagochilin which possesses styptic property. For the first time this genus was investigated and systematized by O.E.Knorring, who has described 27 species growing in the territory of the former USSR, having divided them into 2 sections [1-7]. Later T.I.Tsukervanik has developed the new systematization of this genus, which included 44 species of world flora, having divided them into 3 sections and 6 subsections. According to this systematization, 34 species grow in the territory of the former USSR, 25 species - in Central Asia, and 17 species - in Uzbekistan and Kazakhstan [7.8]. At that, as many as 20 species come under endemic species of plants [9, 10].

Up to the present time a pharmaceutical industry used just one species - *Lagochilus inebrians*. During the Soviet period of country's development this species was cultivated in agriculture and used for production of lagochilin, which in turn was utilized for production of styptic preparation Lagoden. However, as far back as in 1981 I.E.Akopov identified some other species, in which the content of lagochilin was higher than that in *L.inebrians.* Nevertheless, up to date these species remained unstudied.

It is known that tincture of *Lagochilus inebrians* along with the expressed haemostatic effect, has sedative, hypotensive, antispasmodic, anticonvulsant, analgesic, and antiallergic properties. Besides, its therapeutic action is also noted on the animals suffering from acute ionizing radiation, caused by radioactive polonium. Unique property of this plant species consists in fact that its extracts promote acceleration of blood coagulation process at parenteral, enteral and local administration [11]. Subsequently, the "Inebrin" preparation, possessing effective homeostatic effect, has been produced on the basis of a dry *Lagochilus inebrians* extract [12].

Lagochilus setulosus is another most perspective species of this genus. It has been revealed by I.E.Akopov that exactly this species of the given genus contains a significant amount of lagochirzin, which is diterpenoid conditioning its haemostatic effect [11]. Though, further research on *Lagochilus setulosus* has not yet been carried out. Our previous researches revealed its habitats and resources in the wild nature. Besides, we have investigated the elemental and biochemical composition of plant phytomass. Employing aqueous extraction method we have also received a dry extract of *L.setulosus* phytomass, which contains lagochirzin, whose preliminary preparative form was named «Setulin». However, this substance is not yet fully investigated.

In this connection, the purpose of our research was studying of haemostatic properties of Setulin in terms of blood hemostasis in rabbits with the hypocoagulation caused by introduction of heparin. The research aimed at showing that Setulin removes hypocoagulation effect of heparin.

MATERIALS AND METHODS

Studies on thrombelastograph

The investigation was carried out on 18 grey colored rabbits (6 animals in each group) with body weight of 2.5±0.2 kg. Heparin was injected intravenously into rabbit's marginal vein. Investigated preparations were introduced peroral in the doses of 50 mg/kg at time intervals of 5 minutes. A blood sampling for test was carried out over time before introduction of preparations (fate) and in 30, 60, 90, 120 and 180 minutes after the beginning of introduction. The tests were carried out in the thermostat with an open door at a temperature of $+30^0$C. Animals were kept at this temperature at least one hour prior to the beginning of the test. A tip of the tail about 10-12 mm in length was cut off by sharp scissors. The sheet of a filtering paper was brought to a tail cult. The paper was preliminary dried out in the thermostat (until achieving a constant weight) and weighed. Blood, flowing from the tail, was distributed uniformly over a filtering paper. Then filtering paper was dried out and weighed again. Duration of a bleeding was marked on a stopwatch from the moment of inception of the first blood drop through the full termination of a bleeding. The quantity of blood loss was estimated by weight in grams of dry residue. The general trend of coagulation process change under the effect of preparations was estimated by thromboelastograms (TEG) recorded on «Tromb-2» thrombelastograph. The thromboelastograms allowed us to determine the following factors:

R – is the time interval from the beginning of recording to appearance of divergence of the TEG line edges by 1 mm; this indicator is called a time of blood reaction that characterizes I and II phases of blood coagulation process;
K – is the time interval from the end of reaction time to a divergence of TEG line edges by 20 mm. This indicator is called a time of blood clot formation or thromboelastographical constant of thrombin, which depends on concentration of generated thrombin and the amount of fibrinogen;
R/K – is the prothrombin usage constant, which is the ratio of thromboplastin generation rate (R) to amount of formed thrombin (K);
R+K – is the nonspecific coagulation constant. It expresses the total duration of blood coagulation and has almost the same clinical value as the determination of plasma to heparin tolerance, and reflects the content of antithrombin III;
MA – is the maximum amplitude, which is influenced by the concentration of fibrinogen, as well as quantity and quality of platelets;
t – is the blood coagulation constant, which is measured from the end of K period to the maximum amplitude of the TEG and corresponds to the period from the end of visible blood coagulation to the beginning of a blood clot retraction; the shorter this period the more pronounced hypercoagulation and on the contrary;
S – is the syneresis (induration) constant or the chronometric constant, which is measured from the end of K to the maximum amplitude and is equal to K+t; This indicator corresponds to overall phase of fibrin clot formation, i.e. time from beginning of fibrin formation through its completion; it should be noted that nevertheless this parameter is relative, because fibrin formation begins much earlier than the appearance of the first TEG swings; intensity of syneresis is proportional to mass of fibrinogen;
T – is the total blood coagulation constant measured from the beginning of TEG record (plus time of blood preparation) to maximal divergences of TEG edge lines and represents arithmetic sum of R+K+t; reduction of constant T indicates on hypercoagulation, while increase - on hypocoagulation;
E – is the clot elasticity coefficient;
ITP – is the thromb-haemorrhagic potential index (MA/S); and
Ci – is the hypercoagulation index [13.14].

Biochemical indicators of blood

Were defined by following indicators: blood clotting time, determined by Lee-White technique (min); recalcification of plasma – by Kudryashov modification of Berghof and Roka technique (sec); plasma tolerance to heparin – by Sigg's technique (min); prothrombin time (PT) (sec), activated partial thromboplastin time (APTT) (sec) and fibrinogen (g/dl) measured by HumaClotJunior single-channel coagulometer (Germany); clot retraction (min) – by Kotovshhikova's technique [13.15].

Hypocoagulation model was initiated by intravenous injection of 130 units of heparin into rabbit's auricular vein. Preparations were introduced in 5 minutes after injection of heparin [13].
The results obtained were processed statistically [16].

RESULTS AND DISCUSSION

Before testing of Setulin, heparin, which is well-known hypocoagulant, was injected into experimental rabbits' blood. Then in different time intervals blood analysis was conducted on thrombelastograph to register the changes of basic blood parameters. During the test, the results of thromboelastograms were compared to indicators of blood biochemical analysis.

The test data analysis has shown that single intravenous injection of heparin in a dose of 130 units/kg causes the expressed hypocoagulation in 30 minutes that corresponds to lack of deviations in thromboelastograms over a period of 30-60 minutes (Table 1).

Thus, considerable changes of the basic indicators were noted after 90 minutes. Reaction time (R) has increased twice from 63±4.5 to 120±10.0 mm, clot formation time has increased by 2.5 times from 20±1.5 to 64±5.0 mm. Parameter R+K was increased from 83±7.2 to 184±15.0 mm or by 2.2 times. At that, maximum amplitude of MA has decreased by 45% from 78±2.0 to 43±1.0 mm, while hypercoagulation index Ci has decreased by 4.0 times from 0.93±0.08 to 0.23±0.02. This trend continued for 180 minutes in the course of the test and then the hypocoagulative effect of heparin decreased. At that, on thromboelastograms the indicator R

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was equal to 68±4.0 mm, K – to 31±4.0 mm, MA – to 72±1.0 mm, and a hypercoagulation index was equal to 0.73±0.05.

Table 1: Effect of heparin on rabbits' thromboelastogram indicators at a single intravenous injection in a dose of 130 units/kg (M±m; n=6)

Indicators	Test time, minutes					
	Outcome	30	60	90	120	180
TEG: R, mm, %	63±4.5 100	-	-	120±10.0 190	103±10.0 164	68±4.0 108
K, mm, %	20±1.5 100	-	-	64±5.0 320	53±4.0 265	31±4.0 155
R/K, %	3.2±3.0 100		-	1.9±0.1 59	1.9±0.1 59	2.2±0.1 69
R+K, mm, %	83±7.2 100	-	-	184±15.0 222	156±11.0 188	99±10.0 119
MA, mm, %	78±2.0 100	-	-	43±1.0 55	54±1.0 69	72±1.0 92
t, mm, %	100±10.0 100	-	-	100±10.0 100	100±10.0 100	105±10.0 105
S, mm, %	120±11.0 100	-	-	205±15.0 171	225±20.0 188	169±14.0 141
T, mm, %	182±18.0 100	-	-	325±20.0 179	328±20.0 180	234±18.0 129
Ci, %	0.93±0.08 100	-	-	0.23±0.02 25	0.35±0.03 38	0.73±0.05 78
E, %	355±26.0 100	-	-	75.4±3.0 21	117±10.0 33	257±22.0 72.4
ITP, %	3.0±0.2 100	-	-	0.37±0.02 12.3	0.52±0.01 17.3	1.52±0.1 50.7

*P< 0.05 with regard to control

Table 2: Biochemical indicators of blood coagulation at a single intravenous injection of heparin in a dose of 130 units/kg (M±m; n=6)

Indicators	The time from Setulin administration, min					
	Outcome	30	60	90	120	180
Time of blood coagulation, min, %	3.0±0.2 100	23.4±2.0 780	17±1.4 567	10.1±1.0 337	4.1±0.3 137	3.2±0.24 107
Plasma recalcification, sec, %	80±4.0 100	840±60.0 1400	490±20.0 817	206±10.0 343	106±10.0 177	100±10.0 167
Tolerance of plasma to heparin, min, %	4.2±0.3 100	14.7±1.2 350	12.5±1.0 298	7.5±0.4 179	6.1±0.4 145	5.5±0.4 131
Activated partial thromboplastin time (APTT), sec, %	25±1.0 100	84±6.0 336	37±2.0 148	25±1.6 100	25±1.6 100	20±1.0 80
Prothrombin time (PT), sec, %	12±1.0 100	48±0.3 400	32.4±0.2 270	28.8±20.0 240	24±0.2 200	20.0±1.6 170
Amount of fibrinogen, g/dL, %	450±30.0 100	125±10.0 28	275±10.0 61	302±15.0 67	342±15.0 76	625±35.0 139
Clot retraction, min, %	12±2.1 100	45±2.4 375	35±2.0 292	25±1.2 208	20±1.0 167	15±1.5 125

*P< 0.05 with regard to control

The thromboelastogram indicators agree with the biochemical indicators of blood coagulation process (Table 2). In 30 minutes after injection of heparin (control group) blood coagulation time has increased almost by 8 times from 3.0±0.2 to 23±2.0 min, plasma recalcification time has increased by 10 times from 80±4.0 to 840±60.0 sec. At that, APTT, which characterizes the factors of the intrinsic pathway of coagulation (factors XII, XI, IX and VIII) has increased by 3 times from 25±1.0 to 84±6.0 sec. Prothrombin time, characterizing the factors of the extrinsic pathway of coagulation (factors V and VII) has increased by 4 times from 12±1.0 to

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48±0.3 sec, and the amount of fibrinogen has decreased by 3.6 times from 450±30.0 to 125±10.0 g/dl. Tolerance of plasma to heparin, specifying the availability of direct anticoagulants in blood (heparin and antithrombin III) has increased by 3.6 times from 4.2±0.3 to 14.7±1.2 min. High hypocoagulation level was maintained over 120 minutes, further decreasing and approaching the intact indicators in 180-240 minutes.

Table 3: Effect of Setulin on thromboelastogram indicators of rabbits' with hypercoagulation at a single oral introduction in a dose 50 mg/kg (M±m; n=6)

Indicators	The time from Setulin administration, min					
	Outcome	30	60	90	120	180
TEG: R,mm, %	60±3.8 100	165±15.2* 275	95±6.8* 158	63±4.3* 105	30±2.6* 50	35±3.0* 58
K, mm, %	20±2.0 100	105±10* 725	55±4.0 275	34±3.1 171	12±1.0 60	14±1.0 70
R+K, mm, %	80±6.0 100	270±20* 388	150±13.0* 188	97±7.4 121	42±2.8* 53	49±3.6 62
R/K, mm, %	3.0±0.2 100	1.6±0.1* 37	1.8±0.1* 60	1.5±0.1 50	2.5±0.2* 83	2.3±0.2 77
MA, mm, %	73±2.0 100	50±3.0 69	65±2.0 89	70±2.0 98	72±2.0 99	74±2.0 101
t, mm, %	100±10.0 100	100±10.0 100	100±10.0 100	100±10.0 100	100±10.0 100	100±10.0 100
S, mm, %	120±11.0 100	245±22.0 204	155±13.0 129	134±11.0 112	112±11.0 93	114±11.0 95
T, mm, %	180±16.0 100	410±35.0 278	250±20.0 13	196±16.0 109	142±13.0 79	149±13.0 83
Ci, %	0.9±0.1 100	017±0.01 19	0.43±0.03 48	0.74±0.06 82	1.8±0.1 200	1.9±0.1 211
E, %	278±20.0 100	284±20.0 102	284±20.0 100	267±20.0 96	268±21.0 96	259±20.0 93
ITP, %	2.3±0.2 100	0.51±0.03 15	1.15±0.1 50	1.9±0.1 83	2.5±0.2 109	2.7±0.2 217

*P< 0.05 with regard to control

Table 4: Effect of Setulin on biochemical indicators of blood in rabbits with hypercoagulation at a single oral introduction in a dose of 50 mg/kg (M±m; n=6)

Indicators	The time from Setulin administration, min					
	Outcome	30	60	120	180	240
Time of blood coagulation, min, %	2.9±0.2 100	7.0±0.6* 241	4.0±0.3* 138	3.5±0.2* 121	2.3±0.2* 79	1.8±0.1 62
Plasma recalcification, sec, %	102±1.0 100	408±36.0* 404	140±1.0 137	104±1.0 101	90±1.0 88	80±1.0 78
Tolerance of plasma to heparin, min, %	5.6±4.0 100	9.8±0.7* 175	6.8±0.5* 121	5.7±0.4 101	5.0±0.4* 90	4.1±0.3 73
Prothrombin time (PT), sec, %	11.3±1.0 100	28.8±0.6* 255	8.2±0.6* 73	8.6±0.6* 76	7.8±0.7* 69	11.4±0.8 100
Activated partial thromboplastin time (APTT), sec, %	23.4±3.0 100	77±6.6 329	42±3.0 180	20.3±1.6* 87	16±1.2 68	22.1±2.0 94
Amount of fibrinogen, g/dL, %	140±14.0 100	163±14.0 116	206±16.0 147	248±18.0 177	312±26.0 223	167±13.0 119
Clot retraction, min, %	20±2.1 100	35±2.4 175	25±2.0 125	15±1.2 75	10±1.0 50	15±1.5 75

*P< 0.05 with regard to control

As is obvious from the data in Table 3, in 30 minutes after introduction of Setulin in a dose of 50 mg/kg contrastingly to control, the following changes were noted: reaction time R has been increased with regard to an outcome from 60±3.8 to 165±15.2 mm or by 2.8 times; time of clot formation K - from 20±2.0 to 105±10.0 mm or by 5.3 times.; parameter R+K - from 80±6.0 to 270±20.0 mm or by 3.4 times. After 60-90

minutes the expressed hypercoagulative effect was noted in comparison with control group of animals, which accounted to 20-40%, while after 120 minutes complete reduction of the blood coagulation process with hypercoagulation indicators was observed.

According to the data presented in Table 4, biochemical indicators of blood coagulation show, that in 30 minutes after introduction of Setulin in a dose of 50 mg/kg, blood coagulation time became shorter than that in control group by 3.3 times, i.e. it has decreased from 23.4±2.0 to 7.0±0.6 min; time of plasma recalcification has decreased by 2 times from 840±60.0 to 408±36.0 sec; tolerance of plasma to heparin – by 2 times, or from 14.7±1.2 to 9.8±0.7 min.

CONCLUSIONS

Thus, summing up the research outcomes on hemostatic action mechanism of the Setulin, we can make the following conclusions:

- Setulin hemostatic drug excipient has a pronounced hemostatic effect when administered orally at a dose of 50 mg/kg;
- The action mechanism of Setulin hemostatic drug excipient, as a new hemostatic preparation of resorptive effect, towards the plasma hemostasis is related to the fact that it activates generation of thromboplastin and conversion of prothrombin into thrombin due to accelerating contact and phospholipid coagulation releasers (I and II phase of blood coagulation);
- The effect of Setulin hemostatic drug excipient is retained in the heparin-induced hypocoagulation model. At that, even in 60-90 minutes after administration, Setulin completely removes the hypocoagulation effect of heparin.

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