

# The influence of a herbal mixture containing knotweed, motherwort, hawthorn, periwinkle, and horsetail on metabolic processes in rabbits

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The aim of this study was to investigate the effect of the infusion produced from lesser periwinkle (*Vinca minor*), blossoms and fruits of monostillate hawthorn (*Crataegus monogyna*), motherwort (*Leonorus cardiaca*), knotweed (*Polygonum aviculare*) and field horsetail (*Equisetum arvense*) on the carbohydrate–lipid–protein and electrolyte metabolism in blood serum, aortic myocardial tissues, and liver after induction of pituitrin hypertension in rabbits. Induced pituitrin hypertension in rabbits was accompanied by a rise in the levels of atherogenic lipids in blood serum, an increase in the activity of glycolytic enzymes and cholesterol levels in the liver, as well as by a decrease in the activity of alaninaminotransferase in the myocardium, and a rise in the content of transketolase and calcium concentration in the aortic wall. The treatment of pituitrin hypertension in rabbits with a herbal preparation containing knotweed normalized arterial blood pressure and decreased calcium concentration in the aortic wall. In the treatment of hypertension, an infusion made from a herbal preparation containing knotweed decreased hyperlipoproteinemia and hypercholesterinemia, as well as diminished the activity of glycolysis, cholesterol levels in the liver, and the activity of carbohydrates – enzymes of energetic metabolism in the myocardium.

**Key words:** hypertension, knotweed, metabolic processes

## INTRODUCTION

On evaluating human morbidity in the 20th century, the achievements and losses in this field, it can be stated that we enter the new millennium under the conditions of the epidemics of arterial hypertension (AH). The development of AH is significantly influenced by the disturbances in the neural and humoral regulation of the smooth muscle tone in the arterioles of the greater circle [1]. Excessive nervous and emotional tension creates foci of constant excitement in the hypothalamic centers of cerebral cortex, which regulate vascular tone and include into this the reticular formation and the cardiovascular center located in medulla oblongata. This results in catecholamine hypersecretion, vasoconstriction, and disturbances in the metabolic processes. During the recent 10–15 years, the clarification of the mechanism of the damage to cellular structures revealed

the problem of the pathogenesis of AH [1]. For this reason, creation of novel medicines that would influence the pharmacological correction of the metabolic processes of arterial hypertension is highly relevant. New preparations are searched for by combining synthetic compounds with medicinal vegetal stock [2–5].

On the basis of literature sources and known patented prescriptions, we selected for the study a therapeutic vegetal stock characterized by blood pressure-regulating activity. Although some of these raw materials do have their side effects, their usage is continued due to their positive therapeutic effect.

The clinical interest in *Vinca* alkaloids (vinblastine, vincristine, vindensine and vinorelbine) was clearly identified as early as 1965. The alkaloid vincamine is hypotensive, negatively chronotropic, spasmolytic, hypoglycemic and sympatholytic. Scientifically validated studies on the hypotensive effect on humans have not yet been carried out. *Vinca* alkaloids have been shown to cause leucopenia, which was the dose-limiting toxicity, and vincristine is a known

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tubulin inhibitor. Because *Vinca minor* has a drying effect on the tissues of the gastrointestinal tract, it can cause severe constipation.

*Crataegus monogyna* helps in hypertension with myocardial weakness and angina pectoris. Hawthorn leaves, berries and blossoms contain biologically active flavonoid compounds: anthocyanidins and proanthocyanidins (also known as biflavans or procyanidins). *Crataegus* is minimally or negligibly toxic. For *Crataegus monogyna*, the interaction with digitalis glycosides and beta-blockers, related to the potentiation of toxic effects, has been indicated [5].

The composition of *Leonurus cardiaca* phytochemicals: stachydrine, leonurine, glycosides, phytoosterol, diterpenes, flavonoids, caffeic acid, and tannins. This plant has anti-spasmodic, sedative, uterine stimulant, cardio-tonic, diuretic, hypotensive, anti-bacterial, and antifungal effects and also inhibits thyroid activity. Toxicity: relatively safe for long-term use [6].

*Polygonum aviculare* is a safe and effective astringent and diuretic herb used mainly in the treatment of complaints such as dysentery and hemorrhoids. It is also used in the treatment of pulmonary complaints, because the silicic acid it contains strengthens the connective tissue in the lungs. The whole plant is anthelmintic, astringent, cardiogenic, cholagogue, diuretic, febrifuge, haemostatic, lithotryptic, and vulnerary. Recent research has shown that the plant is a useful medicine for bacterial dysentery. *Polygonum* positively acts on the healing of intestinal epithelial wounds and has some antimicrobial activities [7].

The chemical composition of *Equisetum arvense*: acids (ascorbic, ferulic, silicic, malic, caffeic, gallic, pectic, tannic), campesterol, equisetin, equisetinon, alkaloids (nicotine, palustrine), amino acids (niacin) and minerals (magnesium, silicon, silica selenium, calcium, iron, manganese, phosphorus, potassium, aluminum, zinc, chrome, cobalt). Horsetail constitutes one of the most diuretic species of all the plants. It possesses a great capacity to eliminate water from the body and increase urination by up to 30%. At it is a plant with and strong diuretic properties, its internal use can evoke problems with blood pressure. On the other hand, because of the presence of certain components, its prolonged use can be toxic [6].

The aim of this study was to investigate the effect of the infusion produced from lesser periwinkle (*Vinca minor*), blossoms and fruits of monopistillate hawthorn (*Crataegus monogyna*), motherwort (*Leonurus cardiaca*), knotweed (*Polygonum aviculare*), and field horsetail (*Equisetum arvense*) on the carbohydrate-lipid-protein and electrolyte metabolism in blood serum, aortic myocardial tissues, and liver after induction of pituitrin hypertension in rabbits.

## MATERIALS AND METHODS

A mixture of medicinal plants, which included lesser periwinkle (*Vinca minor*), blossoms and fruits of monopistillate hawthorn (*Crataegus monogyna*), motherwort (*Leonurus cardiaca*), knotweed (*Polygonum aviculare*), and field horsetail (*Equisetum arvense*) (further on referred to as herbal mixture No. 1) was used for the investigation of some of the mechanisms of hypotensive activity.

A patented herbal preparation containing blossoms of monopistillate hawthorn (*Crataegus monogyna*), fringes hops (*Humulus lupulus*), leaves of peppermint (*Mentha piperita*), milfoil (*Achillea millefolium*), and roots and rhizomes of valerian (*Valeriana officinalis*) (further on referred to as herbal mixture No. 2) was used as a *control* (comparative) preparation [8]. The influence of herbal preparation No. 1 on some processes of carbohydrate, lipid, protein, and electrolyte metabolism, as well as the indices of aortic tissues, heart, and liver were observed after inducing pituitrin hypertension in rabbits. Twenty chinchilla rabbits weighing 2.5 to 3.5 kg were used in the study (National Veterinary Service pass for work with laboratory animals No. 0006). These animals were differentiated into four groups: Group 1 (controls) – operated animals without any treatment, Group 2 – animals with pituitrin hypertension, Group 3 – animals with prolonged pituitrin hypertension that received orally a mixture made from herbal preparation No. 1, and Group 4 – animals with prolonged pituitrin hypertension that received orally a mixture made from herbal preparation No. 2. Infusions from herbal stock were prepared according to the European Pharmaceutical Requirements [9]. The doses of the preparations were selected with respect to previous dose-dependent effects and on the basis of toxicity studies. Control of arterial blood pressure was performed using cuffs on live animals after passing the external carotid artery through a skin scratch according to Korotkov [1].

Biochemical indices were determined in the presence of marked hypertension (on the 20th day after the discontinuation of pituitrin) when the arterial blood pressure (ABP) was  $175 \pm 4.5$  mmHg (Group 2), in the presence of normal ABP ( $125 \pm 3.5$  mmHg) after a 28-day usage of herbal preparation No. 1 (the dosage was 2.2 ml/kg – Group 3) and following the usage of the patented herbal preparation No. 2 (Group 4). During this period, the blood of the animals was taken for testing, organs were separated, and tissues were homogenized (all operations were performed at a temperature of 0–4 °C). Enzymatic activity was determined in a non-nuclear extract: fructose-1,6-diphosphatase (using the method of Tovarnitsky and Voliuska modified by Ananjev and Obukhova [10]), lactate dehydrogenase (using the spectrophotometric method), creatine phosphokinase (according to Ostrovsky

and Trebukhina) [11, 12], and aspartat- and alaninaminotransferase (by the method of Raitman and Frenkly modified by Kolb and Kamyshnikov [13]). In parallel, we studied the concentration of cholesterol (according to the Liebermann-Burchard reaction) [14], extracting it from tissues using the Blur mixture (method modified by Prokhorov [1]). Serum phospholipids were examined by determining their phosphorus contents (using the reagent mixture produced by Chempor (Czech Republic) for determination of inorganic phosphorus),  $\beta$ -lipoproteids – by applying the Bursstein and Samay turbidimetric method modified by Kolb and Kamyshnikov, and total protein level was determined by Louriv's method modified by Todorov [15]. Calcium levels were determined using the reagent mixture for determination of inorganic calcium (Chempol), and sodium and potassium – using the flame photometry method [16]. All the obtained experimental findings were verified using the methods of variation statistics.

The results are presented as means  $\pm$  S.D. Statistical analysis was performed using Student's t test, and  $p < 0.05$  was taken as the level of significance. We used the Mathcard 5.0 mathematical processing package.

## RESULTS AND DISCUSSION

After daily injections of intravenous pituitrin for 14 days, the animals developed a prominent and constant pituitrin hypertension (Table 1). Arterial blood pressure in rabbits that were operated on without any treatment (Group 1) was  $135.0 \pm 3.4$  mm Hg, in Group 2  $192.0 \pm 4.5$  mm Hg, in Group 3  $189.8 \pm 4.6$  mm Hg, and in Group 4  $184.0 \pm 4.7$  mm Hg. After a 28-day course of treatment with infusion made from herbal preparation No. 1, systolic blood pressure returned to norm and was  $136.0 \pm 4.6$  mm Hg (Group 3), while in case of the usage of infusion made from herbal preparation No. 2 (Group 4), it was  $142.5 \pm 4.4$  mm Hg.

The obtained results showed that the infusion prepared from a herbal mixture that included knotweed had a curative hypotensive effect, and its therapeutic efficiency was higher compared to the infusion prepared from the herbal preparation No. 2.

Biochemical indicators in rabbits were determined on the 28th day after the course of treatment with infusions prepared from herbal mixtures No. 1 and No. 2. (Figs. 1–3).

The findings showed that marked changes in enzymatic activity were seen in the presence of pituitrin hypertension in rabbits. For instance, an increase in fructose-1.6-diphosphataldolase in the presence of experimental pituitrin hypertension in rabbits (Group 2) was seen in the aortic tissues (by 18.4%,  $p < 0.05$ ). In animals with pituitrin hypertension (Group 2), the activity of the main penthose-phosphate enzyme, bypass-transketolase, in the aortic wall increased by 44.7%. Since in many tissues a positive correlation was found between the speed of lipolysis and the activity of penthose cycle enzymes, we may deduce an increased lipid synthesis activity in the aortic wall.

In the aortic wall (Group 3), a reduction in the activity of lactatdehydrogenase (by 28.8%,  $p < 0.05$ ) was observed, which most probably directs the oxidation-reduction potential of the tissues towards aerobization.

Myocardium (Group 3) showed a reduction of lactatdehydrogenase activity by 44.5% ( $p < 0.05$ ). All the findings indicate glycolysis inhibition. At the same time, there was a drop in the intensity of the pethose cycle reactions indicated by a respective decrease in transketolase activity (by 31.0%,  $p < 0.05$ ). Thus, inhibition occurred in the glycolytic change of carbohydrates and the oxidation in the penthose-phosphate cycle. In addition, there was a decrease in alaninaminotransferase activity in the cardiac muscle (by 18.6%,  $p < 0.05$ ) (Group 3), which was induced by a respective change in the levels of free amino acids.

Such metabolic reaction enables the usage of the substances of carbohydrate nature in a way that is more energetically efficient, i.e. aerobic oxidation. There is probably no increase in the influence of ATF, which is indirectly confirmed by a significant decrease in creatinephosphokinase levels (by 33.2%,  $p < 0.05$ ) (Group 3).

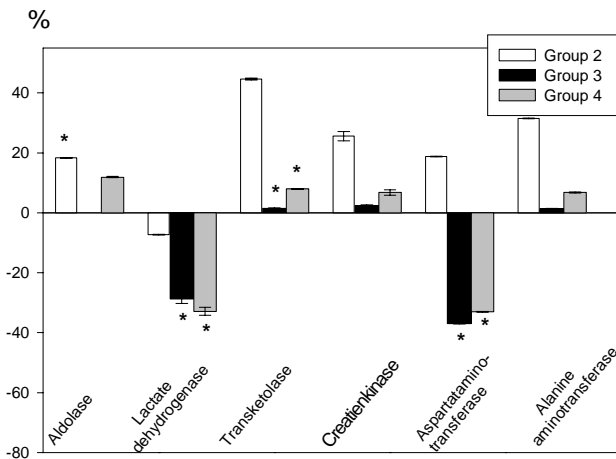
In the liver (group 2), an increase in aldolase (by 38.2%) in the presence of experimental pituitrin hy-

**Table 1. The influence of herbal preparations No. 1 and No. 2 on systemic arterial blood pressure in the course of treatment for experimental pituitrin hypertension**

Group No.	Systolic arterial blood pressure, mmHg				
	Initial	After 7 days	After 14 days	After 21 days	After 28 days
1	$135.0 \pm 3.4$	$135.9 \pm 4.6$	$136.0 \pm 4.2$	$135.8 \pm 4.3$	$136.0 \pm 4.1$
2	$192.0 \pm 4.5^*$	$190.6 \pm 4.6^*$	$190.1 \pm 4.8^*$	$188.3 \pm 4.1^*$	$187.5 \pm 4.7^*$
3	$189.8 \pm 4.6^*$	$168.7 \pm 5.3^*$	$152.5 \pm 4.6^*$	$139.9 \pm 4.6$	$136.0 \pm 4.6$
4	$184.0 \pm 4.7^*$	$174.9 \pm 5.4^*$	$162.8 \pm 5.4^*$	$147.0 \pm 5.5$	$142.5 \pm 4.4$

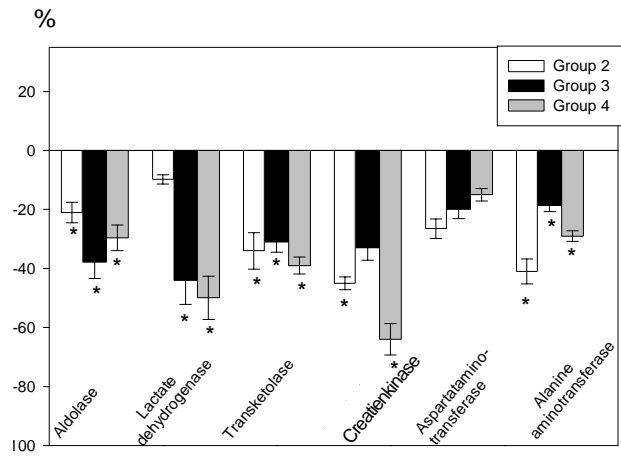
For explanation of group numbers see *Materials and Methods*.

\* The difference is reliable at  $p < 0.05$ .



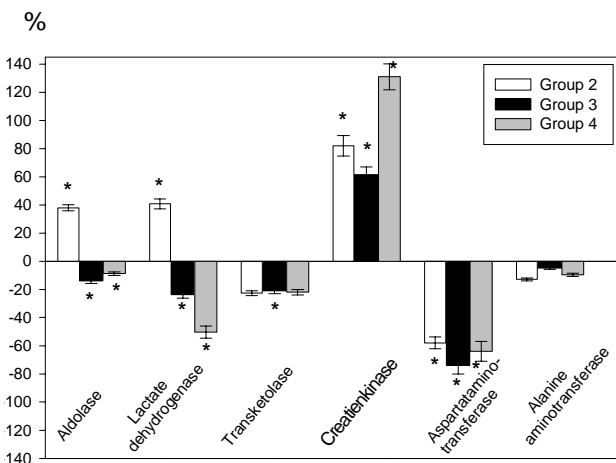
**Fig. 1.** Changes in the activity of carbohydrate metabolism of enzymes, and amino acid metabolism in the aorta in the presence of pituitrin hypertension following the course of treatment with herbal preparations No. 1 and No. 2

For explanation of group numbers see Materials and Methods. \* The difference is reliable at  $p < 0.05$ .



**Fig. 2.** Changes in the activity of carbohydrate metabolism of enzymes, and amino acid metabolism in the myocardium in the presence of pituitrin hypertension following the course of treatment with herbal preparations No. 1 and No. 2.

For explanation of group numbers see Materials and Methods. \*The difference is reliable at  $p < 0.05$ .



**Fig. 3.** Changes in the activity of carbohydrate metabolism of enzymes, and amino acid metabolism in the liver in the presence of pituitrin hypertension following the course of treatment with herbal preparations No. 1 and No. 2.

For explanation of group numbers see Materials and Methods. \* The difference is reliable at  $p < 0.05$ .

pertension in rabbits was seen ( $p < 0.05$ ). In the hepatic tissues, an increase (by 40.7%,  $p < 0.05$ ) in the activity of lactate dehydrogenase was observed, which showed and increased intensity of glycolic processes. Aminotransferase activity in the liver did not differ from the control values, implying that the stimulation of glycolysis was induced by increased losses of hepatocyte energy in the atherogenic synthesis.

The direction of changes in the activity of glycolysis enzymes in the liver (Group 3) was the opposite in the presence of hypertension, *i.e.* the activity of fructose 1,6-diphosphatase decreased by 14.1% and the activity of lactate dehydrogenase by

23.8% ( $p < 0.05$ ), indicating a decrease in the glycolysis tension. It is possibly related to a decrease in energy losses during the synthesis of lipids, as well as to the stimulation of lipolysis and the usage of its products in the citrate cycle. The activity of transketolase in the liver decreased by 20.9% ( $p < 0.05$ ). This indicates repetitions of the intensity of glucose oxidation in the pentose cycle. These shifts conditioned changes in amino acids in liver: alanine aminotransferase activity decreased by 9.7%, and the activity of aspartate aminotransferase by 74.0% ( $p < 0.05$ ), possibly due to a decrease in amino acid balance and an increase in protein synthesis. It is likely that these processes are related to the onset of myocardial hypertrophy caused by the resistance of peripheral blood vessels in the presence of hypertension.

The normalization of arterial blood pressure following the administration of herbal preparations No. 1 and No. 2 was accompanied by a marked rearrangement of metabolic processes induced by pituitrin hypertension (Table 2). The level of atherogenic lipids in blood serum (Group 3) decreased: cholesterol by 60.2% ( $p < 0.05$ ), and  $\beta$ -lipoproteids by 47.3% ( $p < 0.05$ ) as compared to Group 2.

The stimulation of oxidative metabolism allows of the usage of lipid synthesis for the precursors in the aortic wall (Table 3). The reduction of cholesterol levels (Group 3) (by 51.5%,  $p < 0.05$ ) in the aorta is probably related to that, and thus, judging by the reduction of transketolase activity to the norm, the intensity of lipogenesis normalizes. The decrease in cholesterol levels in the aortic wall may be stimulated by the ability of phosphodiesterase inhibitors to significantly reduce the contraction of endothelial cells and the penetration of lipids into blood vessel

**Table 2. Lipids concentration in blood serum in the presence of hypertension**

Group No.	Cholesterol, mmol/l	Phospholipids, g/l	β-lipoproteids, conditional units
1	1.25 ± 0.12	0.92 ± 0.12	9.3 ± 1.3
2	2.36 ± 0.23*	1.28 ± 0.13	24.5 ± 1.8*
3	1.42 ± 0.14	0.93 ± 0.16	11.6 ± 2.84
4	1.34 ± 0.22	0.95 ± 0.17	14.9 ± 2.63

*For note: explanation of group numbers see Materials and Methods.*

*\* The difference is reliable at p < 0.05.*

walls. The concentration of cholesterol in the blood serum of animals with pituitrin hypertension (Group 2) increased by 88.8% (p < 0.05), and the amount of β-lipoproteids increased by 163.4%.

Rabbits with pituitrin hypertension (Group 2) exhibited changes in electrolyte balance, characterized by an increase in sodium levels in blood serum (23.3%, p < 0.05), liver (by 52.49%, p < 0.05), and heart (by 41.1%, p < 0.05) (Table 4). The usage of herbal preparations No. 1 and No. 2 resulted in the normalization of sodium levels in blood serum, aortic tissues, heart, and liver. The activation of oxidative metabolism in the aortic wall is associated with

**Table 3. Cholesterol levels in tissues in the presence of hypertension**

Group No.	Aorta, mmol/kg	Heart, mmol/kg	Liver, mmol/kg
1	5.96 ± 0.61	4.12 ± 0.28	5.32 ± 0.27
2	4.78 ± 0.44	3.82 ± 0.23	12.14 ± 0.24*
3	3.07 ± 0.32*	3.14 ± 0.17	6.51 ± 0.21*
4	3.29 ± 0.24*	3.26 ± 0.16	9.87 ± 0.38*

*For note: explanation of group numbers see Materials and Methods.*

*\* The difference is reliable at p < 0.05.*

**Table 4. Electrolyte concentration in blood serum and tissues**

Group No.	Electrolytes, mmol/kg	Blood serum, mmol/kg	Aorta, mmol/kg	Heart, mmol/kg	Liver, mmol/kg
1	Sodium	84.2 ± 0.22	113.4 ± 7.2	42.3 ± 2.1	26.1 ± 1.42
2		103.8 ± 0.47*	144.3 ± 9.1	59.7 ± 2.6	39.8 ± 1.54*
3		85.8 ± 0.32	115.9 ± 7.2	44.6 ± 2.4	27.3 ± 1.27
4		90.2 ± 0.28	119.8 ± 5.4	47.5 ± 2.7	28.2 ± 1.21
1	Potassium	3.1 ± 0.19	19.7 ± 1.3	30.9 ± 0.7	27.1 ± 1.12
2		2.5 ± 0.12	15.1 ± 1.1	27.8 ± 0.9	28.9 ± 1.57
3		3.0 ± 0.24	19.9 ± 1.9	31.2 ± 1.9	27.4 ± 1.61
4		2.9 ± 0.21	20.8 ± 1.8	31.5 ± 1.7	27.4 ± 1.36
1	Calcium	1.2 ± 0.13	2.7 ± 0.3	2.1 ± 0.4	1.2 ± 0.04
2		1.2 ± 0.11	4.1 ± 0.2*	2.3 ± 0.2	1.6 ± 0.07
3		1.0 ± 0.09	2.5 ± 0.1	2.0 ± 0.3	1.3 ± 0.16
4		0.9 ± 0.11	2.9 ± 0.3	1.9 ± 0.2	1.4 ± 0.13

*For explanation of group numbers see Materials and Methods.*

*\* The difference is reliable at p < 0.05.*

energy losses in vasodilator mechanisms maintained from the activity of the proper calcium pumps. This is confirmed by the drop in calcium levels in the aorta. In addition to that, an increase in calcium levels (by 51.8%, p < 0.05) was observed in the aortic tissues, which is related to persistent vasoconstriction which characterizes hypertension.

Pituitrin hypertension was accompanied by an increase in the levels of atherogenic lipids in blood, of calcium in the aortic wall and of cholesterol in the liver, as well as by a stimulation of the activity glycolytic enzymes, a decrease in the activity of alaninaminotransferase in the myocardium, and an increase in the levels of transketolase, the main enzyme of the penthose-phosphate cycle. The normalization of arterial blood pressure occurred in parallel with a decrease in hyperlipidemia, the inhibition of glycolysis, and a drop in the concentration of cholesterol in the liver.

A comparison of metabolic changes caused by pituitrin hypertension and the changes in metabolic processes in rabbit blood serum and tissues, accompanied by hypotensive effects following administration of the herbal preparation containing knotweed and the control preparation No. 2, may highlight the correlation between certain changes and the functional blood condition in the vessel wall.

Thus, administration of an infusion prepared from a herbal mixture containing knotweed and from a herbal preparation No. 2 normalized ABP, decreased hyperlipoproteinemia, hypercholesterinemia, glycolytic activity, and cholesterol levels in the liver, as well as decreased the activity of the carbohydrates studied (the enzymes of energetic metabolism) in the

cardiac muscle, inhibited glycolysis, and decreased cholesterol levels in the aortic wall.

## CONCLUSIONS

1. Induced pituitrin hypertension in rabbits was accompanied by a rise in the levels of atherogenic lipids in blood serum, an increase in the activity of glycolytic enzymes and cholesterol levels in the liver, as well as by a decrease in the activity of alaninaminotransferase in the myocardium, and a rise in transketolase and calcium concentration in the aortic wall.

2. The treatment of pituitrin hypertension in rabbits by a herbal preparation containing knotweed normalized arterial blood pressure and decreased calcium concentration in the aortic wall.

3. In the treatment of hypertension, an infusion made from a herbal preparation containing knotweed, lesser periwinkle, blossoms and fruits of monopistillate hawthorn, matherwort, and field horsetail decreased hyperlipoproteinemia and hypercholesterolemia, as well as diminished the activity of glycolysis, cholesterol levels in the liver, and the activity of carbohydrates – energetic metabolism enzymes in the myocardium.

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## VAISTAPOLIØ MIÐINIO SU TAKAPOLE, SUKATPOLIØ, GUDOBELE, PIEME IR ASIŪKLIU POVEIKIS TRIUÐIØ METABOLINIAMS PROCESAMS

### S a n t r a u k a

Šio darbo tikslas – ištirti ūpilo, pagaminto iš ūliauþianeiø-jø þiemio þolës, vienapieseiø gudobelio þiedø, vienapieseiø gudobelio vaisio, sukatpolio þolës, rûgties takapolës ir dirviniø asiūkliø þolës, poveiká angliavandeniø, lipidø, baltymø ir elektrolitø apykaitai kraujo serume, aortos miokardo audiniuose bei kepenyse sukëlus triuðiams hipofizio hipertenzijà. Dël hipofizio hipertenzijos kraujo serume padaugëjo aterogeniniø lipidø, padidëjo glikolitinø fermentø aktyvumas ir cholesterolio kiekis kepenyse, sumaþëjo alaninaminotransferazës aktyvumas miokarde, padaugëjo transketolazës ir padidëjo kalcio koncentracija aortos sienelëje. Triuðio hipofizio hipertenzijos gydymas vaistaþoliø miðiniu su rûgtimi takapole normalizavo arteriná spaudimà, sumaþino kalcio koncentracijà aortos sienelëje, sukëlë hiperlipoproteinemijà ir hipercholesterinemijà, taip pat sumaþino glikolizës aktyvumà ir cholesterolio kieká kepenyse, angliavandeniø energetinio metabolizmo fermentø aktyvumà miokarde.