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CARDIOPROTECTIVE PROPERTIES OF TABLETS WITH A THICK EXTRACT OF THE HERB PASTERNAK IN THE CONDITIONS OF ADRENALINE MYOCARDIODYSTROPHY

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Abstract

Considering a rather limited range of herbal preparations that are capable of providing a cardioprotective effect due to a polymodal effect on disturbed metabolic processes in the myocardium, the search and pharmacological studies of new potential phytocardioprotectors are important. Promising in this regard are preparations from the *Pastinaca sativa* (L.).

The aim of this work was to study the cardioprotective properties of a newly created drug - a tablet form of a thick extract of *Pastinaca sativa* herb (TEPPH tablets), developed at the National University of Pharmacy.

Adrenaline-induced myocardial dystrophy was reproduced in rats by a single subcutaneous injection of 0.18% adrenaline hydrochloride solution at a dose of 0.5 mg/kg (0.28 ml/kg) of body weight.

The use of TEPPH tablets at a dose of 100 and 200 mg/kg and the reference drug "Tricardin" contributed to a decrease in the degree of endogenous intoxication, which was manifested by a significant ($p<0.05$) decrease in the serum urea content, respectively by 1.3 and 1.4 and 1.8 times. The investigated agents to a certain extent influenced the cytolytic processes: the activity of the cardiocytolysis marker AsAT was lower than that in the positsve control (PC) group, however, the differences did not reach significance. The antioxidant properties of the studied objects were established: TEPPH tablets in both doses reliably compared with the PC group restored the content of reduced glutathione and catalase activity in the heart homogenate almost to the level of the intact control group and reduced the content of TBA-active products by 1.22 (at a dose of 100 mg/kg) and 1.24 times (at a dose of 200 mg/kg). These changes were reflected in the functional state of the myocardium: under the influence of TEHP in both doses, there is a tendency to a decrease in heart rate and an improvement in atrial conduction, as evidenced by a significant decrease in the systolic index compared with the PC group.

The results obtained indicate the ability of the tableted form of *Pastinaca sativa* herb extract thick to prevent metabolic disorders and functional disorders of cardiac activity in conditions of adrenaline myocardial dystrophy, which justifies the expediency of its further study, in particular, the study of its toxic characteristics.

Key words: adrenal myocardial dystrophy, tablet form of a thick extract of Pastinaca sativa herb, metabolic disorders, cardioprotective activity.

Introduction. Considering a rather limited range of herbal preparations that are capable of providing a cardioprotective effect due to a polymodal effect on disturbed metabolic processes in the myocardium, the search and pharmacological studies of new potential phytocardioprotectors are important. [1, 2, 3, 4, 5].

Promising in this regard are preparations from the Pastinaca sativa (L.). Recent studies have shown that furocoumarins of the herb pasternak have the ability to dilate peripheral vessels and coronary vessels of the heart, eliminate spasms of the bronchi and smooth muscles of the abdominal cavity and have a moderate sedative effect [6, 7]. However, there are only a few drugs based on the herb pasternak, which are indicated for angina, cardioneurosis with the phenomena of vasospasm, spasms of the gastrointestinal tract, kidneys and ureters. [8].

The aim of this work was to study the cardioprotective properties of a newly created drug – a tablet form of a thick extract of Pastinaca sativa herb (TEPPH tablets), developed at the National University of Pharmacy.

Materials and methods. White laboratory rats were selected for the study from ESIAP NUPh vivarium. Animals were kept in a room with controlled microclimate parameters: air temperature +18-22°C, humidity 50-65%, light regime "12 hours day/night" in accordance with the rules of keeping laboratory animals [9]. Animals were treated in accordance with the rules of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes» [10]. The draft study plan was approved by the NUPh Bioethics Commission (protocol № 6 of 08.06.2021). The study was conducted in accordance with the guidelines [11], in compliance with the requirements of Good Laboratory Practice [12].

The experiment had 35 white male rats with an initial weight of 220-250 g, age of 4-4.5 months. The animals were divided into 5 groups: 1 – intact control (IC); 2 – intact control (IC); 3 and 4 groups – animals receiving TEPPH tablets at a dose of 100 mg/kg and 200 mg/kg (in terms of content of pasternak herb extract), respectively; 5 – animals that received the comparison drug (CD) "Tricardin" [13] (1 ml of drops contains: valerian tincture (tinctura Valerianae) (1:5) (extractant ethanol 70 %) – 0,34 ml, hawthorn tincture (tinctura Crataegi) (1:10) (34 ml, hawthorn tincture 70 %) – 0,33 ml, nettle dog (motherwort) tincture (tinctura Leonuri) (1:5) (34 ml, hawthorn tincture 70 %) – 0,33 ml, manufactured by Ternopharm LLC, Ukraine) at a dose of 5 drops/kg. The drug "Tricardin" was used in a dose determined by transferring from the daily dose for humans to rats using the method of Ulanova I.P. [14]. The drug "Tricardin" was chosen as a comparison drug as an analogue for pharmacological activity and origin.

TEPPH was used in tablet formulation in doses of 100 and 200 mg/kg in terms of parsnip content of pasternak herb extract, for which in previous studies the ability to normalize heart rate was found in the event of adrenaline rush [15].

The study drugs were administered intragastrically in the treatment-and-prophylactic mode for 7 days, the last time on the day of the experiment 1 hour before the injection of cardiotoxin.

The pathology was reproduced in all experimental rats except the intact control group. Adrenaline-induced pathology was initiated by a single subcutaneous injection of 0.18% solution of epinephrine hydrochloride at a dose of 0.5 mg/kg (0.28 ml/kg) body weight [11, 15].

The effect of cardiotoxin on the myocardium and test drugs was evaluated by animal survival, myocardial function (ECG study), by the effect on the relative coefficient of heart mass (CHM) according to the formula: $HCM = (m_{organ}\ (z)/M_{animal}\ (z)\ x\ 100\%$, and biochemical parameters in blood serum and heart homogenate.

After 24 hours from administration of epinephrine hydrochloride in animals under mild chloroform anesthesia, the cardiovascular

system was recorded by use of electrocardiograph EK1T 03M, and then removed from the experiment by euthanasia.

The activity of the marker enzyme cardiocytolysis – aspartate aminotransferase (AsAT) was determined in the blood serum using the test kit "Lachema" (Czech Republic) and the level of the product of nitrogen metabolism of urea as an indirect indicator of endogenous intoxication ("Phyllisit-diagnostics", Ukraine). The cardioprotective effect of the drugs was also evaluated by the effect on lipid peroxidation (LPO) and antioxidant protection (AOP) [17]: by the level of active products that interact with thiobarbituric acid (TBA) [18], the content of glutathione (GSH) [19] and catalase activity [20].

Indicators of cardiovascular system were recorded using an electrocardiograph EK1T 03M in the II standard injection. The following indicators were taken into account when interpreting the electrocardiogram: R-R – duration of a complete cardiac cycle; the duration of the interval P-Q, which characterizes the atrioventricular conduction; duration of ventricular QRS complex and electrical ventricular systole – QT interval; voltage of P, T and R. The following indicators were calculated: heart rate (HR, beats/min) as the ratio of time (60 s) to the duration of the cardiac cycle RR and systolic index (SI) as the ratio of the duration of the QT interval to the duration of the cardiac cycle RR (QT/RR,%) [11].

Experimental data were processed by methods of variation statistics (average value and its standard error, $M \pm m$) by use of parametric (one-way analysis of variance ANOVA) and nonparametric methods of analysis (Kruskal-Wallis test). The accepted significance level is $p < 0.05$. The standard STATISTICA software package (version 6) was used to obtain statistical conclusions [21].

Results and Discussion. Adrenaline in high doses has an excessive stimulating effect on the myocardium, which leads to increased oxygen demand, develops hypoxia, ischemia, disorders of plastic and energy metabolism in the myocardium. These processes are the cause of circulatory disorders, metabolic imbalance develops due to the acceleration of metabolism and slow excretion of toxic products of cell life [6–8].

In modeling the pathology, one rat from the PC group died as a result of subcutaneous administration of adrenaline. According to the data (Table 1) it is possible to state that animals from the group of PC developed myocardial infarction, which is characterized by changes in many indicators of the cardiovascular system, determined by ECG analysis 24 hours after the initiation of pathology.

ECG study (Table 1) showed an increase in HR by 15% ($p < 0.05$) relative to IC, which is accompanied by conduction disturbances in the atria, as evidenced by a statistically significant increase in SI according to the IC group, and a decrease of 17% ($p < 0.05$) QRS interval. The cardiostimulatory effect of adrenaline and myocardial stress in rats of the PC group is evidenced by significant changes in the ST segment and a significant increase in the T wave by 257% ($p < 0.05$) relative to IC group. These changes are consistent with the literature data [3, 4].

The development of cytolytic processes in the myocardium of PC rats was accompanied by a significant 2-fold increase in the level of the marker enzyme cytolysis AsAT in the serum relative to group IC (table. 2). Probable increase in CHM indicates the development of myocardial infarction, accompanied by increased alternative processes, tissue damage, cell destruction. In the tissues of the myocardium of animals there is tension in the LPO system, as evidenced by the content of TBA-AP, which is 1.3 times ($p < 0,05$) higher than at the IC group. Depletion of antioxidant protection in the case of adrenaline-induced pathology indicates statistically significant in comparison with the IC group, by 2.2-fold decrease in GSH levels and by 1.8-fold decrease in the content of catalase in heart tissue. The development of endogenous intoxication was indicated by a probable 2-fold increase in urea against the IC group.

According to the results of studies performed on animals that were prophylactically administered TEPPH tablets at doses of 100 mg/kg and 200 mg/kg, there was some normalization of some ECG

parameters: a decrease in HR ($p < 0.05$) and SI ($p < 0.05$) to the level of IC, normalization of the QRS interval, R and T, observed a positive trend to change the ST segment. The comparison drug "Tricardin" showed a similar tendency to adrenaline intoxication, but the cardiac conduction SI remained at the level of the PC group.

The injection of drugs with TEPPH in both doses helped to reduce CHM relative to PC, almost to the level of IC, ie there was a decrease in the severity of alternative processes in the body.

The use of TEPPH drugs at a dose of 100 and 200 mg/kg and the comparison drug "Tricardin" contributed to a decrease in the degree of endogenous intoxication, which was relative ($p < 0.05$) to PC reduction of urea in serum by 1.3, 1.4 and 1.8 times respectively. The studied drugs to some extent influenced the cytolytic processes: the digital activity of the marker of cardiocytolysis AsAT was lower than in the PC group.

The experiment shows antioxidant properties of the studied objects: TEPPH tablets in both doses significantly restored the level of GSH and catalase activity in the homogenate of the heart and reduced the content of TBA-AP by 1.22 (dose 100 mg/kg) and 1.24 times (dose 200 mg/kg) compared with the PC group to the IC group. These changes affect the functional state of the myocardium: under the influence of drugs with TEPPH in both doses there is a tendency to decrease HR and improve conduction in the atria, as evidenced by a significant decrease in systolic index relative to the PC group.

The comparison drug "Tricardin" had a similar effect to tablets with TEPPH on the processes of LPO and AOP in the myocardium, but unlike TEPPH tablets did not contribute to a decrease in SI, which is probably due to the effects of biologically active substances on the cardiovascular function. Biologically active substances contained in the roots and rhizomes of valerian (ester of borneol and isovaleric acid, borneol, isovaleric acid, alkaloids, tannins, sugars), reduce the excitability of the CNS, reduce stress and have antispasmodic properties. Biologically active substances contained in dog nettle grass (motherwort) (essential oil, saponins, tannins, alkaloids), reduce the processes of excitation in the CNS, contribute to the normalization of blood pressure. Biologically active substances contained in hawthorn fruits (flavonoids, choline, acetylcholine, tannins, phytosterols) have antihypertensive, cardioprotective, antispasmodic properties; increase blood circulation in coronary vessels and in vessels of a brain, strengthen contraction of a cardiac muscle and at the same time reduce its excitability [13].

The obtained results testify to the ability of the tablet form of thick extract of the herb pasternak to prevent metabolic and functional disorders of cardiac activity under the conditions of adrenaline myocardial infarction, which justifies the feasibility of its further study, including the study of its toxic characteristics.

Conclusions

1. Therapeutic and prophylactic administration of thick extract of the herb pasternak tablets in doses of 100 mg/kg and 200 mg/kg improves the functional state of the myocardium of rats in adrenaline myocardial infarction, prevents a decrease in the severity of alternative processes in the body and has a beneficial effect on metabolic disorders.
2. Tablets of thick extract of the herb pasternak under the conditions of adrenaline myocardial infarction show antioxidant properties.
3. There were no significant differences in the cardioprotective properties of the injections of thick extract of the herb pasternak tablets in doses of 100 mg/kg and 200 mg/kg.

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Table 1. The effect of TEPPH tablets and comparison drug on the functional state of the myocardium under adrenaline myocardial infarction in rats

Indicators	Experimental conditions				
	Intact control	Positive control	TEPPH tablets, 100 mg/kg	TEPPH tablets, 200 mg/kg	Tricardin, 5 drops/kg
HR, beats/min	383,82±7,95	441,76±8,06 *	395,57±18,42 **	396,43±8,14 **	410,12±8,05 **
SI, %	41,65±1,96	54,51±3,16 *	44,99±2,70 **/***	42,84±1,04 **/***	55,80±2,35 *
QT, s	0,065±0,002	0,074±0,004	0,068±0,003	0,065±0,002	0,072±0,003
PQ, s	0,045±0,002	0,046±0,002	0,038±0,002	0,043±0,002	0,043±0,002
QRS, c	0,018±0,001	0,015±0,001 т*	0,017±±0,001	0,016±0,001	0,016±0,001
R, мВ	0,54±0,058	0,70±0,051	0,48±0,070 **	0,66±0,061	0,63±0,040
P, мВ	0,06±0,007	0,06±0,012	0,08±0,010	0,08±0,016	0,08±0,016
T, мВ	0,07±0,02	0,18±0,02*	0,10±0,02 т**	0,11±0,02 т**	0,13±0,02 т**
Offset of ST, c	0,029±0,003	0,050±0,003*	0,048±0,003*	0,046±0,003*	0,043±0,002*

Notes:

1. * – differences are statistically significant relative to the IC group, p<0,05;
2. ** – differences are statistically significant relative to the PC group, p<0,05;
3. *** – differences are statistically significant relative to the group of animals treated with the drug «Tricardin», p<0,05;
4. т – the value tends to be statistically significant, 0,05<p<0,1.

Table 2. The effect of TEPPH tablets and the comparison drug Tricardin on the course of adrenaline myocardial infarction in rats

Indicators	Experimental conditions				
	Intact control	Positive control	TEPPH tablets, 100 mg/kg	TEPPH tablets, 200 mg/kg	Tricardin, 5 drops/kg
Виживання, %	–	86	100	100	100
СНМ, %	0,31±0,01	0,38±0,01*	0,33±0,01 **	0,34±0,02 **	0,35±0,01 **
Serum					
AsAT, mmol/(year*l)	1,81±0,04	3,88±0,04 *	3,34±0,05 */т**	3,31±0,14 *	3,47±0,15 *
Urea, mmol/l	4,59±0,29	9,08±0,51 */**	7,03±0,36 */**/***	6,52±0,28 */**	5,17±0,33 */**
Heart homogenate					
GSH, µmol/g	3,07±0,73	1,37±0,15 *	1,78±0,25 *	1,88±0,23 *	1,77±0,14 *
TBA-AP, µmol/g	37,2±5,38	48,2±5,36*	39,2±1,85 **	38,8±2,07 **	38,2±2,07 **
Catalase, µcat/(min*d)	67,2±6,19	37,19±5,38*	49,5±2,67	57,3±2,88	51,47±3,17

Notes:

1. * – differences are statistically significant relative to the IC group, p<0,05;
2. ** – differences are statistically significant relative to the PC group, p<0,05;
3. *** – differences are statistically significant relative to the group of animals treated with the drug «Tricardin», p<0,05.