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EFFECT OF NEW HERBAL PREPARATIONS ON SOME INDICATORS OF APOPTOSIS IN RATS WITH ACUTE TOXIC HEPATITIS

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Abstract: *Purpose of the research: a comparative study of the anti-apoptotic effect of catacin, geranyl, cavergal compared to carlsil in a model of acute toxic liver damage with heliotrin. Material and methods. To achieve this goal, studies were conducted on 118 male adult rats. The model of acute toxic damage (ATD) was reproduced by a single subcutaneous injection of heliotrin at a dose of 200 mg/kg of animal body weight in 110 rats, 8 rats made up the intact group. On the 3rd day of the introduction of the toxicant, the surviving animals were divided into 5 groups: 1) ATD + physiological saline at a dose of 0.5 ml/100 g body weight (control) of 20 rats; 2) ATD + carsil (comparison group) 19 rats; 3) ATD + catacin 19 rats; 4) ATD + geranyl of 19 rats; 5) ATD + cover 18 rats. The drugs were administered intragastrically daily in the morning at 100 mg / kg for 6 and 12 days. 24 hours after the final administration of herbal preparations, the animals were killed, the content of cytochrome C and TNF-α was determined in the blood by the enzyme immunoassay. For statistical processing of the results, Excel and OriginPro7.5 software packages (OriginLab Corporation, USA) were used. Results. Determining the level of cytochrome C and TNF-α in the blood of rats with ATD their increase in 34-43 and 7.7-11.1 times. Pharmacotherapy of ATD with carlsilum for 6 and 12 days led to a decrease in the high values of these parameters by 2-2.85 and 1.61-1.98 times, by catacin - 2.75-4.16 and 2.86-3.83 times, geranyl - 3.35-4.44 and 3.28-3.95 times, cavernal - 3-4.32 and 1.58-1.93 times relative to the values of the untreated group of rats. It should be noted that in all groups the level of cytochrome C remained high. Conclusion: experimental pharmacotherapy of ATD with herbal preparations significantly slowed down the processes of apoptosis, this was more pronounced when using catacin, geranyl and cavergal.*

Key words: *acute hepatitis, heliotrin, pharmacotherapy, carsil, geranyl, catacin, cavergal, apoptosis, cytochrome C, tumor necrosis factor-α.*

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I. Introduction

Toxic liver damage is an urgent problem in the world and is associated with the key role of the liver in the processes of detoxification, deposition of endogenous and exogenous substances. The central link in the pathogenesis of toxic liver damage is oxidative stress, mitochondrial dysfunction, impaired calcium metabolism, and the effect of the toxin or its metabolites. Currently, herbal remedies are widely used to treat toxic liver lesions [7, 17]. Hepatoprotectors not only restore metabolic disorders, but also increase the resistance of hepatocytes to damaging agents, inhibit free radical processes, are antihypoxants, protect mitochondria and microsome enzymes from damaging effects, slow down collagen synthesis, etc. [7, 12]. However, the absorption of the drug in enterocytes is low, and high hepatobiliary circulation and excretion somewhat reduce the effectiveness of the action of bioflavonoids. This necessitates the search for new plant hepatoprotectors. Promising in this regard are proanthocyanidins from Geranimum saxatile, consisting of (+) - catechin, (+) - gallocatechin, (-) - epicatechin and (-) epigallocatechin, have low toxicity and are considered as promising substances for the creation of medicines [17]. Polymer proanthocyanidin, isolated by employees of the Institute of Plant Chemistry of Chemical Substances of the Academy of Sciences of the Republic of Uzbekistan from the terrestrial part of the plant [11], has antihypoxic and antioxidant properties [8, 9]. In this regard, the study of the mechanism of their hepatoprotective and antiapoptotic action will allow them to be introduced into clinical practice.

Purpose of the research: a comparative study of the anti-apoptotic effect of catacin, geranyl, caverga compared to carlsil in a model of acute toxic liver damage with heliotrin.

II. Material and methods

To achieve this goal, studies were conducted on 118 adult male rats. The model of acute toxic damage (ATD) was reproduced by a single subcutaneous injection of heliotrin at a dose of 200 mg / kg of animal body weight in 110 rats, 8 rats made up the intact group. Mortality for 1-3 days was 13.6%. On the 3rd day of the introduction of the toxicant, the surviving animals were divided into 5 groups: 1) ATD + physiological saline at a dose of 0.5 ml / 100 g body weight (control) of 20 rats; 2) ATD + carsil (comparison group) 19 rats; 3) ATD + catacin 19 rats; 4) ATD + geranyl of 19 rats; 5) ATD + cover 18 rats. The drugs were administered intragastrically daily in the morning at 100 mg / kg for 6 and 12 days. 24 hours after the final administration of herbal preparations of animals by simultaneous decapitation, they were killed under anesthesia in compliance with the rules outlined by the European Convention for the Protection of Vertebrate Animals.

The animals collected blood, isolated serum. The development of toxic hepatitis was judged by the activity of ALT and AST, the content of bilirubin and its fractions in serum on a MINDRAYBA-88A biochemical analyzer (China) using commercial reagent kits from CYPRESS Diagnostics (Belgium). The contents of cytochrome c and TNF-α were determined by enzyme-linked immunosorbent assay on an ELYZA apparatus (Germany) using rat Bender Medsystems kits. The “sandwich” variant of enzyme-linked immunosorbent assay was used in the kits.

For statistical processing of the results, Excel and OriginPro7.5 software packages (OriginLab Corporation, USA) were used. The significance of differences between the indices of the control and experimental groups was determined by the student coefficient method (t), the significance of differences by the P. indicator. At a significance level of $P < 0.05$, the differences were taken as statistically significant.

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III. Results

According to the literature, depending on the factors inducing apoptosis, two intracellular signaling cascades are distinguished: mitochondrial (cytochrome c output) and receptor (via TNF-α receptors) [6]. In this regard, we studied the content of cytochrome c and the level of TNF-α in rat blood with acute toxic liver damage and during treatment with herbal preparations. The study of the mitochondrial apoptosis pathway by determining the level of cytochrome c in the blood serum of experimental animals with ATD and during treatment with herbal preparations showed a sharp 34-43-fold increase in the level of extra-mitochondrial cytochrome c level with ATD (Table 1). Pharmacotherapy of ATD with carlsil for 6 and 12 days led to a decrease in the high values of this indicator by 2 and 2.85 times, relative to the indices of the untreated group. When using catacin, this decrease was 2.75 and 4.16 times, relative to the values of the untreated group and 1.38 and 1.46 times compared with the comparison group. For the treatment of ATD, the use of geranyl also contributed to a marked decrease in the level of cytochrome c in the blood serum of experimental animals by 3.35 and 4.44 times relative to the values of the untreated group of rats, by 1.67 and 1.56 times, respectively. It should be said that these indicators did not differ significantly from the values of the group of animals with ATD treated with catacin. The kavergal had the same effect, significantly reducing the level of cytochrome c from the indices of the untreated group by 3 and 4.32 times, the values of the comparison group - by 1.49 and 1.51 times, respectively, terms. It should be noted that in all groups the level of cytochrome c remained high.

Table 1

The content of cytochrome c (ng / ml) in the blood of rats with acute toxic hepatitis during treatment with herbal preparations, M ± m

Groups and deadline of the study	The deadline of the study, a day from the start of treatment					
	6 days			12 days		
	M±m	max :min	P_1/P_2	M±m	max :min	P_1/P_2
Intact, n=8	0,11±0,006	0,09 : 0,14		0,11±0,006	0,09 : 0,14	
ОТГ+H_2O, n=10	3,65±0,13	3,1 : 4,3	< 0,001	4,62±0,23	3,7 : 6,0	< 0,001
ОТГ+carsil, n=9-10	1,82±0,08	1,5 : 2,2	< 0,001 < 0,01	1,62±0,07	1,3 : 2,0	< 0,001 < 0,01
ОТГ+ catacin, n=9-10	1,32±0,12	1,0 : 1,8	< 0,001 < 0,001	1,11±0,07	0,8 : 1,5	< 0,001 < 0,001
ОТГ+ geranium, n=9-10	1,09±0,06	0,9 : 1,4	< 0,001 <	1,04±0,07	0,7 : 1,5	< 0,001 <

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			0,001			0,001
ОТГ+kavergal, n=9	1,22±0,11	1,0 : 1,9	< 0,001 < 0,05	1,07±0,09	0,7 : 1,5	< 0,001 < 0,05

Note: P1 - significance of differences between the indices of the experimental and intact groups, P2 - significance of differences between the indices of the treated and untreated groups.

It should be noted that apoptosis induced by binding to the TNF-α family is similar to Fas-R / Fas-L and is carried out with the participation of FRADD protein [6]. The content of TNF-α in the blood serum of experimental animals with ATD also statistically significantly increased by 7.67 and 11.1 times, respectively, the duration of the study (table. 2). Pharmacotherapy of ATD with carlsil for 6 and 12 days contributed to a decrease in high TNF-α value of 1.61 and 1.98 times, however, these parameters remained statistically significantly higher than the values of intact rats by 4.76 and 5.61 times, respectively, terms. The use of catacin for the treatment of ATD had a more pronounced effect: the values f this cytokine significantly decreased 2.86 and 3.83 times relative to the values of the untreated group, 1.78 and 1.94 times compared with the rats treated with the comparison drug carlsil. Despite these positive changes, the level of this cytokine significantly exceeded the indices of intact rats by 2.68 and 2.9 times, respectively, terms.

Table 2

The content of TNF-α (pg / ml) in the blood of rats with acute toxic hepatitis during treatment with herbal preparations, M ± m

Groups and deadline of the study	The deadline of the study, a day from the start of treatment					
	6 days			12 days		
	M± m	max :min	P_1/P_2	M± m	max :min	P_1/P_2
Intact, n=8	3,17 ±0,41	2,0 : 4,9		3,17 ±0,41	2,0 : 4,9	
ОТГ+H_2O, n=10	24,3 ±1,06	21,0 : 29,5	< 0,001	35,2 ±1,92	29,7 : 44,4	< 0,001
ОТГ+carsil, n=9-10	15,1 ±1,42	10,0 : 22,1	< 0,001 < 0,05	17,8 ±1,14	14,1 : 25,0	< 0,001 < 0,01
ОТГ+catacin, n=9-10	8,50 ±0,41	7,1 : 10,0	< 0,001 < 0,001	9,20 ±0,57	5,9 : 11,2	< 0,001 < 0,001
ОТГ+geranium, n=9-10	7,54 ±0,53	5,4 : 9,9	< 0,001 <	8,92 ±0,58	6,9 : 12,0	< 0,001 <

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			0,001			0,001
ОТГ+kavergal, n=9	15,4 ±1,46	10,1 : 22,2	< 0,001 < 0,05	18,2 ±0,85	14,0 : 21,2	< 0,001 < 0,01

Note: P1 - significance of differences between the indices of the experimental and intact groups, P2 - significance of differences between the indices of the treated and untreated groups.

Pharmacotherapy of ATD with geranil for 6 and 12 days on the content of TNF-α had the same positive effect as catacin. The level of this compound significantly decreased 3.22 and 3.95 times relative to the values of the untreated group, 2 and 1.99 times compared with the comparison group, but still significantly exceeded the values of intact rats 2.38 and 2.81 times , according to the timing. The level of TNF-α in the blood serum of rats with ATD treated with kavergal for 6 and 12 days statistically significantly decreased 1.58 and 1.93 times relative to the indices of the untreated group and did not significantly differ from the values of the comparison group. The content of this cytokine significantly exceeded the values of intact rats by 4.86 and 5.74 times, respectively, terms.

Thus, in rats with acute toxic hepatitis caused by the introduction of heliotrin, both the mitochondrial and the receptor pathways of apoptosis of hepatocytes are accelerated. Experimental pharmacotherapy with herbal preparations significantly slowed down the processes of apoptosis, it was more pronounced when using catacin, geranyl and kavergal.

It is known that the toxic effect of heliotrin is associated with the activation of free-radical processes. It is known that the increased formation of reactive oxygen species can trigger the development of apoptosis along the mitochondrial pathway. They can form disulfide bridges between Bax monomers in the cytosol, which leads to the formation of channels in the outer mitochondrial membrane [16]. On the other hand, oxygen free radicals can destroy the bond of cytochrome c with cardiolipin, the mitochondrial membrane phospholipid, causing its hydroperoxidation [13, 14]. Active forms of oxygen, such as peroxynitrite and others, cause an increase in the permeability of mitochondrial membranes. In our opinion, a sharp increase in the level of cytochrome c in the blood of rats with acute heliotrin hepatitis, affecting the permeability of cell membranes, activate proapoptotic mechanisms. So, according to the literature, these apoptosis inducers lead to a sharp decrease in the value of the electrochemical potential of mitochondria ΔμH + and their release of cytochrome c, pro-caspases 2-, 3-, and 9, apoptosis activating factor, endonuclease G, the output of which is controlled by bcl-2 proteins [6]. In the studies of M. A. Savitskaya et al. (2012) on cultured cells of human epidermoid carcinoma A431, it was shown that reactive oxygen species cause dissociation of cytochrome c from cardiolipin and its release into the cytosol, where it triggers caspase activation [10]. According to the authors, cytochrome c does not exit simultaneously from all mitochondria, since even in those cells where the cytoplasm is sufficiently stained with antibodies to cytochrome c, several mitochondria containing cytochrome c are preserved, they are enlarged, have an oval or round shape and exit accompanied by a loss of membrane potential. The parallel increase in the level of TNF-α revealed by us in acute heliotrin liver damage induces apoptosis even more, since mitochondria are an important target for TNF-α initiated signals that lead the cell to death. Induction of proteins of the TNF-α family and their receptors causes aggregation of receptors, the formation of various adapter proteins that activate low-activity caspases [15].

The hepatoprotectors used by us significantly reduced the high levels of cytochrome c and TNF-α. Apparently, this is due to the stimulation of protein biosynthesis and the acceleration of the regeneration of damaged hepatocytes [2, 12]. On the other hand, flavonoids increase the activity of antioxidant enzymes by the restoration of hepatocyte cell membranes, their participation in the processes of molecular transport, cell division and differentiation, and the slowdown of collagen synthesis [4, 12]. A number of studies have shown the presence of antioxidant properties, a decrease in lipid peroxidation processes, and an increase in the energy potential of cells using catacin [1, 3]. The proanthacyanidins that make up Geranil possess neuroprotective, antioxidant, anti-inflammatory, immunostimulating properties, prevent platelet aggregation, and stabilize vascular endothelium [18]. Our previous studies have shown a decrease in the rates of cytolysis, cholestasis, mesenchymal inflammation and liver cell deficiency syndromes, an improvement in the synthetic function of hepatocytes, antioxidant properties in rats with ethanol intoxication, its hepatoprotective activity is superior to the known hepatoprotector carlsil [5], and exhibit antihypoxic properties on a model of carotid artery occlusion [8]. Apparently, the anti-apoptotic properties of catacin and geranil obtained by us are related to their antihypoxic and antioxidant effects.

IV. Conclusions:

1. Acute heliotrin hepatitis is characterized by an increase in the content of cytochrome c and TNF-α in the blood of experimental animals.

2. Hepatoprotectors significantly reduced the high levels of cytochrome c and TNF-α in the blood of rats with acute toxic hepatitis.

References

1. Abdullaev G. R. Influence of catacin on rat lipid peroxidation processes in the dynamics of stress development // Uzbek Biological Journal. 2016. - No. 3. - P. 7-11.

2. Azarova OV, Galaktionova L.P. Flavonoids: a mechanism of anti-inflammatory action // Chemistry of plant materials. - 2012.- No. 4.- P. 61-78.

3. Almatov K. T., Abdullaev G. R. Changes in the energy metabolism of rat liver mitochondria in the dynamics of the development of chronic emotional and pain stress and their correction with catacin // Uzbek Biological Journal. 2016. - No. 2. - P. 20-26.

4. Zverev Y. F. Flavonoids through the eyes of a pharmacologist. Antioxidant and anti-inflammatory activity // Reviews on clinical pharmacology and drug therapy. - 2017.- T.15, No. 4.- P.5-15.

5. Inoyatova F.Kh., Rakhmanov A.Kh., Kurbanova NN, Aslanova A.Kh. Influence of new hepatoprotectors on the detoxifying function of rat liver during its acute toxic damage // Vestnik TMA.- 2018.- No. 3.- P .70-74.

6. Ipatova O.M. Phosphogliv: the mechanism of action and use in the clinic.- M., 2005.- P.116-150.

7. Novikov V.N., Klimkina E.I. Pharmacology of hepatoprotectors // Reviews of clinical pharmacology and drug therapy .- 2005.- T.4, No. 1.- P.2-20.

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8. Norbutaeva D.A., Siddikov D.R., Nishanbaev S.Z., Syrov V.V., Khushbaktova Z.A. Antihypoxic properties of proanthocyanidins from some plants of Uzbekistan // DAN RUz.- 2011.- No. 5.- P.58-60.

9. Norbutaeva D.A., Syrov V.V., Khushbaktova Z.A. Evaluation of the antihypoxic and antioxidant properties of proanthocyanidins from rock geranium // Science and Innovation of the 21st Century. Materials of the first All-Russian Conference of Young Scientists ".- Surgut, 2012.- P.138-142.

10. Savitskaya M.A., Vildanova M.S., Kisurina-Evgenieva O.P., Smirnova E.A., Onishchenko G.E. Mitochondrial apoptosis pathway in A431 human epidermoid carcinoma cells under the action of α-tocopheryl succinate // Acta Narurae.- 2012.- T.4, No.3 (14) .- P. 93-100.

11. Siddikov D.R. Nishanbaev S.Z., Norbutaeva D.A., Babakulov H.M. Secondary metabolites of the aerial part of Geranimumsaxatile // Chemistry of natural compounds.- 2013.- No. 2.- P.289-290.

12. Tarakhovsky Yu.S., Kim Yu.A., Abdrasilov B.S., Muzafarov E.N. Flavonoids: biochemistry, biophysics, medicine. - Pushchino: Synchrobook, 2013.

13. D'Alessio M., De nicola M., Coppola S., Gualandi G., Pugliese L., Cerella C., Cristofanon S., Civitareale P., Ciriolo M.R., Bergamaschi A., et al. // FASeB J.- 2005.- Vol.19, No. 11.- P.1504-1506.

14. Dong L.F., Freeman r., Liu J., Zobalova r., Marin-Hernan-dez A., Stantic M., Rohlena J., Valis K., Rodriguez-Enriquez S., Butcher B., et al. // Clin. cancer res.- 2009.- Vol.15, No. 5.- P.1593-1600.

15. Gogvadze V., Norberg E., Orrenius S., Zhivotovsky B. // Int. J. cancer.- 2010.- Vol. 127, No. 8.- P. 1823–1832.

16. Neuzil J., Wang X.F., Dong L.F., Low P., Ralph S.J. // FeBS Lett.- 2006.- Vol.580, No. 22.- P.5125-5129.

17. Nitesh Kumar, Amita Rai, Neetinkumar D. Reddy, Rekha R. Shenoy, Jayesh Mudgal et al. Improved in vitro and in vivo hepatoprotective effects of liposomal silymarin in alcohol-induced hepatotoxicity in Wistar rats. // Pharmacological Reports.- 2019.- Vol.71.- P.703-712.

18. Shih P.H., Yeh C.T., Yen G.C. Anthocynins induce activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. // J Agric Food Chem. - 2007.- Vol. 55 (23) .- P.9427-9435.

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