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**THE ROLE OF ANTIOXIDANT DEFENSE DISTURBANCES IN PATHOGENESIS
OF THE LATE MANIFESTATIONS OF TRAUMATIC DISEASE AFTER
CRANIO-SKELETAL INJURY AND EFFECTIVENESS OF ITS CORRECTION
BY PHYTOMIXTURE**

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Summary

In a response to cranio-skeletal injury during late manifestations of traumatic disease in the body of experimental animals is proceeded significant decrease of superoxide dismutase and catalase activity in liver tissue. An increasing of ceruloplasmin content in serum from 14 to 35 days of post-traumatic period, the content of SH-group in the liver tissue – after 21 and 28 days is observed. The application of Phytomixture that started on 14th day of experiment, in comparison with animals without correction, accompanied by greater activity in the liver tissue of antioxidant enzymes and lower content in serum of ceruloplasmin in all periods of observation and more content of SH-groups after 28 days in post-traumatic period. After 28-35 days of Phytomixture influence a normalization of blood serum ceruloplasmin appears, after 21-35 days – the normalization of catalase activity and content of SH-groups in liver tissue occurs. This indicates a strong antioxidant effect of Phytomixture in the late period after cranio-skeletal injury.

Key words: cranio-skeletal injury, antioxidant defense, phytotherapy, phytomixture.

Introduction. An important role in the pathogenesis of severe trauma plays hypoxia. The end result of hypoxia is the accumulation of reactive oxygen species, the activation of lipid peroxidation process and the depletion of antioxidant protection that has systemic nature and is observed in tissues and organs distant from the place of direct injury [9]. Neutrophils migrate in the foci of lesion; a protective function of neutrophils is also realized through the extra cellular production of reactive oxygen species (respiratory burst) and intracellular generation of oxygen radicals to eliminate microorganisms in the phagosome [2, 12]. In a number of studies it is shown that activation of lipid peroxidation and antioxidant depletion time corresponds to the maximum system response against inflammation caused by the release of inflammatory mediators in systemic circulation [4, 5, 6]

An important consequence of the intensification of lipid peroxidation (LPO) in conditions of severe trauma is destabilization of cell membranes, which leads to cytolysis processes and endotoxemia, which are the basis of the syndrome of multiple organ dysfunction and failure [3].

Therefore, an important element of complex treatment of traumatic disease, especially during late manifestations of traumatic disease is to find effective means of antioxidant therapy. Moreover effectiveness of phytomedications that exhibit antioxidant activity is not enough studied. Naturally origin of biologically active substances of plant due to absence of side effects makes them perspective a medicament in the correction of antioxidant protection in various pathological processes, including severe trauma [10, 13], that is required a special study.

Aim of study: to clarify the role of antioxidant defense disorders in the pathogenesis of the late manifestations of traumatic disease after cranio-skeletal injury and effectiveness of their correction by phytomixture.

Materials and methods. In the experiments was used 99 nonlinear white rats males with weight of 180-200 g, which were treated in a standard diet of vivarium. All animals were divided into three groups: control and two experimental. The control group included seven

intact animals. In both experimental groups (46 animals in each group) in terms of thiopental sodium anesthesia (40 mg kg^{-1} body weight intraperitoneum) dosed effort modeled by a closed head injury of moderate severity [3] and caused a closed fracture of the femur.

After 14 days of post-traumatic period in the first experimental group of survived animals was performed a correction by applying Phytomixture, which includes arnica herb, couch grass roots, calendula, nettle leaves, birch leaves. From the mixture of herbs was prepared an infusion 1:10 and it was administered one time a day for experimental animals through a tube into the stomach at a dose of 10 ml per kilogram of animal, every day from 14 till 35 day of an experiment.

In the second experimental group in these terms of observations was done an intragastric administration of equivalent volume of saline. An animals that were survived in terms of thiopental sodium anesthesia ($80 \text{ mg} \cdot \text{kg}^{-1}$ body weight ip) in 14, 21, 28 and 35 days after injury were taken out of the experiment by total bloodletting from the heart. In the liver tissue a superoxide dismutase (SOD) [11], catalase [8], and the content of SH-groups were determined [1]. In the blood serum a content of ceruloplasmin (CP) was evaluated [7].

The resulting digital material was processed in the department of systematic statistical studies SHEI «I. Ya. Horbachevsky Ternopil State Medical University Ministry of Health of Ukraine» in the software package STATISTICA («StatSoft Inc.», USA) using the nonparametric criteria of Mann-Whitney.

Results and its discussion. Research results showed that under simulated cranio-skeletal trauma were observed abnormalities of antioxidant system. Thus, the SOD activity of liver tissue (Table. 1) during late manifestations of traumatic disease was significantly lower than in control groups: after 14 days – by 23,2 % after 21 days – by 67,1 %, after 28 days – by 57,1 %, after 35 days – by 49,0 % ($p < 0,05$). The analysis of the studied parameters found that up to 21 days SOD activity in liver tissue decreased, which was statistically significant in comparison with 14 day (57,7 %, $p < 0,05$). Further rate increased after 28 days by 29,9 % higher than the value of 21 days ($p < 0,05$), and after 35 days was higher compared to 21 and 28 days of observation (by 54,7 and 19,1 %, $p < 0,05$), but less than after 14 days (34,6 %, $p < 0,05$). After 35 days an index was also lower on 48,9 % compared with the control group ($p < 0,05$).

In the experimental group, in which a phytomixture was used (see. a table. 1) after 14 days SOD activity in liver tissue did not differ from the experimental group, which was not used phytomixture ($p > 0,05$), and the index was 23,1 % lower than in the control group ($p < 0,05$). After 21 and 28 days, index was at 14 days ($p > 0,05$) and was 37,8 and 23,5 % lower

than in the control. After 35 days he was promoted and became 25,8 % more than after 21 days ($p < 0,05$), but remained in 14,2 % higher than in the control, which was statistically significant ($p < 0,05$).

Comparing of research groups between themselves, it's turned out that after 21-35 days during Phytomixture treatment rate was statistically significantly higher than in the group in which the correction was not carried out: after 21 days - by 2,70 times, after 28 days – by 78,6 %, after 35 days – by 67,9 % ($p < 0,05$).

The analysis of the activity of catalase of liver tissue showed (Table. 1), that after cranio-skeletal injury, after 14 days an index do not differ from the control group ($p > 0,05$). But after 21-35 days it decreased and became statistically significantly lower than the control group (respectively 37,0, 33,9 and 34,8 %, $p < 0,05$) and compared to 14 day of observations (by 31,4, 28,1 and 29,0 %, $p < 0,05$).

In the experimental group, which used phytomixture after 14 days of post-traumatic period the catalase activity in the liver tissue did not differ from the experimental group of injured animals, where phytomixture was not used ($p > 0,05$).

Further the rate changed vibrationally, but the control group and the previous observation period practically did not differ ($p > 0,05$).

Comparing of research groups between themselves showed that after 21-35 days catalase activity of liver tissue during treatment by Phytomixture was significantly greater than in the group of animals in which Phytomixture not used: after 21 days – by 61,0 %, after 28 days – by 41,8 %, after 35 days – by 49,2 % ($p < 0,05$).

In its turn, the content of SH-groups in liver tissue under the influence of cranio-skeletal injury after 14 days (Table. 1) has no difference from the level of the control group ($p < 0,05$). After 21 and 28 days the index became a significantly lower than in controls (respectively 24,7 and 21,2 %, $p < 0,05$). After 35 days the index increased up to control values ($p > 0,05$). A characteristic feature in the dynamics is decrease after 21-28 days, that was significantly less than 14 days (respectively 23,1 and 19,6 %, $p < 0,05$). Up to 35 days index increased in this group, that was significantly greater than after 21 days (20,0 %, $p < 0,05$).

The group, which used phytomixture (Table. 1), an index after 14 days were not significantly different from the comparison group and the control group ($p > 0,05$). After 21-35 days it remained at 14 days ($p > 0,05$) and not significantly different from the control group ($p > 0,05$). The analysis of this index shows that it reached the minimum value in 21 days, further increased and after 35 days became statistically significantly greater than 21 and 28 days (respectively 18,0 and 9,9 %, $p < 0,05$).

The comparison of research groups showed that after 21 and 35 days was not found significant differences in amount of SH-group content in the liver tissue between the experimental groups ($p>0,05$), but after 28 days index was significantly higher in the group of traumatized animals, which used phytomixture (13,9 %, $p<0,05$).

As can be seen from the Table. 1, content in the blood serum of ceruloplasmin under the influence of cranio-skeletal injury, compared to the control group during the late manifestations of traumatic disease was higher: after 14 days – by 72,5 % after 21 days – by 89,0 % after 28 days – 71,6 %, after 35 days – by 30,9 % ($p<0,05$). In the dynamics of the studied parameters characteristic was that contents within 14-28 days in serum securities was almost on the same level with the tendency to increase after 21 days. However, after 35 days rate significantly decreased compared with all previous observation period (respectively 24,1, 30,7 and 23,7%, $p<0,05$).

In terms of application of Phytomixture (Table. 1) in 14 days index was almost the same in comparison to the group, in which was not applied Phytomixture ($p>0,05$) and 68,4 % higher than the level of the control group ($p<0,05$). Further index decreased and after 28-35 days reached the level of control ($p>0,05$). In these conditions after 21 and 28 days index was substantially less than 14 days (respectively 23,9 and 31,2 %, $p<0,05$), but after 35 days – compared to 14 and 21 days (by 37,9 and 18,4 %, $p<0,05$).

Comparison of experimental groups between themselves showed that after using Phytomixture, CPU content in blood serum of 21, 28 and 35 days was significantly lower than in the group of animals without correction (respectively 32,2, 32,5 and 20,2 %, $p<0,05$).

Thus, during the late period manifestations after cranio-skeletal injuries compared to the control group observed a significant reduction in liver tissue SOD activity, catalase and increasing of the CP content in serum in all periods of observation, content of SH-groups in liver tissue after 21 and 28 days post-traumatic period. Thus, during the late manifestations of traumatic disease the formation of reactive oxygen species is not going down, which leads to decreased activity of the main enzymes that are able to neutralize them as in liver tissue and serum depletion and content of SH-groups. The last belong mainly to glutathione, which neutralize hydrogen peroxide despite due glutathione peroxidase, is able to reduce the level of endotoxemia due to the formation of joined up hydrophilic compounds [4].

Table 1 – Indicators of antioxidant protection in the liver tissue and serum in the late manifestation of cranio-skeletal injury and efficiency of Phytomixture (M±m)

Corection	Control (n=7)	Cranio-skeletal trauma			
		14 day (n=7/7)	21 day (n=6/9)	28 day (n=7/8)	35 day (n=6/9)
SOD in the liver tissues, conv. units. ·mg ⁻¹					
Without corection	0,415± 0,015	0,324± 0,014*	0,137± 0,006*	0,178± 0,008*	0,212± 0,008*
Phytomixture		0,319± 0,018*	0,283± 0,010*	0,318± 0,017*	0,356± 0,011*
p		>0,05	<0,05	<0,05	<0,05
Catalase in the liver tissues, conv. units. ·mg ⁻¹					
Without corectin	1,080± 0,044	1,001± 0,029	0,687± 0,024*	0,720± 0,038*	0,711± 0,022*
Phytomixture		1,030± 0,027	1,106± 0,060	1,021± 0,043	1,061± 0,046
p		>0,05	<0,05	<0,05	<0,05
SH-grups in the liver tissues, mmoll·kg ⁻¹					
Without corection	1,461± 0,088	1,43± 0,09	1,10± 0,06*	1,15± 0,05*	1,32± 0,07
Phytomixture		1,36± 0,06	1,22± 0,07	1,31± 0,03	1,44± 0,05
p		>0,05	>0,05	<0,05	>0,05
Ceruloplasmin in blood serum (g·l ⁻¹)					
Without corection	7,46± 0,25	12,87± 0,49*	14,10± 0,64*	12,80± 0,77*	9,77± 0,65*
Phytomixture		12,56± 0,59*	9,56± 0,49*	8,64± 0,55	7,80± 0,35
p		>0,05	<0,05	<0,05	<0,05

Notes:

1. * – differences in relation to the control group statistically significantly (p<0,05);
2. p – the probability of differences between groups of animals injured Phytomixture correction and without correction;
3. n = number of animals in the group without correction / Number of animals in the group of correction by Phytomixture.

Application of Phytomixture in comparison with animals without correction accompanied by greater activity in the liver tissue SOD, catalase and lower content in serum ceruloplasmin in all periods of observation and more content SH-groups after 28 days of post-traumatic period. It should be noted that after 28-35 days under the influence of Phytomixture came a normalization of serum ceruloplasmin after 21-35 days of observation at the level of the control group were in liver tissue catalase activity, the content of SH-groups.

Obtained results indicate that the Phytomixture has pronounced antioxidant activity, which is obviously linked to the presence of natural bioantioxidant and other biologically active substances that can stimulate recovery processes in the body that requires further study.

Conclusions. 1. In a response to cranio-skeletal trauma, during the late manifestations of traumatic disease in the body of experimental animals was observed a significant reduction of SOD activity in liver tissue, catalase and ceruloplasmin content was increased in blood serum from 14 to 35 days of post-traumatic period, and SH-group content in the liver tissue – after 21 and 28 days.

2. Application of Phytomixture, started from the 14th day of the experiment, compared with animals without correction, accompanied by greater activity in the liver tissue SOD, catalase and lower content in serum of ceruloplasmin in all periods of observation and increased content of SH-groups after 28 days of post-traumatic period. After 28-35 days under the influence of Phytomixture comes to normalization of serum ceruloplasmin, after 21-35 days – activity of catalase and content of SH-groups in the liver tissue.

Prospects for further research. In a future research it is supposed to expand the range of research systemic effects of Phytomixture in conditions of severe injury.

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