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# *Glutatione system in the liver of animals with acute toxic hepatitis receiving Echinacea purpurea tincture*

System glutationowy wątroby zwierząt z zapaleniem toksycznym wątroby po podaniu nalewki z *Echinacea purpurea*

## INTRODUCTION

Due to peculiarities of the molecular mechanisms of tetrachlormethan ($CCl_4$) action upon the hepatocyte subcellular membranes (microsomal activation, lipid peroxide oxidation as the mechanism of catalytic peculiarities disorders of the membrane-linked enzymes), toxic hepatitis is considered to be a model of molecular pathology of the membranous structures [12]. Disturbance of the membrane structure and functions is a key intracellular process in the development of aetiologically different pathological processes.

Preparations of medicinal plants are known to be widely used for the treatment of hepatobilliary system diseases. In this respect investigation of medicinal preparations made of Echinacea Purpurea is rather perspective [1, 3]. That is why taking into account everything mentioned above, we undertook the task to study the influence of Echinacea purpurea tincture (E.P.t) upon the content of reduced glutathione and enzyme activity of the antioxidant protection in the liver of rats under conditions of experimental toxic hepatitis.

## MATERIAL AND METHODS

White rats with body weight 140–160 g were received a double dose of $CCl_4$ intragastrically (diluted to 50% with olive oil; 0.025 ml/kg) [12]. Experimental animals were divided into two groups: I – animals with hepatitis and not treated; II – animals with hepatitis receiving E.P.t. The tincture made of a dried root of Echinacea Purpurea was introduced intragastrically every day in the dose of 0.25 ml/kg. The liver of animals of the I–II groups was examined in dynamics 3, 7 and 14 days later after the last $CCl_4$ introduction and compared with the results of intact group of animals. The liver was rapidly removed, weighed and homogenized in appropriate buffers for determination of glutathione concentration and glutathione system enzyme activities.

The reduced form of glutathione (GSH) was measured in the liver homogenates by the method of Meshchishen [9]. Glutathione-S-transferase (G-S-T), glutathione reductase (GR), glutathione peroxidase (GPx) and glucose-6-phosphate dehydrogenase (G6PD) activities, and total protein concentration were determined according to Habig [4], Beutler [2], Mannervick [8], Kornberg [5] and Lowry [6], respectively.

Values are presented as mean±SEM and were compared by a Student test. $P$-value <0.05 was considered statistically significant.

## RESULTS

The research showed that acute toxic hepatitis induced activation of some indices of the body glutathione system. Thus, there was found increased activity of G6PD (by 27.4%), GR (by 31.5%) and GPx (by 29.1%) and the content of GSH (by 43.7%) in the liver of rats after $CCl_4$ intoxication (Table 1). These changes are a protective, compensatory body reaction to a substantial increase of lipid peroxide oxidation (LPO) after $CCl_4$ poisoning. It should be noted that under toxic hepatitis the activity of G-S-T in the liver decreases by 26.9% in comparison, with the indices of intact animals.

Oral administration of E.P.t. during 3 days with the therapeutic aim improved the activity of G-S-T in the liver of rats afflicted with hepatitis.

Table 1. *Echinacea purpurea* tincture influence upon the state of indices of the liver glutathione system under condition of toxic $CCl_4$ hepatitis (M±m, n=10–25)

| Days | Groups of animals | Examined indices (measurement units) | | | | |
|---|---|---|---|---|---|---|
| | | GSH (μmol/g liver) | GPx (nmol/ min/mg protein) | GR (nmol/ min/mg protein) | G-6-PD (nmol/min/ mg protein) | G-S-T (nmol/min/ mg protein) |
| Control | | 7.00±0.04 | 180.9±2.7 | 3.78±0.04 | 8.77±0.05 | 60.3±1.1 |
| 3 | hepatitis | 10.06±0.21** | 233.6±4.4** | 4.97±0.16** | 11.17±0.08** | 44.1±1.9** |
| | III- hepatitis + E.P.t. | 8.53±0.16** | 208.8±4.9** | 5.18±0.13** | 10.82±0.06** | 48.2±2.1** |
| 7 | II- hepatitis | 5.45±0.11** | 235.5±5.6** | 4.85±0.18** | 10.02±0.18** | 47.6±1.3** |
| | III- hepatitis + E.P.t. | 7.51±0.14* | 193.1±3.8* | 4.92±0.19** | 8.99±0.11 | 56.5±2.3 |
| 14 | II- hepatitis | 7.68±0.12** | 195.8±3.4* | 4.25±0.16* | 8.93±0.12 | 54.3±1.7* |
| | III- hepatitis + E.P.t. | 7.08±0.08 | 185.5±3.1 | 3.71±0.18 | 8.73±0.07 | 59.6±1.3 |

*Possible changes in comparison with intact animals ($P<0.05$).
**Possible changes in comparison with intact animals ($P<0.01$).

The 7-th day of the experiment revealed that treating animals with this preparation rather improved quick normalization of the antioxidant enzyme activity in the liver of rats in comparison with animals which were not treated. Under the influence of E.P.t. on the 7-th day of the experiment the activity of G6PD and G-S-T in the liver of rats became normal and the activity of GPx and the content of GSH decreased by 18% and 11.1% in comparison with the indices of untreated animals.

A fortnight's administration of the preparation promoted complete normalization of the activity of all the examined enzymes decreased the content of reduced glutathione, whose level was close to control level.

## DISCUSSION

Acute toxic hepatitis caused by $CCl_4$ is characterized by vivid disorders of the functioning of the liver glutathione protective system. First of all, it is revealed by a sharp reduction of GSH content and inhibition of G-S-T activity. But GPx activity in the liver of experimental animals increased during all the dates of the research. By means of GPx and GSH various peroxide compounds become harmless ($H_2O_2$, lipoperoxides). They are produced in great amounts in case of $CCl_4$ intoxication. This is to explain an increased activity of GPx and decreased content of GSH in the liver of animals afflicted with toxic hepatitis. Decreasing GSH content induced a compensatory increasing activity

of G6PD, which supplies NADP H-reduced equivalents to GR and ensures increasing activity of this enzyme, which promotes reducing glutathione from its oxidized form. Sulfhydryl group GSH, being a good nucleophylic agent, can join various xenobiotics and separate metabolic products with the participation of G-S-T and take part in the protective functions of the body. $CCl_4$ poisoning is characterized by conjugation disturbance of GSH with cellular metabolites and $CCl_4$ waste products, which results in disorder of the body detoxication function.

Lesions of the membranes induced by $CCl_4$ and the products of its metabolism lead to enzyme solubilization of the protoplasmatic, microsomal, lysosomal, mitochondrial and other membranes and their lesions [11, 12]. Besides, in the development of pathological process in case of $CCl_4$ intoxication a substantial role is played by disorder of the process of enzyme activation of oxygen reactive intermediators, which promotes further production of free radicals and reveals their hepatotoxic activity.

Thus, the character of expression and prognosis of pathological process development in the liver can be closely connected with the functional state of the protective antioxidant system and the process of reactive oxygen species (ROS) generation. The question concerning pharmacological regulation of the mechanisms of formation and utilization of ROS is of certain practical importance.

Administration of E.P.t. on the background of $CCl_4$ intoxication found a positive influence upon the liver functional state. It is proved by earlier normalization of enzyme activity of the glutathione system under the influence of E.P.t. in comparison with the indices of untreated animals. Antioxidant and membrane-stabilizing properties of E.P.t. form the basis of a positive therapeutic effect of the preparation in case of acute toxic hepatitis [3]. These properties are stipulated by the presence of such biologically active substances as polyphenols, phlavonoids, alkaloids, caffeine acid, betain, vitamin C, macro- and microelements [1], which take part in the inhibition of LPO reaction and stimulate opportunities of the body antioxidant system and stimulate GSH synthesis [ 7].

Hepatoprotective action of E.P.t. is stipulated by polyphenol compounds and phlavonoids, which link free radical and inhibit activated processes of LPO [10]. Thus, intoxication of animals by $CCl_4$ causes deep changes in the functioning of the liver glutathione system. Treating animals with E.P.t. stabilizes the development of LPO processes in the hepatocytes and favours complete normalization of enzyme activity of the glutathione protective system, which forms the basis of its positive therapeutic effect.

In the case of toxic hepatitis administration of E.P.t. appeared to be the most effective during two weeks in comparison with other terms of its use. But to prove these suppositions further research should be conducted with a longer use of the preparation and in the case of other diseases as well.

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SUMMARY

Toxic hepatitis increases glutathione concentration and activities of glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and decreases activities of glutathion-S-transferase in the liver. In animals with toxic hepatitis treatment with *Echinacea purpurea* tincture decreases glutathione concentration and enzyme activities, which is probably associated with inhibition of free radical oxidation under the influence of this medicine. In the case of toxic hepatitis administration of E.P.t. appeared to be the most effective during two weeks in comparison with other terms of its use.

STRESZCZENIE

Ostre toksyczne uszkodzenie hepatocytów wywołane tetrachlormetanem cechuje się zaburzeniem funkcji systemu obronnego wątroby: obniżeniem składu odnowionego glutationu, podwyższeniem aktywności glutationoperoksydazy, glutationoreduktazy, dehydrogenazy glukozo-6-fosforanu i hamowaniem aktywności glutationo-S-transferazy. Leczenie zwierząt wyciągiem z jeżówki purpurowej zmniejsza stężenie glutationu i aktywność enzymów, co jest prawdopodobnie związane z hamowaniem utleniania wolnorodnikowego. W ostrym toksycznym uszkodzeniu hepatocytów najbardziej efektywne okazało się zastosowanie wyciągu z jeżówki purpurowej w okresie dwóch tygodni.