EFFECT OF POLYPHENOL EXTRACTS ON GLUTATHIONE PEROXIDASE ENZYME ACTIVITY IN CONDITIONS OF TOXIC HEPATITIS

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ABSTRACT

In this study, the inhibitory effects of helmar-1 and helmar-2 polyphenol extracts isolated from the helichrysummaracandicum plant on the activity of the antioxidant glutathione peroxides enzymes in a toxic hepatitis model were compared with silymarin.To induce toxic hepatitis, the subcutaneous area of the abdomens of the experimental animals were injected twice a week with a 50% solution of carbon tetrachloride in olive oil at a dose of 1 ml/kg. After an increase in the levels of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed in the blood plasma,which indicates toxic hepatitis, the helmar-1 and helmar-2 polyphenol extracts and silymarin were administered subcutaneously at a dose of 20 mg/kg once daily for 15 days for pharmacocorrection.

KEYWORDS: *Helichrysummaracandicum, Glutathione Peroxidase, Malondialdehyde, Mitochondria, Superoxide Dismutase, Silymarin, Toxic Hepatitis*

INTRODUCTION

Under conditions of oxidative stress, an increase in the amount of active forms of oxygen (ROS) in the mitochondria is manifested by the occurrence of damage at the cellular level. This leads to an imbalance in pro- and antioxidant systems through the overproduction of free radicals or disruption of the body's antioxidant capacity **[1,2].**

Catalase (CAT), peroxidase (POD), glutathione peroxidase (GPx), and superoxide dismutase (SOD) are enzymes that have antioxidant effects in biological and biochemical systems. The

antioxidant protection system protects the cell against oxidative damage of free radicals or other reactive molecules. Therefore, antioxidant enzymes such as CAT, POD, glutathione peroxidase (GPx), and SOD are of great importance in this defense system **[3].**

Poisoning of the body with toxicants is one of the main factors leading to the development of liver disease. It is known that the introduction of CCl_4 in experimental toxic hepatitis leads to an increase in LPO in hepatocytes and a significant decrease in the activity of antioxidant enzymes SOD, catalase, glutathione peroxidase **[3].**

In the context of toxic hepatitis, it is important to identify disorders of the functional activity of the liver mitochondria and antioxidant defense system, as well as their pharmacological correction with plant compounds. To this end, the effect of polyphenol extracts isolated from Helichrysum maracandicum on the activity of the enzyme glutathione peroxidase in mitochondria under conditions of toxic hepatitis caused by CCl_4 was studied comparatively with that of silymarin.

Materials and methods

Currently, there are many animal models of toxic hepatitis. One of the classical methods is the CCl_4-induced toxic hepatitis model. Experiments were carried out on 40 white male rats weighing 180-200g. Twice a week, CCl_4 was injected subcutaneously into the abdominal cavity at dose of 1 ml/kg (in olive oil as the vehicle) to induce toxic hepatitis. The animals were divided into 4 groups:

I control group (n = 10);

Group II CCl_4 (1 ml/kg) (n = 10);

Group III CCl_4 + *helmar-1* (20 mg/kg) (n = 10);

Group IV CCl_4 + *helmar-2* (20 mg/kg) (n = 10);

Group V CCl_4 + silymarin (20 mg/kg) (n = 10);

14 days after CCl4 injection in rats, after an increase in ALT and AST in the blood, group II animals were given olive oil once a day (1 ml / kg), group III was given helmar-1, group IV was given helmar-2 polyphenols, and group B was given silymarin daily once injected at a dose of 20 mg/kg for 10 days. The ALT and AST levels in the blood plasma of pharmacotherapeutic animals of Group III, IV and Group V were determined every 3 days and experiments were performed after approaching control values.

In the normal and toxic hepatitis groups, rats were isolated by differential centrifugation of the liver mitochondria **[4].**

The GP activity in the reaction medium (containing 2 ml of phosphate buffer (0.05MpH 8,0), 0,2 мл of 1 mM EDTA, 0.5 ml of 7.5mM oxidized glutathione, 0.2 ml of hemolysate, and 0.1 ml of 1,2 mM NADF.N) was determined at a wavelength of 340 nm after 10 min of reaction at 37°C due to the decrease in NADF.N [**5]**. Here, 1g of NADF.N protein is expressed as micromoles per minute. The content of mitochondrial proteins was determined by the method of Lowry modified by Peterson **[6].** The results obtained were processed using the Origin 6.1 program by calculating the arithmetic mean (M), standard error (m), and confidence index (p). A p value <0.05 was considered an indicator of a significant difference.

Results

In toxic hepatitis caused by CCl_4, there is an increase in prooxidant factors in the liver mitochondria as a result of increased levels of fatty acids as a result of membrane LPO. At this time, an imbalance of enzyme-dependent antioxidant defense systems occurs in the cell and mitochondria. In the context of toxic hepatitis, the activity of antioxidant enzymes SOD and catalase in liver tissue may change, as well as the activity of glutathione peroxidase. There is a growing interest in compounds that have the potential to enhance the antioxidant defense system of the organelle to the harmful effects of free radicals in the mitochondria in a pathological condition. In our next experiment, the effect of helmar-1 and helmar-2 polyphenol extract on the activity of glutathione peroxidase, another antioxidant enzyme, in the setting of toxic hepatitis was studied.

According to the results, glutathione peroxidase activity in the liver mitochondria of healthy group I rats was 80.11±1.75 mM/min mg protein. The activity of liver mitochondrial glutathione peroxidase enzyme activity in group II rats caused by toxic hepatitis was found to be 60.88 ± 1.40 mM/min mg protein, a decrease of 24.0±2.1% compared to the control group (Figure 1). When pharmacotherapy of animals with group III and IV caused by toxic hepatitis with helmar-1 and helmar-2 polyphenol extracts, respectively, their liver mitochondrial glutathione peroxidase enzyme activity was 74.51±3.16 mM/min mg protein and 75.98±3.56 mM/min mg protein. This indicates that glutathione peroxidase activity was restored to 22.3±1.20% and 24.8±1.17% compared to group II (Figure 1).

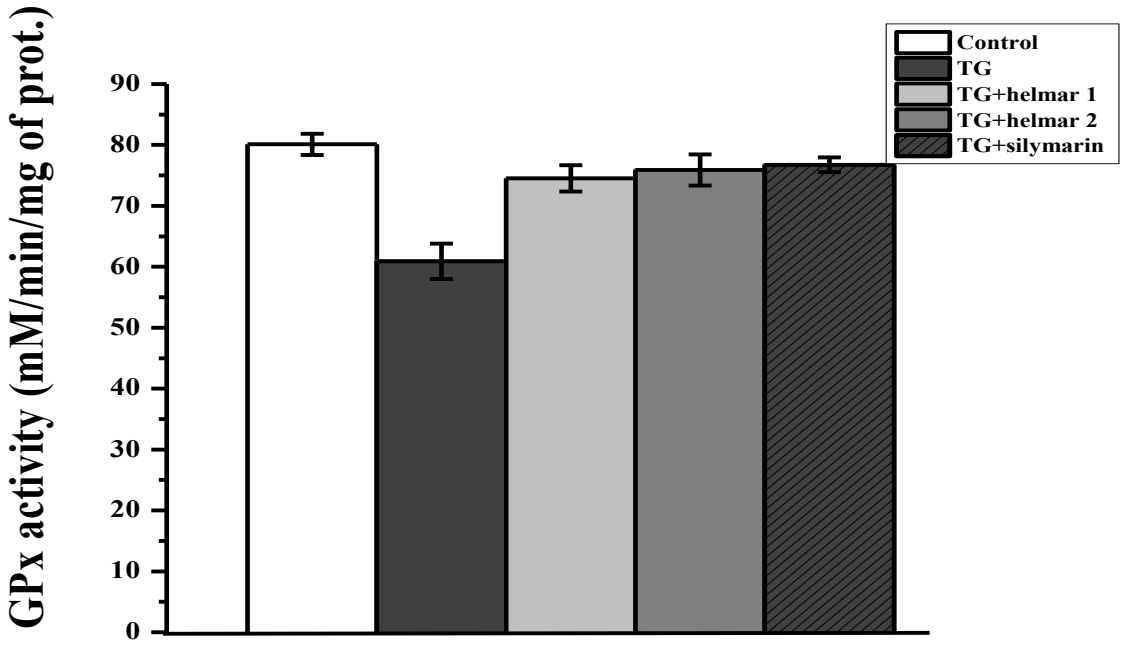

Figure 1. Effect of helmar-1 and helmar-2 extract (mM/min mg protein) on liver mitochondrial glutathione peroxidase activity in toxic hepatitis.

Note: - Statistical analysis Variability of differences between toxic hepatitis and toxic hepatitis + drug groups * P<0.05:

Thus, in toxic hepatitis caused by CCl_4, it was found that hepatic mitochondria restored the enzyme activity by acting closely on the glutathione peroxidase activity of helmar-1 and helmar-2 polyphenol extracts silymarin.

Disorders of lipid metabolism in the context of toxic hepatitis are associated with oxidative stress, which is manifested by the formation of ROS and DNA mutations in the mitochondria under its influence, as well as disruption of bioenergetic processes.

CONCLUSIONS:

In the conditions of toxic hepatitis, the activity of the antioxidant enzyme gutationperoxidase of rat liver mitochondria is reduced.

Liver mitochondrial gutation peroxidase activity in toxic hepatitis has been shown to restore antioxidant activity by regenerating natural polyphenol extracts at doses of helmar-1 20 mg / kg and helmar-2 20 mg / kg.

Based on the results obtained, it is possible to create further hepatoprotective drugs from helmar-1 and helmar-2 polyphenol extracts.

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