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Research Article

DETERMINATION OF THE EFFECT OF POLYPHENOLS ON CERTAIN BIOCHEMICAL CHANGES (ALT, AST, ALKALINE PHOSPHOTASE AND TOTAL PROTEIN) IN THE CONDITIONS OF TOXIC HEPATITIS

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Mallayeva Mavjudaxon Maxramovna
Teacher At The Department Of General Hygiene And Ecology At Samarkand State Medical University, Uzbekistan

ABSTRACT

To evaluate the hepatoprotective activity in the serum of experimental toxic hepatitis model rats treated with polyphenolic compounds, total protein, alkaline phosphatase, AST, ALT were determined by Cypress Diagnostica (Belgium) test kit. Blood was collected from animals, centrifuged at 3000 revolutions/min for 12 minutes, serum was isolated and biochemical indicators studied.

KEYWORDS

Toxic hepatitis, ALT, AST, alkaline phosphatase, protein content, effect of polyphenols, catalase, spectrophotometric, colorometric.

INTRODUCTION

Materials and research methods: the most effective and classic method of studying the changes in physiological processes occurring in liver cells, especially mitochondria, and the mechanisms of action of various biologically active compounds on it in the conditions of toxic hepatitis is SSI4 intoxication of animals. These research methods are mainly conducted in vivo. There are 2 types of animal intoxication: acute and chronic toxic hepatitis. In acute toxic

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hepatitis, the toxicant selected for intoxication is injected subcutaneously twice a day in a relatively high dose.

In this case, acute toxic hepatitis is quickly called and studied using several methods of studying changes in the mitochondria of animal livers. Researches were conducted in male white rats (Rattus vulgaris L.) weighing 140-200 g. The experiments were carried out in accordance with the "Rules for the use of experimental animals", as well as the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental Research or for Other Scientific Purposes.

Tetrachloromethane (SCl4) solution in 50% olive oil was challenged by parenteral administration at a dose of 2 ml/kg 2 times in 1 day [17]. Medicines Slimarin (pharmacological trade name Karsil) 50 mg/kg, rutan 10 mg/kg, gossitan 10 mg/kg were administered orally in a dose of 10 mg/kg within 7 days after hepatitis was induced.

The experimental animals were divided into 5 groups: 1) healthy group, 2) experimental toxic hepatitis (SCl4-infected toxic hepatitis), 3) group Slimarin (Carsil) 50 mg/kg, 4) group rutan 10 mg/kg, 5) group gossitan The amount of total protein, alkaline phosphatase, alanine and aspartate aminotransferase (ALT, AST) in serum after 10 mg/kg. 7 days was determined using the test kits produced by Cupress Diognostica Biochemical Tests (Belgium).

At the end of the experiment, the animals were anesthetized with chloroform, decapitated and studied to study their pathological changes. Extraction of liver tissue homogenate We isolated 150-200 grams of rat liver tissue homogenate by differential centrifugation [54]. The rat was first immobilized, then the liver was removed from the body and placed in an ice-cold isolation medium. The composition of the separation medium is as follows: sucrose 250 mM, tris-NSI 10 mM, eDTA 1 mM, pH 7.4. After washing in chilled saline to obtain the homogenate, a 5-g sample of rat liver tissue was placed in 5-10 ml medium containing 0.85% NaCl and 50 mM KH2PO4 (pH 7.4 at 4°C). Mechanical pressed through the press. It is homogenized with a Polytron type homogenizer for 90 seconds. The homogenate was centrifuged at 3000 g for 15 min and stored at minus 4°. In order to evaluate the hepatoprotective activity in the serum of rats treated with polyphenolic compounds in an experimental toxic hepatitis model, total protein, alkaline phosphatase, AST, ALT were determined by Cypress Diagnostica (Belgium) test kit. Blood was collected from animals, centrifuged at 3000 rpm for 12 minutes, serum was separated and biochemical parameters were studied.

Determination of total protein concentration:

Total protein concentration in blood serum was determined by the biuret method. The protein forms a colored complex with copper ions in an alkaline medium. (Table 2.1.1).

1.1- table.

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Add to test tubes	Sample experience, ml	Calibration sample, ml	Blank sample, ml
Working reactive	5,0	5,0	5,0
Serum	0,1	–	–
Calibrator	–	0,1	–
Distilled water	–	–	0,1

Alanine aminotransferase activity in serum was determined by the single Reitman-Frenkel method [5]. As a result of transamination under the influence of alanine aminotransferase (ALT) enzyme, amino acids are transferred from alanine to α-ketoglutarate. ALT activity is proportional to the amount of pyruvate dinitrophenylhydrazones formed in an alkaline medium and was determined by colorometric method (Table 1.2).

1.2-table.

Sample composition	Sample experience	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
Solution 2,4- DNFG, ml	0,25	0,25
Serum, ml	–	50

Aspartate aminotransferase activity in serum was determined by the single Reitmann-Frenkel method.

1.3-table.

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Sample composition	Sample experience	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
Solution 2,4- DNFG, ml	0,25	0,25
Serum, ml	–	50

Alkaline phosphatase activity in blood serum was determined by the unique Bassey method [59]. The amount of p-nitrophenol formed is proportional to the activity of the enzyme and is determined photometrically. (Table 1.4)

1.4-table.

Composition of samples	Sample experience	Blank sample
Working reagent, ml	0,2	0,2
Serum, ml	20	–
Incubate at 37°C for 30 minutes		
Diluted reagent No. 2 (Sodium hydroxide solution), ml	2,0	2,0
Serum, mkl	–	20

Reagent 1 (glycine buffer) and Reagent 3 (p-Nitrophenylphosphate) are mixed in a ratio of 4:1. The samples were mixed and photometered at a wavelength of 405 nm. Enzyme activity is calculated using a calibration curve. The activity

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of superoxide dismutase and catalase was determined in the liver tissue homogenate.

Determination of SOD enzyme activity (KF 1.15.1.1) Misra and J. Fridovich [62]. conducted according to the method. The principle of the method is based on nitrotetrazolium blue (NTK) for superoxide anions, which are formed as a result of aerobic action and reduce the amount of NADN phenosine metasulfate (FMS). (Table 2.1)

1.5-table.

	Control	Experience	Reminder
TRIS-EDTAbuffer. rN=7.4	0.05 ml	-	
Homogenate	-	0.05 ml	
Reagent 1	2.0 ml	2.0 ml	10 minutes 37^0S
Reagent 2	0.1 ml	0.1 ml	5 minutes 25^0S

The principle of spectrophotometric measurement of catalase activity is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts [3] The reaction was started by adding 0.1 ml of homogenate to 2 ml of 0.03% hydrogen peroxide solution. 0.1 ml of distilled water was added to the blank sample instead of serum. The reaction was stopped after 10 min by adding 1 mL of 4% ammonium molybdate. Color intensity was measured in a spectrophotometer at a wavelength of 410 nm relative to a control sample in which 2 mL of distilled water was added instead of hydrogen peroxide.

Table 1.6.

	Control	Experience	Reminder
N_2O_2	2 ml	2 ml	-
Homogenate	-	0,1 ml	10 minutes 37^0S
N_2O	0,1ml	-	
$(NH_4)_6Mo_7O_{24}$	1ml	1ml	-

The amount of protein in the samples was determined by the biuret reaction [6]. In order to disrupt the mitochondrial membrane, 0.9 ml of 2 N KOH and 10 ml of deoxycholic acid were added

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to 0.1 ml of the mitochondrial suspension. After the protein was completely dissolved, 4 ml of biuret reagent was added to the solution and left for 30 minutes at room temperature. At the same time, control samples were prepared (1 ml of 2 n KOH+10 ml of DOX+4 ml of biuret reagent). We carried out calorimetry in cuvettes with a thickness of 10 mm at a wavelength of 540 nm. We determined the amount of protein by calorimetry of bovine serum albumin (BSA) 10 mg/ml standards and using a calibrated curve.

Obtained results and comments: statistical analysis of obtained experimental results and drawing of pictures were carried out using the computer program OriginPro 8.6 (Microsoft, USA) in Hypothesis Testing one-sample t-test. The results of 5 experiments were calculated as the arithmetic mean value based on ± standard deviation. The difference between the values obtained from control, experiment and experiment+study material was calculated by t-test. Statistical reliability was calculated according to Student's criterion. In this case, values of $R<0.05$ represent statistical reliability.

Summary. In the course of our research, we used modern biochemistry and molecular biology methods, namely - the method of creating a toxic hepatitis model, the method of isolating animal tissue homogenate and mitochondria, determining the amount of LPO product MDA in the liver tissue homogenate, determining biochemical indicators in blood serum, protein quantitative determination, antioxidant system enzyme activity determination methods were used.

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