ISSN NO: 2770-2936
June 2024

Certain Biochemistry Of Blood In Toxic Hepatitis(Alt, Ast, Alkaline Phosphatase And Total Protein Amount) Determination Of The Effect Of Polyphenols

Mallayeva Mavjudaxon Makhramovna
Samarkand State Medical University,General hygiene and ecology
teacher of the department

Abstract: Total protein, alkaline phosphatase, AST, ALT were determined by the Cypress Diagnostica (Belgium) test kit to evaluate the hepatoprotective activity in the serum of experimental toxic hepatitis model rats treated with polyphenol compounds. Blood was collected from animals, centrifuged at 3000 revolutions/min for 12 minutes, serum was separated and biochemical indicators studied.
Key words: toxic hepatitis, ALT, AST, alkaline phosphatase, protein content, effect of polyphenols, catalase, spectrophotometric, colorometric

Materials and research methods: the most effective and classical method of studying the changes of 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 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) group healthy, 2) group experimental toxic hepatitis (toxic hepatitis infected with SCl4), 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 the blood 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 a homogenate, a 5 g rat liver tissue sample 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°. An experimental toxic hepatitis model was determined by Cypress Diagnostica (Belgium) Cypress Diagnostica (Belgium) test kit for total protein, alkaline phosphatase, AST, ALT to evaluate the hepatoprotective activity in the blood serum of rats treated with polyphenol compounds. Blood was collected from the animals, centrifuged at 3000 rpm for 12 minutes, serum was separated and biochemical indicators 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 environment. (Table 2.1.1).

ISSN NO: 2770-2936
June 2024

Add to test tubes	Test sample, ml	Calibration sample, ml	Empty sample, ml
Working reactive	5,0	5,0	5,0
Serum	0,1	–	–
Calibrator	–	0,1	–
Distilled water	–	–	0,1

Add to test tubes Test sample, 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

Sample composition	Experience is an example	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
solution2,4– DNFG, ml	0,25	0,25
Serum, ml	–	50

Aspartate aminotransferase activity in serum was determined by the single Reitmann-Frenkel method. Table 1.3.

Sample composition	Experience is an example	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		

ISSN NO: 2770-2936
June 2024

solution2,4– 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 produced is proportional to the activity of the enzyme and is determined photometrically. (Table 1.4)

Sample composition	Experience is an example	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
solution2,4– DNFG, ml	0,25	0,25
Serum, ml	–	50

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 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)

	Nazorat	Tajriba	Eslatma
TRIS-EDTAbuffer. rN=7.4	0.05 ml	-	
Gomogenat	-	0.05 ml	
Reagent 1	2.0 ml	2.0 ml	10 daqiqa 37^0S
Reagent 2	0.1 ml	0.1 ml	5 daqiqa 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

ISSN NO: 2770-2936
June 2024

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.

	Nazorat	Tarjiba	Eslatma
N_2O_2	2 ml	2 ml	-
Gomogenat	-	0,1 ml	10 minut 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 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 at room temperature for 30 minutes. 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 Studentt'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, that is, 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 methods were used to determine the enzyme activity of the antioxidant system.

List of used literature:

CERTAIN BIOCHEMISTRY OF BLOOD IN TOXIC HEPATITIS(ALT, AST, ALKALINE PHOSPHOTASE AND TOTAL PROTEIN AMOUNT) DETERMINATION OF THE EFFECT OF POLYPHENOLS

Mallayeva Mavjudaxon Makhramovna
Samarkand State Medical University,General hygiene and ecology
teacher of the department

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

Key words: toxic hepatitis, ALT, AST, alkaline phosphatase, protein content, effect of polyphenols, catalase, spectrophotometric, colorometric

Materials and research methods: the most effective and classical method of studying the changes of 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 hepatitis, the toxicant selected for intoxication is injected subcutaneously twice a day in a relatively high dose.

ISSN NO: 2770-2936
June 2024

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) group healthy, 2) group experimental toxic hepatitis (toxic hepatitis infected with SCl4), 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 the blood 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 a homogenate, a 5 g rat liver tissue sample 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°.

An experimental toxic hepatitis model was determined by Cypress Diagnostica (Belgium) Cypress Diagnostica (Belgium) test kit for total protein, alkaline phosphatase, AST, ALT to evaluate the hepatoprotective activity in the blood serum of rats treated with polyphenol compounds. Blood was collected from the animals, centrifuged at 3000 rpm for 12 minutes, serum was separated and biochemical indicators 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 environment. (Table 2.1.1).

Add to test tubes	Test sample, ml	Calibration sample, ml	Empty sample, ml
Working reactive	5,0	5,0	5,0
Serum	0,1	–	–
Calibrator	–	0,1	–
Distilled water	–	–	0,1

Add to test tubes Test sample, 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

ISSN NO: 2770-2936
June 2024

Sample composition	Experience is an example	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
solution2,4– DNFG, ml	0,25	0,25
Serum, ml	–	50

Aspartate aminotransferase activity in serum was determined by the single Reitmann-Frenkel method.
Table 1.3.

Sample composition	Experience is an example	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
solution2,4– 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 produced is proportional to the activity of the enzyme and is determined photometrically. (Table 1.4)

Sample composition	Experience is an example	Blank sample
Substrate buffer solution, ml	0,25	0,25
Blood serum, ml	50	–
Incubate in a water bath at 37ºC for 60 minutes		
solution2,4– DNFG, ml	0,25	0,25
Serum, ml	–	50

ISSN NO: 2770-2936
June 2024

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 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)

	Nazorat	Tajriba	Eslatma
TRIS-EDTAbuffer. rN=7.4	0.05 ml	-	
Gomogenat	-	0.05 ml	
Reagent 1	2.0 ml	2.0 ml	10 daqiqa 37^0S
Reagent 2	0.1 ml	0.1 ml	5 daqiqa 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.

	Nazorat	Tarjiba	Eslatma
N_2O_2	2 ml	2 ml	-
Gomogenat	-	0,1 ml	10 minut 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 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 at room temperature for 30 minutes. 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 Studentt'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, that is, 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

ISSN NO: 2770-2936
June 2024

biochemical indicators in blood serum, protein Quantitative methods were used to determine the enzyme activity of the antioxidant system.

List of used literature:

1. Pentyuk A. A., Moroz L. V., Palamarchuk O. V. Porazheniya pecheni xenobioticami //Sovremennye problemy toxicologii - 2001; – No. 1: – C. 8.
2. Eidi A. Hepatoprotective effects of pantothenic acid on Carbon Tetrachloride-induced toxicity in rats //EXCLI Journal.-2012. - No. 1. - P. 748-759.
3. Rumboldt Z., Vavro H., Špero M. Exogenous Toxins and CNS Injuries // Clinical Neuroradiology-2019.P-1–39.
4. Zobnin Yu.V. Toksicheskoe povrejdenie pecheni u detey //Sibirsky meditsinsky zurnal(Irkutsk).,2017,№-4.S.37-53.
5. Muriel P. Role of free radicals in liver diseases // Hepatol Int.-2009. – V.3(4): – P.526–536.
6. Shiryaeva A., Arkadeva A., Emelyanova L., Sakuta G., Morozov V. Superoxide anion production by the mitochondrial respiratory chain of hepatocytes of
7. rats with experimental toxic hepatitis //J. Bioenergy.
8. Biomembr. 2009.–V.41.–P.379–385.
9. Turdieva O.M., Pozilov M.K., Rakhmatullaeva
10. M.M., Abdulladjanova N.G. Effect of gossitan and getasan polyphenols on the passive ion permeability of the liver mitochondria membrane in toxic hepatitis // Pharmaceutical Journal - 2021. - No. 2. - P. 146-151.
11. Shabbir A. Rhus coriaria linn, a plant of medicinal, nutritional and industrial importance: a review //J Anim Plant Sci. – 2012. – T. 22. – No. 2. – S. 505-12.
12. Shabbir A. Rhus coriaria linn, a plant of medicinal, nutritional and industrial importance: a review //J Anim Plant Sci. – 2012. – T. 22. – No. 2. – S. 505-12.
13. Schneider W.S., Hageboom D.H, Pallade G.E. Cytochemical studies of mammalian tissues: the isolation of cell components by differential centrifugation//Cancer.res.-1951.-P.1-22.
14. Brierley GP. Passive permeability and energy-linked ion movements in isolated hearth mitochondria// Ann. N. Y. Acad. Sci. - 1974. - V. 227. - P. 398-411.
15. Vladimirov Yu.A. Rol narushenyi svoystv lipidnogo sloya membrany v razvitii patologicheskikh protsesov. Pat. physiol. I'm an expert. Med. 1989; 4: 7-91.
16. Pozilov M.K., Ergashev N.A., Asrarov M.I.,
17. Rakhmatullaeva M.M., Aminov S.N. The effect of glucorasmulin on the passive permeability of mitochondrial membranes in conditions of experimental diabetes // Pharmaceutical journal. - Tashkent, 2016. -
18. #3. - B. 99-103