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SOME IMPORTANT BIOCHEMICAL PARAMETERS IN CLINICAL VETERINARY TOXICOLOGY

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ABSTRACT

Biochemistry and medicine are inextricably linked. Health depends on the harmonious balance of biochemical reactions that take place in the body, and the disease – reflects abnormalities or abnormalities in biomolecules, biochemical reactions or processes.

Progress in biochemical theory has shed light on many fields of medicine, the study of disease, contributing to the enrichment and further development of biochemistry. The biochemical approach in veterinary medicine is fundamental to discovering the causes of intoxication and prescribing therapy. Clinical veterinary toxicology as a system of specialized knowledge studies the causes, mechanism of interactions of poisons, clinical signs, diagnosis and treatment of animal poisoning.

Key words: biochemical parameters in animals, animal poisoning, clinical toxicology.

Introduction

Under the present conditions of animal husbandry, prerequisites are created for poisoning not only with chemicals accumulated in soils of industrially polluted areas such as heavy metals, but also with herbicides, pesticides, insecticides, etc., Plants containing alkaloids, cyanglycosides and other toxins. molds are also a common cause of intoxication.

Biochemistry is the science of the chemical foundations of life. This also determines the enormous importance of biochemical laboratory testing for timely diagnosis and monitoring of the course of poisoning.

The theoretical goals of biochemistry are to study at the molecular level the composition, structure and functions of cellular components, the chemical reactions and processes that take place in cells and their regulation, and to explain their importance to the body in norm and pathology.

The main biochemical parameters to be monitored for intoxication are abnormalities in blood, urine and liver.

Factors affecting the effects of poisonous substances: factors that influence the action of poisonous substances are the dose, physical and chemical form of the poison, depends on the exposure whether it is single or repeated, depends on the animal, size, age, sex and health status of animals.

Blood. Diseases of any organ or system in the body affect the composition of the blood – pathological changes in the blood can interfere with the function of the organs. Of great importance in clinical toxicology is the biochemical study of blood.

It is most commonly used for blood serum or plasma.

It should be borne in mind that platelets and erythrocytes secrete significant amounts of enzymes, such as acid phosphatase, lactate dehydrogenase, aldolase, etc., when clotting, which is why it is best to detect them in the blood plasma.

Blood is biochemically tested for: blood sugar, total protein, creatinine, uric acid, ammonia, amino acids, lipids, bile pigments (chromoproteins) – bilirubin, calcium, phosphorus and more.

• *Blood sugar.* Carbohydrates are the main energy source for animals and humans (except ruminants), which is why blood glucose testing is one of the most commonly performed biochemical studies in clinical laboratory practice. Usually, the term blood glucose refers to only blood glucose. However, the blood contains other sugars – galactose, fructose, maltose, etc., but because their amount is under normal physiological conditions is too low, practically they do not affect the concentration of blood sugar.

• *Total protein in the blood.* Proteins are high molecular weight nitrogen-containing organic compounds that are extremely important for the formation and maintenance of the structure and function of cells in the body. The total protein in the blood serum largely depends on the functional state of the liver in which the albumins, fibrinogen, prothrombin and various clotting factors are formed.

• *Creatinine.* Creatinine is creatine anhydride. A constant dynamic balance is established between the two compounds in the body. Creatinine is mainly formed in the muscles of creatine phosphate by the separation of one molecule of water without enzymatic involvement. The released creatinine is excreted in the blood and is then excreted in the urine. Its level is constant and depends only on the kidney, respectively on glomerular function.

• *Uric acid.* Uric acid is 2,6,8-trihydroxypurine and is a major product of the exchange of purine bases that are part of complex proteins, nucleoproteins.

• *Ammonia.* It is formed by the breakdown of proteins, amino acids, purines, pyrimidines and other nitrogen-containing compounds. In blood and other biological fluids, ammonia is mainly found in the form of NH4 + ion. For the most part, ammonia turns into urea.

• *Amino acids.* The amino acids in the blood besides being bound are also in the free state. Free amino acids are present in all tissues and body fluids. They are in constant equilibrium in the individual body spaces.

• *Lipids.* They are a collective term that includes a group of naturally occurring organic compounds of different structure but with similar physicochemical properties. The lipids include triglycerides, cholesterol (free and ester), fatty acids, phospholipids and more. Lipids have important physiological functions in the body. They form the lipid matrix of cell membranes and organelles, and are crucial in energy metabolism.

• *Bile pigments.* Bile pigments, bilirubin and its derivatives – urobilinogen bodies, are derived from hemoglobin, which is composed of a protein part – globin and a prosthetic group (ferroprotorphyrin ring, heme). Under the influence of the enzyme biliverdin reductase, biliverdin is reduced to bilirubin. This is the so-called. blood or free bilirubin, which is a normal component of blood serum. Because it does not directly react with the Ehrlich diacetate, it is also known as "indirect bilirubin".

• *Calcium.* Calcium is a widespread cation in the body of animals. It is located in the intra- and extracellular space. In the blood, calcium is in the form of protein-bound, ionized, and non-ionized. It participates in the processes of blood clotting, muscle contraction, capillary permeability, in the construction of cell membranes, etc. Hypercalcemia is observed with an overdose of vitamin D.

• *Phosphorus.* In the blood and in the blood serum, phosphorus is included in the form of two groups of compounds – organic and inorganic phosphates. Phosphates are the most common intracellular anions. They are involved in the synthesis of macro-energetic compounds (ATP and creatine phosphate), in the processes of glycolysis and glycogenesis, in the composition of DNA, RNA, phospholipids and cell membranes.

• *Magnesium.* Magnesium is a very common intracellular cation. About 60% of its content is localized in the bones, about 30% in the intracellular fluid, and a very small amount in the extracellular space. Magnesium is rich in muscle, nervous system and liver. It is localized in the mitochondria in cells and is an activator of oxidative phosphorylation, as well as of all ATP-dependent biochemical reactions.

For the correct and prompt diagnosis of intoxication, monitoring of abnormalities in some urine parameters is of great importance. The urine response is determined by the amount and ratio of the salts present and the related acids (pH). Important indicators are proteins, hemoglobin, cysteine, glutathione and bile pigments.

As the largest gland with parenchymal tissue in the vertebrate body, the liver has important and multifaceted functions for the living organism, making it the central organ of metabolism It processes synthesis; the breakdown and elimination of carbohydrates, proteins and fats; bile synthesis and secretion. It serves as a depot for vitamins, glycogen, proteins, minerals and more. The liver neutralizes and eliminates much of the exogenously released and endogenously formed toxic substances.

Methods for the determination of enzymes. The so-called optimized methods are mainly used to achieve unification of reagents, methods and results. To determine enzyme activity, these methods use optimal standard conditions for substrates, cofactors, activators, buffers, etc. that are precisely defined quantitatively and qualitatively. If the results are obtained at a measurement temperature other than 25 ° C, which is assumed to be standard, appropriate correction factors shall be used to obtain comparable results.

Sources of error in enzyme assays:

Incorrect receipt of study material. Blood for enzyme testing should be taken in the patient's calm state and after physical rest. In patients in a state of excitement and after exercise, the values of muscle enzymes, such as LDH, ASAT, aldolase, and others, may increase. The serum should be clear, with no signs of hemolysis, as hemolysis increases the levels of LDH, ASAT, Che and more. enzymes.

Material storage conditions. The tested biological material (serum, plasma) should be fresh, sera without hemolysis. It is desirable that the enzyme determination be carried out immediately after serum excretion. If this is not possible, the serum may be refrigerated at +2 ° C to +4 ° C for several hours. Serum freezing should be avoided since significant freezing of enzymatic activity results in freezing. Some enzymes exhibit relatively high thermal stability, allowing them to be stored for longer periods of time, even at room temperature.

Interaction of toxic substances with enzymes. In many cases, when the mechanism of action of the poisons is elucidated, the inhibition of the effect of some specific enzymes is found. Enzyme inhibition can be irreversible and reversible, specific and non-specific. The interaction of the poisonous substance with the enzymes impairs oxidative phosphorylation, results in lethal synthesis, and blocks the use of oxygen by the tissues. A classic example of irreversible enzyme inhibition by toxic substances is the blocking of acetylcholinesterase by organophosphorus compounds that form a covalent bond with the enzyme's active site. At the synapse region, endogenous acetylcholine accumulates, which is no longer hydrolyzed to choline and acetic acid. Reversible enzyme inhibition occurs when antimetabolites compete with endogenous substances when blocking enzyme centers – thus many drugs act (Dilov, P et al. 2005).

Enzymes. In clinical toxicology, much attention is paid to the activity of the following enzymes: Acetylcholinesterase (ACHE), Aminotransferases – L-aspartate: 2-oxoglutaraminotransferase (ACAT), L-alanine: 2-oxoglutarate-aminotransferase (AAT); Gamma-glutamyltransferase (GGT), Lactate dehydrogenase (LDH), Glutamate dehydrogenase (GLDH), Sorbitol dehydrogenase (SDH), Creatine kinase (CK).

The crucial importance of enzymes for animal intoxication stems from the fact that virtually all metabolic reactions in the body are catalyzed by enzymes. At the level of the enzyme molecules, the regulation of metabolic processes and the maintenance of normal homeostasis of the body are realized. Increasing or decreasing the enzyme activity in a particular unit of the metabolic process alters the rate of flow of the relevant chain, which is followed by a specific metabolic disorder.

Discussion

In his research (Todorov, T. 2007) on the acute toxicity, tolerability and clinical efficacy of enrofloxacin (dosage forms) in dogs and cats. The clinical and chemical analyzes of the blood, at the end of the experiment, the increase of the total protein and in the three experimental groups, respectively, to 80.11 ± 2.315 g / l, 81.85 ± 4.983 g / l and 81.758 ± 3,410 g / l but was unreliable from the control measurement – 84.83 ± 4,368 g / l ($p > 0.05$). The same author, when determining blood sugar levels, found a trend toward stable euglycaemia in all treated animals, regardless of the dose administered. In blood studies, after internal administration in therapeutic, three- and five-fold increased doses of Biofloxavet C tablets, the enzyme activity of ALAT, ASAT and AP was not statistically significant for 7 days (Todorov, T. 2007).

In the local and general tolerability test of Biofroxavet D tablets in dogs – the level of total protein in all experimental animals was in the lower limit of the reference range. The same author, when tested for local and general tolerability of Biofloxavet 5% solution for injection in dogs and cats, found some fluctuations in the mean urea values, but these were within the reference range. In the study, the enzyme activity of AP did not observe changes relative to baseline levels, but in animals treated with the 5ED50 dose it recorded more than twice lower enzyme activity compared to control animals (Todorov, T. 2007).

In his studies of subchronic oral 35-day toxicity of zinc methionate and zinc sulfate in broiler chickens, he found a decrease in albumin values in both control and experimental groups (3). This trend was most pronounced in chickens receiving zinc sulfate. This author observed the highest values of creatinine (50.9 mmol / l) in group VI treated with zinc sulfate at a dosage of 600 ppm and the lowest (41.9 mmol / l) in group III treated with zinc methionate at a dosage of 600 ppm (Ivanova, S. 2016).

In studies with experimentally reproduced acute intoxication with triazole fungicides diniconazole, triadimenol and triticonazole in birds, rabbits and pigs treated with increasing doses (Binev, R. 2005). This author observes that these intoxications take place with increasing amounts of macronutrients – calcium, inorganic phosphorus and magnesium in the blood.

(Binev, R. 2005) found that after treatment the calcium values increased at the 48th hour – 3.85 ± 0.28 mmol/l ($p < 0.05$) at the first, 4.31 ± 0.32 mmol/l ($p < 0.05$) in the second and 4.75 ± 0.38 mmol/l ($p < 0.01$) in the third group (Binev, R. 2005). The same trend was established at the 72nd hour. Similar changes were detected in the level of inorganic phosphorus at the 48th hour. In rabbits, the maximum calcium values were 12.3 hours ± 3.33 ± 0.28 mmol/l ($p < 0.05$) in the first group, and in the second group the increase in inorganic phosphorus was 10 hours 3,54 ±

0.32 mmol/l ($p < 0.05$). The highest levels in the amount of magnesium were recorded at the 10th hour, at the first group – 2.13 ± 0.18 mmol/l ($p < 0,05$) and at the third group at the 4th hour – 2.24 ± 0.27 mmol/l ($p < 0.05$). The highest levels in the amount of magnesium observed in the third group at the 4th hour were 2.24 ± 0.27 mmol/l ($p < 0.05$). The treated pigs also observed similar changes with respect to inorganic phosphorus at the 8th hour, respectively – 3.88 ± 0.36 mmol/l ($p < 0.05$) in the second group and 3.98 ± 0.36 mmol/l. ($p < 0.05$) third group. The highest levels in the amount of magnesium were recorded at the 6th hour – 1.68 ± 0.15 mmol/l (Binev, R. 2005). The observed increase in the levels of macronutrients according to Binev, R, is due to their decreased excretion in the urine (oliguria or anuria), as well as hypofunction of the thyroid gland, which affects the metabolism of macronutrients (Binev, R. 2005).

The same author reported in a study of the amount of biochemical parameters urea, creatinine and uric acid that the amount of urea was significantly increased in birds and rabbits under the influence of diniconazole. It proves that the increased urea level is due to the nephrotoxic effect of diniconazole. In the ASAT enzyme activity study (Binev, R. 2005) observed elevated levels in all three experimental rabbit groups at the 2nd hour after treatment.

The ASAT values in the first and second groups reach the highest values at the 8th hour. A similar trend was observed in ALAT values, with the highest values in the first group observed at the 10th hour – 98.2 ± 7.6 U/l ($p < 0.001$). In the study of LDH values the author observed the highest values in the first group at the 12th hour – 1880 ± 104 U / l ($p < 0.001$), at the second group at the 10th hour – 2134 ± 162 U/l ($p < 0.001$) and in the third group at the 4th hour – 2010 ± 126 U/l ($p < 0.001$). In the treated pigs, the author registered an increase in ASAT activity at the 2nd hour in all three experimental groups (Binev, R. 2005). The results of Binev, R show an increase in the levels of ALAT (over 4 times), ASAT (over 15 times) and LDH (over 20 times, relative to control animals). His studies show a steady upward trend in all experimental animals (birds, rabbits and pigs). Increased values of biochemical parameters ALAT, ASAT and LDH can be interpreted by the toxic effect of triazole fungicides on the liver parenchyma. These data closely correlate with the author's established hypoproteinemia with hyperalbuminemia, hyperglycemia, hypercholesterolemia and bilirubinemia (Binev, R. 2005).

Blood sugar is increased by poisoning with strychnine, alcohol, nicotine, caffeine, mercury salts and more. Hypoglycaemia can occur with intoxication. An increase in creatinine has been observed in severe intoxication associated with acute and chronic renal failure. Endogenous uric acid elevation has been observed with intoxication. The amount of ammonia in the blood increases when poisoning with urea and other toxicosis. Hyperaminoacidemia has been observed with phosphorus poisoning, chloroform. An increase in bilirubin is observed with phosphorus, copper and arsenic poisoning, with some types of horsetail poisoning (Dilov, P. et al. 2005).

Acetylcholinesterase (Che, AchE)

Serum cholinesterase is a collective term for a large number of similar enzymes and isoenzymes that are synthesized mainly in the liver and are excreted in the blood. It must be distinguished from the so-called. true cholinesterase, which is mostly present in the erythrocytes and the nervous system. Inhibition of Che by Organophosphorus Insecticide (FOI) is associated with the phosphorylation of its esterase pole, which is durable and practically irreversible

(Wilson, B 1998, Wilson. B 1999). Carbamate insecticides inhibit (Che) permanently (reversibly) and incompletely (Dilov, P. et al. 2005). Che activity is most strongly inhibited in brain tissue and kidney cells, and decreased in liver cells. In one study, the brain and kidneys were much more

affected by the effect of diazinon compared to the liver. Major changes were observed in physiological, biochemical and histochemical parameters in animals when treated with diazinon at a concentration of 3mg dissolved in 1 liter of water for 10s. and total plasma protein. Increased levels of cholesterol and decreased levels of body weight and cytochrome P-450 have also been observed (Mona, A et al. 2007).

Zhelev, I. has done extensive studies on the toxic effects of carbofuran in pigs and broiler chickens (Zhelev, I. 2005). His research focuses entirely on cholinesterase activity. It found a significant inhibition of Che ($p < 0.001$) at 1 hour after carbofuran treatment in both study groups, and in pigs treated at a dose of 18.0 mg / kg m, the activity of this biochemical indicator was inhibited by about 85%, relative to the control group of animals (Zhelev, I. 2005). Zhelev, I. examined the activity of Che in whole blood, and so in blood plasma. It found at the 3rd hour after treatment a significant reactivation in Che values that were significantly reduced (between 29.6 and 68.6%) compared to control animals (Zhelev, I. 2005).

Zhelev, I. examined the values of brain cholinesterase in chickens (Zhelev, I. 2005). The same author found at 1 hour after treatment that carbofuran had an inhibitory effect on Che in the brains of broiler chickens ($p < 0.001$). The inhibition of this biochemical indicator in the group treated with 12.0 mg / kg m. Carbofuran was 66.3% and in the groups treated with the higher doses the inhibition of Che was 75.4%, 78.1% and 86.2% respectively, compared with the control group of animals (Zhelev, I. 2005).

In his experiments with experimentally reproduced acute intoxication with triazole fungicides diniconazole, triadimenol and triticonazole in birds, rabbits and pigs, Binev, R, examined cholinesterase activity. His studies prove that this biochemical indicator does not show changes in values in all experimental animals (Binev, R. 2005).

Therefore, these data indicate that preparations of the triazole group have no anticholinesterase effect. This could be used to differentiate from intoxication caused by organophosphorus compounds or dithiocarbamate fungicides. Binev, R, proves that triazole fungicides cause hypothyroidism – a decrease in the levels of hormones T3 and T4. His studies also show that rabbits are more sensitive than hens to intoxication with the triazole fungicides diniconazole and triadimenol (Binev, R. 2005).

Transferases

In clinical toxicology, aminotransferases have a particularly broad clinical diagnostic significance. They catalyze the transfer of amino groups from amino acids to keto acids.

L-aspartate: 2-oxoglutarataminotransferase (ASAT)

The enzyme catalyzes the transfer of amino groups from aspartate to the keto acid α-ketoglutarate. ACAT, as well as other transaminases, are found in almost all tissues of the animal body. ACAT has the highest activity in heart muscle, liver, skeletal muscle, kidney, pancreas, lungs, erythrocytes and adipose tissue. An increase in ASAT has been reported in toxic liver damage, including overdose and misuse of veterinary medicinal products. ASAT is useful as an indicator of liver and / or muscle damage in large and small animals. Medications: Anticonvulsants may lead to increased ASAT activity, which is considered secondary to hepatocellular damage in dogs. Corticosteroids generally do not lead to increased ASAT activity unless they cause damage to the hepatocellular system (in dogs). Plasma activity of liver enzymes are sensitive indicators of liver damage in sheep and cattle from F.hepatica (Qian Yang et al. 1998, Rahman, M 2001, Shashi, K 2007).

Alanine aminotransferase (ALAT) is an intracellular cytoplasmic enzyme. It is widespread in various tissues of the body, most of which is found in the liver and kidneys. ALAT catalyzes the transfer of amino groups from alanine (forming pyruvate) to 2-oxoglutarate (forming glutamate). It is a key enzyme in gluconeogenesis. Its presence in plasma is the result of normal or pathological functioning of the tissues. It is an important indicator as a specific indicator of hepatocellular damage in dogs and cats. Medications: primidone, phenobarbitone, dilantin can increase ALAT activity up to 4 above normal values. These drugs have been shown to induce ALAT synthesis, and the increase in ALAT activity is considered to be secondary to hepatocellular necrosis. Corticosteroids raise ALAT to approximately 2–3 above normal values. Studies by several scientists suggest that plasma levels of liver enzymes are indicators of liver damage caused by subclinical fasciolosis in water buffaloes (Qian Yang et al. 1998). Administration of erythromycin as a base or various salts can be followed in up to 10% of cases to significantly increase serum ALT and ASAT serum transaminases (Vfluksela, M et al. 1988).

Synthetic pyrethroids that resemble pyrethrins in structure are widely used as insecticides for the control of agricultural pests, for external decontamination and in many other sectors. Pyrethroids are low toxic substances – LD 50 orally is 60 to 10,000 mg/kg m. Poisoning is possible when animals are treated externally with sprays or solutions (dogs, cats, sheep).

Pyrethroids are highly toxic to insects, and fish and aquatic arthropods are relatively sensitive to them. The reason is that these pesticides are metabolized more rapidly than the body. In a study done in fish (Cyprinus carpio), significantly higher levels of ASAT and ALAT aminotransferases were observed in all tissues, at all sublethal concentrations. All changes in ASAT and ALAT activity affect nitrogen metabolism and, accordingly, most of the biochemical reactions. Many scientists have reported that increased levels of aminotransferases may result from tissue damage based on toxic stress (Casida, J 1980, Neelima, P et al. 2013, Raju, T et al. 1985, Wells, R et al. 1986). Aspartate aminotransferase is an important indicator of nitrogen metabolism and very often used as a biochemical parameter for stress (Rahman, M 2001, Gabriel, V et al. 2005, Shobha, R et al. 2001, Naveed, A et al. 2004).

For the diagnosis of nephrotoxicity in the dog, many enzymes that are tested in urine are used: γ-glutamyl transferase (GGT) and NAG are used as biochemical indicators. These enzymes are suitable because they are relatively stable at room temperature and can be measured in the urine without first removing the enzyme inhibitors. Many other enzymes are also used as biochemical indicators: lactate dehydrogenase (LDH), P-glucuronidase (P-GLU), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), acid phosphatase (ACP), alanine aminopeptidase (ALP), P-galactosidase (PGAL) and lysozyme (muramidase) (Frances, A et al. 1998). Determination of isoenzyme forms of enzymes such as LDH; ALP and NAG can help determine the origin of the increase in enzyme activity. These enzymes can also be used as subcellular markers of renal impairment due to their location in the cytosol, lysosomes and mitochondria (Frances, A et al. 1998). Urinary enzymes can be studied in dynamics, thus monitoring the recovery process, since enzymes vary depending on the degree of kidney disease (Rivers, B et al. 1996). Enzymes are relatively sensitive, so other biochemical parameters in the urine such as creatinine, glucose, total protein, and others (Matteucci, E et al. 1993) must be investigated for accurate diagnosis.

In liver diseases, very suitable traceable biochemical markers are alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) because they are specific to this organ, with good test accessibility such as blood collection and subsequent analysis. Therefore, these enzymes are widely used in preclinical studies, and can be used as major biochemical parameters for damage to

many organs and systems in humans, monkeys, mice, rats, dogs and other animals (Josef, O et al. 2008).

Gamma Glutamyltransferase (GGT); a synonym for gamma-glutamyltranspeptidase, is an enzyme involved in the exchange of amino acids. It catalyzes the transfer of a gamma-glutamyl residue from a gamma-glutamyl peptide to an amino acid, another peptide. It is mainly accumulated in the kidneys (GGT level is 7000 times higher than in serum), liver (200–500 times higher) and pancreas.

In cells localized to the membrane, lysosomes and cytoplasm. It is considered as a good indicator of liver damage by pyrolysidine alkaloids (Dilov, P et al. 2005). Gamma-glutamyltransferase is a typical microsomal enzyme whose synthesis is strongly induced by some drugs, for example antipyretics, antibiotics and anticonvulsants. GGT content in mercury and mercury poisoning is increased in the urine (Dilov, P et al. 2005). In the diagnosis of acute and chronic kidney disease, a number of enzymes must be analyzed. In a study in dogs with chronic kidney disease, no increase in GGT or ALP was observed, but an increase in ALP was observed, which was associated with acute kidney disease (Heiene, R et al. 1991).

Lactate dehydrogenase (LDH) is a complex quaternary structure enzyme made up of four subunits (tetramer). The enzyme is represented by five isoforms that catalyze the same reaction but have different tissue expression and localization. Lactate dehydrogenase is a widely represented intracellular enzyme in the class of oxidative reductases. This enzyme catalyzes the nodal reversible reduction of pyruvate obtained in glycolysis to L-lactate under anaerobic conditions in the presence of an electron donor (NADH). Of greater diagnostic importance is the study of LDH isoenzymes. Increases in this enzyme are observed in toxic hepatitis. The lactate dehydrogenase enzyme has five isoforms, allowing it to be used for differential diagnosis between progressive myopathy and non-progressive neuro-muscular disease. The relationship between the individual isoforms for the purpose of accurate diagnosis can be traced (Brancaccio, P et al. 2010, Stadhouders, A et al. 1994). Bipyridyl derivatives have a specific organotropic effect on the lungs and significantly affect the isoenzyme profile of LDH, significantly decrease LDH 2, and LDH 1 completely disappears (Stoyanov, K et al. 1989). Glutamate dehydrogenase (GLDH, GDH) is mainly found in the liver and its location is in the mitochondria. Participates in urea metabolism. It catalyzes the reversible chemical reaction of converting glutamate to α-oxoglutarate.

The reaction that glutamate dehydrogenase catalyzes is:

$$\text{L-glutamate} + H_2O + NAD^+ \rightleftarrows \text{2-oxoglutarate} + NH_3 + NAD.H + H^+$$

The incorporation of ammonia into animal organisms occurs under the action of the enzymes glutamate dehydrogenase and glutamine synthetase. Glutamate plays a major role in the metabolism of nitrogen compounds, as it serves both as an acceptor and a donor of nitrogen in organic form. Increased levels of GDH in blood serum have been observed in various types of poisoning.

Sorbitol dehydrogenase (SDH) – The activity of SDH is highest in the liver, with insignificant amounts found in the kidneys and testes. SDH appears as a liver-specific enzyme. Localized in the cytoplasm of cells.

Creatine kinase (CK), (ATP, creatine phosphotransferase). Creatine kinase is an enzyme that catalyzes the synthesis and breakdown of creatine phosphate.

$$\text{Creatine} + ATP \leftrightarrow \text{Creatine phosphate} + ADP$$

The brain, the myocardium, the skeletal muscles, followed by the thyroid, the lungs and the kidneys, have the highest creatine kinase activity. The biological role of the enzyme lies in providing

creatine phosphate as a major source for ATP synthesis. Three isoforms of creatine kinase have been demonstrated in humans. The first isoenzyme consists of two subunits. It is located in the brain, lungs and prostate gland. The second isoenzyme is composed of one subunit, and is present at most in the myocardium. The third isoenzyme is composed of two subunits, and is located in transversely grooved muscle (Wallimann, T et al. 1992). According to other scientists, creatine kinase has at least five isoforms – three isoenzymes are located in the cytoplasm and the other two isoenzymes are located in the mitochondria (Stadhouders, A et al. 1994). According to the same author, the enzyme is used to diagnose different types of myopathies. Creatine kinase is an important biochemical indicator, and is used for all organs and systems (Wallimann, T et al. 1994). It is most commonly studied in animals with suspected myocardial infarction (heart attack), various muscle disorders, muscular dystrophy, and kidney injury (Moghadam-Kia, S et al. 1016). Strychnine poisoning is observed increase in creatine kinase levels but is not strictly specific (Dilov, P et al. 2005).

Alkaline phosphatase (ALP) is an enzyme (hydrolase) that catalyzes the hydrolysis of various phosphate esters with a pH optimum in the alkaline region. It is found in almost all organs, with a relatively higher concentration in the epithelial cells of the intestine, in the proximal renal tubules, bones (in osteoblasts – cells involved in bone formation), bile capillaries, liver, placenta, spleen and leukocytes. Increased levels of alkaline phosphatase in blood serum are important for the diagnosis of bone and liver disease. The values of jaundice are especially high due to obstruction of the biliary tract by various disease processes (mechanical jaundice).

In mixed types of hepatic impairment, an increase in GPT, GOT and ALP (rats and dogs) has been observed, which is related to histopathologic results (Hilaly, J et al. 2004, Fujii, 1997). Increased serum levels of ALP, CK, LDH have been observed with herbicide poisoning (fatty acid phenoxy derivatives) (Dilov, P et al. 2005). When poisoned with copper and copper compounds, the levels of the major liver enzymes – ASAT, LDH, SDH – increase in the blood serum.

Conclusion

Animal poisoning is still relevant today. There are many cases described in European countries (Belgium, France, Greece, Italy and Spain) in the last decade. Most cases were observed in horses and dogs, these being mostly younger animals. The majority of incidents in domestic animals are due to poisoning by insecticides, rodenticides (anticoagulants and strychnine). Horses and cats were the most susceptible to plant toxins. There are information on herbicide intoxication, metals, household products, veterinary and human health products, which have been isolated cases (Amacher, E., 2002, Berny, P et al. 2010, Raimon, G et al. 2010, Raimon, G et al. 2010, Norberto Ruiz-Suárez et al. 2015, Jennifer Pareja-Carrera et al. 2014, Jaime Rodríguez-Estival et al. 2012, Nebbia, C 2001).

Appropriate biochemical parameters must be used to accurately diagnose animal poisoning, and interpretation of all results is very important.

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