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POSSIBLE MECHANISMS OF METABOLIC CHANGES OF BIOCHEMICAL METABOLISM IN THE CONDITIONS OF EXPERIMENTAL ALLOXAN-INDUCED DIABETES MELLITUS (DM)

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ABSTRACT: Alloxan diabetes mellitus (DM) simulates acute metabolic stress in a different direction, whose starting point is primary lysis of β-cells in the pancreas. Disorders in the system of the major insulin-induced regulatory mechanism occur, initiating imbalance in the system of a living organism generally. Primary changes occur on the level of metabolic ways of the cells of the pancreas and liver: redistribution of mitochondrial and microsomal processes of oxidation with possible synthesis of new isoenzymatic forms of important metabolic enzymes, disturbance of synthesis of regulatory proteins and, as a result, degenerative changes in the cellular structure (lysis, necrosis and apoptosis). Despite the numerous and even contradictory points of view, the theory of cell lysis with the increase of transaminases activity is still predominant. Cytolytic syndrome leads to the destruction of liver cells, the concentration of serum GOT increases dramatically (probably due to the mitochondrial fraction). Under conditions of alloxan-induced diabetic rat model intensification of oxidative stress processes occur provoking changes, which are typical for chronic hepatitis. Alloxan diabetes serves as a classic example of free radical pathology. The next probable link involved in this pathogenesis will be nervous system and general cerebral dysfunction of the body. Numerous disputable data are the evidence of neuroendocrine regulation of the triple system where enzymatic activity or concentration of metabolic indices is the result of many cascade reactions or associated parallel conductors in the system of involved organs, first of all the pancreas and liver. Alloxan diabetes is an example of prior activation of the enzymatic systems. It serves to ensure metabolic changes under conditions of increased generation of hydroxyl radicals and disorders of transmembrane transport both between organelles inside the cells and in the intercellular space.

INTRODUCTION: The diabetes mellitus still remains nowadays an urgent issue despite the high social-economic development, which does not prevent Diabetes Mellitus (DM) type 1 and 2.

On the contrary, it is caused by the modern lifestyle, which includes unsystematic, irregular sleeping and eating regimes with bad habits together only stimulate the development of DM diseases and complications following it. According to the statistics database of people suffering from DM worldwide, which steadily grows. Based on International Diabetes Federation forecasts, there will be 552 million people suffering from it in year of 2030 and 642 million in 2040. The same data shows 1.198.5 thousands of people in Ukraine suffering, which is 2.99 % of population [1, 2].

One of the most urgent problems of modern diabetology at the moment is that it has not discovered a clear molecular mechanisms of inheritance of predisposition to DM type 1. There is no single generally accepted theory that can explain the data obtained in this area regarding the formation of this heterogeneous disease, which is a combination of genetic predisposition, immune reactivity, and influence of environmental factors.

To investigate DM, numerous models of this disease have been applied including surgical, chemical, endocrine, immune, genetic model, they can be applied to various groups of animals: rodents (linear and nonlinear), primates, pigs, cats and dogs [3, 4, 5].

In modern experimental diabetology streptozotocin, alloxan and dithizone induced diabetic rat models are the most preferable. According to statistical data during the period from 1996 to 2006 streptozotocin and alloxan were used as chemical factors to investigate metabolic aspects in the development of disease in 69% and 31% of cases respectively [6, 7, 8, 9].

Numerous publications of recent years show a consistently high interest of these models concerning the issue of forming different complications on the background of DM type 1 and 2, such as macrovascular disease, neuropathy, retinopathy, nephropathy, except this alloxan model provokes oxidative stress. According to modern conceptions, this one exactly is an essential moderator of these pathological states with the frequency of their appearance, which significantly exceeds the registered data.

It should be noted that neither alloxan nor streptozotocin-induced diabetic rat models obtained a considerable assessment of action against the ground of anti-diabetic and anti-neuropathic drugs. Therefore, the modern experimental studies of alloxan-induced DM are aimed at studying of pathological influence of this diabetic model on the system of organs, the forecasting of eventual metabolic changes on the DM's backgrounds, the simultaneous studies of medicines' and medical drugs' impact on the course of these processes [10-13].

One of the disadvantages of the chemical model of diabetes mellitus is a high probable manifestation of toxicity not only on the pancreas as the major target, but other organs and systems as well. Although, on the other hand, it enables to examine the most prevailing signs of DM on the body and form probable models of development concerning further complications against the ground of endocrine disorders [14, 15].

Therefore, investigations of DM models in association with different pathologies of other systems and organs correspond to topical issues of studying metabolic ways and links of a wide spectrum of biochemical, pathophysiological, pharmacological, and neuroimmunoendocrine aspects in the pathogenesis of this disease [16].

RESULTS AND DISCUSSION:
Characteristic of Alloxan-Induced DM: Alloxan model has enough supporters and adversaries of its application for experimental medicine since the history of its establishment was dated back to the beginning of the 50's in the 20th century (Dunn & Mc Lechie, 1943). What are advantages and disadvantages of its usage? An observed data gives a reason to talk about the development of oxidative stress model, as not only a single result of pancreatic tissue's damages but as well as an outcome of fundamental metabolic disorders of liver, adrenal glands and nervous system, which are the reflection of essentially diverse and many-sided effects [17].

The alloxan (mesoxalylurea is chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione) is a decay product of uric acid, from the outside, a white crystalline substance, which has a diabetogenic effect only when parenteral method of administration is used to study chemical experimental model of DM. It should be noted that the substance causes selective necrosis of the islets of Langerhans, including β-cells, without hitting the external zone of enzymatic secretion and allows to simulate DM without pancreatectomy [18, 19]. Alloxan is to be characterized as a weak acid similar to glucose, it is transported through the plasma membrane into cytosol of pancreas' cells and liver with assistance of GLUT2 transport system, but it does not inhibit its activity in any single case. In reverse, hyperglycemia and hypoinsulinemia stimulate gene expression of liver protein membrane-transport system of GLUT2 [19].

Analysis of scientific literature has found disputable data concerning the content of different biochemical indices in the blood plasma and serum of the examined animals under conditions of alloxan-induced diabetic rat models. For example, an average glucose content in rats was 232.2 ± 11.34 mg/dl evidenced by one author (Barysheva E.V., 2015) [20], 142.2 ± 12.60 mg/dl presented by the group of other scientists (Telushkin P. K. *et al.*, 2014) [21] and even within the range of 630.0 mg/dl (Poliakova V. V, 2013) [22], that can be indicative of a moderately pronounced hyperglycemia in the experimental animals and even hyperglycemic coma **Table 1**.

TABLE 1: THE AVERAGE LEVEL OF GLUCOSE IN THE BLOOD DEPENDING ON THE DOSE OF ALLOXAN AND THE DAY OF THE STUDY IN ANIMALS ACCORDING TO NEXT AUTHORS

No.	Author	Experimental animal	Control level of glucose (mg/dl)	Dose of alloxan (mg/kg)	Day of the study	Level of glucose during alloxan-induced DM
20	Barysheva E. V.	white nonlinear rats, males	104.4	170	30	232.2*
21	Telushkin P. K.	white nonlinear rats	95.4	135	15	142.2*
24	Bukchari S. S.	white nonlinear rats	79.2	100	7	95.4
				200		277.2*
				300		356.4*
				400		mortality
36	Lin H.	female New Zealand rabbits	95.4	100	28	286.2*
42	Nolasco E. L.	male Wistar rats	109.8	$42_{intravenous}$	10	552.6*
48	Kumar V.	male Swiss albino mice	76.7	150	3	220.0*
64	Ismail Z. B.	mixed breed dogs	73.0	120	28	327.0*

Note: the number (No.) corresponds to the numbering in the list of references;
alloxan-induced diabetes stimulated by means of intraperitoneal administration if there are no other marks;
* - indicate a significant difference between control and experiment levels; to converse concentration of glucose from mg/dl into mmol/L: mmol/L = mg/dl÷18

Gwarso M. Y. and co-authors (2014) consider the minimal threshold concentration level of sugar in blood 200 mg/dl for reaching the model of alloxan DM [23], according to the other group ≥250 mg/dl.

It should be noted that according to the literary data glucose level in the blood of animals with alloxan-induced diabetic rat models depends on the individual dose and the method of administration of alloxan (intravenous, intraperitoneal, subcutaneously). According to different literary evidence intraperitoneal administration in the following doses: 100 mg/kg [24, 25], 150 mg/kg [26], 160-170 mg/kg [20, 27] 200 mg/kg [28, 29, 30] of body weight in different solvents are preferred, which are the models to study a long-term toxic action of the drug. As a rule, purified water, 0.9% NaCl solution and acetate buffer (pH=4.5) are used as solvents to prepare alloxan for injections. It should be noted that even inconsiderable overdosage of alloxan is a probable cause of general intoxication of the body, especially for the excretory system of the kidneys that can be a cause of death during the first days of the experiment especially by using alloxan within the dose of 300-500 mg/kg of the animal's weight [16, 31].

The experimental dose for animals should be chosen very carefully to prevent excessive damage to the pancreas with the probability of high lethal outcome [24]. Therefore, it is obligatory to differentiate between sub compensated and decompensated states in the models of DM [32].

In rats and mice, two forms of the disease are found:

✓ Acute form resulting in death of animals during the first day of the experiment or in the period of 3-5 days after the drug administration due to stressful reverse of hypoglycemia (as a result of β-cells lysis of the pancreas with simultaneous release of extremely high amount of insulin) into hyperglycemia, due to damage and necrosis of insulin-producing cells when insulin is absent, or as a result of toxic alloxan action on other systems and organs;

✓ Long-term form of diabetes observed during 44 days in mice and 3-6 months in rats with adjustment to the alloxan-induced diabetic rat model.

It is an interesting fact that after diabetes of various duration even recovery of certain animals was observed (individual heterogeneity of individuals) [33], which is a partial regeneration of the β-cells population of the pancreas that did not experience destruction due to toxic action of alloxan. This can be explained not only by the size of the dose administered but also by the high resistance of young animals to the diabetic effect of alloxan and influence [34], for example, hormones (gastrin, epidermal growth factor [35], oxytocin [36]).

In addition, scientists characterize different phases of DM development. The majority of authors differentiate three clear phases in the development of alloxan-induced diabetic rat model replacing one another: short hyperglycemic (2-4 h) followed by hypoglycemic (4-8 h), and a long-term secondary hyperglycemic again. Another group of authors differentiates one more additional short hypoglycemic phase with maximum duration of 30 min preceding the primary hyperglycemic phase, but it was not found in all the experimental animals [37].

Initial momentary hyperglycemia is characterized by a maximal deficiency of insulin with simultaneous inhibition of glucokinase activity, making inclusion of phosphorylated glucose form into further metabolic ways impossible. It is reflected in functions of hepatocytes: alloxan disturbs the major metabolic regulation reactions of energetically important carbohydrate metabolism. Certain scientists evidence concerning 90% decreased activity of the liver glucokinase, disorders of its immunoreactivity and probability of pathogenesis development on the basis of primary effect on the liver, but not on the pancreas. Interestingly, the inhibition of glucokinase is achieved by the one-minute influence of the alloxan. The interaction of alloxan and glucokinase occurs with the formation of disulfide bonds, resulting in inactivation of the enzyme [25, 38].

In its turn, it decreases the level of glucose oxidation and involvement of ATP into this process: at the moment of initial hyperinsulinemia a short-term peak increase of this high-energy compound occurs, resulting in inhibition of the signal system involved in the stimulation of insulin secretion. So in terms of initial alloxan-induced diabetic rat model, the specific activity of total glycogen and intermediate products in the synthesis is sharply reduced.

It should be noted that the alloxan may also inactivate other thiol-enzymes, such as phosphofructokinase, aconitase, and Ca^{2+}/calmodulin-dependent protein kinase. However, precisely glucokinase, which plays a key role in glucose metabolism, has the greatest susceptibility to deactivation from all of thiol-enzymes [34]. In contradistinction to hexokinase, it acts only on D-glucose, it is not inhibited by the reaction product of glucose-6-phosphate (G-6-P) and has a high index of Km (the Michaelis constant). Diabetic patients have a significantly reduced activity of glucokinase, which leads to negative consequences: the pancreas does not produce enough insulin, the level of glucose remains high, and the synthesis of glycogen in the liver is significantly reduced because of low activity of a key enzyme.

Activity of Transaminases: A wide range of content in the studied parameters of blood serum or plasma for the selected model of alloxan matrix, also it is related to the average value of urea concentration, total protein, creatinine, the activity of enzymes [39, 40]. But the vast majority of data is characterized by a tendency to a significant increase in the activity of enzymes in terms of alloxan-induced DM **Table 2**.

It is known that serum glutamate pyruvate transaminase (GPT or ALT) EC [2.6.1.2], glutamate oxaloacetate transaminase (GOT or AST) EC [2.6.1.1], and γ-glutamyltransferase (γ-GT) EC [2.3.2.2] play a key role in the homeostasis of the liver and are the markers of hepatocellular damage [41]. It should be noted that the maximum activity of enzymes in plasma characterizes the simultaneous damage of a significant number of liver cells (tissue hypoxia, necrotic changes) [42]. While for the pancreatic cells there are more specific mechanisms of lysis and further apoptosis.

If the primary intracellular role of GPT is believed to be its participation in the combination of the metabolism of amino-, ketoacids, and tricarboxylic acid cycle, whereas in the case of more studied enzyme – GOT – there are several features to consider.

Firstly, it is involved in the urea cycle and malate-aspartate shunt across the mitochondrial membranes for the operation of the system NAD^+/ NADH.

TABLE 2: THE CHANGE IN ENZYME ACTIVITY IN BLOOD SERUM AND PLASMA WITHIN CONDITIONS OF ALLOXAN-INDUCED DM ACCORDING TO DIFFERENT AUTHORS

No.	Author	Experimental animal	Dose of alloxan (mg/kg)	Day of experiment	GPT control/ experiment (U/L)	GOT control/ experiment (U/L)	γ-GT control/ experiment (U/L)	Alkaline pH control/experiment (U/L)
11	Helal E.G.E.	male albino rats of Sprague Dawely	120	3	37/53*	22/33*	22/29*	-
19	Lucchesi A. N.	male Wistar rats	42 intra-venous	14	30/96*	36/87*	84/70	68/76
20	Barysheva E. V.	white nonlinear rats	170	30	45/107*	163/192*	-	-
39	Ahmad B.	Wistar male albino rats	100	7	75/117*	115/275*	-	142/194*
40	Umar S. A.	Wistar rats of both sexes	150	3	12/37*	16/42*	-	75/126*
64	Ismail Z. B.	mixed breed dogs	120	28	9/21	8/20	-	43/53

Note: the number (No.) corresponds to the numbering in the list of references; indicated average values of enzymes activity in groups of experimental animals; alloxan-induced diabetes stimulated by means of intraperitoneal administration if there are no other marks. * - indicate a significant difference between control and experiment

The above mentioned metabolic pathway is localized inside the cell; consequently enzymes need to be in a cell to perform its physiological role. It is pertinent to note that, at the moment, there is no known natural inhibitor of transaminases, which could contribute to regulation of enzyme activity and to explain the lack of correlation between concentration and enzyme activity. In addition, there is no data regarding the ratio of the activity of GOT and its immunoreactivity [43].

On the one hand, increased activity of GOT will be indicative of cellular lysis, on the other hand, increased activity of GOT might be associated with the necessity to eliminate the products of glycolysis from the organs being under conditions of hypoxia or increased energy losses by the liver.

Direct participation of GOT is known in transmembrane transmission of reduced potentials through the mitochondrial membrane and NADH regeneration formed due to glycolysis in malate-aspartate shunt. In this respect, increased activity of GOT is indicative of not only cellular lysis but of metabolic hyperactivity to ensure necessary biochemical mechanisms and physiological functions. One of the evidence to prove this theory is appearance of additional form of malatedehydrogenase (MDH₃) [EC 1.1.1.37] in animals with experimental diabetes as compared to the control group, where only two isoforms are present: MDH_1 and MDH_2. MDH_3 must be a peroxide isoenzyme of the liver cells synthesized due to oxidative stress to ensure the processes of glyoxylate shunt [44].

For another author, the activity of mitochondrial MDH, in condition of alloxan-induced DM, increases almost four times in comparison with the control group [33]. As to GPT activity, even under conditions of pronounced cytolytic syndrome of hepatocytes its mitochondrial fraction is not practically found in the blood according to certain scientific evidence [43].

In addition to determining the activity of GOT and GPT, it will be important also to assess changes in the activity of γ-GT in terms of alloxan DM. γ-GT is a microsomal and mitochondrial enzyme that characterizes the processes of oxidative stress of any nature, including those that arise as a result of diabetic ketoacidosis and general intoxication [45].

γ-GT is mainly localized in mitochondria of the liver cells, enabling to refer the diagnostics of this enzyme in the blood plasma to the processes of liver damage. γ-GT is localized in great amounts on the cellular membranes with high functional ability to secrete and reabsorb (pancreas, bile ducts).

A moderate increase of γ-GT activity is found in the case of pancreatitis and liver damages in contrast to DM. According to literature sources the level of γ-GT and CRP (C-reactive protein) are diagnostic markers of glycemic control [46].

In general, a slight increase in activity of γ-GT is not associated with impaired metabolism of bile acids, cholesterol, and lipids. Firstly, in case of damage of secretory cells of the bile ducts γ-GT activity increases at least 5-10 times. Secondly, lipid metabolism in rats (about 70% of alloxan model's studies are carried out on rats) differs from that of humans, due to the availability of special bile acids: α- and β- muricholic acids. They can accelerate metabolism of cholesterol, and their content is about 40% of all the bile acids in addition to anatomical absence of the gallbladder [47]. Of course, the following probable link of further damage, under conditions of diabetic oxidative stress, will be lipid metabolism as transport form between different systems of organs and sources of energy, but it will take place later.

On the other hand, the concentration of endogenous antioxidant – glutathione depends on the membrane γ-GT, which catalyzes the decomposition of glutathione in glutamine cycle, therefore an increase in its activity in the liver tissue and blood serum is a direct consequence of oxidative stress, as demonstrated by the positive correlation between increased activity of γ-GT, superoxide dismutase (SOD) [EC 1.15.1.1] and catalase [EC 1.11.1.6] [48]. If at the early stage of diabetes development (4 weeks) increased activity of hepatic catalase and SOD is observed due to high concentration of H_2O_2, then after six weeks a depletion of first line of antioxidant defense occurs. The high activity of SOD is related to the activation of γ-GT, on the other hand, exactly the increased activity of hepatic γ-GT can exacerbate oxidative stress, which is accompanied by increase of free-radical pathology [49]. As follows, it is possible to justify such numerous, diverse and even opposite changes in clinical-biochemical parameters of metabolic changes. Of course, the underlying model of alloxan-induced DM is to be observed in combination with the mechanism of cytotoxic effects on the pancreas, which leads to generation of reactive oxygen species and the "response" of the body to this stage.

One of the main causes of toxic damage of β-cells is considered to be a formation of hydroxyl radicals: alloxan at the presence of intracellular thiols, especially glutathione, is able to generate hydrogen peroxide with the following accumulation of oxygen active forms. Formation of hydroxyl radicals as the final reaction is responsible for the destruction of β-cells of the pancreas [4, 17, 20].

In case of oxidation-reduction reactions with the formation of active oxygen forms of the cycle are prevented, the development of alloxan-induced diabetic rat model can be prevented as well because neither alloxan molecule itself nor the product of its reduction – dialuric acid – are cytotoxic for insulin-producing cells. It should be noted that formed dialuric acid is oxidized to alloxan again. And it is this oxidation-reduction cycle that is a constant source of generation of superoxide radicals which are able to cause fragmentation of the nuclear apparatus of β-cells of the pancreas, due to repeated cascade reactions of disproportion.

At the beginning of the chemical models' study of DM Okamoto K. (1950) formulated the previous hypotheses, "zinc theory" of diabetes due to the action of the chemical agent – dithizone. The reason for ditizone diabetes is blocking the activity of metal ions since they are elements of the active centers of enzymes involved in the synthesis of insulin. The leading role among them belongs to zinc, the predominant content in the pancreas was observed in the area of β-cells insulin production [50, 51, 52, 53].

Studies show that during the development of streptozotocin DM, on the background of oxidative stress, the number of metalloproteins in the liver increases, but that is not correlated with the direct content of metals in tissues (with the exception of Zn). So metalloproteins can be used as a biomarker of oxidative stress that leads to peroxidation of the hepatocyte membrane and is a marker of the level of gene expression of metalloproteins [54].

By the way, alloxan as streptozotocin reduces gene expression of m-RNA in transport systems of liver cells, such as multi-drug resistance protein (Mdr) and multidrug resistance-associated protein (Mrp), organic anion transporters (Oatps) and organic

cation transporters (Octs), which leads to the disturbance of their functioning for the neutralization of toxic action of xenobiotics on the body [55].

If alloxan, dithizon and streptozotocin have a selective mechanism of damage of the same cells, and therefore, their patterns of lesions may have similar metabolic pathways. Patterns of lesions are based on the alternative ways of exchange in terms of DM: polyol, hexosamine and pentose phosphate pathways.

Recent studies indicate two important signaling systems in β-cells: the first for the secretion of insulin – CD38- cyclic-ADP-ribose (cADPR) and the other is the regenerating gene protein or Reg-receptor system for β-cell regeneration. Critical activation of both regulatory systems leads to excessive consumption of NAD^+, which leads to the depletion of both systems and subsequent cell damage [37, 50]. This is attributed to the violation of the mechanism of ingress of Ca^{2+} into the cell, leading to depolarization of membranes and the destruction of β-cells.

The analysis of literature sources concerning histological examinations of the liver samples from the experimental group of rats is indicative of the fact that administration of alloxan-induced DM manifested by morphological changes of the tissues: sign of toxic damage of the structure, necrosis of the liver cells, adipose and protein dystrophy similar to the processes of chronic hepatitis [30, 33, 56].

According to the results of numerous studies, pathomorphological changes occurring in the case of alloxan-induced diabetic rat model in the islet part of the pancreas and liver cells are associated with pathologic transformation of the main ways of the cellular metabolism [48, 57]. Changes occur in the functions of the key enzymes of Krebs cycle or tricarboxylic acid cycle (TCA), and in the enzymes of glyoxalate way, they are peculiar for animal organisms [56, 58].

A considerable increase of GOT activity under conditions of our experiment can be indicative of transient damage of both: the pancreas and liver. There is evidence that in case of alloxan-induced diabetic rat model, more pronounced disorders of the activity of proteolytic enzymes occur from the side of components of the elastolysis system into the direction of intensification of protease activity due to diabetic ketoacidosis. The consequence of such violations is tissue damage, which might possibly occur in the proteinase/anti-proteinase activity system by reducing the level of α1-proteinase inhibitor.

Alloxan administration provokes primary short-term hyperglycemic reaction, which cause is found in the indirect effect of the nervous system with its important links as the pituitary gland and adrenal glands [59, 60].

Primary hyperglycemia is a sign of adrenal glands reaction under circumstances of stress and β-cells lysis. There is certain evidence concerning hyper compensatory innervation of the pancreas as the organ of internal and external secretion. In case of administration of numerous chemical toxic substances with a selective action, including alloxan, certain degenerative changes of the nerve endings in the area of Langerhans islets are found [61, 62]. Hyperglycemia under conditions of insulin absence promotes activation of sorbitol way of metabolism with decreased formation of reduced glutathione due to redistribution of NADPH into the side of glucose aldehyde group reduction and its further transformation, especially along the way of the peripheral innervation including the pancreas.

Exhaustion of the level of NADPH makes the formation of reduced glutathione worse [63]. Therefore, the cycle is closed, and this circulation damages antioxidant balance causing oxidative stress with accumulation of free radicals.

According to literature sources and our views, alloxan might manifest general neurotoxic but not nephrotoxic action in the period of body adaptation to DM. However, activation of TCA can inhibit urea synthesis, which leads to intoxication, first of all, the brain intoxication with possible disorders of pH developing into acidosis (diabetic ketoacidosis). It is proved by the data concerning increased concentration of glucose, glutamate, aspartate, GABA, taurine and decreased activity of glutamine synthetase in the cerebral cortex under conditions of alloxan-induced diabetic rat model [60].

On the other hand, the nutritional introduction of taurine into an experienced animal, per os, as an intermediate product of the exchange among SH-containing proteins and peptides improves the energy and reduces oxidative stress, enhances the sensitivity of tissues to insulin [22]. This can be explained by the fact that sorbitol, as an alternative product of glucose metabolism, reduces the content of taurine, which is able to regenerate neurons.

Damage of the renal tissue is probably reduces amount of absorbed oxygen by 8-10%, this rest is used for oxidative processes. Death of experimental animals is direct evidence of it, but only during the first days of DM modeling (up to five days) due to the general processes of body intoxication. Also, nephrotoxic action of alloxan is known at early terms after its administration, associated with dystrophy and necrosis of certain areas of tubules and with overgrowth of the gently fibrous connective tissue (especially in cases of inconsiderable overdosage of alloxan). There is an opinion that it is the body reaction to general toxic effect of the agent, which is lasting the first days after its administration, especially when using large doses of alloxan (usually more than 400 mg/kg of animal weight) [8, 24, 64].

Ratio of Enzymes: Despite the numerous and even contradictory points of view, the theory of cell lysis with the increase of transaminases activity is still predominant. Cytolytic syndrome leads to the destruction of liver cells, the concentration of GOT increases dramatically (probably due to the mitochondrial fraction). There Ritis coefficient (GOT/GPT ratio) is increasing excessively over norm after alloxan administration, as a result of the signs of liver damage appear enzymologically. According to the literature, while modeling alloxan-induced DM, acute form develops on the 10th day and on 21th day – chronic form of this type of DM [65]. Such peak increase of GOT activity and hyperbolization of De Ritis ratio are markers of mitochondrial dysfunction of the liver cells and chronization of hepatotoxic processes.

High values of De Ritis ratio are indicative of active involvement of substrates in TCA and generalization of the main metabolic way. At the same time, general ways of carbohydrate metabolism become slow resulting in their gradual

exhaustion and imbalance. The 1.77 times increase of succinate dehydrogenase (SDH) [EC 1.3.5.4] activity in the condition of alloxan-induced DM is a result of substantial activation of TCA for the improvement of ATP and NADH delivery to the cells, can be seen as a prove for that [33]. It is indicative of a stressful character of the influence and overloading of the central metabolic ways, to ensure vital activity of the major systems of organs.

Changes in activity of transaminational enzymes indicate the state of the homeostatic function of the liver. Under circumstances of alloxan-induced diabetic rat model intensification of oxidative stress processes occur provoking changes characteristic for chronic hepatitis. The increase of activity of GOT and γ-GT happens practically in the same way, therefore their correlation with GPT activity is characterized by considerable growth. In comparison with the previous enzymes a little less increase of SGPT activity might characterize disorders in circulatory functioning of glucose-alanine cycle due to the activation of gluconeogenesis processes.

These are the ratios of serum γ-GT/GPT and serum GOT/GPT activity that characterize chronization of pathological processes in the liver [41, 66].

Oxidative Stress: It enables to follow liver damage resulting from the activation of free radical pathology under conditions of an alloxan-induced diabetic rat model. Insulin insufficiency promotes peroxide oxidation processes. Activation of inflammatory processes leads to increased formation of thiols with release of glutamine residues. It is evidenced by the data concerning increased activity of oxidative stress enzymes: superoxide dismutase (SOD) [E 1.15.1.1] and catalase [39, 48, 49], malone aldehyde content, glutathione peroxidase [23, 67], and metabolites of inflammatory mediators – "cysteine" leukotrienes, C4 and D4 in particular [68]. This chain reaction of oxidative pathology promotes development of general inflammatory process and vascular damage. The recent studies are indicative of the activity of γ-GT which is closely connected with such wide-spread metabolic diseases as cardio-vascular pathology and also type 1 and 2 diabetes mellitus [46, 67, 69].

From the data given in **Table 3**, there is a trend of decrease in the activity of antioxidant enzymes, because the conditions of alloxanian DM cells are characterized by extreme sensitivity to oxidative stress [39].

TABLE 3: THE ACTIVITY'S CHANGE OF ENZYMES OF ANTIOXIDANT PROTECTION IN THE CONDITIONS OF ALLOXAN-INDUCED DIABETES ACCORDING TO DIFFERENT AUTHORS

No.	Author	Experimental animal	Dose of alloxan (mg/kg)	Day of experiment	SOD control/ experiment	Catalase control/ experiment	GT-Red control/ experiment	GT-Px control/ experiment
15	Lucchesi, 2013	male Wistar rats (liver)	$42_{intravenous}$	30	10.7/6.8*	77.5/47.8*	---	10.6/5.8*
39	Ahmad B.	Wistar male albino rats (blood)	$100_{intraperitoneal}$	3	5.4/2.7*	674.4/315*	---	6.4/3.0*
48	Kumar V.	Swiss albino mice (blood)	$150_{intraperitoneal}$	3	6.1/3.9	72.0/48.8	---	---
57	Shah N. A.	male rats Sprague Dawley (pancreas), (liver)	$120_{intraperitoneal}$	3	6.8/1.8* 11.4/4.2*	7.8/2.7* 9.0/3.3*	- -	83.3/39.0* 99.6/42.0*
64	Sushko O.	white nonlinear rats (pancreas)	$150_{intraperitoneal}$	40	20.8/12.9*	5.9/3.2*	0.76/0.49*	47.1/37.8*

Note: No. means the number in the list of references; Activity of catalase in tissues: was calculated in terms of nmol H_2O_2 consumed/min per mg protein; in blood: IU/L. The activity of SOD in tissue: U/mg of protein; in blood: IU/L, GT-Red – glutation reductase, $\mu mol/min \times mg$ of protein; GT-Px – glutathione peroxidase, $\mu mol/min \times mg$ of protein; * - indicate significant difference between control and experiment levels

Since, the processes of gluconeogenesis and TAC are activated in the liver, in the nervous system due to sorbitol hyperosmolarity polyol, hexosamine ways are activated contrary to glycolytic one. TCA activation and excess of electrons promote activation of superoxide radicals and oxidative stress resulting in the destruction of cells.

Alloxan diabetes serves as a classical example of free radical pathology. The next probable link involved in this pathogenesis will be the nervous system and general cerebral dysfunction of the body [17, 60].

Under conditions of alloxan-induced diabetic rat model against the ground of stable hypoinsulinemia in the dynamics of pathological process development, a vector direction of metabolic processes is found for the preservation of hyperglycemia. Hyperglycemia is directly associated with increased activity of SGOT in the liver tissue evidenced by a number of studies [27, 59, 70]. Increased synthesis of glucocorticoids activates glucose synthesis from carbohydrate-free components by means of induction of mRNA synthesis of key enzymes of gluconeogenesis and aminotransferase. It is proved by twice as much increased index of the adrenal mass in the experimental animals under conditions of diabetes modeling as compared to the control data [8]. According to literary sources SGOT activity in the liver homogenates increases directly proportionally since the 9th to the 20th day of the experiment, in the dynamics of development of alloxan-induced diabetic rat model reaching the peak value in the period of formation of DM's chronic form [59].

Absolute insufficiency of insulin and hyperglycemia occurring due to alloxan diabetes are able to cause complex metabolic and osmotic disorders that, in their turn, are able to activate adrenocortical system [59].

Factor analysis found disorders of inter-systemic relations of the adrenocortical and renin-angiotensin systems in experimental diabetes which indicates disintegration of regulatory systems [27]. This disintegration can be a result of uncontrolled oxidative stress. It should be noted that due to insulin absence and general hyperglycemia we can observe the hypoglycemic effect in the insulin-dependent tissues (liver, muscles, adipose tissue) and "hyperglycocytosis" condition in the insulin-independent tissues (nervous tissue, vascular endothelium).

CONCLUSION: It should be noted that alloxan-induced diabetic rat model causes clinical and morphological type 1 DM as the majority of scientists state, but considering numerous ways and signs of its action reviewed above and according to the opinion of other authors it may possess signs of both type 1 and type 2 DM, and is an important instrument for testing pharmacological agents [34, 71, 72]. After all, excess glucose leads to synthesis of fatty acids and facilitates fatty degeneration of the liver with subsequent manifestation of steatosis and cirrhosis, which can be associated with type 2 diabetes on the background of cellular oxidative stress in alloxan action [11, 19].

On the other side, DM is a reflection of disorders in general endocrine mechanisms. Metabolic changes will differ in insulin-dependent and insulin-independent tissues due to diametrically opposite values of glucose content in them. It is an example of close interaction of the nervous, immune and endocrine systems underlying the basis of the hypothalamic regulation of one of the important factors of maintaining homeostasis – insulin.

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