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Effects Of New Derivatives Of 1, 2, 3 – Triazoles On Liver Glycogene And Some Biochemical Indicators In The Blood Of Alloxan-Induced Diabetic Rats

[1]Muydinov Izzatillo Ismatullayevich ,[2]Zaynabiddinov Anvar Erkinjonovich, [3]Pzilov Mamurjon Komiljonovich , [4]Kyrgyzov Fazliddin Bakhtoyarovich , [5]Komilov Kamariddin Urinovich , [6]Rakhimov Rakhmatilla Nurillayevich

[1]*Doctoral student of the Department of Human and Animal Physiology. Address: University street 129, Andijan region, Uzbekistan - 170100*

[2] *Doctor of Biological Sciences, Head of the Department of Human Physiology and Life Safety of Andijan State University.Address: University street 129, Andijan region, Uzbekistan – 170100,*

[3]Pzilov Mamurjon Komiljonovich- 3*Doctor of Biological Sciences, Senior Researcher at the Laboratory* molecular biophysics *of Institute of Biophysics and Biochemistry at the National University of Uzbekistan. Address: 100174, Tashkent city, Almazar district, Students town, University St., 174.*

[4]Kyrgyzov Fazliddin Bakhtoyarovich -4 *Doctoral student of Andijan Institute of Mechanical Engineering. Address: Bobur shah Street 56, Andijan region, Uzbekistan-170119*

[5]Komilov Kamariddin Urinovich - 5Candidate of Technical Sciences, Associate Professor, Department of Chemistry, Chirchik State Pedagogical Institute *of Tashkent region, Address: 111700, Chirchik city, Amir Temur avenue, 104*

[6]Rakhimov Rakhmatilla Nurillayevich- *6*Doctor of Philosophy (PhD) in Chemical Sciences, Senior Researcher at the Laboratory of practical experimental technology of Institute of Bioorganic Chemistry named after A.S. Sadikov. Address: 100125 Tashkent city, Mirzo Ulugbek district, Mirzo Ulugbek avenue, 83.

ABSTRACT

Impacts of heterocyclic compounds - new derivatives of 1, 2, 3 – triazoles to the amount of glycogen in the liver homogenate, the amount of glucose in the blood plasma, activity of aspartate aminotransferase (Al AT) and alanine aminotransferase (As AT) of the healthy and alloxan-induced diabetic rat were studied in *vivo* experiments. New derivatives of 1, 2, 3 - triazoles were administered to diabetes-induced animals once daily for 10 days through the *per os* method. It was determined that the amount of glycogen in their liver was almost close to the control (intact) level after pharmacotherapy of diabetes-induced rats with new derivatives of 1,2,3-triazoles. New derivatives of 1,2,3-triazoles were found to be able to restore AsAT and AlAT activity, which play a key role in carbohydrate metabolism in animal liver homogenates in the diabetes-induced model.

KEY WORLD: liver homogenate, alloxan, ISO-25, ISO -27 ва ISO -31, glycose, glycogen, AsAT, AlAT.

Introduction

Nowadays there is a growing interest in heterocyclic compounds because of the fact that they can restore tissue and cell disorders in various pathological conditions, and there is a need to study their mechanisms of action. The synthesis of species of heterocyclic compounds belonging to different pharmacological groups that are not toxic to tissues and cells and studying their biological activity is one of the important tasks facing modern pharmacology and physiology[16]. In recent years, the study of the biological activity of triazoles has become an important research object [12]. Currently, many types of researches are being conducted to screen the biological activity of five-membered heterocyclic compounds containing three nitrogen atoms[17].

New derivatives of 1, 2, 3-triazoles are one of such classes of heterocyclic compounds that stands out in the structure, diversity, high biological activity, and low toxicity [11]. Thereby, these derivatives of 1, 2, 3-triazoles called ISO-25, ISO-27 and ISO-31 may have cardioprotective, hepatoprotector, neurodegenerative, and antidiabetic properties. In pharmaceuticals, 1, 2, 3-triazoles are widely used against inflammation [3,5]. In addition to this, 1, 2, 3-triazoles have biologically active properties and are considered active against microbes, leishmaniasis, and diabetes [2,7].

The antioxidant and antiradical activity of new derivatives of 1,2,3-triazoles called ISO-25, ISO-27, and ISO-31 and their effect on the amount of glycogen in liver tissue, blood glucose, As AT, and Al AT enzyme activity changes under diabetic condition, was investigated. Glycogen and glucose production function of the liver plays a special role in the activity of the organism[1,2]. This is based on the fact that on the one hand, it synthesizes glycogen from glucose in the blood, on the other hand, it breaks down glycogen into glucose and releases it into the bloodstream, depending on the energy needs of the body. In this regard, many scientific laboratories around the globe are currently conducting intensive scientific researches. Our experiments are also an integral part of scientific research on the restoration of cellular disorders using heterocyclic compounds and the evaluation of the mechanisms of their action under diabetic condition. Therefore, the synthesis of new derivatives of 1, 2, 3-triazoles, the determination of their structure, and the search for substances with biological activity on their basis is an actual task[5].

The aim of our study was to determine the effect of new derivatives of 1,2,3-triazoles called ISO-25, ISO-27, and ISO-31 on the amount of blood glucose, glycogen in liver homogenate, AsAT and AlAT activity in alloxan-induced diabetic rats.

MATERIALS AND METHODS.

The research was performed on male, white, non-breeding rats with a weight of 180–200 gr. Alloxan monohydrate solution was used to create experimental diabetic conditions in animals. In order to induce diabetes, alloxan monohydrate solution was injected once at a dose of 150 mg/kg into the subcutaneous area of the abdomen after one day of starvation[6]. The experimental animals were divided into five group:

I – control;

II – alloxan diabetes 150 mg/kg (n=6);

III –alloxan diabetes + ISO-25 40 mg / kg (n = 6);

IV -alloxan diabetes + ISO-27 15 mg / kg (n = 6);

V - alloxan diabetes + ISO-31 25 mg / kg (n = 6);

Animals in groups II, III, IV, and V were injected once a day by dissolving 150 mg/kg alloxan monohydrate in physiological saline (0.2 ml / 100 mg). After twelve days since the injection of alloxan monohydrate in rats, when the blood glucose level exceeded 11 mmol / l, new products of 1,2,3-triazoles were administered once a day to animals in group III from ISO-25, to animals in group IV from ISO-27, and to animals in group V from ISO-31 for 10 days through *per os* method. The amount of glucose in the blood was determined by the method of glucose oxidase ("Glucose - enzymatic-colorimetric test", Cypress diagnostic, Belgium). Blood glucose is calculated according to the following formula:

$C = (E0 / Ek) \cdot 101$

C - the amount of glucose in mmol/l;

E0 - the optical density of the experimental sample;

Ek - the optical density of the calibration sample;
10 - the amount of glucose in the calibrator in mmol/l
The amount of glycogen in the liver was determined by the anthrone method. The freshly obtained animal liver (0.5 g) is placed into a test tube containing 3 ml of 30% caustic potassium and kept in a boiling water bath for 20-30 minutes to make a homogeneous solution. Then, 4 ml of 96% ethyl alcohol was added into the solution, mixed well with a glass rod, and held in a water bath for another 30-40 seconds. The solution is cooled down in the water and centrifuged at 3000 rpm for 15 minutes. After that, the precipitate was dissolved in distilled water, poured into a 100 ml flask, and, mixed well, bringing the volume of the solution to 100 ml. 1 ml of this solution and 1 ml of standard glucose solution were collected in two separate flasks, and 1 ml of distilled water was sampled into two other flasks and used as a control. Later, 6 ml of anthrone reagent was mixed with all samples and placed in a boiling water bath for 10 minutes. After cooling in cold water, the standard and experimental samples compared to control were calorimetered in cuvettes of 660 nm 10 ml with a red filter. The activity of AlaT and AsaT in the serum of experimental animals was performed using a generally accepted method. Pyruvic acid is formed by the transition reaction of amino groups under the influence of enzymes that ensure the transition of amino groups of aminotransferases from amino acids to keto acids. When 2,4-dinitylphenylhydrozine (2,4-DNFG) is added to this mixture, pyruvic acid hydrazone was formed, which gives color in an alkaline environment. The obtained color is directly proportional to the amount of pyruvic acid. Plasma glucose and glycogen, ASaT, ALaT in liver tissue levels were determined at a wavelength of 540 nm using a Roche Hitachi 912 Chemistry Auto-Analyzer (GMI Inc., MN, USA) device.

RESULTS AND DISCUSSIONS

In the experiments, the alloxan-induced diabetic animals groups were administered new derivatives of 1,2,3-triazoles ISO-25 (40 mg/kg), ISO-27 (15 mg/kg) and ISO-31 (25 mg/kg) orally for 10 days. Blood glucose levels of pharmacotherapeutic animals were checked every 3 days. According to the results, the control group did not show any dynamics and was 5.7 mmol/l for glucose in the blood of animals. Twelve days after alloxan administration, blood glucose levels in animals groups of II, III, IV, and V were found to have exceeded 11 mmol / l.
10 days of administration of the test substances to these animals in groups III, IV, and V, resulted in their blood glucose level to be 12.3 mmol/l, 7.7 mmol/l, and 10.5 mmol/l, respectively (Fig-1). At this time, it was detected that the blood glucose level of animals in group II, (alloxan-induced diabetes) reached 18.5 mmol / l (Table 1). New derivatives of ISO-25, ISO-27, and ISO-31 of 1,2,3-triazoles showed hypoglycemic properties by reducing the amount of glucose in the blood plasma of alloxan-induced diabetic rats. In this case, the hypoglycemic property of the product ISO-27 was clearly manifested. Hyperglycemia occurs in insulin deficiency, increased excitability in the upper nervous system, increased hormonal functions of the thyroid gland, adrenal cortex, increased levels of glucocorticoids in the blood causing increased glucose production from proteins as well as inhibiting the utilization of glucose in tissues. Moreover, hyperglycemia is observed when the adrenocorticotropic hormone is released more than necessary from the pituitary gland stimulating the processing and secretion of glucocorticoids by the adrenal cortex[14]. The hypoglycemic activity of ISO-27 in hyperglycemic conditions may affect to increase glucose adsorption on cell membranes and increase the function of glucose transporters. Glycogenesis processes in the liver

play an important role in the maintenance of glucose homeostasis in blood plasma[1]. Glycogen is an important reserve polysaccharide for the body, which can be up to 20% in the liver. In diabetic conditions, depletion of glycogen is observed due to the violation of glycogen synthesis in the liver. Decreased insulin secretion in type I diabetes results in decreased glycogen synthesis in the liver and muscles. Some flavonoids have been found to reduce the activity of the antioxidant enzymes catalase, glutathione, and superoxide dismutase in liver tissue when given orally at 10 mg/kg for 10 days in rats (alloxan-induced diabetes). It also reduces the activity of serum cholesterol and hepatic glucose-6-phosphatase due to the pharmacotherapy of animals with flavonoids in conditions of hyperglycemia[8]. The blood glucose levels of the experimental animals were checked every 3 days. The animals were decapitated and the amount of glycogen in their liver tissue was determined after the blood glucose level diminished and came close to the control values. The effect of new derivatives of 1,2,3-triazoles such as ISO-25, ISO-27, and ISO-31 on the amount of glycogen in the liver tissue of rats in alloxan-induced diabetes is presented in Table 1 below.

Table-1

Effect of new derivatives of 1, 2, 3-triazoles such as ISO-25, ISO-27, and ISO-31 on the amount of glucose of blood plasma and glycogen in liver tissue in alloxan-induced diabetic rat (M ± m).

№	Animal groups	The amount of glycogen (mg / 100 g for body weight	The amount of glucose (mmol / l)
I	Control (Intact)	781,8±41,4	5,7±0,8
II	Alloxan diabetes	389,2±20,1**	18,5±1,4**
III	Alloxan diabetes + ISO-25	467,4±22,5*	12,3±1,1*
IV	Alloxan diabetes + ISO-27	639,2±32,6*	7,7±0,9*
V	Alloxan diabetes + ISO-31	537,6±28,8*	10,5±1,0**

The results showed that the amount of glycogen in the liver of animals of group II caused by alloxan-induced diabetes was reliably diminished by 50.2% compared to the control group (group I). Impaired glycogen synthesis in the liver in alloxan-induced diabetes may be associated with decreased glycogen synthase activity and decreased glucose oxidation due to a deficiency in the pyruvate dehydrogenase complex. However, when rats in group III, IV, and V were performed pharmacotherapy with new derivatives of 1,2,3-triazoles such as ISO-25, ISO-27, and ISO-31, their liver glycogen levels increased compared to the alloxan-induced diabetes group (Table 1). Oral administration of ISO-25 to animals' in-group III revealed a 10% increase for glycogen in their liver tissue in comparison to diabetic group. While ISO-27 and ISO-31 products were administered orally to group IV animals, their glycogen levels in the liver climbed by 31.9% and 18.9%, respectively, in contrast to the alloxan diabetic group (group II) (Table 1). It was determined that a new derivative of ISO-27 of 1,2,3-triazoles effectively increased the amount of liver glycogen in alloxan diabetes. In this case, one of the mechanisms of restoration of blood glucose concentration in diabetic animals after the adoption of a new product ISO-27 may occur because

of the restoration of carbohydrate metabolism between blood and liver due to changes in glycogenesis processes.

Liver tissue cells are very sensitive to various endogenous and exogenous factors, and the response to them occurs primarily through changes in enzymatic system activity in hepatocytes [4]. In addition to this, the activity of enzymes in the blood and liver may change. Of these enzymes, aspartate aminotransferase (AsAT) and alanine aminotransferase (AlAT) are found in many tissues, and their main activity is represented in the liver [15]. Therefore, the appearance of AsAT and AlAT activity in the blood indicates the presence of pathological changes in the liver. In our next experiment, a reliable change in the activity of the enzymes AsAT and AlAT in the liver homogenate of animals in alloxan diabetes, and restoration of these changes due to new derivatives of 1,2,3-triazoles was observed. In liver homogenate, changes in the activity of AsAT and AlAT enzymes were indicated to have a pronounced value, that is, the activity of AsAT and AlAT was taken as a 100% indicator of control (Fig-1 and 2). The results showed a sharp increase in the activity of the enzyme AsAT in the liver homogenate of animals in alloxan diabetes group by 75.5 ± 7.5% compared to the control group. New derivatives of 1,2,3-triazoles in animals of groups III, IV and V caused by alloxan diabetes were administered ISO-25 (40 mg / kg), ISO-27 (15 mg / kg) and ISO-31 (25 mg / kg) orally during the 10 day, the activity of the enzyme AsAT in their liver homogenate declined by 19.9 ± 2.1%, 51.1 ± 4.4 and 33.2 ± 2.4%, respectively (Fig. 1).comparing to the diabetic group.

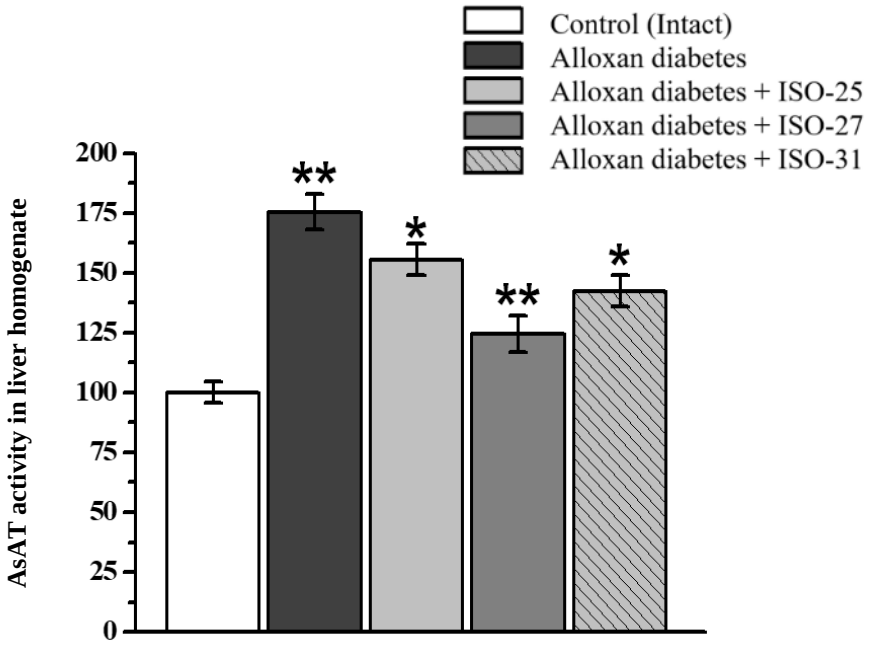

Figure 1. Influence of new derivatives of 1,2,3-triazoles ISO-25, ISO-27 and ISO-31 on the activity of AsAT in the liver homogenate of healthy and diabetic animals (in all cases * P <0.05; ** P <0.01; n = 4-5).

Continuing our experiments, the effect of new derivatives of 1,2,3-triazoles on the activity of the enzyme AlAT in the liver tissue of alloxan-induced diabetic rats was also studied. AlAT enzyme activity in the liver homogenate of animals of the alloxan diabetic group went up sharply by 115.4 ± 9.5% than control sample. Animals in groups III, IV, and V received oral administration of the

drug substances ISO-25 (40 mg / kg), ISO-27 (15 mg / kg), and ISO-31 (25 mg / kg) for 10 days and showed a decrease in the activity of the AlAT enzyme in the liver homogenate. In this group, results suggested that under the influence of ISO-25, the activity of the enzyme AlAT in the liver homogenate of diabetic rats decreased by 41.7 ± 3.5% compared to group II (group II). After new derivatives of ISO-27 and ISO-31delivered to animals in groups IV and V under this condition, their AlAT enzyme activity in the liver homogenate was 80.2 ± 6.8% and 64.9 ±, respectively.

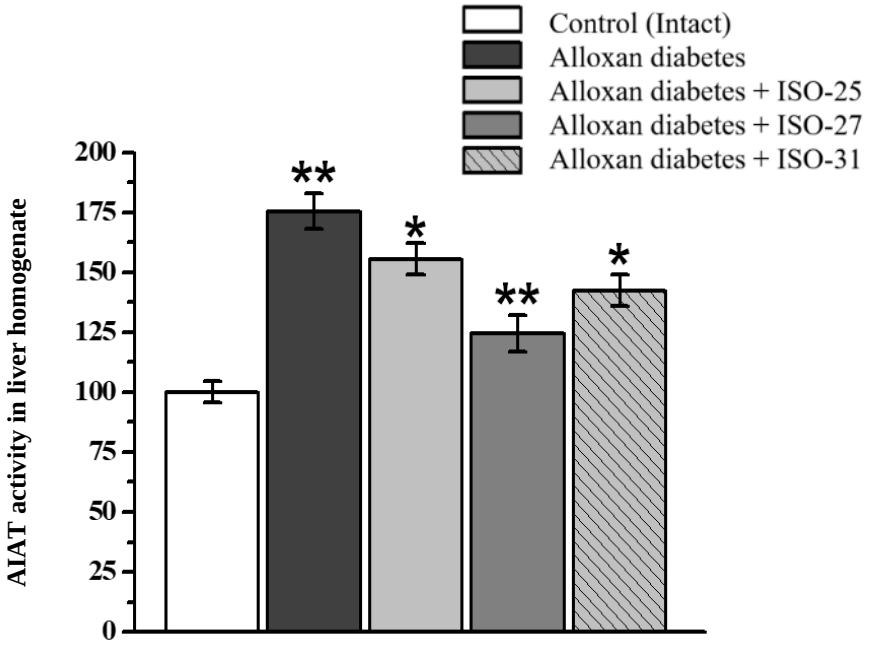

Figure 2. Influence of new derivatives of 1,2,3-triazoles ISO-25, ISO-27 and ISO-31 on AlAT activity in liver homogenate of healthy and diabetic animals (in all cases * P <0.05; ** P <0.01; n = 4-5).

Hence, the increase in AsAT and AlAT activity in the liver under experimental diabetes may be associated with disruption of the antioxidant system in the animal body. It is stated that in the destruction of the membrane structures of hepatocytes are mainly products of lipid peroxidation [4]. Thus, new derivatives of 1,2,3-triazoles were found to be able to restore the activity of AsAT and AlAT, which play a key role in the metabolism of carbohydrates in the liver homogenate of animals in the ISO-25, ISO-27 and ISO-31 alloxan diabetes model. Because of the treatment performed, it is deteremined that the experimental group restores the function, structure and stability of the cell membrane, which forms the basis of hepatocytes in animals. In the future, the development of such research will serve as a fundamental basis for the development of new antidiabetic and hepatoprotective drugs against diabetes.

CONCLUSION

1. Triazoles reliably reduced the amount of glycogen in the liver in alloxan diabetes, and the efficacy of ISO-27 from these triazoles was evident.
2. New derivatives of triazoles have shown hypoglycemic activity by lowering blood glucose levels in alloxan diabetes. The hypoglycemic activity of ISO-27 triazole was noted to be effective.

3. New derivatives of triazoles have been shown to reduce the activity of the enzymes AsAT and AlAT in liver homogenate in alloxan diabetes.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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