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# CHANGE OF ANTIOXIDANT SYSTEM IN DIABETIC MODEL RAT LIVER CELLS AND ITS CORRECTION WITH COMPLEX COMPOUNDS

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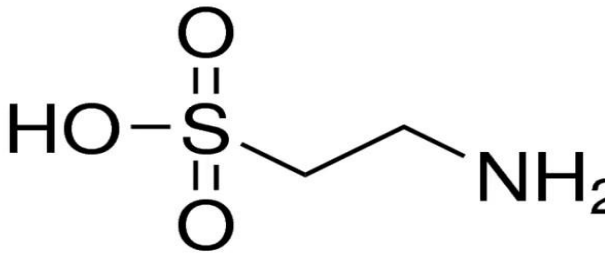
**Abstract:** Diabetes is the most common disease associated with metabolic disorders. Diabetes mellitus is characterized by dysfunction of pancreatic beta cells. Taurine is a β-amino acid that is widely distributed in mammalian tissues and is not involved in protein synthesis. The purpose of this study was to study the effect of a complex combination of taurine and Schiff's base derivative on the antioxidant system in the liver of model rats with diabetes. 12 male white rats were divided into 4 groups: Control, diabetic (QD), diabetic treated with Metformin (QD+Metformin), diabetic treated with Taurine and Schiff base (QD+Tau/Schiff base). Rats were injected subcutaneously for 3 days at a dose of 140 mg/kg of alloxan to induce diabetes. It was observed that blood glucose content and daily water intake increased during two weeks, as well as body weight decreased. The 3rd and 4th groups with diabetes were treated with metformin and complex combination for 1 week. Treatment with taurine reduces the decrease in liver catalase and protein content and the increased levels of glutamyl transferase (Gt), alanine aminotransferase (AlAt) and aspartate aminotransferase (AsAt) in the blood. In addition, it was found to reduce the level of malondialdehyde (MDA), a secondary metabolite of lipids in the liver and blood. These results indicated that taurine and schiff base complex are effective in alleviating diabetes by reducing oxidative stress and blood glucose levels.

**Key words:** Diabetes, metformin, taurine, Schiff's base, alloxan, antioxidant, lipid peroxidation, liver, alanine aminotransferase, aspartate aminotransferase, malondialdehyde

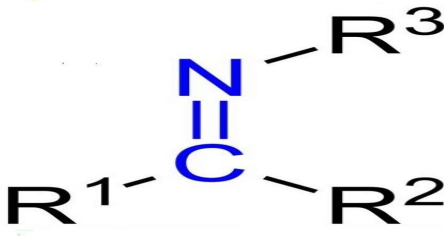
## INTRODUCTION

More than half a billion people worldwide live with diabetes, affecting men, women and children of all ages in every country, and this number is expected to more than double to 1.3 billion in the next 30 years. The current global prevalence is 6.1%, making diabetes one of the top 10 causes of death and disability. It is projected to rise to 16.8% by 2050. This means an increase from 529 million to 1.3 billion. Approximately 3.5 million people worldwide die each year due to diabetes. Almost all of the 16 risk factors studied were found to be associated with type 2 diabetes (QD2) [1,11]. And type 1 diabetes (QD1) accounts for 5% to 10% of all diabetes (depending on the region of the world). It occurs suddenly due to acute illness and destroys insulin-producing beta cells in the pancreas through autoimmune diseases leading to complete deficiency [5]. Type 2 diabetes is associated with pancreatic β-cell damage and decreased insulin production [9]. Disorders of carbohydrate, lipid and protein metabolism play a key role in the complications of diabetes [6]. Diabetes is a metabolic disease that is usually accompanied by an increase in the level of free radicals and a decrease in the concentration or activity of antioxidants [3]. Oxidative stress is an imbalance between the systemic expression of reactive oxygen species and the biological system's ability to rapidly neutralize reactive mediators or repair damage. Hyperglycemia can increase oxidative stress through several pathways. The main mechanism is intracellular reactive oxygen species (ROS) produced by the proton electromechanical gradient produced by the mitochondrial electron transport chain and induced by hyperglycemia, which leads to increased superoxide production [8]. Taurine is a conditionally essential amino acid, which makes up 0.1% of the human body weight, is not used in protein synthesis and is never added to muscle proteins, and therefore appears in the body as a free

molecule or simple peptides [7, 12]. Although taurine is unable to directly scavenge classical ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide, there are many studies showing that it is an effective inhibitor of ROS generation [10]. Hyperglycemia and hyperinsulinemia decrease taurine transporter activity, depriving the affected cell of a potentially important endogenous substance. Serum taurine depletion is associated with many oxidative stress pathologies, and taurine supplementation has been shown to improve these pathologies and their complications. Various physiological functions and roles are modulated by taurine, including: antioxidant, osmoregulation, membrane stabilization, bile acid conjugation, neuromodulation, detoxification, and regulation of calcium homeostasis. Clinically, prophylactic and therapeutic taurine supplementation has been shown to be beneficial in a wide range of oxidative stress-induced pathologies and clinical conditions, including: hepatotoxicity and liver disease, alcoholism, Alzheimer's disease, developmental delay, retinal degeneration, and diabetes [ 10,12].




Chemical structure of taurine




Schiff base

is a compound with the general structure R $_2$ C=NR and is considered a subclass of imines, (imines are compounds consisting of a carbon-nitrogen double bond) which are either secondary aldehydes or secondary ketimines depending on their structure. Schiff bases are the most popular organic compounds. They are used as dyes and pigments, catalysts, steps in the synthesis of organic compounds and stabilizers of polymers. Schiff bases exhibit a wide spectrum of biological activities such as antibacterial, antifungal, antimalarial, anti-inflammatory, antiproliferative, antiviral and antipyretic. In addition, it has a carrier property [13]. In our study, it is aimed to further increase the efficiency of taurine by using its carrier properties.

## 2. MATERIALS AND METHODS

### 2.1 Animals

The use of laboratory animals was carried out in accordance with the International Organization for Medical Sciences Council's code of ethics for animal experiments and the norms confirmed by the glucose oxidase method. Experiments were carried out on male rats weighing 210-290 g, kept in standard vivarium conditions.

### 2.2 Experimental groups

Male white rats were divided into the following 4 groups (n=3): Group 1 healthy (control), group 2 diabetic (QD), group 3 diabetes treated with Metformin 150 mg/kg (QD+Metformin), group 4 was treated with 50 mg/kg of diabetes+Taurine and Schiff's base complex (QD+Tau/Schiff's base). Animals were monitored for 2 weeks and groups 3, 4 were treated for 1 week.

### 2.3 Creating a diabetes model

In groups 2, 3 and 4, alloxan was used to create a diabetes model. It was injected subcutaneously at a dose of 140 mg/kg for 3 days. After 3 days, their blood glucose levels were measured. The blood glucose level of the rats was higher.

### 2.4 Measurement of blood glucose

A glucometer from Satellit was used to measure blood glucose. Blood was collected from the tail tip of the rats. A test stick was attached to the glucometer device and blood was drawn into the special part. The glucometer showed the amount of glucose in the blood of rats within 5 seconds. Blood glucose levels were measured before the start of the experiment and after 2 weeks.

**2.5 Quantification of MDA**

When measuring the degree of lipid peroxidation, the amount of its secondary product, malondialdehyde, is measured. 1 ml of tissue homogenate was carefully taken and mixed with 2 ml of TCA-TBA-HCl solution and heated in a water bath for 15 minutes. After cooling, the precipitate was separated by a centrifuge, and the remaining part was measured in a spectrophotometer at a wavelength of 535 nm.

**2.6 Determination of protein content**

The amount of protein in the liver homogenate was measured by the Lowry method, in which $Cu^{2+}$ ions form a complex with peptide bonds in an alkaline medium and become a $Cu^{2+}$ complex. Monovalent copper ions react with Folin's reagent (phosphomolybdic acid with phenol) to form an unstable product that turns molybdenum blue, with a maximum absorption at 750 nm. The increase in absorbance at 750 nm is proportional to the protein concentration.

**2.7 Determination of catalase activity**

The color intensity is measured in a spectrophotometer at a wavelength of 410 nm against a sample containing 2 ml of $H_2O$.

| Reagents | Control | Experience | Reminder |
|---|---|---|---|
| $N_2O_2$ | 2 ml | 2 ml | - |
| Serum or liver homogenate | - | 0.1 ml | 10 minutes $37^0$ C |
| $N_2O$ | 0.1 | - | |
| $(NH_4)_6Mo_7O2_4$ | 1 ml | 1 ml | - |

Catalase activity in blood serum and tissues is determined by the number of catalase and is found by the following formula [1].

(μkat/l) $E= (A_{control} - A_{experiment}) *V*t*22.2$

**2.8 Measurement of glutamyl transferase**

2 different reagents are needed to measure GGT. 1-Reagent Tris Buffer 100 mmol/L, NaCl 5 mmol/L, glycylglycine 125 mmol/L, 2-Reagent Tris Buffer 100 mmol/L, L-gutamyl-3-carboxy-4-nitroaniline. Simple is stable for 7 days at 2-8 C.

1-Reagent 100 ml, 2 reagent 50 ml, sample amount 25 ml, main wavelength 405-420 nm, temperature 37 C, passage 1 cm, test time 60-120 sec, absorbance line 0 -2A [11].

**RESULTS OBTAINED AND THEIR ANALYSIS**

**The amount of glucose in the blood.**

In our experiments, we found that the blood glucose level of diabetes model animals changes. The amount of glucose in the blood of healthy animals is equal to 5.6 mmol/ml. It was noted that the amount of glucose in the blood of diabetic model rats created with alloxan increased by 15.5 mmol/ml compared to a healthy animal. 150 per body weight of sick animals for 7 days after that mg/kg Metformin and 20 mg/kg taurine and Schiff base complex were administered. At the end of the experiments, the amount of glucose in the blood of the animals was checked. Glucose levels were found to decrease by 7.2 mmol/ml in metformin-treated animals compared to model animals. It was found that the amount of glucose in the animals

injected with the complex compound was lower by 6.7 mmol/ml compared to the diseased animals.

**Figure 1**

**Changes in the amount of glucose in the blood of alloxan diabetes model animals and the effect of the complex compound on it (M±m, n=3)**

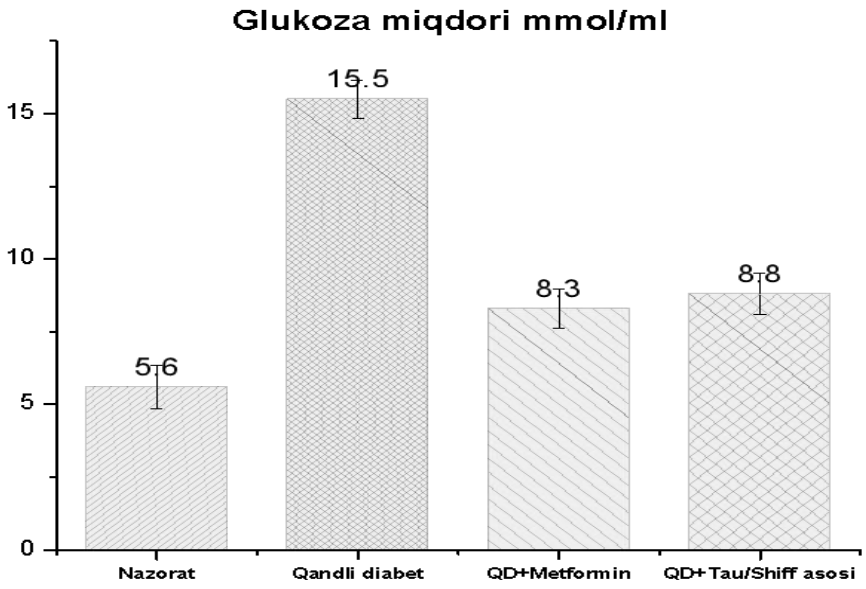
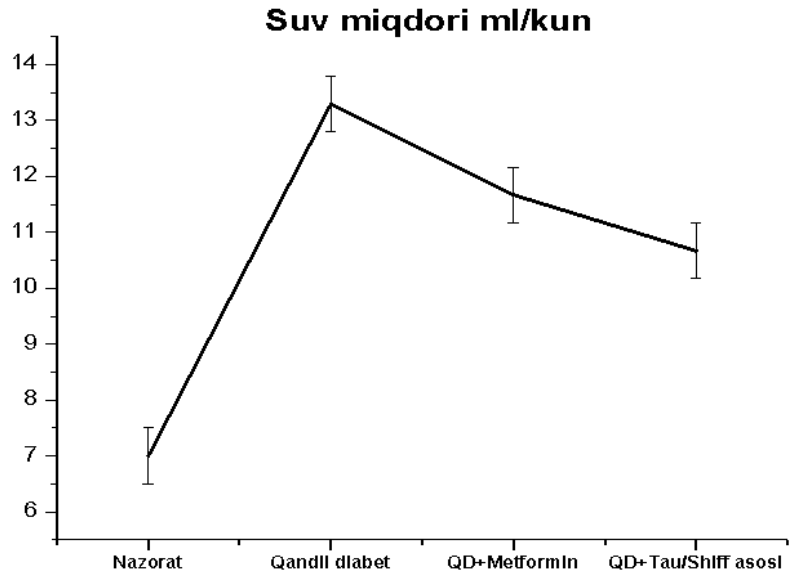



**Figure 2**

**Changes in the daily water intake of alloxan diabetes model animals and the effect of complex compounds on it (M±m, n=3)**

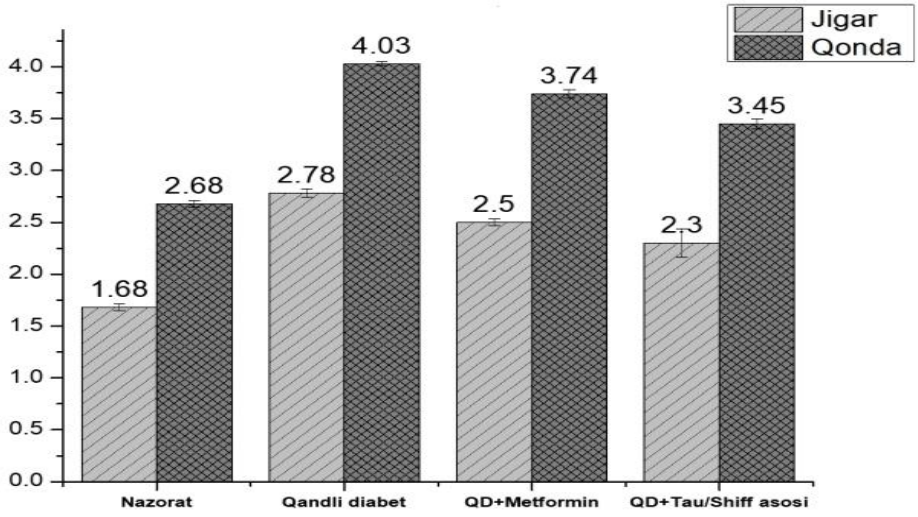



**Change in the amount of MDA**

liver and blood was higher in diabetic animals compared to healthy ones, but after 7 days of treatment, it was decreased under the effect of metformin and taurine and schiff bases. In addition, we can see that our complex has a more effective effect than metformin.

**Figure 3**

**Changes in the amount of MDA in the liver homogenate and blood of alloxan diabetes model animals and the effect of the complex compound on it (M±m, n=3)**



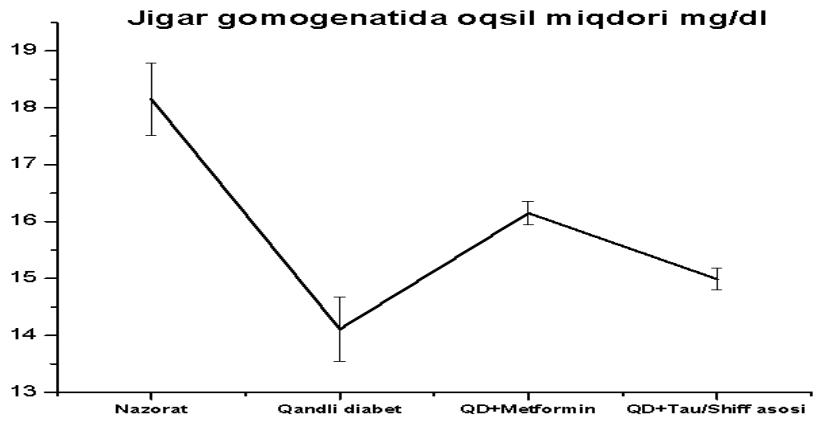

**Changes in the amount of protein**

It was noted that the total amount of proteins in the liver of healthy animals is 18.5 mg/dl, and the total amount of proteins in the liver of diabetic model rats is 14.11 mg/dl. At the end of the experiments, the livers of the animals were isolated and their total protein content was

determined. We can see that total protein was 16.15 mg/dl in metformin-treated animals. When we administered taurine and Schiff's base derivative to the alloxan model, it was found that the total protein content in his liver was equal to 14.99 mg/dl.

**Figure 4**

**Changes in the amount of protein in the liver homogenate of alloxan diabetes model animals and the effect of the complex compound on it (M±m, n=3)**




**Changes in the amount of catalase**

We can see that the amount of catalase decreased in the diabetic group and increased in the metformin group after 1 week of treatment. In the effect of our complex drug, the amount of catalase was found to be much higher than in the group treated with metformin.

**Table 1**

**Changes in the amount of catalase in the liver homogenate and blood of alloxan diabetes model animals and the effect of the complex compound (M±m, n=3)**

| Experimental groups | Catalase µKat/mg protein (in liver homogenate) | Catalase µKat/mg protein (in blood) |
|---|---|---|
| Control | 50.40 ± 1.43 | 37.92 ± 0.74 |
| QD | 39.57 ± 0.35* | 22.9 ± 1.11 |
| QD+Metformin | 44.77 ± 1.08 | 28.11 ± 0.84 |
| QD+Taurine and Schiff base derivatives | 47.13 ± 0.73* | 30.13 ± 0.95* |

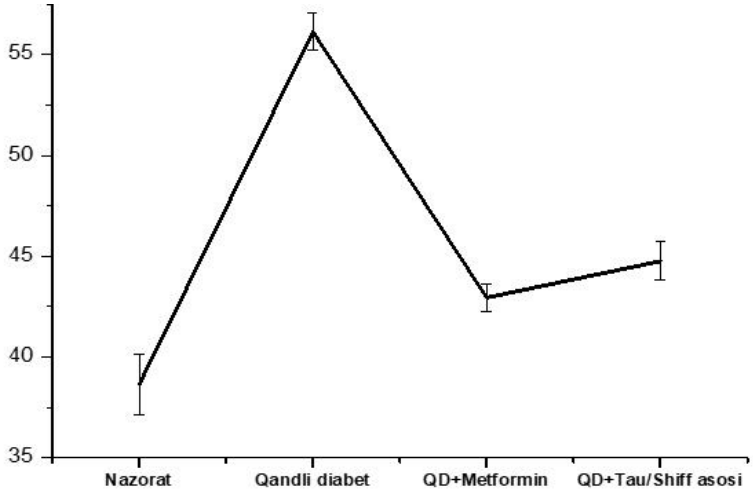
**Determining the amount of GG**

It was noted that the amount of GGT in the liver homogenate of diabetic model rats increased up to 17.5 Ed/l compared to healthy rats. After that, Metformin and the complex compound were administered to sick animals for 7 days. At the end of the experiments, the amount of GGT in the liver homogenate of the animals was checked. It was found that the amount of GGT decreased by 42.95 Ed/l in Metformin-treated animals. It was found that the amount of GGT decreased by 42.76 Ed/l in animals administered a complex of derivatives of taurine and Schiff's bases.

**Figure 2**

**glutamyl transferase (GGT) in the blood of alloxan diabetes model animals and the effect of the complex compound on it (M±m, n=3)**

**Amount of Glutamyl transferase in blood (Ed/l)**




Streptazocin and alloxan substances are mainly used to create a diabetes model. We used alloxan throughout the experiment. Because alloxan has a similar structure to glucose, it enters the cell through the GLUT4 receptor and generates ROS, which causes pancreatic beta cells to die and diabetes. The use of alloxan in the creation of a diabetes model is also widely used in other studies [2]. Taurine treatment for one week reduced blood glucose levels in rats, indicating its antidiabetic properties. In addition, the reduction of hyperglycemia caused by beta cell dysfunction also indicates the antioxidant properties of taurine. Our results also confirm the study by Joydeep Das et al [7]. In their studies, taurine was found to protect pancreatic beta cells and modulate insulin sensitivity and insulin secretion. As a result, blood glucose levels decreased due to increased insulin secretion [7].

As a result of diabetes, various organs, such as the liver, heart, and kidneys, are disturbed, and as a result of this, a person has related diseases. Diabetes initially causes various changes in liver function. For example, protein biosynthesis decreases, activity of antioxidant enzymes decreases. The main reason for this is the origin of ROS. During our experiment, protein biosynthesis decreased in diabetic rats. In addition, catalase enzyme activity also decreased. And through taurine, protein biosynthesis was restored and we can see that enzyme activity also increased significantly compared to diseased animals. Some experiments have shown that taurine's antioxidant properties depend on the Nrf 2 pathway. The Nrf 2 pathway tries to fight against oxidative stress in early diabetes and increases the synthesis of antioxidant enzymes, resulting in increased activity. Taurine affects this Nrf 2 pathway and increases the activity of antioxidant enzymes. In addition, Nrf 2 pathway activation of GSH-px increases GH synthesis and decreases lipid peroxidation in inflamed liver. This reduces the amount of MDA, which is the main indicator of lipid peroxidation [4,10]. Since GGT is located on the outer surface of most cells and mediates the uptake of glutathione, an important component of the intracellular antioxidant defense, we can see that it is also reduced [11]. Based on these studies, it can be said that the antioxidant property of taurine is more effective in its complex with Schiff's bases, and in the future, this complex can serve as a basis for creating antidiabetic drugs.

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