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Effect of combination therapy with diazepam and glibenclamide on serum lipids and glucose in type 2 diabetic rats

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ABSTRACT

Background and Objective: Several studies have found that diazepam decreases serum glucose. Meanwhile, glibenclamide is commonly used in diabetes treatment. The objective of this study was to survey the treatment impact of diazepam and glibenclamide on blood glucose and serum lipids in mice with type II diabetes.

Materials and Methods: In this study, 32 male rats were divided into four groups, i.e. diabetes, glibenclamide, diazepam and combination of glibenclamide and diazepam. Diabetes was inducted using 60 mg/kg of streptozocin injection at single dosage. Medicinal dosage was 0.285 mg/kg for glibenclamide and was 1 mg/kg for diazepam and 50 percent of above mentioned dosages for complex therapeutic dosage. Treatment was continued for 16 days after positivity of diabetes. The level of blood glucose in serum and lipid profile of the rats were surveyed at the end of the study.

Results: Therapeutic combination of diazepam and glibenclamide caused a significant reduction of serum glucose in comparison with control group at 9 and 16 days ($p<0.05$). Combined treatment also caused more significant increase of serum HDL and serum HDL to LDL ratio ($p<0.05$), while these changes were not observed in the groups that were treated using glibenclamide or diazepam, alone.

Conclusion: The combined treatment of glibenclamide and diazepam caused the improvement in controlling the serum glucose and can appropriately change the level of serum HDL in diabetic rats.

1. Introduction

Diabetes mellitus type 2 is one of the most common endocrinology diseases including a heterogeneous group of disorders which is characterized with varying degrees of insulin resistance, impaired insulin secretion and increase glucose production (1). The total number of people with diabetes mellitus type 2 is predicted to rise from 171 million people in 2000 to 366 million people in 2030 that shows the number of people with diabetes is increasing. Moreover, in the future the prevalence of diabetes mellitus type 2 is expected to rise more than diabetes mellitus type 1 due to obesity and physical inactivity (2).

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Diazepam is a benzodiazepines and GABA agonist that is prescribed for treatment of anxiety. According to the researches, diazepam and GABA agonist drugs elevate insulin resistance and decrease the blood glucose levels (3); also diazepam treatment decreases phospholipid fatty acid composition in particular chains. However, the combination of diazepam with anti-diabetic drugs improves serum glucose, insulin, C-peptide and liver glycogen and improves biomarkers of oxidative stress (5). Glibenclamide in a class of sulfonylureas is an insulin secretion stimulants drug and it is commonly prescribed in the treatment of diabetes type 2. Glibenclamide acts by increasing insulin release and decreasing blood sugar levels from the beta cells in the pancreas and other tissues including liver, muscle and fat (6). Today, several pharmaceutical treatments is used to help better control of diseases including diabetes. According to several studies, diazepam treatment decreases blood glucose level (5,3). Thus, due to high prevalence of anxiety disorders, and regarding the low cost and high availability of diazepam, in respect of better control of many diseases through several pharmaceutical treatment (lower dose of medicines), we aimed at studying the effect of the combination of Diazepam and Glibenclamide and comparing individual combinations on the control of blood sugar level. In respect of the hypothesis of the research we can prescribe diazepam and glibenclamide with lower dose levels or less volume of glibenclamide in the patients who take any type of diazepam.

2. **Materials & Methods**

In this study, 32 male adult Wistar rats weighing from 195 to 220 g were selected from Razi Institute. The rats were non-diabetic with blood glucose level lower than 150 mg/dl and they adapted to the new condition and housed 3 to 4 per cage with controlled temperature, light cycle of 12 hours dark/ light cycle and unrestricted access to standard water and food (Pars Co.). The current research was conducted according to the Guide for the Care and Use of Laboratory Animals (NIH) and Research Center Principles of Shahed University.

To induce diabetes mellitus type 2, the rats received a single intraperitoneal injection of a freshly prepared solution of streptozocin (STZ; Sigma chemicals) dissolved in physiological saline (60 mg/kg body weight). STZ destructed pancreatic cells and decreased insulin, then the rats were considered as diabetic (in the early 16 days of injection, beta cells did not destroyed out, so the induced diabetes was considered as diabetes mellitus type 2). The blood glucose was measured by glucometer on the third day after the STZ injection and glucose values were above 250 mg/dL.

The rats were randomly divided into 4 groups: group 1: normal control, group 2: diabetic receiving glibenclamide (0.285 mg/kg) intraperitoneally (Toliddaru Co.), group 3: diabetic receiving diazepam (1 mg/kg) intraperitoneally (Chemidarou), group 4: diabetic receiving both glibenclamide (0.143 mg/kg) and diazepam (0.5 mg/kg) from 3rd day to 16th day (after proving diabetes).

2.1. Analysis of serum parameters:

On the 4th, 9th and 16th day of STZ injection, serum glucose levels was measured as the main criterion for measuring the impact of anti-diabetic drugs. Moreover, on the 16th day after opening the peritoneal cavity of anesthetized animals using blood from the animal heart and separating blood serum using marking test tubes in a centrifuge for 10 minutes at 3000 rpm, glucose levels and lipid profiles, namely, cholesterol, triglycerides, and HDL-cholesterol were calculated using appropriate kits (Zistmohiti Co.) by spectrophotometric assay and measuring serum LDL with Fried-Wald formula (LDL=Chol-HDL-TG/5).

2.2. Statistical analysis

Collected data were analyzed with SPSS19 software to determine the normal and abnormal data. The normal data was analyzed with ANOVA and abnormal data was analyzed with Kruskal-Wallis test. The mean of individual data of the groups was compared and evaluated with Tukey's test. Significance level was $p<0.05$ and relevant diagrams were drawn with Microsoft Excel 2007.

3. Results

3.1. The effect of treatment with glibenclamide, diazepam and combination therapy on serum glucose level

According to the figure 1, on the 4th day after STZ injection, comparing the diabetic control group and 3 diabetic groups (diabetic group receiving glibenclamide, diabetic group receiving diazepam, and diabetic group receiving both glibenclamide and diazepam), there was no significant serum glucose decrease. On the 9th and 16th day after STZ injection, comparing diabetic control group with 2 diabetic groups (diabetic group receiving glibenclamide and diabetic group receiving both glibenclamide and diazepam), there was a significant serum glucose decrease ($p<0.05$). However, comparing 2 mentioned groups, there was no significant serum glucose decrease, and only in diabetic group receiving diazepam, there was no significant serum glucose decrease.

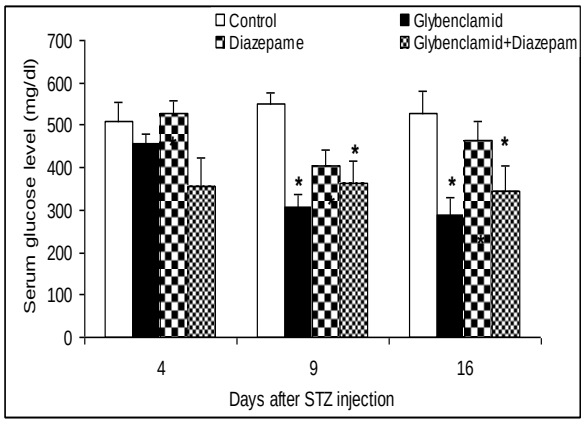

Figure 1. Comparing serum glucose levels on the 14th, 9th and 16th day after STZ injection in the groups of current research

3.2. The effect of treatment with glibenclamide, diazepam and combination therapy on serum lipid level

According to table 1, comparing diabetic control group with 3 diabetic groups (diabetic group receiving glibenclamide, diabetic group receiving diazepam and diabetic group receiving both glibenclamide and diazepam), there was not a significant decrease in serum triglyceride levels. In the current research, there was not a significant decrease in serum cholesterol levels comparing diabetic control group with 3 diabetic groups (diabetic group receiving glibenclamide, diabetic group receiving diazepam, diabetic group receiving both glibenclamide and diazepam). Furthermore, there was not a significant reduction in serum LDL when comparing diabetic control group with 3 diabetic groups. With respect to table 1, comparing diabetic control group with diabetic group receiving glibenclamide and diabetic group receiving diazepam there was a significant increase in serum HDL ($p<0.05$). However, there was not a significant increase in diabetic group receiving only glibenclamide or diazepam. Moreover, there was a significant increase in serum HDL/LDL ($p<0.05$) comparing diabetic control group with diabetic group receiving both glibenclamide and diazepam. However, there was no a significant increasing in diabetic groups receiving both glibenclamide and diazepam.

Table 1. Serum cholesterol, triglyceride, LDL, and HDH levels and HDL/LDL ratio in the groups of the current research

Group tests	Serum parameter (mg/dl)				
	Cholesterol	TG	LDL	HDL	HDL/LDL ratio
Control	78.22 ± 6.83	204.09 ± 19.52	62.33 ± 8.69	15.88 ± 4.44	0.37
Glibenclamide	81.00 ± 8.15	161.83 ± 28.85	70.12 ± 6.65	18.66 ± 4.27	0.24
Diazepam	68.85 ± 5.70	165.53 ± 55.17	64.50 ± 10.76	16.75 ± 5.22	0.45
Diaz+Gli	71.23 ± 6.92	127.37 ±42.45	54.40 ± 10.47	29.60 6.36 *	1.55

** P<0.05 (versus diabetic)*

4. Discussion

In the current study, streptozocin (STZ) was injected to the rats to induce diabetes. In this respect, STZ effects on destroying beta cells within 16 days to induce diabetes mellitus type 2 that decreased insulin is still secreting similar to diabetes mellitus type 2 (7). Furthermore, there was a significant reduction of serum glucose levels in the diabetic rats receiving glibenclamide on the 9^{th} and 16^{th} day. These results are similar to the results of "Ebrahimifakhar" studies-glibenclamide decreases blood sugar levels due to insulin secretion from Langerhans islets (9). Meanwhile, "Chien" studies displayed that there was a greater reduction of fasting plasma glucose and HbA1C in the patients who received the combination therapy of metformin and glyburide comparing with the patients who received metformin or glyburide individually (10).

Although, "Rayisi's" researches showed that pre-surgery administration of a single dose of intravenous diazepam helps to maintain homeostasis and decrease blood glucose due to anxiety of surgery (11), according to "Ardekani" study, alprazolam is a benzodiazepine of the diazepam class, as a supporting drug to control diabetes mellitus type 2, helps to decrease fasting blood glucose (12), also this researcher displayed that alprazolam helps to decrease blood glucose due to increasing plasma insulin levels and reducing resistance to insulin (13), "Gomez" in a study found that diazepam and GABA agonists elevate blood insulin levels and decrease blood glucose levels in different ways (3), and "Schroeder" showed that comparing the group receiving diazepam 6-12 and control group, decreasing plasma glucose concentrations in the first group is greater than control group (14). However, according to "Yadama" studies, diazepam causes a dose-dependent and transient hyperglycemia (15), but in the current study it is found that receiving diazepam after diabetes induction did not decrease blood glucose that was similar to " Shcaira " researches (16), but combination therapy of glibenclamide and diazepam (half-dose) significantly decreased serum glucose levels that was similar to "Garabadu" (17) and " Mohamed " (5) studies. In the previous studies, increasing sensitivity to insulin was taking into account as diazepam effects (17) and according to glibenclamide effects on increasing insulin secretion from Pancreas beta cells, it seems that receiving diazepam after diabetes induction is able to decrease blood glucose only when there is induced insulin by glibenclamide (half-dose), but diazepam is not able to decrease blood glucose individually. Studies show that increasing blood glucose due to diabetes induction with streptozotocin leads to obvious and undesirable changes in serum lipids and lipoproteins. This could explain the undesirable level of serum lipids in diabetic rats in the current research.

In this study, as " Mohamed " reports (5), receiving glibenclamide decreased triglyceride levels -not significantly- and diazepam individually did not significantly decrease triglyceride levels, but in "Garabadu" reports there was a significant decreasing of triglyceride levels (17). Furthermore, in two recent researches on combination therapy of 'diazepam and glimepiride" and "diazepam and metformin", serum triglyceride decreased significantly, but in the current study serum triglyceride did not decreased significantly. Moreover "Ristic" found that diazepam individually increases saturated and unsaturated fatty acids but it significantly decreases phospholipid fatty acid composition in the chain of 22:6n-3, 22:5n-3, n-3 (4).

In the current study, in the diabetic groups there was an increase of serum cholesterol and LDL cholesterol levels and decrease of HDL cholesterol levels and receiving glibenclamide did not decrease serum cholesterol and LDL cholesterol levels significantly. Furthermore receiving diazepam and combination therapy did not help to decrease serum cholesterol and LDL cholesterol levels while combination therapy increased it which is probably due to diazepam effect on plasma fatty acids LDL/HDL and HDL ratio (4).

Taken together, the combined treatment of glibenclamide and diazepam improved controlling the serum glucose and can appropriately change the level of serum HDL in diabetic rats.

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