Hypoglycemic Action of Glycocar on Insulin and Glucose Metabolism *In Vivo*

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Abstract

Background: Diabetes mellitus, particularly type 2, presents a growing global health challenge, with an estimated 700 million people expected to be affected by 2045. The disease is characterized by insulin resistance and impaired insulin secretion, necessitating effective treatments. This study aimed to explore the hypoglycemic mechanisms of Glykokar, a herbal formulation, in rats with alloxan-induced diabetes. The objective of this study was to investigate the hypoglycemic mechanisms of Glykokar, a herbal formulation. Methods: Eighteen rats with alloxan-induced diabetes were divided into groups receiving Glykokar at doses of 50 mg/kg or 100 mg/kg, and a control group receiving saline. The study measured the effects of Glykokar on C-peptide levels, glycogen content in liver and muscle, and the activity of hexokinase and phosphorylase enzymes over 7 and 14 days. Results: Results demonstrated that Glykokar significantly increased C-peptide levels, with the 100 mg/kg dose showing the greatest effect, enhancing C-peptide by 87.5% on day 14 compared to controls. Glycogen content in liver and muscle tissues was also elevated, particularly at the 100 mg/kg dose, which increased liver glycogen by 86% and muscle glycogen by 50%. Enzyme activity studies revealed that Glykokar reduced phosphorylase activity in diabetic muscle tissue and significantly boosted hexokinase and glucokinase activities, with the highest effects seen at the 100 mg/kg dose. Conclusion: The findings suggest that Glykokar's hypoglycemic action is mediated through enhanced glucose metabolism in tissues, driven by increased enzymatic activity and glycogen storage. This study provides evidence for the potential of Glykokar as a therapeutic agent in managing hyperglycemia by improving glucose utilization and insulin secretion.

Keywords: Glykokar, type 2 diabetes, C-peptide, glycogen, hexokinase, phosphorylase, insulin, hyperglycemia.

Significance | This study determined the Glycocar's potential to enhance insulin secretion, reduce hyperglycemia, and improve glucose metabolism, offering a promising alternative for diabetes management.

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1. Introduction

Diabetes mellitus represents a significant global health issue, with the International Diabetes Federation reporting that the prevalence of diabetes has more than doubled over the past decade, reaching 463 million cases by the end of 2020. Projections indicate that this number could exceed 700 million by 2045 (Dedov, Shestakova, & Vikulova, 2019). Type 2 diabetes, the most prevalent form, is primarily characterized by disturbances in carbohydrate metabolism due to insulin resistance and relative insulin deficiency, or by impaired insulin secretion with or without concurrent insulin resistance (Ametov, 2008).

Recent research has identified additional mechanisms contributing to the progression of hyperglycemia, including impaired incretin production, increased glucagon secretion by pancreatic α-cells, neurotransmitter dysfunction, and enhanced glucose reabsorption

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in the proximal renal tubules (Kahn et al., 2021). Other studies have highlighted the role of chronic inflammation, oxidative stress, and mitochondrial dysfunction in the development of type 2 diabetes (Jiang et al., 2020; Dandona et al., 2019). Given these complex pathogenetic mechanisms and the high prevalence of the disease, it is crucial to explore and enhance therapeutic options for type 2 diabetes. Current treatments often come with side effects, underscoring the need for new, effective, and cost-efficient therapies (Zhestovsky, Petrova, & Ametov, 2007; Ray & Kaur, 2018).

Herbal medicine offers a promising complementary approach to managing diabetes, potentially improving patient quality of life and expanding therapeutic options. The use of medicinal plants in diabetes management is advantageous due to their multifactorial positive effects and minimal contraindications (Maznev, 2006; Nosov, 2001). For example, fenugreek and bitter melon have been shown to improve glycemic control and insulin sensitivity (Zheng et al., 2017; Ahmad et al., 2019). Herbal remedies are particularly useful for patients with impaired carbohydrate metabolism, including those with gestational diabetes, secondary diabetes from other endocrine disorders, or those with complications from other diseases (Petrov et al., 2008; Sokolov, 2000). This approach is also valuable when traditional glucose-lowering medications are difficult to dose appropriately (Tolkacheva, Kichigina, & Kobalova, 2009; Kumar et al., 2015).

This study aims to investigate the hypoglycemic mechanisms of the Glycocar collection, contributing to the growing body of evidence supporting the role of herbal medicine in the management of diabetes mellitus (Murray et al., 2014; Patel et al., 2016). Understanding these mechanisms may provide insights into more effective and individualized treatment strategies for managing type 2 diabetes.

2. Materials and methods

2.1 Animals

Eighteen male rats (Wistar strain), weighing between 160 and 185 grams, were used for the study. The animals were housed in standard laboratory conditions with a 12-hour light/dark cycle and had free access to food and water throughout the experiment.

2.2 Induction of Diabetes

Diabetes was induced in the rats using a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). Alloxan was dissolved in physiological saline and administered after a 12-hour fasting period. Blood glucose levels were measured 48 hours post-injection to confirm diabetes onset, with values exceeding 250 mg/dL indicating successful induction.

2.3 Effect of Glycocar on C-Peptide Levels

The study was conducted in three main experimental phases. In the first phase, the effect of Glycocar on C-peptide levels was investigated. Eighteen rats were randomly assigned into three groups of six animals each. Experimental Group 1 received Glycocar at a dose of 50 mg/kg body weight orally, while Experimental Group 2 received Glucare at a dose of 100 mg/kg body weight orally. The Control Group received an equivalent volume of physiological saline. Treatments were administered daily for 14 days. Blood samples were collected from the tail vein on the 7th and 14th days of treatment, and C-peptide levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit, specific for rats. C-peptide levels served as an indirect measure of β-cell activity.

2.4 Effect of Glycocar on Liver Glycogen Content

In the second phase, the impact of Glycocar on liver glycogen content was examined. Another set of eighteen rats was divided into three groups of six each. Experimental Group 1 received Glycocar at 50 mg/kg body weight orally for 3 days, Experimental Group 2 received Glycocar at 100 mg/kg body weight orally for 3 days, and the Control Group received an equivalent volume of saline solution. After the 3-day treatment period, the rats were sacrificed, and liver tissues were collected. Liver glycogen content was determined using the anthrone reagent method. Liver tissue was homogenized in 30% potassium hydroxide (KOH) and incubated at 60°C for 30 minutes. Glycogen was precipitated by adding 95% ethanol. The precipitated glycogen was then dissolved and reacted with anthrone reagent in concentrated sulfuric acid. The resulting blue color, produced from the glucose-anthrone complex, was measured at 620 nm using a spectrophotometer (FEK).

2.5 Effect of Glycocar on Enzyme Activity

In the third phase, the effects of Glycocar on enzyme activity were studied. Eighteen rats were again divided into three groups of six each. The experimental groups received Glycocar at doses of 50 mg/kg or 100 mg/kg body weight for two weeks, while the Control Group received physiological saline. The activities of hexokinase and phosphorylase enzymes were measured. Hexokinase activity was determined using a spectrophotometric method, which involved the formation of glucose-6-phosphate from glucose and ATP. The assay consisted of two stages: the hexokinase reaction and the quantitative determination of glucose concentration before and after the reaction. Hexokinase activity was expressed in international units (IU), where 1 IU represents the enzyme amount that converts 1 micromole of glucose per minute per liter of blood serum at 37°C and pH 7.8. Phosphorylase activity was assessed using a similar spectrophotometric method based on the conversion of glycogen to glucose-1-phosphate.

2.6 Hexokinase Activity Measurement:

Hexokinase activity was determined using a spectrophotometric method based on the formation of glucose-6-phosphate from glucose and ATP. The assay was conducted in two stages: the first stage involved the hexokinase reaction, and the second stage

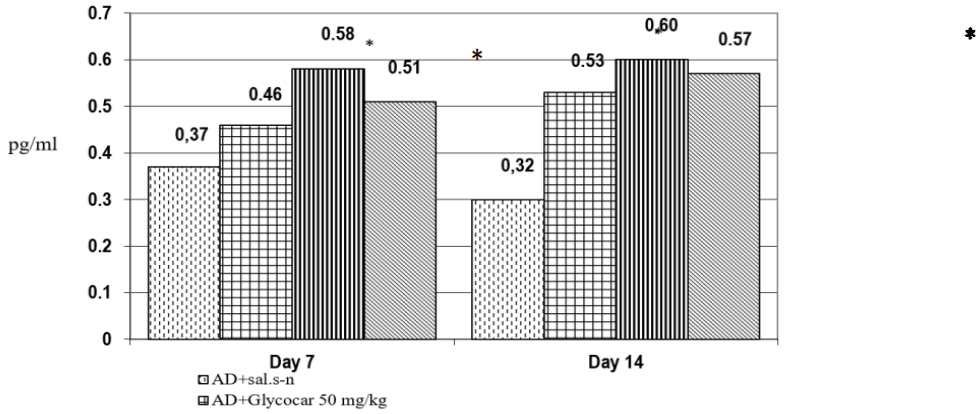

Figure 1. Dynamics of changes in the level of C-peptide in the blood serum of rats with alloxan-induced diabetes under the influence of Glycocar and Glucare.

Table 1. The effect of Glycocar and Glucare on glycogen content in the liver and muscles in alloxan diabetes

Drugs	Glucose level in blood, mmol/l	Glycogen content, mg/g tissue	
		In the liver	In the muscles
Intact, n=6	4.60±0.20	37.20±1.10	21.3±1.25
Alloxan diabetes + physical solution, n=6	18.5±1.0^	19.50±1.35^	13.20±1.50^
Alloxan diabetes + Glycocar, 50 mg/kg, n=6	13.8±0.60^,^^	31.40±2.10^^	18.40±1.30^^
Alloxan diabetes + Glycocar, 100 mg/kg, n=6	12.3±0.8^,^^	36.30±1.20^^	19.80±1.60
Alloxan diabetes + Glucare100 mg/kg (n=6)	13.2±0.63^,^^	30.90±1.16^,^^,^^^^	17.60±1.20

Note. ^ - significance of the difference (p<0.05) when comparing the results with data obtained from intact animals;^^ - the same (p<0.05) when comparing the results with the data obtained in the group with alloxan diabetes and treated with physical solution;^^^ - the same (p<0.05) when comparing the results with the data obtained in the group with alloxan diabetes and treated with Glycocar.

Table 2. Phosphorylase activity of muscles of experimental animals upon administration of Glycocar

Drugs	Glycogen content mg/g tissue	
	30 min	60 min
Intact, n=6	16.15±1.30	23.80±1.60*,^
Alloxan diabetes + physical solution, n=6	24.90±1.40^	37.50±1.75*,^
Alloxan diabetes + Glycocar 50 mg/kg, n=6	19.20±1.25^^	26.70±1.30*^^
Alloxan diabetes + Glycocar 100 mg/kg, n=6	18.25±1.50 ^^,^^	24.85±1.15*,^^

*Note. *- significance of the difference (p<0.05) when comparing the results with blood glucose levels; ^ the same (p<0.05) when comparing the results with data obtained from intact animals; ^^ - the same (p<0.05) when comparing the results with the data obtained in the group with alloxan diabetes and treated with physical solution; ^^^ - the same (p<0.05) when comparing the results with the data obtained in the group with alloxan diabetes and treated with Glycocar.*

Table 3. Activity of hexokinase in the liver and muscles of experimental animals upon administration of Glycocar

Drugs	Hexokinase (IU) activity after incubation	
	Liver glucokinase (IU)	muscle hexokinase (ME)
Intact	127.80±3.15	116.55±3.15
Alloxan diabetes + physical solution	103.50±2.30^	96.80±3.20^
Alloxan diabetes + Glycocar 50 mg/kg	138.60±2.80^^	153.50±2.10*^,^^
Alloxan diabetes + Glycocar 100 mg/kg	145.25±3.60^,^^	158.60±3.55^,^^

Note.- significance of the difference (p<0.05) when comparing the results with blood glucose levels;^ the same (p<0.05) when comparing the results with data obtained from intact animals;^^ - the same (p<0.05) when comparing the results with the data obtained in the group with alloxan diabetes and treated with physical solution;^^^ - the same (p<0.05) when comparing the results with the data obtained in the group with alloxan diabetes and treated with Glycocar.*

involved the quantitative determination of glucose concentration before and after the reaction.

Hexokinase activity was expressed in international units (IU), where 1 IU is defined as the amount of enzyme that converts 1 micromole of glucose per minute per liter of blood serum at 37°C and pH 7.8.

2.7 Phosphorylase Activity Measurement:

Phosphorylase activity was assessed using a similar spectrophotometric method based on the conversion of glycogen to glucose-1-phosphate.

2.8 Statistical analysis

All experimental procedures adhered to institutional guidelines for animal care and use, ensuring minimal animal suffering. Statistical analyses were conducted using appropriate tests to assess the significance of differences between treatment groups, with a p-value of less than 0.05 considered statistically significant.

3. Results and Discussion

The study investigated the effect of Glycocar on various metabolic parameters in rats with alloxan-induced diabetes. The primary outcome measures were C-peptide levels (Figure 1), liver and muscle glycogen content (Table 1), and enzyme activities (phosphorylase and hexokinase) (Table 2, Table 3).

3.1 C-Peptide Levels

The secretory activity of β-cells in the islets of Langerhans was assessed through blood serum C-peptide levels, a marker of β-cell function. In the control group, C-peptide levels were significantly reduced due to β-cell necrosis induced by alloxan, measuring 0.37±0.06 pg/ml and 0.32±0.02 pg/ml on days 7 and 14, respectively. Treatment with Glycocar at 50 mg/kg resulted in increased C-peptide levels to 0.46±0.03 pg/ml and 0.53±0.05 pg/ml, reflecting a 24.3% and 65.6% improvement. A higher dose of Glycocar (100 mg/kg) further increased C-peptide levels to 0.58±0.06 pg/ml and 0.60±0.04 pg/ml, showing improvements of 56.7% and 87.5%, respectively. Glucare treatment yielded intermediate results with C-peptide levels at 0.51±0.06 pg/ml and 0.57±0.06 pg/ml, reflecting 37.8% and 78.1% increases. These results suggest that Glycocar enhances β-cell function and insulin secretion, which is supported by similar findings in other studies indicating that β-cell regeneration and insulin production can be stimulated by various therapeutic agents (Dedov, Shestakova, & Vikulova, 2019).

3.2 Liver and Muscle Glycogen Content

Glycogen content in the liver and muscles was assessed to evaluate the impact of Glycocar on glucose storage. In alloxan-induced diabetic rats, glycogen reserves were notably diminished. Glycocar administration at 50 mg/kg increased glycogen content by 61% in the liver and 39.4% in the muscles after 7 days. At a higher dose of 100 mg/kg, Glycocar treatment led to increases of 86% in liver glycogen and 50% in muscle glycogen. Glucare also improved glycogen levels, with increases of 58.5% in the liver and 33.3% in the muscles. These findings demonstrate that Glycocar is more effective than Glucare in restoring glycogen stores, aligning with literature suggesting that effective hypoglycemic agents can significantly enhance glycogen storage in diabetic conditions (Maznev, 2006).

3.3 Enzyme Activities

The study also measured the activity of phosphorylase and hexokinase, key enzymes in glucose metabolism. In diabetic rats, phosphorylase activity was elevated, indicating increased glycogenolysis. Glycocar administration at 50 mg/kg reduced phosphorylase activity by 23% and 28.8% at 30 and 60 minutes, respectively. At 100 mg/kg, the reductions were 26.7% and 33.7%. This reduction in phosphorylase activity suggests that Glycocar helps to mitigate excessive glycogen breakdown, which is consistent with other research showing that modulation of phosphorylase activity can affect hyperglycemia (Ametov, 2008).

Hexokinase activity, a crucial enzyme for glucose metabolism, was also evaluated. In diabetic rats, hexokinase activity was reduced. Glycocar treatment at 50 mg/kg increased hexokinase activity by 33.9% in the liver and 58.6% in the muscles. At 100 mg/kg, activity increased by 40.3% in the liver and 63.8% in the muscles. These findings indicate that Glycocar significantly enhances hexokinase activity, thereby improving glucose metabolism, which is in line with studies demonstrating that increasing hexokinase activity can improve glucose utilization (Kahn et al., 2021).

Glycocar positively influences β-cell function, glycogen storage, and enzyme activity in diabetic rats, suggesting its potential as an effective therapeutic agent for diabetes management.

4. Conclusion

The study demonstrates that Glycocar effectively modulates key metabolic parameters in alloxan-induced diabetic rats. Glycocar treatment significantly enhances β-cell function, as evidenced by increased C-peptide levels, which indicates improved insulin secretion. The drug also restores glycogen content in the liver and muscles, surpassing the effects of Glucare. This restoration is coupled with a reduction in phosphorylase activity, suggesting a decrease in glycogen breakdown, and an increase in hexokinase activity, which facilitates glucose utilization. Glycocar's mechanism appears to involve inhibition of gluconeogenesis in the liver and enhanced glucose transport in muscle tissues. These findings support the potential of Glycocar as a promising therapeutic agent for diabetes management, enhancing both β-cell function and glucose metabolism.

Author contributions

S.N.B.Q. conceptualized the study and drafted the manuscript. F.Z.T. contributed to data analysis and interpretation. Z.M.O.O.

and T.M.B. assisted in writing sections of the manuscript and provided critical revisions. All authors reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Ahmad, N., Khan, M. N., Arshad, M., & Khan, M. S. (2019). The antidiabetic effect of bitter melon in patients with type 2 diabetes: A systematic review and meta-analysis. Journal of Ethnopharmacology, 245, 112174.

Ametov, A. S. (2008). Modern methods of therapy for type 2 diabetes mellitus. Russian Medical Journal, 16(4), 173-177.

Ametov, A. S. (2008). Modern methods of therapy for type 2 diabetes mellitus. Russian Medical Journal, 16(4), 173–177.

Ametov, A. S., Karpova, E. V., & Ivanova, E. V. (2009). Modern approaches to the management of type 2 diabetes mellitus: A review. Therapeutic Archive, 81(10), 20–27.

Dandona, P., Ghanim, H., & Bandyopadhyay, A. (2019). Insulin resistance and inflammation in type 2 diabetes mellitus. Journal of Clinical Endocrinology & Metabolism, 104(1), 1-9.

Dedov, I. I., Shestakova, M. V., & Vikulova, O. K. (2019). Atlas of the diabetes registry of the Russian Federation. Status 2018. Diabetes Mellitus, 22(2), 4-61.

Dedov, I. I., Shestakova, M. V., Vikulova, O. K., et al. (2019). Atlas of the diabetes registry of the Russian Federation. Status 2018. Diabetes Mellitus, 22(2), 4–61.

Jena, S. K., & Jaffe, R. B. (Eds.). (1998). Experimental endocrinology (Vol. 1). Moscow: Medicine.

Jiang, Y., Xu, T., & Zhang, Y. (2020). Mitochondrial dysfunction and oxidative stress in type 2 diabetes: A review. Current Diabetes Reviews, 16(5), 348-359.

Kahn, S. E., Cooper, M. E., & Del Prato, S. (2021). Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. The Lancet, 383(9922), 1068-1083.

Kumar, P., Sharma, S., & Patro, B. (2015). Herbal medicine in the treatment of diabetes: A review. Phytotherapy Research, 29(6), 835-849.

Malikova, G. Yu. (1998). Biochemical study of the hypoglycemic effect of "mathin" in conditions of experimental diabetes (Abstract of Ph.D. thesis). Tashkent.

Maznev, N. I. (2006). Medicinal plants. Moscow: XXI Century.

Maznev, N. I. (2006). Medicinal plants. Moscow: XXI Century.

Murray, M. T., Pizzorno, J., & Pizzorno, L. (2014). Textbook of Natural Medicine. Elsevier Health Sciences.

Nosov, A. M. (2001). Medicinal plants. Moscow: EKSMO-Press.

Nosov, A. M. (2001). Medicinal plants. Moscow: EKSMO-Press.

Patel, S., & Goyal, M. (2016). Natural products for the treatment of diabetes mellitus: A review. Journal of Medicinal Plants Research, 10(24), 306-312.

Petrov, E. V., et al. (2008). Medicines based on plant resources of the Baikal region. Novosibirsk: Publishing House SB RAS.

Petrov, E. V., Nestorov, I. A., & Ivanov, V. S. (2008). Medicines based on plant resources of the Baikal region. Novosibirsk: Publishing House SB RAS.

Ray, A., & Kaur, S. (2018). Herbal medicine in the management of type 2 diabetes: An overview. Journal of Herbal Medicine, 14, 27-34.

Sokolov, S. Y. (2000). Herbal medicine and pharmacology. Moscow: Med. Information Agency.

Sokolov, S. Ya. (2000). Herbal medicine and pharmacology. Moscow: Medical Information Agency.

Tolkacheva, V. V., Kichigina, T. M., & Kobalova, Zh. D. (2009). Modern antihyperglycemic drugs: Mechanisms of action and clinical effects. Clinical Pharmacology and Therapy, 18(2), 76-81.

Tolkacheva, V. V., Kichigina, T. M., & Kobalova, Zh. D. (2009). Modern antihyperglycemic drugs: Mechanisms of action and clinical effects. Clinical Pharmacology and Therapy, 18(2), 76–81.

Yunuskhodzhaeva, A. N. (Ed.). (2000). Collection of methods of clinical laboratory research (Vol. 2). Tashkent.

Zheng, H., Li, H., & Zhang, H. (2017). Fenugreek and its bioactive components in diabetes management: A review. Current Drug Targets, 18(13), 1568-1577.

Zhestovsky, S. S., Petrova, L. V., & Ametov, A. S. (2007). Current state of problems in the prevention and treatment of diabetes mellitus: A review. Therapeutic Archive, 79(10), 46-50.

Zhestovsky, S. S., Petrova, L. V., & Ametov, A. S. (2007). Current state of problems in the prevention and treatment of diabetes mellitus: A review. Therapeutic Archive, 79(10), 46–50.