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Gene expression of *Gck* in alloxan-induced diabetic male rats: potential therapeutic role of beta-aminobutyric acid

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Diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia. This study aimed to evaluate the protective effect of beta-aminobutyric acid (BABA) on alloxan-induced diabetes in male rats. Furthermore, this study examined the crucial role of the *Gck* gene in diabetes development, elucidating its characteristics and expression levels in liver tissue by studying its effect on glucose, ALT, AST, MDA, GlP, GlP-1, and GCK pathways. For modeling diabetes mellitus, thirty adult male rats were randomly assigned to six groups of five individuals each. The rats were 6–8 weeks old and weighed 140–160 g. Positive control was treated with 2 mL normal saline, while the negative control was treated only with 50 mg/kg alloxan, and the other four groups were treated with 50 mg/kg of alloxan, then 50, 100, 150, 200 mg/kg of BABA respectively. Enzyme-linked immunosorbent assay (ELISA) was utilized to evaluate the serum levels of glucose, ALT, AST, MDA, GlP, GlP-1, and hepatic glycogen GCK, and hepatic *Gck* mRNA levels had been measured via the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) technique in thirty adult male albino rats. Hepatic damage was stimulated in six groups for the research using alloxan. The addition of BABA at four different concentrations lasted for one month. Blood and tissue samples were collected at the end of the experimental period, following overnight fasting. All assays were performed in technical triplicates to ensure data reliability and reproducibility. Control− showed significantly higher levels of glucose, liver enzymes ALT and AST, and the oxidative stress marker (MDA), with a decrease in metabolic activity markers (GlP, GlP-1, GCK) compared with Control+. Treatment with BABA at increasing doses (50, 100, 150, 200 mg/kg) led to a gradual improvement in all studied markers. Glucose, ALT, AST, and MDA levels significantly decreased, while GlP, GlP-1, and GCK significantly increased. The best results were achieved at the 200 mg/kg dose (Group D), where values approached normal levels. This study indicates the effectiveness of BABA in reducing liver damage and oxidative stress and improving metabolic balance in the alloxan-induced diabetes model.

Keywords: diabetes; BABA; alloxan; ELISA; *Gck* gene; histopathological examination.

Introduction

Diabetes (T2D) is one of the most prevalent chronic diseases worldwide. It is characterized by a metabolic disorder resulting from impaired insulin production or response, leading to elevated blood sugar levels and serious long-term complications (Ghasemi & Jeddi, 2023; Arif et al., 2025). Although numerous drug treatments are available, there is a growing interest to researching new, more effective treatments with fewer side effects, particularly those based on bioactive and natural compounds (Amrolahi et al., 2022). Impaired function and programmed cell death of pancreatic beta cells are key mechanisms in the development of diabetes mellitus, leading to persistent insulin deficiency and chronic hyperglycemia. Therefore, studies recommend protecting and promoting the proliferation of beta cells while also exploring alternative sources for their regeneration. It is worth noting that alpha and beta cells share a common embryonic origin within islet endocrine cells, mature through the same pathway, and work together to regulate glucose levels (Zhang et al., 2024).

Alloxan, which is chemically known as 5,5-dihydroxyl pyrimidine-2,4,6-trione, is an organic compound, a urea derivative, a carcinogen and cytotoxic glucose analog. The compound has the molecular formula $C_4H_2N_2O_4$ and a relative molecular mass of 142.06. Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of both pure compounds and plant extracts in studies involving diabetes (Setiawati et al., 2024). Alloxan selectively affects pancreatic beta cells responsible for insulin secretion, and this effect is the basis for the induction of diabetes in animal models. Alloxan has a chemical structure similar to glucose, enabling it to enter beta cells via the glucose transporter type 2 (GLUT2), which is abundant in these cells. Once inside the cell, alloxan undergoes a series of chemical reactions that produce free radicals (ROS), such as hydrogen peroxide and hydroxyl ions, leading to severe oxidative stress within the cell (Abdulkareem et al., 2015). This stress directly damages cell components, such as DNA, lipids, and proteins, causing apoptosis or necrosis. Alloxan also inhibits the activity of glucokinase, the first step in glucose sensing and insulin secretion, impairing the cell's ability to perform its functions even before complete death.

Beta-aminobutyric acid (BABA) has emerged as a promising compound that may play an important role in regulating metabolic and immune responses, making it a potential treatment option for diabetes (Thamer & Jasim, 2021). Studies have shown that BABA has the ability to stimulate protective mechanisms at the cellular and molecular levels, helping mitigate damage associated with high blood glucose levels and improve insulin sensitivity (Al-Dulaimy & Jasim, 2022). β-aminobutyric acid (BABA) acts as a potent immunomodulator by inducing a "defense primed" state in cells, enhancing the immune response to oxidative stress caused by hyperglycemia. BABA exerts antioxidant effects by elevating the activity of defense enzymes such as SOD, CAT, and GPx, contributing to the reduction of oxidative damage in liver and pancreatic tissue (Tiwari & Singh, 2024). Glucose-dependent insulinotropic polypeptide (GIP), an intestinal hormone secreted by K cells in the small intestine upon food intake, plays a pivotal role in promoting insulin secretion from the pancreas (Miao et al., 2013). Glucagon-like peptide-1, or GLP-1, is an incretin hormone released by cells in the small intestine following food consumption. It plays a crucial role in controlling blood sugar levels by stimulating the pancreas to release insulin in response to increased glucose, thereby aiding in the reduction of blood sugar levels (Müller et al., 2019). Studies have confirmed that GLP-1 promotes the regeneration of beta cells both *in vitro* and *in vivo* and contributes to their differentiation and the formation of new cells. This is likely related to

the role of the GLP-1/GLP-1R signaling pathway in the ex[pansion and differentiation of pancreatic endocrine cells during developmental stages (Ghorbani et al., 2019). Glucagon-like peptide-1 enhances insulin secretion and suppresses glucagon release, thereby contributing to a better blood sugar regulation. As a critical marker, pre-proglucagon plays a significant role in researching glucose metabolism disorders and in the development of treatments targeting the GLP-1 and glucagon pathways (Khorrami et al., 2019). This study focused on assessing the therapeutic potential of BABA in male rats with alloxan-induced diabetes by investigating its impact on key molecular pathways involved in glucose regulation and inflam-mation.

Glucokinase (GCK), a member of the hexokinase family and also known as hexokinase IV, is a crucial component of glucose metabolism, essential for maintaining normal glucose levels. It was first discovered in the livers of mice and later in the pancreas of obese mice during the 1960s. Hepatic glucokinase is a key enzyme in glucose uptake, and its reduced activity is associated with the development of insulin resistance and type 2 diabetes (Matschinsky & Wilson, 2019).

This research provides an in-depth analysis of the fundamental role of the *Gck* gene in the initiation and progression of diabetes, focusing on its expression pattern, regulatory mechanisms, and associated single-gene alterations in alloxan-induced diabetic male mouse models.

Materal and methods

Ethics approval. The Experimental protocols and animal use (protocol number CHREC/11/2022) have been authorized by the Institutional Animal Ethics Committee, and all procedures adhered strictly to the ethical standards established by Anbar University.

To model diabetes mellitus, thirty adult male albino rats were randomly assigned to six groups of five individuals each. The animals were 8–10 weeks old, in good health, and weighed 140 to 160 g. After the acclimatization period, the rats were given three doses of alloxan, one every three days. The rats also received oral therapeutic doses of β-aminobutyric acid, four doses every three days, and one dose via daily gavage for 30 days. The treatments administered were tailored to the specific requirements of the experiment:

– Control (+): positive control group treated with 2 mL normal saline;

– Control (-): negative control group treated with 50 mg/kg of alloxan;

– Group A: group treated with alloxan in a concentration of 50 mg/kg of body weight, then 50 mg/kg of BABA;

– Group B: group treated with a concentration of 50 mg/kg of alloxan, then 100 mg/kg of BABA;

– Group C: group treated with a concentration of 50 mg/kg of alloxan, then 150 mg/kg of BABA;

– Group D: group treated with a concentration of 50 mg/kg of alloxan, then 200 mg/kg of BABA.

Collection of blood samples. After completing the designated 60-day experiment period, the animals were subjected to a night of fasting lasting 10 hours. Subsequently, their final weight was measured. Then, the animals were anesthetized using a chloroform solution. Blood samples measuring 10 mL were collected from each animal through cardiac puncture using specialized syringes. From these sample, 5 mL were placed into test tubes without anticoagulants. The serum was acquired by centrifugation at 2,500–3,000 rpm. It was then preserved at a temperature of –20 °C in fresh plastic containers until specific biochemical examinations were conducted (Müller et al., 2019). The remaining 5 mL of blood was placed in Trizol tubes and kept frozen at –20 °C for molecular analysis.

Biochemical analysis. Glucose levels were measured using a commercial kit (Glucose-TR, Spinreact Company, Spain). Blood samples were centrifuged at 3,000 rpm for 5 min. Sera were collected, and the levels of ALT, AST, and bilirubin were measured using a Cobas Mira Plus CC Chemistry Analyzer (Switzerland).

The enzyme-linked immunosorbent assay (ELISA) was utilized to evaluate the serum concentrations of MDA, GIP, and GIP-1. The antibodies for MDA, GIP, and GIP-1 were precoated on the plates of the ELISA. The chromogenic response in substrate solutions is related to the concentration of MDA, GIP, and GIP-1 upon placing the samples on plates. The cessation of the process was achieved through the introduction of a stop solution, followed by the quantification of absorbance at a wavelength of 450 nm (Malik et al., 2023). The levels of MDA (Cat. No.: CK-bio-15273, Sensitivity: 0.1 ng/mL), GIP (Cat. No.: CK-bio-26417, Sensitivity: 1.0 pg/mL), and GIP-1 (Cat. No.: CK-bio-14680, Sensitivity: 10.0 pg/mL) were measured in serum using a kit (Sunlong, China). The levels of GCK Mouse PYGL/glycogen phosphorylase were measured using an ELISA Kit (MOFI01076).

Estimation of gene expression of Gck RNA by QRT-PCR. Extraction of serum total RNA, including *Gck* RNA, from the diabetic rats, as well as the healthy groups (control), was performed using the PAXgene Blood RNA Kit (Qiagen, Cat. No. 762174) following the manufacturer's instructions. The RNA samples were subjected to RNA quantitation and purity assessment using the NanoDrop 2000/2000c spectrophotometer (Thermo Fisher Scientific, USA). Reverse transcription (RT) was carried out using the SuperScript™ IV First-Strand Synthesis Kit (Thermo Fisher, Cat. No. 18091050, USA) according to the manufacturer's manual. Quantitative real-time PCR (qRT–PCR) was carried out via the QIAGEN Rotor Gene Q Real-time PCR System (Germany). The PCR primers were employed to target the *Gck* gene, which was normalized to an endogenous control reference beta-actin gene, as illustrated in Table 1. The goal of the quantitative PCR technique was to determine the cycling threshold (Ct) using the 2xqPCR Master Mix Kits. Each reaction was performed twice. To achieve a final volume of 20μL, the following components were added: 10 μL GoTaq qPCR Master Mix (Promega, USA), 1 μL (0.1 μM) from each primer, 4 μL of cDNA sample, and nuclease-free water. The thermal profile consisted of an initial step at 94 °C for 5 minutes (one cycle), followed by 40 cycles involving denaturation at 94 °C for 5 minutes, annealing at 58 °C for *Gck* for 15 seconds and extension at 72 °C for 20 seconds. The relative gene expression was calculated by comparing cycles for each target PCR. Cycle threshold values were converted to relative gene expression levels using the 2−ΔΔCT method.

Table 1
The primers used in the present study

Primer	Sequence (5′→3′ direction)	Product size, bp	Tm °C
	Gck:NM-012560.2		
Forward	AGTATGACCGGATGGTGGAT	120	58
Reverse	CCGTGGAACAGAAGGTTCTC		
	Beta-actin		
Forward	GATTACTGCCCTGGCTCCTA	150	58
Reverse	TCATCGTACTCCTGCTTGCT		

Histopathological examination. After drawing blood samples, the animals were dissected directly by making an incision in the abdominal cavity from the bottom upwards towards the heart, then the livers were removed after removing the fatty tissue and the surrounding connective tissue. Then, they were washed with distilled water to remove the blood, dried on filter paper, and weighed. These tissues were preserved in a 10% formalin solution. Following mounting, a 5 μm slice of each tissue was cut and subsequently immunostained with hematoxylin and eosin. Two pathologists performed the histopathological evaluation using a semi-quantitative scale, classifying the lesions as follows: normal – 0, mild – less than 25%, moderate – 25–50%, and severe – more than 50% of the affected tissue area (Khorrami et al., 2019).

Statistical analysis. The Statistical Packages of Social Sciences-SPSS (2019) program were used to detect the effect of difference groups. Parameters and gene expression (fold change) were studied and the least significant difference (LSD) was used for comparison of the means.

Results

Table 2 presents the mean ± SD of various biochemical and physiological parameters measured across the experimental groups of rats.

The measured parameters included blood glucose concentration (mg/dl), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, malon-dialdehyde (MDA), glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1).

The groups A, B, C, D treated with BABA at concentrations of 50, 100, 150, and 200 showed a significant statistical decrease in glucose levels, as 9.67 ± 0.50, 7.45 ± 1.12, 6.88 ± 2.15, 4.55 ± 0.90 mg/dL were recorded, respectively.

The results showed a significant difference ($P < 0.05$) in the studied liver parameters of the rats with the disorder. The ALT, and AST showed higher mean levels in Control– (138.9 ± 6.1 and 140.8 ± 28.9 mg/dL), compared with Control+ (80.0 ± 3.4 and 100.2 ± 3.1 mg/dL, respectively), as shown in Table 2.

Malondialdehyde is a well-established biomarker of oxidative stress resulting from lipid peroxidation. In the current study, the results showed a significant increase ($P = 0.001$) in the MDA levels in Control– (6.57 ± 0.17 mg/dL), compared with Control+ (2.27 ± 0.15 mg/dL). The groups treated with BABA at different concentrations (Group A (50 mg/kg), Group B (100 mg/kg), Group C (150 mg/kg), and Group D (200 mg/kg)) showed a gradual and significant decrease in MDA levels, which measured 4.00 ± 1.93, 3.01 ± 0.76, 2.81 ± 0.41, and 2.59 ± 0.22 mg/dL, respectively. This progressive reduction reflects a dose-dependent response of BABA in mitigating oxidative stress.

Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 are two of the most important incretins that play a role in blood sugar regulation, and their levels in the blood reflect the metabolic status and activation of insulin secretion. The results presented in Table 2 demonstrate statistically significant differences in the levels of both GIP and GLP-1 among the different groups ($P = 0.001$). Control– exhibited lower values of 30.6 ± 6.4 for GIP and 250.2 ± 7.8 for GLP-1, compared with Control+, which exhibited higher levels of GIP and GLP-1, accounting for 60.2 ± 2.0 and 300.1 ± 2.1, respectively. The groups treated with BABA after alloxan administration showed a gradual increase in the levels of both indicators. Group A (with 50 mg/kg BABA) was observed to have levels of 40.4 ± 35.1 for GIP and 360.5 ± 4.5 for GLP-1, while Group D (with 200 mg/kg BABA) exhibited values of 55.4 ± 10.6 for GIP and 275.4 ± 4.6 for GLP-1.

Table 2
Mean levels of parameter in studied rats groups

Groups	Glucose, mg/dL	ALT, mg/dL	AST, mg/dL	MDA, mg/dL	GlP, mg/dL	GlP-1, mg/dL	GCK, mg/dL
Control (+)	3.40 ± 1.20^{a}	80.0 ± 3.4^{a}	100.2 ± 3.1^{a}	2.27 ± 0.15^{a}	60.2 ± 2.0^{c}	300.1 ± 2.1^{d}	11.5 ± 0.1^{d}
Control (-)	12.50 ± 2.05^{c}	138.9 ± 6.1^{e}	140.8 ± 28.9^{bc}	6.57 ± 0.17^{b}	30.6 ± 6.4^{a}	250.2 ± 7.8^{a}	5.1 ± 0.4^{a}
Group A	9.67 ± 0.50^{bc}	120.5 ± 5.1^{d}	132.0 ± 9.9^{c}	4.00 ± 1.93^{a}	40.4 ± 5.1^{ab}	360.5 ± 4.5^{ab}	7.0 ± 0.6^{b}
Group B	7.45 ± 1.12^{b}	108.0 ± 2.5^{c}	129.3 ± 4.6^{c}	3.01 ± 0.76^{a}	44.8 ± 6.4^{b}	265.2 ± 3.1^{b}	9.6 ± 0.7^{c}
Group C	6.88 ± 2.15^{b}	95.0 ± 2.0^{b}	120.7 ± 3.2^{c}	2.81 ± 0.41^{a}	48.5 ± 10.3^{b}	270.2 ± 3.1^{bc}	10.8 ± 0.9^{cd}
Group D	4.55 ± 0.90^{ab}	90.0 ± 7.1^{b}	113.3 ± 2.5^{b}	2.59 ± 0.22^{a}	55.4 ± 10.6^{bc}	275.4 ± 4.6^{c}	13.1 ± 1.1^{e}
P	0.001	0.001	0.001	0.001	0.001	0.001	0.001

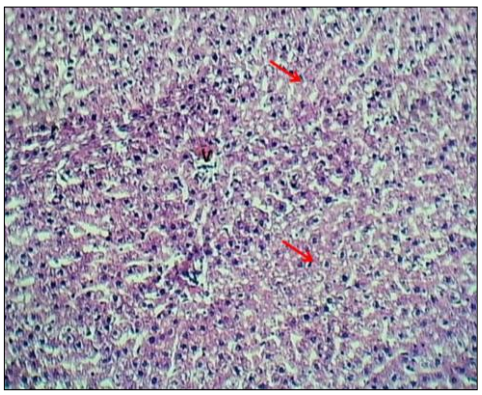
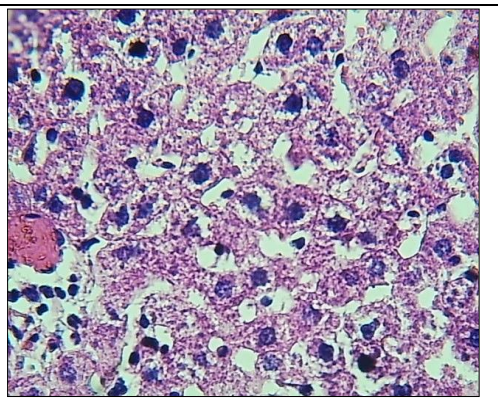
Mean expression levels of the Gck gene in various groups of the studied rats. Based on the experimental details and qPCR results for glucokinase (*Gck*) expression in an alloxan-induced diabetic rat models, the result revelead an increase in the *Gck* expression with fold change > 1, (4.36, 2.21, 2.79, 3.69, 4.10, and 5.60) in all the studied groups (Control+, Control–, A, B, C, and D), relative to the baseline control (1.000), as illustrated in Table 3.

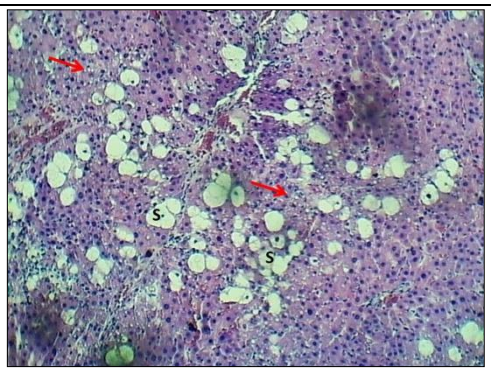
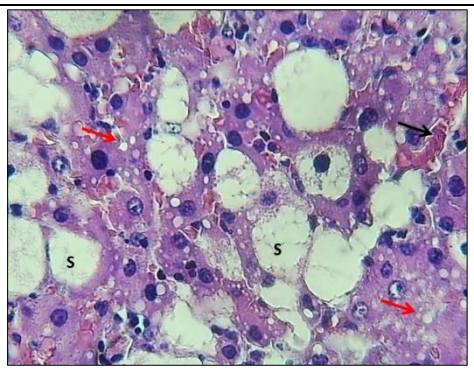
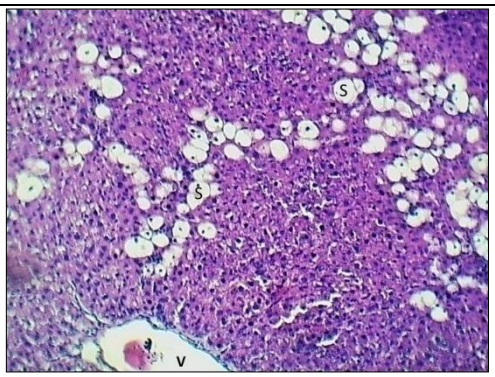
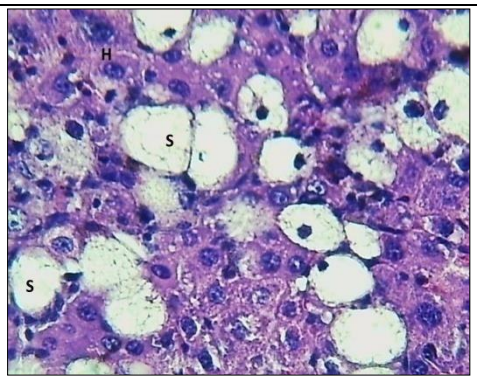
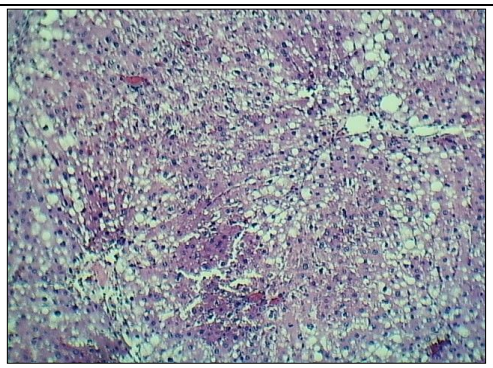
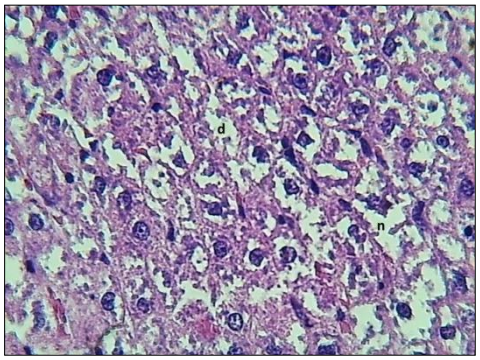
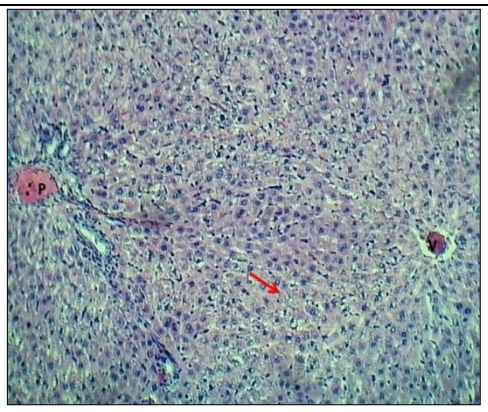
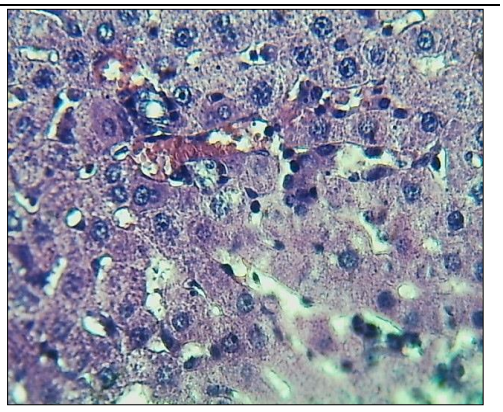
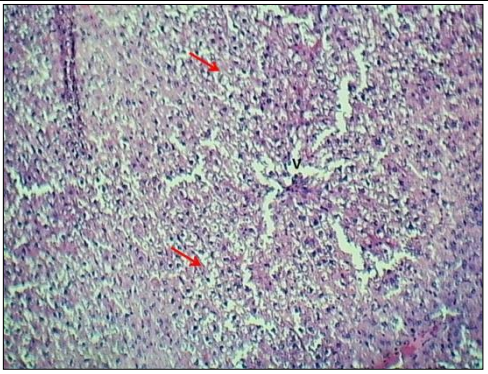
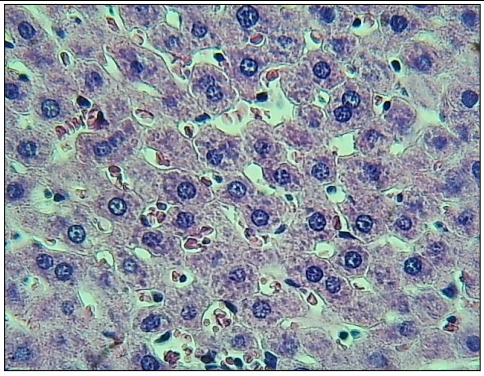
Histological study. The present study demonstrated the effect of alloxan and BABA on liver tissues in the dose groups: Histopathological features of the liver cortex and medulla in the Control+ group showed normal appearance of the liver glomeruli and tubular lining cells. Meanwhile, one case from the Control− group showed congestion and severe steatosis of hepatocytes (red arrows), numerous scattered hypertrophied fat cells (S), and congestion (black arrows). Group A showed dilatation with congestion of the central vein (V), and mild steatosis in hepatocyte areas (arrows). Group B exhibited a section of hepatocyte swelling in the hepatic lobule with slight necrosis (N). Group C was observed to have normal hepatocytes (arrow), with slight congestion of the central vein (V) and the trigeminal portal vein (P). Group D presented with slight sinusoidal congestion, hypercellularity of Kupffer cells, and normal hepatocytes (H).

Table 3
Mean levels of liver enzyme in studied rats groups

Groups	Means Ct of *Gck*	Means Ct of B-actin	ΔCt	ΔΔCt	Fold of gene expression
Control+	24.24	18.25	5.99	–2.124	4.360
Control-	28.23	21.26	6.97	–1.144	2.210
A	30.73	24.10	6.63	–1.484	2.798
B	26.85	20.62	6.23	–1.884	3.690
C	28.53	22.45	6.08	–2.034	4.096
D	31.85	25.48	6.37	–1.744	5.595
Baseline control	28.46	20.35	8.11	0.000	1.000

Table 4
The effect of alloxan and BABA on liver tissues in the dose groups

Concentrations	100^{X}	400^{X}
Control+		

Concentrations	100X	400X
Control -		
A		
B		
C		
D		

Discussion

Declining pancreatic β-cell function and depletion aggravate the course of diabetes mellitus. The enhancement of endogenous β-cell proliferation appears to have a significant therapeutic potential by decelerating the course of diabetes mellitus or potentially reversing it. This study assessed the genesis of neonatal β-cells in the diabetic mice subjected to alloxan administration. β-Aminobutyric acid (BABA) has been successfully used to prime stress resistance in numerous rats, and the current study was designed to investigate the effect of BABA as a possible treatment diabetic male rats. The results of the current study showed that the induction of diabetes by alloxan may increase the glucose levels in blood serum of rats, which is consistent with the study conducted by Yao et al. (2022). Alloxan plays an important role in raising blood sugar levels, leading to diabetes in the laboratory animals. Alloxan has an inhibitory role in many cellular processes, such as affecting many enzymes through the oxidation of their thiol group. These enzymes are functionally important for glucose analysis, namely phosphofructokinase, hexokinase, aconitase, and calmodulin-dependent protein kinase, among others (Mistry et al., 2023). It is noteworthy that the mechanism of alloxan entry into cells is through the same glucose transporters, because alloxan has the same molecular shape as glucose (Ighodaro et al., 2017).

Liver enzymes are important biomarkers for assessing treatment response in diabetic patients, as their normal levels reflect improved liver function. This improvement is a good sign that the liver has regained its functional balance. Our results revealing that the induction of diabetes by alloxan increased the activity of the liver enzyme AST in blood serum of the rats are consistent with the study conducted by Elderbi et al. (2025). This is due to the fact that the increase in the AST enzyme results from liver cellular damage and thus leads to an increase in the levels of this enzyme. The increase in the levels of the AST enzyme is also due to the resulting enlargement of the liver cells and the stimulation of the endoplasmic reticulum to produce a larger amount of this enzyme to suit the cell size (Fikry et al., 2025).

The results of this study also showed that the induction of diabetes by alloxan increased the level of the ALT enzyme in blood serum of the diabetic rats. Our results are in agreement with the findings of Alaebo et al. (2022), who (through liver enzyme tests) found elevated ALT levels in the diabetic animals. The main reason behind it is the production of free radicals that incease the levels of these enzymes, as these radicals play a role in the breakdown and necrosis of liver cells, and then cause the release of these enzymes into the bloodstream. These radicals also cause cirrhosis and damage to the liver tissue, and then the loss of enzyme receptors on the epithelial cells lining the bile duct and around the central vessel. In addition, they lead to intensified release of these enzymes outside the cells (Martemucci et al., 2022).

Amino acids such as BABA can help fight many diseases and have the ability to reduce liver tissue damage (Al-Dulaimy & Jasim, 2025). This may be attributed to the acids' protective effect, removal of toxins from the liver. Thus, amino acids can treat liver disorders, as it works to reduce fat peroxidation and restore the state of antioxidants to a normal state (Lee & Kim, 2019). Another reason is their ability to synthesize and replenish the glutathione compound, which plays a role in the protection against reactive oxygen species produced during salt stress (Mahmud et al., 2020). Research has shown that beta-aminobutyric acid plays a beneficial function in accelerating wound healing and reducing inflammation. In addition, BABA stimulates B-cells to generate Igg and IgM antibodies, which in turn activate the complement system and facilitate communication between innate and acquired immunity (Thamer & Jasim, 2021). The BABA compound also plays its role in protecting cells from toxicity. Carbon tetrachloride induces the trapping of free radicals and increases the levels of antioxidant enzymes (Hamad & Jasim, 2024). Moreover, recent studies have demonstrated that the amino acid has a role in increasing the number of white blood cells and lymphocytes and increasing the production of IgG in rats treated with the amino acid BABA (Park et al., 2022), thus enhancing the immunity of the animals.

In the alloxan-induced diabetes model, tissue damage is caused by oxidative stress via abnormally increased production of free radicals (ROS), leading to lipid peroxidation, DNA damage, decreased activity of antioxidant enzymes such as SOD, and increased MDA content, a marker of lipid damage (Eldib et al., 2025). This is indicated by the results of the current study. Studies have shown that treatment with BABA restores oxidative balance in the liver by elevating SOD levels and reducing MDA and DNA damage, reflecting its ability to mitigate oxidative stress and protect hepatocytes. Beta-aminobutyric acid is likely to act as a potent non-protein antioxidant, contributing to reduced cell membrane damage and tissue recovery by supporting cellular antioxidant defense systems and DNA regeneration.

Glucokinase (GCK) is an enzyme involved in the regulation of glucose metabolism, particularly in liver cells and beta cells. It controls the levels of all the glucose-containing proteins involved in metabolism by phosphorylating them. This regulation is important for blood sugar (Chee & Dalan, 2024). Increased expression of glucokinase (GCK) leads to increased insulin secretion by the pancreas, which helps increase glucose storage and convert it into energy instead of remaining in the blood, thus improving blood sugar regulation (Haddad et al., 2024). Amino acids such as BABA are able to mitigate damage to hepatic tissue and can assist in the battle against a wide variety of disorders. Compared with those in the control negative group that was treated with 50 mg/kg of alloxan alone, the ratios of the mRNA expression of *Gck* in the damaged hepatic tissues of the rats were greater. On the other hand, the hepatic-damaged rats were observed to have an increase in the relative gene expression ratios of *Gck*. The results confirmed the successful induction of diabetes by alloxan (negative control group – 2.21-fold) compared with the healthy group (positive control – 4.36-fold). The BABA treatment showed a dose-dependent restoration of glucokinase (*Gck*) gene expression, with the 50 mg/kg dose (Group A) achieving a slight improvement (2.80-fold), the 100 mg/kg dose (Group B) producing a significant improvement (3.69-fold), and the 150 mg/kg dose (Group C) restoring gene expression to the level of the healthy group (4.10-fold), indicating that it is the optimal therapeutic dose. This is in agreement with the study conducted by Ali & Jasim (2025). Also, we observed an unexpected increase (5.60-fold) in Group D (200 mg/kg) with an anomaly in the control gene (β-actin) that requires verification. In conclusion, BABA is a promising candidate for diabetes treatment at a dose of 150 mg/kg.

Conclusion

The results of this study suggest that the nonprotein amino acid BABA has the potential to reduce pancreatic beta cell damage induced by alloxan, and may alleviate diabetes-related markers by restoring levels of GlP, GlP-1, and GCK. Moreover, BABA modulated the expression of *Gck* gene. However, further studies, particularly in human models, are necessary to validate these findings.

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