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Therapeutic Role of Beta-Aminobutyric Acid in Male Rats with Streptozotocin-Induced Diabetes: Insights into GIP, GLP-1, SCube1/2, Resistin, and Preproglucagon Gene Expression

Shams Husham Al-Naqqash, Mohammed Abbas Jasim*
Biology Department, College of Education for Women, Al-Ramadi 30001, Al-Anbar, Iraq

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Abstract

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia. This study aimed to evaluate the protective effect of beta-aminobutyric acid (BABA) on streptozotocin (STZ)-induced diabetes in male rats, by studying its impact on glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), signal peptide, CUB and EGF-Like domain-containing protein 1/2 (SCube1/2), and Resistin (RETN) pathways. Thirty adult male rats with diabetes mellitus were randomly assigned to six groups of five rats each, aged 6-8 weeks. Healthy with weights (140 to 160 grams), positive control treated with 2ml normal saline, negative control groups treated only with streptozotocin, groups treated with 50, 100, 150, 200 mg/kg of streptozotocin (STZ), then BABA, respectively. β-aminobutyric acid was administered twice weekly via oral gavage for 30 days following STZ induction at varying concentrations across the groups. Enzyme-linked immunosorbent assay (ELISA) was used to evaluate serum levels of GIP, GLP-1, SCUBE1 and SCUBE2, and RTN. The expression levels of the resistin (*RES*) gene and the preproglucagon (*GCG*) gene were assessed using the reverse transcription-quantitative polymerase chain reaction (qRT-PCR) technique. At the end of the experiment periods, blood was collected after overnight fasting. Triplicate technical sampling for all assays was studied. The results showed decreases in GIP, GLP-1, SCube ½, and RTN levels. Though treatment with BABA significantly enhanced those parameters, leading to elevated GIP and SCube 1/2 and reduced RTN, demonstrating a therapeutic effect that positively affects metabolic function. Group C1 treated with STZ followed by 150mg/kg BABA showed the highest GCG expression level (3.70635), which was significantly higher than that of other groups. On the other hand, A2 treated with STZ and 100 mg/kg BABA showed a decline in *GCG* expression with a fold change of 0.00458. Furthermore, the B1 and C1 groups displayed significantly higher *RES* expression levels (7.26015 and 3.09513, respectively) than the control groups. These findings support further investigation of BABA as a novel therapeutic compound for modulating metabolic and inflammatory pathways in type 2 diabetes

Keywords: Diabetes Mellitus, Male rats, BABA, Streptozotocin, Gene expression. Resistin gene, and Preproglucagon gene.

*Email: mohammed.a.jasim@uoanbar.edu.iq

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دور الحامض الاميني بيتا امينو بيوتارك كعلاج محتمل لذكور الجرذان المصابة بداء السكري: التأثير في مسارات GIPو GLP-1و SCube1/2 و RTN و التعبير لجينَيْ *Resistin* و *Preproglucagon*

شمس هشام النقاش, محمد عباس جاسم*

قسم علوم الحياة، كلية التربية للبنات، جامعة الأنبار، الأنبار 31001, العراق

الخلاصة

داء السكري، خلل أيضي وظيفي يلاحظ فيه زيادة مستويات السكر، استهدفت الدراسة تقييم التأثير الوقائي للحامض الاميني بيتا امينو بيوتارك اسيد (BABA) لذكور الجرذان المصابة بداء السكري المحفز بالستربتوزوتوسين (STZ) ، بدراسة التأثير على المسارات الايضية GIP, GLP-1, SCube1/2, RTN، فضلاً عن دراسة التعبير الجيني لها. شملت الدراسة 30 ذكراً طبيعية ومحفزة تجريبياً بداء السكري، قسمت عشوائياً الى ستة مجاميع بواقع خمسة لكل مجموعة بأعمار 6-8 أسابيع بوزن تراوح بين 140-160غم. مجموعة الضابطة الأولى معاملة بمحلول الملح الفسلجي 2مل، المجموعة الثانية معاملة فقط بالستربتوزوتوسين، أما المجاميع الأخرى فعوملت بالستربتوزوتوسين وتراكيز 50، 100، 150، 200 ملغم\ كغم من وزن الحيوان على التوالي. استخدمت تقنية الامتصاص المناعي المرتبط إنزيمياً (ELISA) لتقدير مستويات GIP وGLP-1 وSCube1/2 و RTNفي المصل، فضلاً عن تقدير التعبير الجيني للجينين *Resistin* و*Preproglucagon* بواسطة تقنية التفاعل التسلسلي المبلمر الكمي بالاستنساخ العكسي (qRT-PCR). أظهرت النتائج انخفاض في مستويات GIP وGLP-1 وSCube1/2 وRTN عن الطبيعي للحيوانات المصابة بالسكري، في حين أدت معاملتها بال BABA إلى تحسين مستوياتها إلى مستويات تقرب من الطبيعي، مما برهن إمكانية الحامض الاميني لإستعادة الحالة الايضية الطبيعية لها، وظهرت أعلى مستوى هي المجموعة C1 المعاملة 150ملغم\كغم BABA مع ارتفاع مستوى التعبير الجيني إلى 3.70635، وأظهرت اقل مستويات عند استخدام التركيز 100 ملغم\كغم BABA (التغاير اقل بـ 0.00458 مرة) مقارنة بالطبيعي. كذلك أزهرت المجموعتين B1 وC1 زيادة معنوية في مستويات تعبير جين RES (7.26015 و3.09513) على التوالي مقارنة بالمجاميع الضابطة. تقترح النتائج الدور العلاجي الواعد لمعالجة داء السكري النوع الثاني عن طريق رفع مستوى التعبير الجيني للبروتينات المرتبطة بالوظائف الوعائية والاستجابات الالتهابية.

1. Introduction

Diabetes is a severe chronic disease that arises when insulin generation is insufficient or the generated insulin cannot be used in the body, resulting in a long-term metabolic disorder. Diabetes affects an estimated 537 million adults worldwide between the ages of 20 and 79 (10.5% of all adults in this age range) [1]. By 2030, 643 million people will have diabetes globally, increasing to 783 million by 2045. According to the IDF ^{10}th edition, the incidence of diabetes has been rising in Southeast Asia (SEA) nations for at least 20 years, and current estimates have outperformed all previous forecasts [2].

Streptozotocin (STZ) is the most widely used diabetogenic agent in experimental animals to create models of type 1 and type 2 diabetes (hybrid), depending on diet and dose [3]. Short-chain fatty acids (SCFA) have emerged as promising compounds that may play an important role in regulating metabolism, making them a potential treatment option for diabetes [4]. Research indicates that BABA can activate protective mechanisms at both cellular and molecular levels, aiding in mitigating damage associated with elevated blood glucose levels while enhancing insulin sensitivity [5]. 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 exhibits antioxidant effects by elevating the activity of defence enzymes, such as SOD, CAT, and GPx, thereby reducing oxidative damage in liver and pancreatic tissue.
Previous studies indicated that BABA interacts with cellular signalling pathways, including those involved in tissue inflammation, thereby reducing the expression of inflammatory cytokines (TNF-α and IL-6). Moreover, BABA supports the production of phenols and other secondary metabolites that alleviate chronic oxidative stress. BABA doesn't directly target free radicals, but it resets physiological responses in a protective and stimulatory manner. Glucose-Dependent Insulinotropic Polypeptide (GIP), a hormone secreted by K cells in the small intestine upon food intake, plays an essential role in promoting insulin secretion from the pancreas [6-9].

Glucagon-like peptide-1 (GLP-1), an incretin hormone released from the small intestine cells after food ingestion. It plays a crucial role by releasing insulin after stimulating the pancreas' response, regulating blood sugar levels [10]. GLP-1 affects in vivo and in vivo regeneration of β-cells by differentiation and neogenesis, due to the role of GLP-1 / GLP-1R signaling in the differentiation of pancreatic endocrine cells upon their development [11].
SCube1 and SCube2 are surface proteins that regulate various processes, including vascular development, inflammation, and cell signaling [12].

In particular, SCube1 has been linked to thrombosis and vascular inflammation; it contributes to the risk of cardiovascular complications in diabetic individuals [13]. Correspondingly, SCube2 regulates inflammatory pathways, thereby disrupting glucose metabolism and contributing to insulin resistance [13]. Increased levels of SCube 1/2 may serve as indicators of diabetic progression, positioning these proteins as biomarkers for disease severity assessment [14].

The preproglucagon genes may encode glucagon and other hormones (such as GLP-1) related to maintaining blood glucose balance [15]. GLP-1 increases insulin secretion during glucagon release, thereby improving blood sugar regulation. Preproglucagon plays a significant role in glucose metabolism disorders and in the improvement of treatments targeting those pathways [16].
BABA can cure alloxan-induced diabetes by enhancing DNA fragmentation [5]. This study aimed to evaluate the potential therapeutic effects of BABA on Sprague-Dawley diabetic rats induced with STZ, focusing on molecular pathways regulating glucose.

1.2 Materials and methods:

1.2.1 *Animals*

Six groups of Sprague Dawley male rats (5 rats each) with 8-10 weeks. All rats were in good health, with weights ranging from 140 to 160 grams. The experimental protocols and animal use (protocol number CHREC/11/2022) have been authorised by the Institutional Animal Ethics Committee, and all procedures have adhered strictly to the ethical standards established by Anbar University. After the acclimatization period, the rats received three doses of streptozotocin, administered one every three days. The rats also received oral therapeutic doses of β-aminobutyric acid, which was administered twice weekly via oral gavage for 30 days following STZ induction at varying concentrations across the groups:
Control (+): Positive control groups treated with 2 mL of normal saline
Control (-): Negative control groups treated only with streptozotocin.

Groups A, B, C, and D**:** treated with STZ and a concentration of 50, 100, 150, and 200 mg/kg of body weight, then BABA, respectively.

1.2.2 Collection of blood samples

After completing the designated 60-day experimental period, the animals were subjected to a 10-hour period of starvation overnight. Subsequently, their final weight was measured. Following this, the animals were anesthetized using a chloroform solution. 10 ml of blood samples were collected from each animal through cardiac puncture using specialized syringes. Divide this blood into 5 ml and place it into test tubes without anticoagulants. The serum was acquired by centrifugation at 2500 -3000 revolutions (rpm). It was then preserved at a temperature of -20°C in fresh plastic containers until specific biochemical examinations were conducted [11]. The remaining 5 ml of blood was placed in Trizol tubes and kept frozen at −20°C for molecular analysis.

1.2.3 ELISA test

The enzyme-linked immunosorbent assay (ELISA) was utilized to evaluate the serum concentrations of glucose-dependent insulinotropic polypeptide (GIP), (GIP-1), resistin (RETN), signal peptide, CUB, and EGF-like domain-containing protein 1 (SCUBE1), signal peptide, CUB, and EGF-like domain-containing protein 2 (SCUBE2). The antibodies for GIP, GIP-1, RETN, SCUBE1, and SCUBE2 were precoated on the ELISA plates. The chromogenic response in substrate solutions is related to the concentration of GIP, GIP-1, RETN, SCUBE1, and SCUBE2 after the samples are placed 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. The level of GIP(Cat No:CK-bio-26417, Sensitivity: 1.0pg/Ml), GIP-1(Cat No:CK-bio-14680, Sensitivity: 10.0pg/ml), RETN(Cat No:CK-bio-15273, Sensitivity:0.1ng/mL), SCUBE1(Cat No:CK-bio-15317, Sensitivity: 0.1ng/mL), and SCUBE2(Cat No:CK-bio-26416, Sensitivity: 10.0pg/ml), were measured in serum by using (Sunlong, China).

1.2.4 Molecular study

1.2.4.1 Genomic RNA Extraction

The RNA was extracted from the blood of rats as well as from apparently healthy groups (control) using TransZol Up Plus RNA (Transgen) following the manufacturer's instructions. Subsequently, the quality of the samples for further usage was evaluated by measuring the concentration of the extracted RNA using a Quantus Fluorometer. (Thermo Fisher Scientific, USA). The RNA was then stored at -80 °C for further analysis**.**

1.2.4.2 cDNA synthesis

The total RNA underwent reverse transcription into complementary DNA (cDNA) using QIAGEN Rotor gene Q Real-time PCR System (Germany). The EasyScript One-Step gDNA Removal and cDNA, Synthesis SuperMix Kit, as provided by TransGen Biotech Co in China, within a reaction volume of 20 µl, following the manufacturer's guidelines. As well as primers that were specific for genes under study, a negative RT control was used to ensure the purity. The cDNA was then ready for downstream analysis by qPCR.

1.2.4.3 Quantification of GCG and RES genes by Quantitative Real Time PCR (qRT–PCR)

For the quantification of *GCG* and *RES*, quantitative PCR was performed using primer sets targeting the *GCG* and *RES* genes, normalised to the endogenous reference GAPDH gene, as shown in Table 1. Primers were carefully designed, and their specificity was verified using the Primer BLAST tool on the National Centre for Biotechnology Information (NCBI)

website. 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 µl, 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. Furthermore, to optimize the PCR process, annealing temperatures were systematically optimized. This was achieved using a gradient thermal cycler (Eppendorf, Germany) with a range of temperatures, as specified in the program outlined in Table 2.

Table 1: Primers used for gene expression of *GCG, RES*, and *GAPDH* genes

Primer	Sequence (5′→3′ Direction)	Product Size (bp)	Tm °C
GCG			
Forward	ACAAAATCTTGAGCACGGCG	275	60
Reverse	CACTACTGCACGGGACCTT		62.2
RES			
Forward	CCCAGCTCCAGAGCCATTAG	129	63.5
Reverse	GAAAGAAGCCCCCGAAAAGC		62.4
Beta actin			
Forward	CACGGCATTGTAACCAAC	1125	56.1
Reverse	GAGCGCGTAACCCTCATAGA		60.8

Table 2: The thermal profile of *GCG, RES*, and *GAPDH* gene expressions

Steps	Temperature (°C)	Duration	Repeat cycle
Enzyme activation	94	5 min	1
Denaturation	94	5 sec	35
Annealing	58	15 sec	
Extension	72	20 sec	
Dissociation	55 ºC-95 ºC		1

1.2.4.4 Data analysis of RT-qPCR

The expression of *DNMT-1* was assessed using the double delta Ct (threshold cycle) (ΔΔCT) analysis (folding change), with the *GAPDH* serving as the housekeeping reference gene, which was calculated according to the Levaic equation [17], using the following calculations:

$$\Delta CT = CT\ target\ gene - CT\ of\ reference\ gene \quad [17]$$
$$\Delta\Delta CT = \Delta CT\ of\ each\ sample - average\ control\ \Delta CT$$
$$Folding\ change = 2 - \Delta\Delta Ct$$

1.2.4.5 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, 2019) program was used to detect the effect of differences between groups/isolates on study parameters. LSD (LSD-Least Significant Difference) was used to compare the means in this study significantly.

1.3 Results

1.3.1 GIP levels

Figure 1 shows GIP levels among the diabetic induced rat groups using Mean ± SE, with statistical differences indicated via LSD and P value. The results show group A recorded the

highest GIP level (97.43 ± 35.06) compared to the control+ group (positive control group), which recorded 75.19 ± 2.02.

The control group (negative control) recorded the lowest GIP level (39.58 ± 6.36), indicating a significant decrease compared with the other groups, especially A and control+. Groups B, C, and D recorded (52.89 ±6.41), (53.45 ±10.34), and (55.35 ±10.63), respectively, meaning they were not significantly different from each other or from the Control+ group.

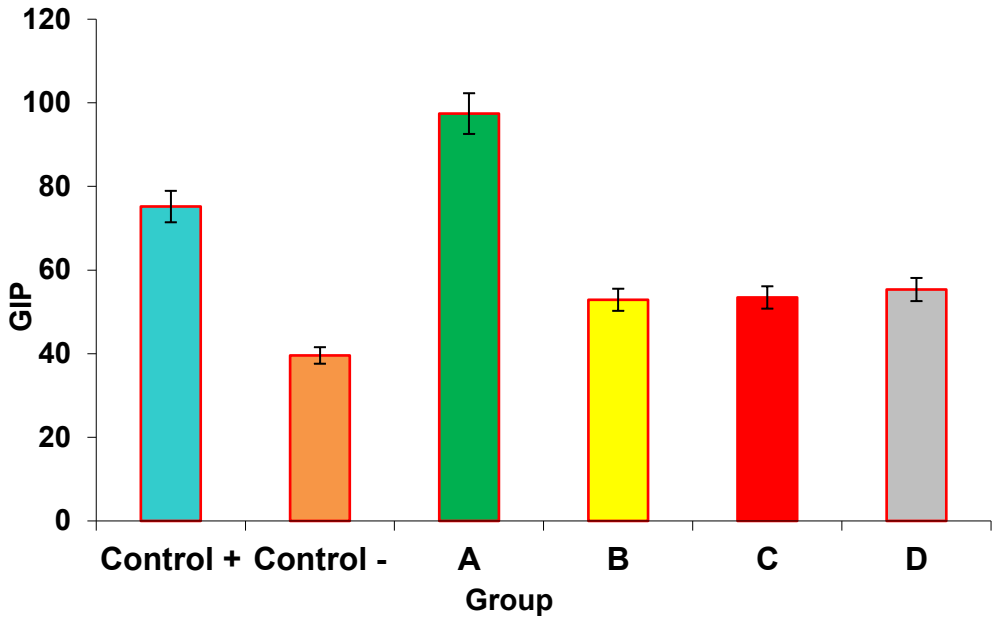


Figure 1: Comparison between difference groups in GIP

Figure 2 shows the levels of GLP-1 in the groups of induced diabetic rats. The results indicate that the negative control group (Control-) recorded the lowest GLP-1 levels (256.24 ± 17.78), while group A (50 mg/kg STZ) recorded the highest GLP-1 levels (355.45 ± 104.46), suggesting that a low dose of BABA may help stimulate GLP-1 production or improve the body's response to it. Group D (200 mg/kg STZ+BABA) recorded levels close to those of the positive control group (335.41 ± 64.55). Groups B and C (100 and 150 mg/kg STZ+BABA) recorded the lowest GLP-1 levels (214.23 ± 3.08), compared to the positive control group.

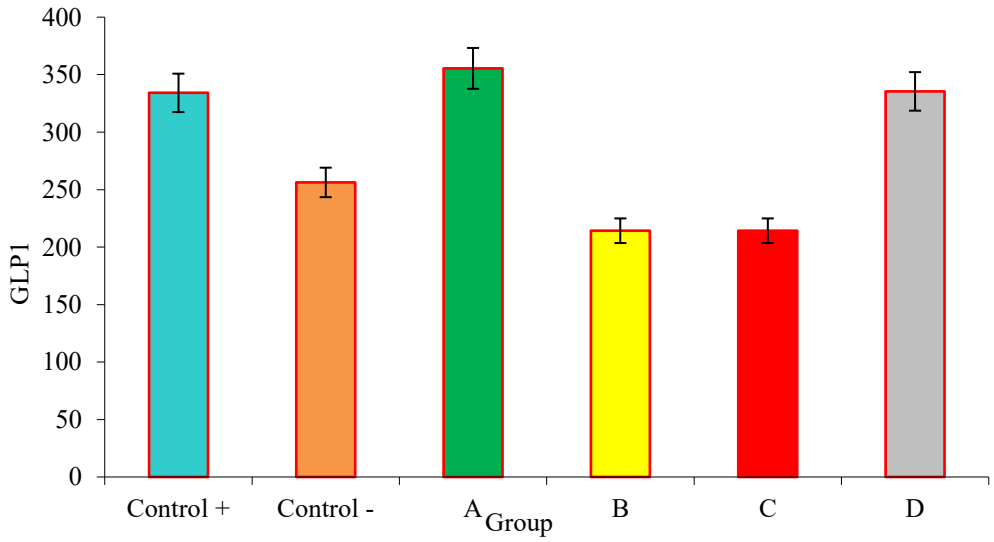


Figure 2: Comparison between difference groups in GLP1

Figures 3 and 4 compare the mean levels of SCUBE1 and SCUBE2 across the different groups. The results were expressed as Mean ± SE. The highest level of SCUBE1 was recorded in the positive control group (Control+) at 12.66 ± 0.24, and the lowest level was recorded in the negative control group (Control-) at 6.51 ± 0.31. Group A showed a relatively high value (11.86 ± 1.41). Groups B, C, and D recorded levels lower than Control+, but higher than Control-, with values ranging from 7.34 to 9.24, indicating variation in response to treatment across the groups. The results indicate the presence of statistically significant differences at the P=0.0014 level.

The highest level of SCUBE2 was recorded in group A (697.86 ± 226.94), compared to the Control+, which recorded 417.03 ± 20.33, the Control- recorded the lowest level (246.18 ± 33.58), groups B, C, and D recorded average levels ranging from 467.95 to 604.47, and there were no significant differences between them and the Control+. The LSD value for SCUBE2 was 411.43 at P=0.0498, indicating the presence of significant differences between some groups, but less pronounced than SCUBE1.

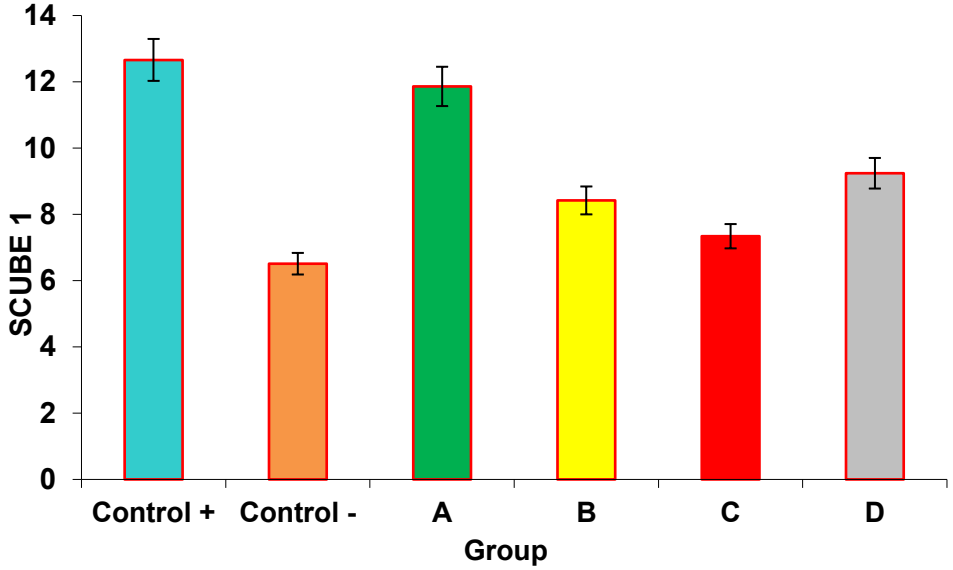


Figure 3: Comparison between difference groups in SCUBE 1

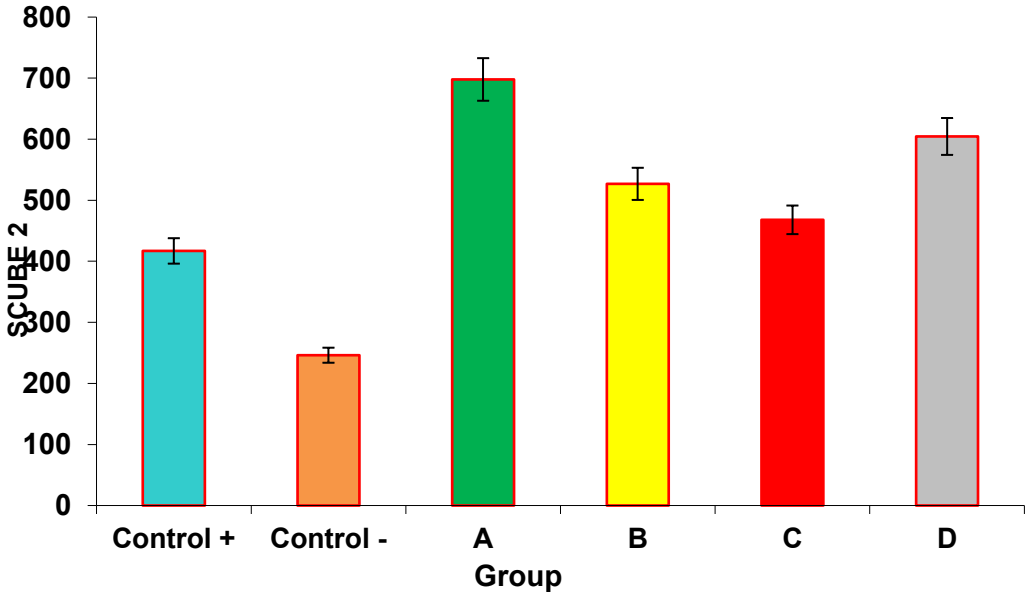


Figure 4: Comparison between difference groups in SCUBE 2

1.3.2 RENT levels

RENT is a hormone associated with insulin resistance and involved in inflammatory processes in the body. Figure 5 shows RENT levels in diabetic-induced rat groups. The results in Figure 2 show that the Control+ recorded the highest level (6.13 ± 0.02), indicating a significant increase in RENT levels compared to the other groups. The Control recorded the lowest level (3.31 ± 0.28), which may reflect the effect of the disease on reducing RENT levels compared with the Control+. Group A recorded a level (4.96 ± 1.21), indicating that the treatment used may have a partial effect on regulating RENT. Groups B, C, and D recorded levels (3.71 ±0.78), (3.78 ±0.53), and (3.69 ±0.61), respectively. There is no significant difference between them and Control-.

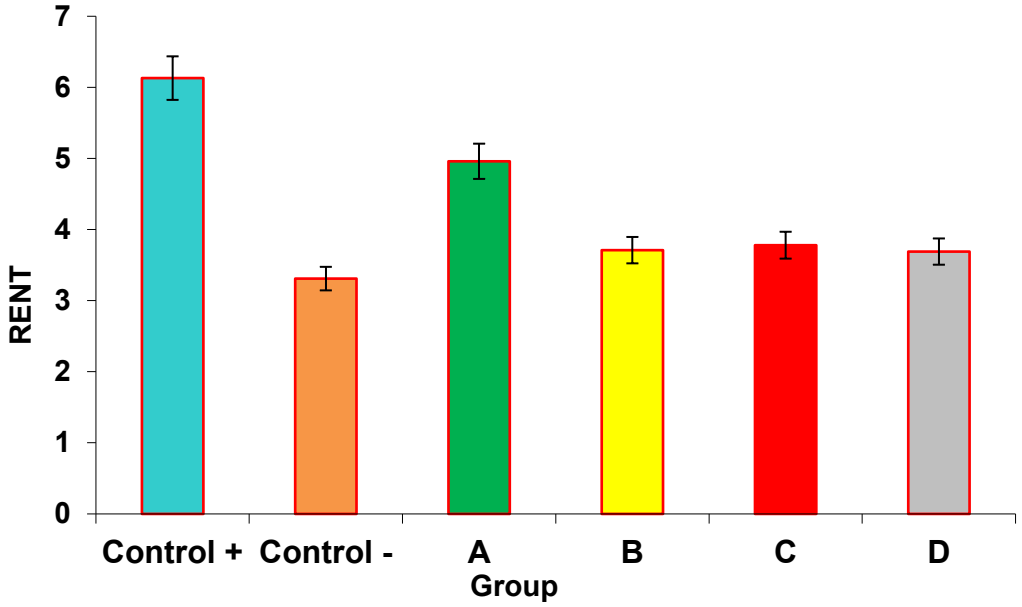


Figure 5: Comparison between difference groups in RENT

1.3.3 GCG and RES gene expression

The fold of the *GCG* gene and *RES* expression was calculated as a ratio of $2^{-\Delta\Delta CT}$ of the experimental groups (A1, A2, B1, B2, C1, C2, D1, and D2) to that of the control group (NEG1and NEG2). According to the study results, there were highly statistically significant differences in *GCG* and *RES* expression levels among the different experimental groups of male rats with diabetes mellitus. In the present study, it was found that only the C1 group, which was treated with a concentration of 150 mg/kg of body weight STZ, then BABA, had notably higher *GCG* level (3.70635) than the positive and negative control groups, which were treated with normal saline and streptozotocin, respectively. While all of the other isolates showed a decrease in gene expression, with the greatest decrease in isolate group A2, with a fold (0.00458), which was treated with a concentration of 100 mg/kg of body weight streptozotocin and then BABA. Similarly, *RES* expression levels were notably increased in the B1 and C1 groups, which were treated with STZ at concentrations of 100 mg/kg and 150 mg/kg, respectively, based on body weight, followed by BABA at (7.26015, 3.09513, respectively), compared to the positive and negative control groups. For a more detailed overview of the fold change values for each patient group, refer to Table 3 (GCG) and Table 4 (*RES*).

Table 3: Fold change of *GCG* gene expression in different experimental groups using the Livak formula (a,b,c refers to significance at P-value (0.0001)

Groups	Positive control			Treatments			ΔΔCt	Fold change
	Mean Ct GADPH	Mean Ct GCG	ΔCt	Mean Ct GADPH	Mean Ct GCG	ΔCt		
A1	17.69	25.05	7.36	13.6	27.13	13.53	6.17	0.01388 ±0.004 c
A2	17.69	25.05	7.36	13.37	28.5	15.13	7.77	0.00458 ±0.001 c
B1	17.69	25.05	7.36	23.05	31.47	8.42	1.06	0.47963 ±0.052 b
B2	17.69	25.05	7.36	17.66	29.93	12.27	4.91	0.03326 ±0.005 c
C1	17.69	25.05	7.36	19.94	25.41	5.47	-1.89	3.70635 ±0.632 a
C2	17.69	25.05	7.36	12.43	25.73	13.3	5.94	0.01628 ±0.004 c
D1	17.69	25.05	7.36	16.06	27.73	11.67	4.31	0.05042 ±0.013 c
D2	17.69	25.05	7.36	15.14	28.15	13.01	5.65	0.01992 ±0.007 c
NEG1	17.69	25.05	7.36	14.36	25.6	11.24	3.88	0.06792 ±0.142 c
NEG2	17.69	25.05	7.36	13.13	27.34	14.21	6.85	0.00866 ±0.002 c
L.S.D. (P-value)	--	--	--	--	--	--	--	0.271 ** (0.0001)

Table 4: Fold change of *RES* gene expression in different experimental groups using the Livak formula (a,b,c refers to significance at P-value (0.0001)

Groups	Positive control			Treatments			ΔΔCt	Fold change
	Mean Ct GADPH	Mean Ct RES	ΔCt	Mean Ct GADPH	Mean Ct RES	ΔCt		
A1	17.69	25.28	7.59	13.6	27.1	13.5	5.91	0.01663 ±0.005 c
A2	17.69	25.28	7.59	13.37	26.71	13.34	5.75	0.01858 ±0.007 c
B1	17.69	25.28	7.59	23.05	27.78	4.73	-2.86	7.26015 ±0.688 a
B2	17.69	25.28	7.59	17.66	26.65	8.99	1.4	0.37893 ±0.071 c
C1	17.69	25.28	7.59	19.94	25.9	5.96	-1.63	3.09513 ±0.522 b
C2	17.69	25.28	7.59	12.43	26.39	13.96	6.37	0.01209 ±0.004 c
D1	17.69	25.28	7.59	16.06	27.85	11.79	4.2	0.05441 ±0.017 c
D2	17.69	25.28	7.59	15.14	27.04	11.9	4.31	0.05041 ±0.016 c
NEG1	17.69	25.28	7.59	14.36	25.5	11.14	3.55	0.08537 ±0.024 c
NEG2	17.69	25.28	7.59	13.13	27.21	14.08	6.49	0.011125 ±0.005 c
L.S.D. (P-value)	--	--	--	--	--	--	--	0.3877 ** (0.0001)

1.4 Discussion

β-Aminobutyric acid (BABA) has been successfully used to prime stress resistance in numerous rats; the current study was designed to investigate the effect of BABA as a possible treatment for diabetic male rats. The current study was designed to investigate the impact of BABA as a potential treatment for diabetic male rats. STZ is commonly used to induce diabetes in mice by destroying the pancreatic cells responsible for insulin production, resulting in elevated blood sugar levels.

GIP may play a role in protecting pancreatic beta cells from damage. GIP stimulates pancreatic beta cells to secrete insulin, which helps lower blood sugar levels. This effect is known as the "incretin effect," as it works in conjunction with GLP-1 to enhance insulin secretion in a glucose-dependent manner, and its anti-apoptotic effect on beta cell survival is mediated by STZ inhibition [18]. Results indicate that STZ destroys 90% of beta cells, affecting glucose-induced insulin secretion without causing hyperglycemia [19]. The

hyperclucagonemia plays a vital role in elevating blood glucose levels. Moreover, these findings indicated that at low STZ concentrations (50 mg/kg), β-cells are partially damaged, which may explain the moderate changes in GIP levels compared to the control. The results in Figure 1 indicate that BABA treatment improved glucose tolerance by increasing insulin secretion during the glucose tolerance test in STZ-untreated rats (Control +). Additionally, BABA treatment significantly increased active GIP and GLP-1 levels. The results of the current study are consistent with a study conducted by which found that the amino acid BABA plays a protective role in improving GIP levels. At high STZ levels, BABA treatment failed to improve glucose and insulin secretion responses in rats injected with high STZ doses, as STZ destroys beta cells in the pancreas, leading to a severe decrease in insulin production and elevated blood sugar levels. These results declare that GIP induces insulin secretion in STZ rats treated with BABA [20]. The results supported by studies using Gcgr-/-Glp1r-/-, in both models the deficient in GLP-1 and GIP activity exhibiting hyperglymecaemia upon STZ treatment, highlighting the importance of GLP-1 and GIP on glycemic control under STZ- induced β-cells suppression [21, 22].
Reticulon (RTN) is a cellular stress-related protein that interferes with cellular responses to damage, particularly in cells of the pancreas, liver, and nervous system. Many factors may affect RTN levels, including cytotoxic substances (such as STZ) and protective agents (such as BABA). In groups treated with STZ alone (Control -) STZ initially increases RTN levels as a stress response to damaged pancreatic and liver cells, stimulating RTN protein expression as a defense mechanism. As cellular damage continues, RTN levels subsequently decline due to cell death and loss of the ability to respond to stress. However, when BABA is administered after STZ, it reduces oxidative stress and cell death, thereby helping maintain RTN levels within a balanced range compared with the STZ-alone group. This is because BABA protects cells from excessive damage [23].

The study results also indicate the negative effects of STZ on (SCube 1 and SCube 2), as it disrupts the gene expression of proteins associated with vascular injury. The Control-, which was exposed only to STZ, showed the lowest levels of SCube 1 (6.51 ± 0.31) and SCube 2 (246.18 ± 33.58), indicating that STZ caused damage to cellular function.
This reduction reflects increased cellular damage and a reduced cell's ability to express these proteins, which are crucial for healing and cellular repair. STZ causes severe oxidative stress, leading to the spread of inflammation and increasing SCube1 and SCube2 in the initial stages of the inflammatory response [24].
In BABA-treated groups, SCube1 and SCube2 levels increased gradually, indicating the ability of BABA to enhance the gene expression of these proteins, aid in repairing STZ-induced damage, and improve the inflammatory and regenerative responses [25].
GLP-1 commonly synthesized from preproglucagon, which is encoded by the glucagon gene (*GCG*) [26]. Our results displayed that *GCG* gene expression was elevated in diabetic male rats after BABA injection. This increase in gene expression may be due to the interaction of BABA with gene-regulating mechanisms, stimulation of protein production associated with metabolic responses, or reduction of insulin resistance [27]. BABA may act as a regulatory factor, enhancing GCG gene expression, improving pancreatic β-cell function, or regulating blood glucose levels [28].

Numerous mechanisms can affect *GCG* gene expression in diabetic male rats. The first is modulation of transcription factors: BABA may enhance the activity of transcription factors that bind to the *GCG* gene promoter region, thereby increasing transcription [29]. The other mechanism may involve epigenetic modifications, as BABA may alter histones or DNA methylation, exposing genes for expression [30]. The administration of STZ damages

pancreatic β-cells, leading to insulin deficiency and elevated blood glucose levels, then to diabetes [31]. This situation can adversely affect the rats' health, resulting in reduced physical activity and increased fatigue. Subsequently, BABA was given to the rats. It may exhibit anti-inflammatory properties and protect pancreatic cells.
As a result, BABA may improve rat's health, aiding in the restoration of their energy levels and activity.

Conclusion:

The study results suggest that BABA has the potential to heal β-cell damage induced by STZ and may restore levels of SCube1, SCube3, GIP, and RNT. However, BABA controlled the expression of *GCG* and *RES* genes. Furthermore, further studies, particularly in humans, are necessary to validate these findings.

Statements on compliance with ethical standards

The experimental protocols have been authorized and issued by Ethical Committee no.22, University of Anbar.

Disclosure and conflict of interest

Conflict of Interest: The authors declare that they have no conflicts of interest.

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