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THE EFFECTS OF *Gymnema sylvestre* EXTRACT SUPPLEMENTATION ON EXPRESSION OF GENE ASSOCIATED WITH GLUCOSE HOMEOSTASIS IN DIABETIC MOUSE MODEL

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ABSTRACT

Gymnema sylvestre, a traditional medicinal herb known for its anti-diabetic properties, has been widely used to manage hyperglycemia by various mechanisms, including the inhibition of glucose absorption, stimulation of insulin secretion, and enhancement of pancreatic beta-cell regeneration. In the context of the increasing prevalence of diabetes, particularly in Vietnam, this study investigates the molecular effects of *G. sylvestre* extract (gymnemic acid) on gene expression associated with glucose homeostasis in diabetic mouse models induced by streptozotocin. The expression levels of key regulators involved in glucose transport, insulin production, and inflammatory responses, such as *GLUT2*, *MAFA*, *INS2*, *PDX1*, *NFKB*, and *GCK*, were evaluated using RT-qPCR analysis. Our findings indicated that diabetic mice, receiving 250 mg of *G. sylvestre* extract per kg of body weight daily for 6 weeks, exhibited significant improvements in glucose regulation when compared to their counterparts on a high-fat diet. This result was evidenced by increased gene expression related to insulin secretion and glucose uptake, alongside a decrease in body weight and blood glucose levels in diabetic mice. These beneficial outcomes can be attributed to the role of gymnemic acid in supporting various processes, including competitive inhibition of glucose absorption, enhancement of insulin secretion, protection and regeneration of pancreatic beta-cells, and improvement of lipid metabolism. Collectively, these findings highlight the potential of *G. sylvestre* as a nutrigenomic intervention in diabetes management through its regulation of key metabolic pathways.

Keywords: Diabetes mellitus, *Gymnema sylvestre*, gymnemic acid, *Mus musculus*, RT-qPCR analysis.

INTRODUCTION

Gymnema sylvestre, a medicinal herb originating from India, Africa, Australia, and Vietnam, has served as a fundamental element of traditional medicine for more than 2,000 years and is commonly known as "Gurmar" and the "Sugar destroyer" herb (Tiwari *et al.*, 2014). The leaves are rich in anti-diabetic gymnemic acid, which plays a key role in managing diabetes, a chronic metabolic disorder characterized by elevated blood glucose levels. This compound functions by inhibiting intestinal glucose absorption, stimulating insulin secretion, and promoting beta-cell regeneration (Briscoe, 2006; Tiwari *et al.*, 2014). In Vietnam, diabetes is identified as the seventh primary cause of mortality, with statistical analyses indicating that its prevalence doubled over a decade, rising from 2.7% in 2002 to 5.4% in 2012, and further increasing to 7.3% by 2020 (Ngoc *et al.*, 2020). The chronic high blood sugar levels associated with diabetes can result in various serious health issues, such as cardiovascular disorders, neurological impairment, ocular and renal complications, and increased mortality risk in individuals lacking adequate treatment.

Dietary interventions have proven highly effective in alleviating diabetes symptoms through enhancement of metabolic outcomes, which include improved glycemic control, optimized lipid profiles, and reduced oxidative stress (Imam *et al.*, 2012; Weng *et al.*, 2019). Nguyen *et al.* (2024) found that providing a diet consisting of germinated Huyet Rong red rice (GRR) to streptozotocin (STZ)-induced diabetic mice, formulated by substituting carbohydrate content with GRR flour, significantly reduced body weight and upregulated the expression of *GLUT*, *NFKB1*, *IRS1*, and *GSK-3*, which are crucial in glucose transport, insulin signaling, and the responses to inflammatory as well as oxidative stress responses. Similarly, Pothuraju *et al.* (2014) demonstrated that supplementation with *G. sylvestre* extract, which was abundant in gymnemic acids, supports glucose homeostasis by downregulating the expression of genes involved in lipogenesis and fatty acid production, such as *Sterol regulatory element-binding protein 1c* (*SREBP1c*) and *Carbohydrate response element-binding protein* (*ChREBP*). Likewise, an 8-week administration of *G. sylvestre* in STZ-induced diabetic mice enhanced insulin secretion and decreased blood glucose concentrations by protecting liver, kidney, and pancreatic tissues against oxidative damage (Tiwari *et al.*, 2014). These findings highlight the potential of dietary modifications involving *G. sylvestre* to modulate metabolic pathways and attenuate the progression of diabetes.

The present study aims to investigate the effects of *G. sylvestre* extract, particularly its gymnemic acid content, on the expression of genes involved in glucose homeostasis pathways in diabetic mouse models. We first generate two groups of diabetic mice through administration of STZ to high-fat diet (HFD)-induced obese mice. Subsequently, the impact of *G. sylvestre* supplementation on the expression of key diabetes-related genes, including *Glucose transporter-2* (*GLUT2*), *MAF bZIP*

transcription factor A (*MAFA*), *Insulin-2* (*INS2*), *Pancreatic and duodenal homeobox 1* (*PDX1*), *Nuclear factor kappa B* (*NFKB*), and Glucokinase (*GCK*), was evaluated using RT-qPCR. This research seeks to elucidate the molecular mechanisms underlying the therapeutic potential of *G. sylvestre*, providing insights into the interplay between nutrition and gene expression to support future nutrigenomic strategies for diabetes management in humans.

MATERIALS AND METHODS

Animals and plant materials

Thirty-six male Swiss albino mice (*Mus musculus* var. albino) aged 6–8 weeks (weighing 20 ± 2 g) were sourced from the National Institute of Hygiene and Epidemiology (NIHE) and kept in standard plastic cages with sterile rice husk bedding. They were maintained under controlled conditions, including a 12-hour light/12-hour dark cycle, a temperature of 24 ± 2°C, and humidity levels of 60 to 70%. All procedures followed the ethical guidelines set by the Ethics Committee of the Institute of Biology.

Gymnema sylvestre (Retz.) R. Br. ex Sm. was provided by medicinal herb gardens in Phu Luong district, Thai Nguyen province. *G. sylvestre* was harvested at the optimal maturity, neither too young nor too old, to ensure the highest active compound content. From planting to harvesting, it takes approximately 6 to 8 months, and a single planting can be harvested for over 10 years. Each year, it can be harvested 4 to 5 times, from April to December, approximately every 2 months.

Plant extract

Leaves of *G. sylvestre* were cleaned by removing damaged and wilted parts and dirt and then thoroughly washed, air-dried, and roughly ground into small pieces. Extraction of *G. sylvestre* leaves was conducted based on protocols described by Ahamad *et al.* and Saeed *et al.* with some minor modifications (Ahamad *et al.*, 2014; Saeed *et al.*, 2022). In brief, *G. sylvestre* material was soaked in ethanol at a ratio of 1:1 (w/v) for 24 hours at room temperature, followed by ultrasonic-assisted extraction at 200 W and 40°C for three sessions of 30 minutes each. The extracted solution was filtered three times to remove insoluble materials and concentrated under reduced pressure using rotary evaporation to yield a semi-solid extract.

Post ultrasonic-assisted extraction of *G. sylvestre*, the crude extract was fractionated by different types of solvents (chloroform, ethyl acetate, n-butanol, and n-hexane) in accordance with the protocol of Srinivasan and Perumal (2020) with some minor modifications. The gymnemic acid content of each *G. sylvestre* fraction was analyzed by the HPLC Alliance TM e2695 (Waters, USA) using an Xbridge BEH-C18 column according to the manufacturer's guidance. The fractions of *G. sylvestre* extract were stored at 4°C for subsequent experiments.

Experimental design

Induction of obesity in mice

Mice were divided into three groups. Group 1 (n = 12; control) was fed with a standard regular diet (SRD), and Groups 2 and 3 (n = 12 each) were fed with a high-fat diet (HFD; 40% fat, 30% carbohydrate and 30% protein)

containing a soybean and coconut oil mixture (1:2; v/v) as the source of fat for 8 weeks. All mice were allowed to drink water *ad libitum* during all experiments.

Induction of diabetic mouse models

Post 8 weeks, mice in Group 1 were injected with 0.1 M citrate buffer (pH: 4.5), while mice in Groups 2 and 3 were injected intraperitoneally with 35 mg/kg body weight of streptozotocin (STZ; Sigma, USA) dissolved in 0.1 M citrate buffer (Nguyen *et al.*, 2024). Groups 1-3 were provided access to their respective diets and maintained under the same housing conditions.

Dietary changes

Diabetic mice from Group 3 were daily fed with 250 mg *G. sylvestre* extract (DTC) per kg body weight and maintained at the same housing conditions for 6 weeks. Subsequently, the intraperitoneal glucose tolerance (IPGTT) test was conducted again to measure the blood glucose level. For all groups, body weight was measured at 2-week intervals. Mice of all groups were sacrificed at the end of the experiment, and liver tissues were harvested for subsequent experiments.

Intraperitoneal glucose tolerance test

The IPGTT test was conducted 10 days after the STZ injection. Mice were fasted overnight, and their blood glucose levels were measured using a glucometer (Accu-Chek Instant, Roche, Switzerland) and recorded before (baseline) and after a glucose injection (50%, 2 g of glucose/kg body mass) at 30-, 60-, and 90-minute intervals. Mice with a blood glucose level higher than 11.1 mmol/L were considered diabetic.

Complementary DNA synthesis and RT-qPCR analysis

Total RNA was isolated from homogenized pancreatic tissues using the Qiagen RNeasy kit (Germany) according to the manufacturer's protocol. The RNA concentration and purity were assessed with a NanoDrop™ spectrophotometer (USA). Complementary DNA (cDNA) was synthesized from 1.0 µg of total RNA using the NEB ProtoScript® II First Strand cDNA Synthesis Kit (Vietnam) following the provided protocol. The resulting cDNA samples were stored at -20°C for subsequent experiments.

RT-qPCR analysis was conducted using a Qiagen Rotor-Gene Q system (USA) with the NEB Luna® Universal One-Step RT-qPCR Kit (Vietnam). Each 10 µL RT-qPCR reaction contained 1X Master Mix, 1.0 mM of each primer, and 50 ng of cDNA. The amplification process included an initial step at 95°C for 10 minutes (1 cycle), followed by 40 cycles of 95°C for 15 seconds and 60°C for 40 seconds. Primers used were detailed in Table 1. The reference genes, *RPL13A* and *UBC*, were employed for normalization of the RT-qPCR data, as described by Chhabra *et al.* (2021) and Yan *et al.* (2016). The analysis was performed in triplicate, and the results were evaluated using the Livak's ΔΔCt method (Livak & Schmittgen, 2001).

Statistical analysis

Data analysis was performed using the Student's t-test method in Microsoft Excel. A *p*-value below 0.05 was considered statistically significant.

Table 1. List of primers used for RT-qPCR analysis.

Primer	Sequence (5'-3')	Amplicon (bp)	Gene name	Accession number
GLUT2	F: ATCACCGGAACCTTGGCTTT R: CAGCTTTCCGGTCATCCAGT	151	Glucose transporter 2	NM_031197.2
MAFA	F: CTCCTCGCTCATTCGCTCTG R: GGACCAACACGCAGGTAAGT	116	MAF bZIP transcription factor A	NM_194350.2
INS2	F: CAGCAAGCAGGAAGGTTATTGT R: CAGGTGGGAACCACAAAGGT	133	Insulin 2	NM_001185083.2
PDX1	F: ATTCTTGAGGGCACGAGAGC R: CTGGTCCGTATTGGAACGCT	222	Pancreatic and duodenal homeobox 1	NM_008814.4
NFKB	F: GAAATTCCTGATCCAGACAAAAAC R: ATCACTTCAATGGCCTCTGTGTAG	194	Nuclear factor kappa B	NM_008689.3
GCK	F: CAACTGGACCAAGGGCTTCAA R: TGTGGCCACCGTGTCATTC	133	Glucokinase	NM_010292.5
RPL13A	F: CTGCCCCACAAGACCAAGAG R: GGACCACCATCCGCTTTTTC	100	Ribosomal protein L13A	NM_009438.5
UBC	F: AGCCCAGTGTTACCACCAAGA R: TAAGACACCTCCCCCATCACA	117	Ubiquitin C	NM_019639.4

RESULTS AND DISCUSSION

Gymnemic acid content from *Gymnema sylvestre* extracts

The results from Table 2 show that the crude ethanol extract of *G. sylvestre* contains 6.9 mg of gymnemic acid per 100 g of plant material. Among the solvent fractions derived from this extract, only the N-butanol fraction (Fraction 4) exhibited a detectable and significantly higher gymnemic acid content of 15.8 mg per 100 g of plant material, whereas the N-hexane, chloroform, and ethyl acetate fractions showed no detectable gymnemic acid. The findings suggest that gymnemic acid exhibits greater solubility in the moderately polar N-butanol solvent compared to nonpolar or less polar solvents such as N-hexane, chloroform, and ethyl acetate.

The elevated level of gymnemic acid content in the N-butanol fraction suggests that this method of solvent fractionation effectively enhances the concentration of gymnemic acid from the crude ethanol extract. This was consistent with previous studies indicating that the polarity of solvent significantly influences the extraction efficiency of gymnemic acid, where polar solvents like ethanol and butanol demonstrate greater effectiveness in comparison to nonpolar solvents (Nunta *et al.*, 2023; Rai *et al.*, 2023). The lack of gymnemic acid in the nonpolar fractions (N-hexane and

chloroform) may be due to its poor solubility, a characteristic feature of polar saponin glycosides, in nonpolar solvents (Halilu, 2023).

Table 2. Gymnemic acid content in different *G. sylvestre* extracted fractions.

	Solvent	Gymnemic acid (mg/100 g)
Crude extract	Ethanol	6.9
Fraction 1	N-hexane	ND
Fraction 2	Chloroform	ND
Fraction 3	Ethyl acetate	ND
Fraction 4	N-butanol	15.8

ND: not detected

Induction of diabetic mouse model by STZ injection

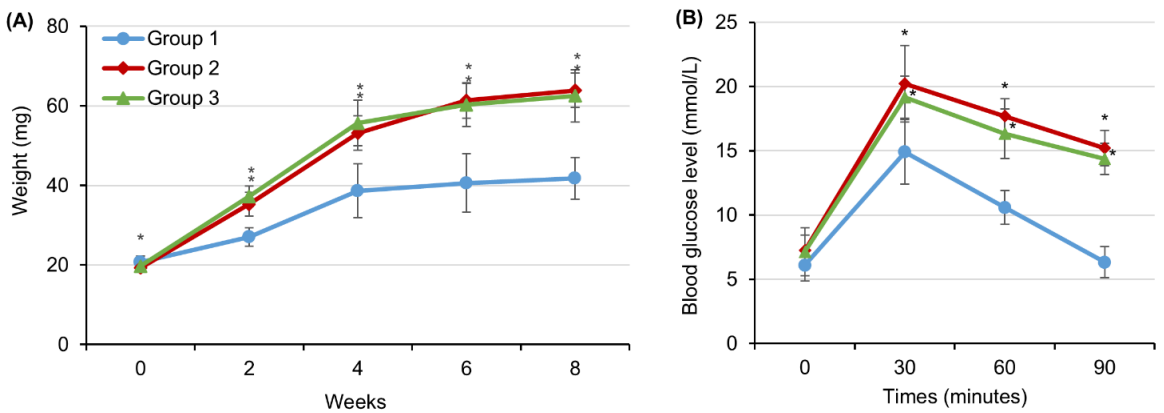

Figure 1. Measurement of mouse weight over 8 weeks of obesity induction using an HFD diet (A) and blood glucose level of mice in three experimental groups after diabetic induction by injecting STZ at a concentration of 35 mg/kg body weight (B). Mice in Groups 2 and 3, fed with the HFD diet, were significantly heavier than those in the control Group 1. The IPGTT results reveal that the blood glucose level of mice in Groups 2 and 3 remained at a diabetic level (above 11.1 mmol/L), while the blood glucose level of mice in Group 1 returned to a normal level. An asterisk denotes a statistically significant difference between the experimental and control groups at each time interval (p-value < 0.05).

Following an 8-week period of HFD diet consumption, it was clear that mice in Groups 2 and 3 exhibited a markedly higher increase in body weight in comparison to those on the SRD (Figure 1A). The mice in the control Group 1, which received the SRD, exhibited a weight gain from 20.7 g to 41.8 g, representing an approximate doubling of their weight. In contrast, the mice in Groups 2 and 3 that were fed a HFD diet reached final weights of 63.9 g and 62.5 g, displaying an increase of 3.31- and 3.17-fold, respectively, from their initial weights. This significant increase in body weight highlights the obesogenic impact of the HFD diet. These results align with previous

studies suggesting that diets rich in saturated fats, including butter, lard, and ghee, contribute more significantly to obesity and elevated blood glucose levels than those high in unsaturated fats such as olive or sesame oil (He *et al.*, 2020; Heydemann, 2016). Therefore, the mice in Groups 2 and 3 that were fed with a HFD diet were considered suitable for the induction of diabetes through STZ injection.

Ten days following the injection of STZ solution, an IPGTT test was conducted to evaluate the blood glucose levels in all experimental groups of mice. Figure 1B reveals that glucose levels in the SRD-fed control Group 1 increased from 6.1 mmol/L at baseline to a peak of 14.9 mmol/L at 30 minutes, then declined to 6.3 mmol/L by 90 minutes. In contrast, Groups 2 and 3 displayed compromised glucose clearance, reaching peak concentrations of 20.2 and 19.2 mmol/L at 30 minutes and maintaining high levels of 15.2 and 14.4 mmol/L at 90 minutes, respectively. The glucose levels of mice from Groups 2 and 3 were observed to stay above 11.1 mmol/L, a threshold that indicates a diabetic condition, following STZ injection (Akinlade *et al.*, 2021; Skovsø, 2014). Together, these data suggest that the mice subjected to the HFD diet developed diabetic conditions, thereby establishing them as suitable diabetic models for subsequent dietary intervention and RT-qPCR analyses.

Evaluation of the specificity of RT-qPCR primers

Before conducting RT-qPCR analysis, the primers employed in this study underwent validation through agarose gel electrophoresis and RT-qPCR melting curve analysis. As depicted in Figure 2, all eight primer pairs evaluated successfully amplified the cDNA template, producing a single amplicon of the anticipated size for each target, such as *GLUT2* (151 bp), *MAFA* (116 bp), *INS2* (133 bp), *PDX1* (222 bp), *NFKB* (194 bp), *RPL13A* (100 bp), *UBC* (117 bp), and *GCK* (133 bp). The specificity of these primer pairs was further confirmed by melt curve analysis. Melt curve results revealed a single peak for each pair of primers, confirming that only the intended target sequences were amplified without non-specific products. Taken together, these results demonstrate that the chosen primers were well-suited for RT-qPCR analysis in this study.

Expression of genes involved in glucose homeostasis by RT-qPCR

Complementary DNA from control mice (Group 1) and STZ (35 mg/kg)-induced diabetic mice subjected to the HFD (Group 2) and DTC diets (Group 3) was used to evaluate the expression levels of *GLUT2*, *MAFA*, *INS2*, *PDX1*, *NFKB,* and *GCK* using RT-qPCR analysis (Figure 3). Our study revealed that expression of *GLUT2* significantly decreased by 3.2-fold in Group 2 diabetic mice, which were fed HFD. However, the expression of *GLUT2* recorded a 2.2-fold reduction in mice that were subjected to the DTC for 6 weeks. In pancreatic beta-cells, GLUT2 is an insulin independent glucose uniporter, which plays a crucial role in glucose homeostasis via processes of glucose-stimulated insulin secretion (GSIS). Similar to our finding, our data are comparable with previous reports, showing that diabetes causes a decrease of 50-70% in the expression of *GLUT2* in mouse pancreatic beta-cells (Hrovatin *et al.*, 2023; Low *et al.*, 2021). Pancreatic beta-cell destruction and dedifferentiation induced by

STZ injection are believed to be causes for the marked reduction in expression of *GLUT2* in the pancreas (Dludla *et al.*, 2023). In addition, the reduction in the transcript abundance of *GLUT2* has also been caused by the oxidative stress from chronic hyperglycemia (glucotoxicity and lipotoxicity), which was caused by the reduction in the expression of *MAFA*. *MAFA* is a gene that encodes a transcription activator that functions to regulate the expression of *GLUT2* by binding to the enhancer and promoter regions (Low *et al.*, 2021; Ono & Kataoka, 2021).

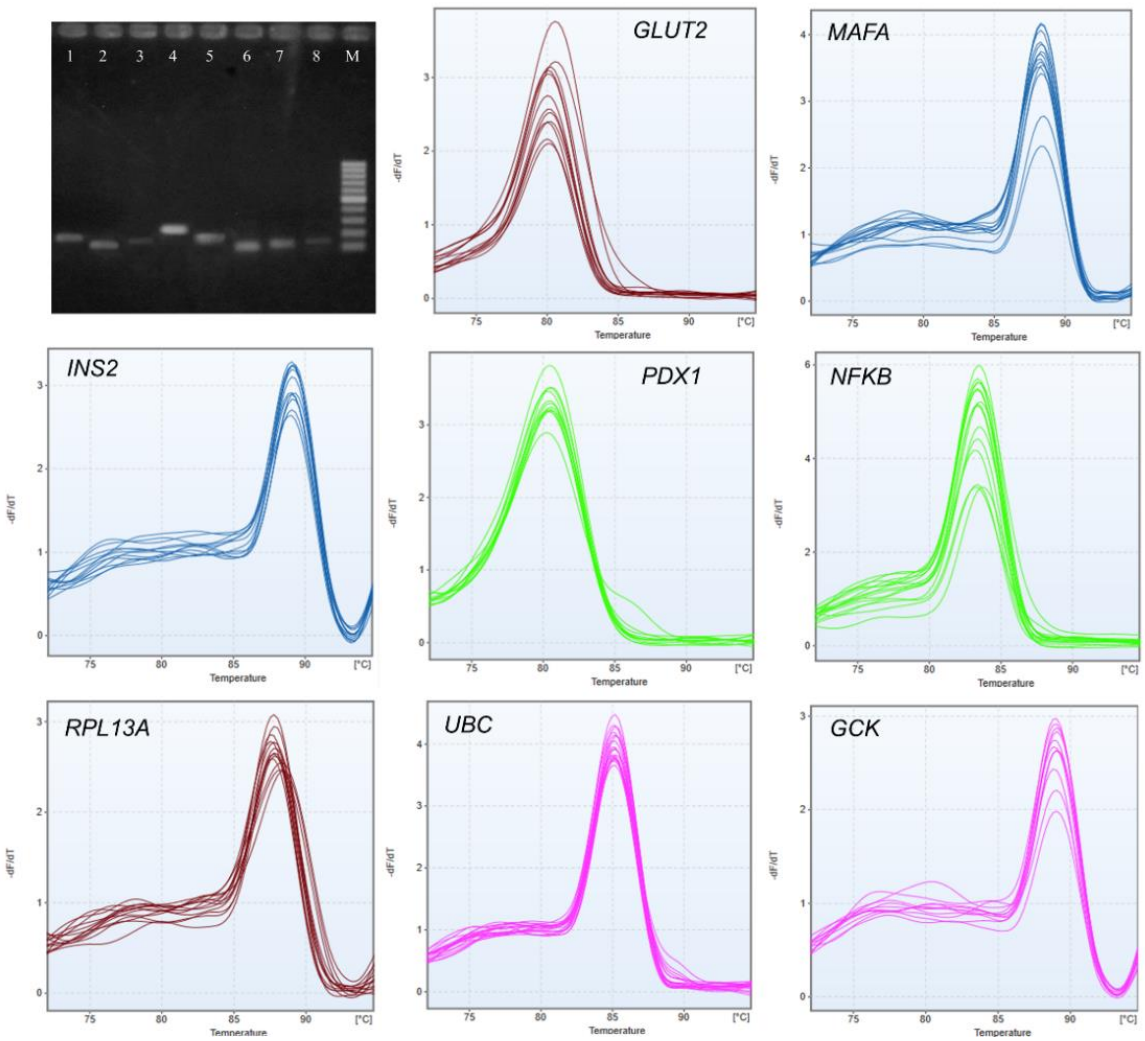

Figure 2. Evaluation of the specificity of primers by gel electrophoresis and RT-qPCR melt curves. All PCR products appear to have a single product at the expected size on the agarose gel, and RT-qPCR generates a single melt curve peak for all assessed pairs of primers. M: GeneRuler™ 100 bp DNA ladder; 1-8: *GLUT2*, *MAFA*, *INS2*, *PDX1*, *NFKB, RPL13A, UBC*, and *GCK*.

Besides the regulatory functions of MAFA, transcription factor PDX1 has been found to play a crucial role in the process of glucose homeostasis. MAFA and PDX1 have also been found to cooperatively regulate the expression of insulin genes, including INS2.

By binding to the A-box region on the promoter of *INS1*, PDX1 recruits MAFA and works cooperatively to drive the expression of *INS2* in response to the elevation of glucose in the bloodstream. PDX1 has also been known as a master regulator that regulates *GCK*, a gene encoding for a glucose sensor for the GSIS process, in pancreatic tissues. Since PDX1 and MAFA have been found to be deeply involved in regulating beta-cell maturation and function as well as insulin gene expression, the loss of beta-cell mass caused by STZ resulted in a severe reduction in the transcript abundance of *MAFA* and *PDX1* in the pancreas of diabetic mice (Liang *et al.*, 2022; Ono & Kataoka, 2021). Our RT-qPCR results reveal that the expression level of *MAFA* significantly decreased in diabetic mice, with those in Group 2 having a more substantial reduction (7.1-fold) than their counterparts in Group 3 (reduction of 2.7-fold). A similar trend of expression was found in *PDX1*. Particularly, the expression level of *PDX1* strongly dropped 9.1-fold in pancreatic tissues of diabetic mice in Group 2, while it decreased to a lesser level in Group 3, reducing by 3.5-fold (Figure 3).

As expected, the decrease in the expression of genes encoding PDX1 and MAFA, a transcription regulator of insulin gene expression, suppressed the expression of GCK, a gene encoding for a glucose sensor, and *INS2*, a gene that encodes an insulin precursor, in the murine pancreatic tissues. Under healthy conditions, GCK then catalyzes the phosphorylation of glucose into G6P, which enters the Krebs cycle in the form of pyruvate to produce adenosine triphosphate (ATP). The release of newly synthesized ATP elevates the adenosine diphosphate (ADP) ATP/ADP ratio, resulting in K^+ efflux and the opening of Ca^{2+} channels to elevate cytosolic Ca^{2+} levels. As a result, it turns the *INS2* on and activates the secretion of insulin granules from pancreatic β-cells (Abu Aqel *et al.*, 2024). The expression of *GCK* was maintained at a stable level until the body reaches the normal blood glucose level. In diabetic mice, however, the transcript abundance of *GCK* was strongly suppressed due to the reduction in expression of its master regulator (PDX1) as well as diabetic conditions, including glucotoxicity induced by beta-cell dysfunction (Lu *et al.*, 2018), insulin signaling impairment, feedback inhibition by glucose metabolites (glucose-6-phosphate, pyruvate), and inflammation and oxidation stress via NFKB activation (Abu Aqel *et al.*, 2024; Bensellam *et al.*, 2012). Figure 3 reveals that the transcript abundance of *INS2* of mice in Groups 2 and 3 was decreased by 9.9- and 5.1-fold, respectively. The impaired GSIS conditions also strongly impact the expression of *GCK* in pancreatic tissues, evidenced by a great reduction in its transcription abundance, decreasing by 3.1- and 1.7-fold in mice from Groups 2 and 3, respectively.

In this study, the expression of *NFKB* has been found to significantly increase in mice from Groups 2 and 3 by 3.2- and 2.3-fold, respectively (Figure 3). NFKB is a pivotal transcription factor that plays a central role in promoting pancreatic inflammation, and oxidative stress impacting insulin secretion (insulin resistance and deficiency) and contributing to the overall metabolic dysfunction (Eldor *et al.*, 2006). Its activation in pancreatic β-cells and surrounding tissues results in the production of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, which intensify the β-cell dysfunction and death (Eldor *et al.*, 2006; Meyerovich *et al.*, 2018).

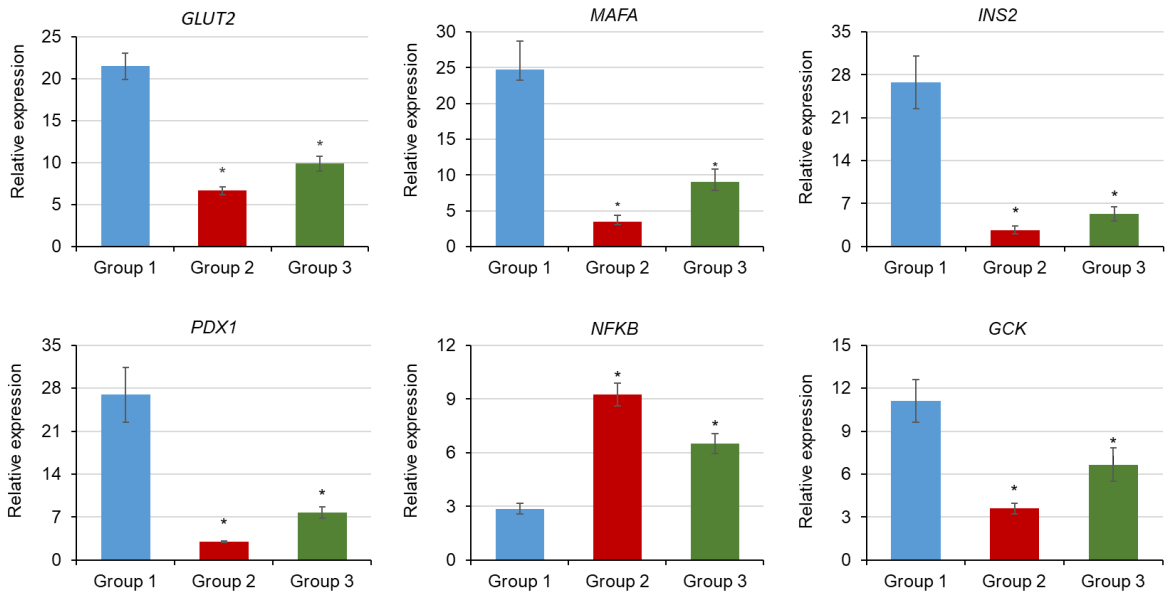

Figure 3. Evaluation of expression of genes associated with glucose homeostasis in control and diabetic mice by RT-qPCR analysis. Groups 1, 2, and 3 are control mice, HFD-fed diabetic mice, and DTC-fed diabetic mice, respectively. An asterisk denotes a statistically significant difference between the experimental and the control group at each time interval (p-value < 0.05).

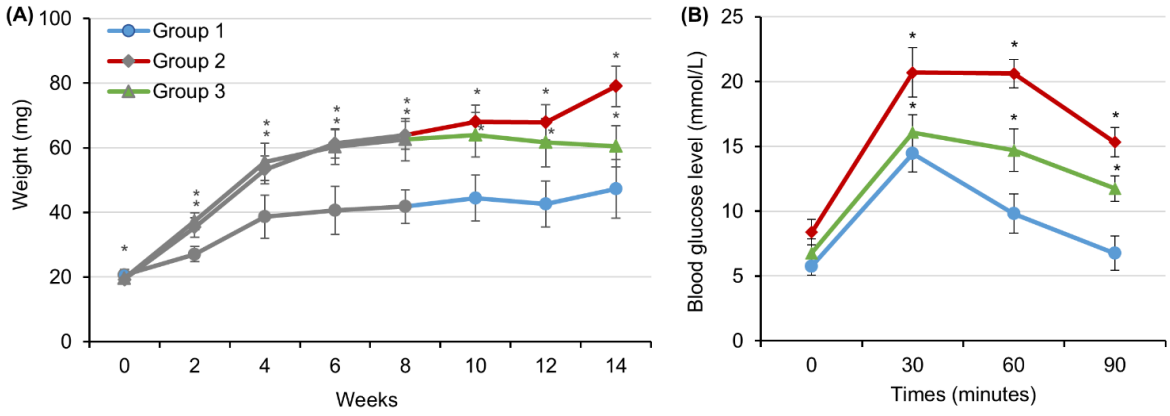

Figure 4. Measurement of mouse weight after diabetes induction (after week 8 to week 14; highlighted in different colors) (A) and blood glucose level of mice in three experimental groups at week 14 by IPGTT test (B). An asterisk denotes a statistically significant difference between the experimental and control groups at each time interval (p-value < 0.05).

Collectively, the RT-qPCR-derived data indicate that there were clear improvements in Group 3 mice, which were administered with the DTC at a concentration of 250 mg/kg of body weight for 6 weeks, in terms of glucose homeostasis in comparison with their counterparts in Group 2, which were fed the HFD diet during the period of the experiment. A possible explanation for the alleviation of diabetic symptoms in mice of Group 3 could be the effects of gymnemic acid, a group of triterpenoid saponins

derived from *G. sylvestre*. Gymnemic acid has been proven to alleviate diabetic symptoms in mouse models through multiple mechanisms, including inhibition of glucose absorption by competitively binding to glucose receptors due to its structural resemblance and lowering the blood glucose level, stimulation of insulin secretion to enhance glucose uptake, regeneration and/or protection of pancreatic beta-cells to improve insulin production capacity, and improvement of lipid metabolism to reduce levels of triglycerides and cholesterol and enhance insulin sensitivity (Chen *et al.*, 2023; Kannan *et al.*, 2022; Li *et al.*, 2019).

These data supported the results of the weight measurement (from week 8 to 16) and IPGTT test. Figure 4 reveals that blood glucose levels across three groups of mice were monitored over 90 minutes. Initially, Group 1 exhibited the lowest baseline glucose at 5.8 mmol/L, followed by Group 3 at 6.79 mmol/L and Group 2 at 8.4 mmol/L. After 30 minutes, blood glucose peaked sharply in all experimental groups assessed, with Group 2 reaching the highest at 20.7 mmol/L, followed by Group 3 at 16.07 mmol/L, and Group 1 at 14.5 mmol/L. At 60 minutes, the blood glucose slowly dropped in all groups. By 90 minutes, the blood glucose in Group 1 closely restored to a normal level at 6.8 mmol/L, and Group 3 reached 11.76 mmol/L. In contrast, the blood glucose level of mice in Group 2 persisted at a high level of 15.3 mmol/L, indicating the slowest blood glucose restoration at the end of the experiment.

In conclusion, the present study demonstrates that dietary supplementation with *G. sylvestre* extract rich with gymnemic acid was capable of modulating the expression of genes associated with glucose homeostasis in diabetic mouse models. These results provide valuable insights into the potential of *G. sylvestre* extract as a functional food component in the context of nutrigenomic strategies for diabetes management. Nonetheless, further investigations are required to confirm the therapeutic efficacy of *G. sylvestre* extract in alleviating diabetic symptoms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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