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The Potential Hepatoprotective Effect of *Vaccinium arctostaphylos* L. Fruit Extract in Diabetic Rat

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Abstract

Objective: *Vaccinium arctostaphylos* has traditionally been employed in Iranian folk medicine to treat diabetes. However, the precise molecular mechanisms underlying its antidiabetic properties remain incompletely understood. The current experiment intended to explore the modulatory effects of *V. arctostaphylos* fruit ethanolic extract (VAE) on biochemical and molecular events in the livers of diabetic rats.

Materials and Methods: In this experimental study, male Wistar rats were randomly assigned to four groups: normal control, normal rats with VAE treatment, diabetic control, and diabetic rats with VAE treatment. Following 42 days of treatment, the impact of VAE on diabetes-induced rats was assessed by measuring various serum biochemical parameters, including insulin, free fatty acids (FFA), tumor necrosis factor-α (TNF-α), reactive oxygen species (ROS), and adiponectin levels. The activities of hepatic carbohydrate metabolic enzymes and glycogen content were determined. Additionally, expression levels of selected genes implicated in carbohydrate/lipid metabolism and miR-27b expression were evaluated. H&E-stained liver sections were prepared for light microscopy examination.

Results: Treatment with VAE elevated levels of insulin and adiponectin that reduced levels of FFA, ROS, and TNF-α in the serum of diabetic rats. VAE-treated rats exhibited increased activities of hepatic glucokinase (GK), glucose-6-phosphate dehydrogenase (G6PD), and glycogen concentrations, in conjunction with decreased activities of glucose-6-phosphatase (G6Pase) and fructose-1,6-bisphosphatase (FBPase). Furthermore, VAE significantly upregulated the transcription levels of hepatic insulin receptor substrate 1 (*Irs1*) and glucose transporter 2 (Glut2), while considerably downregulated the expression of peroxisome proliferator-activated receptor gamma (*Pparg*) and sterol regulatory element-binding protein 1c (*Srebp1c*). VAE remarkably enhanced the expression of miR27-b in the hepatic tissues of diabetic rats. Abnormal histological signs were dramatically normalized in diabetic rats receiving VAE compared to those in the diabetic control group.

Conclusion: Our findings underscore the hypoglycemic and hypolipidemic activities of *V. arctostaphylos* and assist in better comprehension of its antidiabetic properties.

Keywords: Diabetic Rat, Liver, miR27-b, *Vaccinium arctostaphylos*

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Introduction

Diabetes mellitus is a serious metabolic disorder that is defined by elevated levels of blood glucose. This condition primarily stems from deficient insulin secretion, insulin action, or both which may arise from impaired metabolism of carbohydrates, proteins, and lipids in target tissues (1).

As the central metabolic organ, the liver serves a crucial biochemical function in maintaining glucose and lipid homeostasis and is profoundly impacted by diabetes. Chronic hyperglycemia and hyperlipidemia lead to inflammation and liver damage due to augmented oxidative stress and development of reactive oxygen species (ROS) (2). Accumulating evidence suggests that oxidative stress in the liver can initiate molecular alterations by influencing the expression of genes and micro-ribonucleic acids, potentially manifesting as liver dysfunction (3). Hepatic microRNAs (miRNAs) have emerged as critical regulators of glucose and lipid metabolism (4).

MiRNAs are small, approximately 22-nucleotide-long, evolutionarily conserved noncoding RNA molecules that negatively regulate the expression of target genes at the posttranscriptional level. They bind to mRNAs through base pairing to complementary sites, consequently leading to mRNA destabilization or translational repression. Expression profiling studies of miRNAs have confirmed their dysregulation during the onset and progression of a plethora of diseases, such as diabetes and metabolic disorders. Due to the pivotal roles of miRNAs in various aspects of glucose homeostasis and lipid metabolism, they are considered novel therapeutic targets and biomarkers

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for the prognosis, diagnosis, and management of diabetes and its complications (5).

MiR-27b, a member of the miR-27 family, is functionally associated with multiple biological and pathogenic processes including angiogenesis, oxidative stress, inflammation, lipid metabolism, and adipogenesis (6). It has been shown that miR-27b is significantly upregulated in the serum of children with new-onset type 1 diabetes (7) and patients with type 2 diabetes (8). Notably, miR-27b is among the most profuse miRNAs in the liver. MiR-27b-induced alterations in hepatocytes contribute to various signaling pathways relevant to glucose and lipid metabolism and insulin sensitivity (4, 9). Interestingly, it has been shown that different dietary habits can influence the circulating and tissue miRNA signatures. Similarly, recent research on the molecular biology of diabetes has demonstrated that the incorporation of antidiabetic herbs into the diet alleviates diabetes by altering miRNA expression (10, 11).

There is abundant evidence for the identification of medicinal plants possessing antidiabetic properties, and several noteworthy findings have emerged in this field. However, the search for hypoglycemic phyto-bioactive compounds with free radical scavenging and antioxidant capabilities for the development of antidiabetic therapeutic agents remains ongoing. *Vaccinium arctostaphylos* is the only species of the *Vaccinium* genus (Ericaceae family) in northern regions of Iran, especially Ardebil province (known as *Qare-qat* and *Cyah-gileh* in Persian). The berries of *V. arctostaphylos* have traditionally been used in Iranian folk medicine as a remedy for diabetes, hypertension, and hyperlipidemia (12, 13). These effects are attributed to the potential antioxidant action of the fruit of *V. arctostaphylos*, owing to the presence of substantial quantities of flavonoids and anthocyanins (14). It has been reported that malvidin-3-O-β-glucoside has an inhibitory effects on pancreatic α-amylase (15).

These characteristics sparked our interest in acquiring comprehension of the diverse parameters that contribute to the mediation of antidiabetic activity. Accordingly, the main goal of this study was to examine the effects of *V. arctostaphylos* fruit on liver-related biochemical, molecular, and histological changes in an experimental rat model of diabetes. Furthermore, we aimed to investigate the role of miR-27b in hepatic lipid metabolism in this model. Additionally, we determined whether *V. arctostaphylos* effects were mechanistically driven by modulating expression of mir-27 and related genes.

Materials and Methods

Preparation of *V. arctostaphylos* fruit extract

Fresh and ripe fruits of *V. arctostaphylos* were collected from Ardabil Province, northwest of Iran. The specimen was identified by experts at the Central Herbarium of the Department of Botany, University of Tehran (Tehran, Iran). The fruits were rinsed thoroughly with distilled water for removing any adhering dust and air-dried in shaded areas at room temperature. The berries were then finely powdered and soaked in ethanol three times for 12 hours. The resulted ethanolic phase was filtered through Whatman No. 1 filter paper (Whatman, USA). The filtrate was concentrated at 40°C using a rotary evaporator (IKA, Germany) and freeze-dried to powder on an alpha 1-2 LDplus freeze dryer (Martin Christ, Germany) (16). The obtained extract, known as *V. arctostaphylos* ethanolic fruit extract (VAE), was stored in the dark at 4°C until further use.

Experimental animals

In this experimental study, healthy male albino Wistar rats (*Rattus norvegicus*) weighing 180-220 g and approximately two months old were provided by the School of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran). They were acclimatized to the new laboratory environment for seven days prior to the commencement of the experiment. The rats were housed in a well-maintained and pathogen-free animal house under standard vivarium conditions (humidity 55 ± 5%; ambient temperature of 25 ± 2°C; constant 12-hour/12-hour light/dark cycle). They were fed standard chow pellets of known composition and provided clean drinking tap water ad libitum throughout the research period. The present study was reviewed and received approval from the Research Ethics Committees of the College of Sciences, University of Tehran (IR.UT.SCIENCE.REC.1402.005).

Induction of experimental Diabetes mellitus

Type 1 diabetes was induced by subcutaneous injection of freshly prepared alloxan monohydrate (Sigma Aldrich, USA) solution (120 mg/kg body weight, in cold 0.1 M citrate buffer, pH=4.5) to 18-hour fasted rats. An equal volume of citrate buffer without alloxan injected to non-diabetic group of rats for simulation of drug injection (17). Six hours after alloxan administration, a 20% glucose solution was injected intraperitoneally. The animals were then given access to a 5% glucose solution for the next 24 hours to overcome drug-induced fatal hypoglycemia (18). 72- hour post-alloxan treatment, hyperglycemia was confirmed using a portable glucometer (On Call Plus Blood Glucose Meter, USA), and rats with elevated blood glucose levels (>250 mg/dl) were deemed to be diabetic (17).

Experimental design

A total of 32 rats were stochastically aliquoted into four groups, each containing eight animals. These groups either were treated with vehicle (distilled water) alone or VAE as follows:

Normal control (NC): Healthy rats treated with vehicle alone.

Normal+VAE (N+VAE): Healthy rats treated with VAE at 400 mg/kg body weight.

Diabetic control (DC): Diabetic rats received vehicle.

Diabetic+VAE (D+VAE): Diabetic rats received VAE at 400 mg/kg body weight.

VAE was dissolved in distilled water (1 ml/rat) at the desired concentration immediately prior to each administration. VAE was administered by oral gavage to the respective groups for 42 consecutive days. The optimal dose of VAE (400 mg/kg body weight) was selected based on previous findings from our laboratory (16). During the experimental period, rats were monitored for physiological parameters, such as food and water intake, body weight, and blood glucose levels, at weekly intervals to investigate the stability of the diabetic condition. At the end of the treatment period, all rats were subjected to 12 hours of overnight fasting. Next, the rats were anesthetized intraperitoneally with a combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) solution and humanely sacrificed (19). Thereafter, blood samples were collected via intracardiac puncture for biochemical analyses. Serum was prepared by coagulating blood samples at room temperature for 15-30 minutes and centrifugation at 1500 × g for 15 minutes. The supernatant sera were stored in aliquots at a temperature of -80°C. Immediately after dissection, tissue samples were separated, rinsed in ice-cold saline, frozen in liquid nitrogen, and kept at -80°C for subsequent biochemical and quantitative polymerase chain reaction (PCR)-based gene expression analyses. A portion of the tissues were fixed in 10% formalin (Merck, Germany) and subjected to histopathological examination.

Biochemical analysis

Serum insulin levels were estimated with a commercial rat insulin ELISA kit in accordance with the guidance provided by the manufacturer (Demeditec, Germany). The concentration of serum-free fatty acids (FFA) was quantified using an EnzyChrom™ free fatty acid kit (BioAssay Systems, USA). ELISA was performed to evaluate ROS levels in the serum of the experimental groups (MyBioSource, USA). The levels of serum and hepatic tumor necrosis factor-α (TNF-α), a proinflammatory cytokine, were measured in accordance with the manufacturer's protocol (RayBiotech, USA). A commercially available kit was used to determine adiponectin levels in the serum samples (Abcam, UK).

Determination of hepatic carbohydrate metabolic enzymes and glycogen

A portion of the harvested liver tissue underwent the process of homogenization in Tris-HCl buffer (100 mM, pH=7.4) centrifuged at 3000 × g for 10 minutes at the temperature of 4°C. The resultant supernatant was used to evaluate the carbohydrate-metabolizing enzyme activity. The protein content of the supernatant was estimated by the Bradford method (20). Hepatic glucokinase (GK), glucose-6-phosphate dehydrogenase (G6PD), glucose-6-phosphatase (G6Pase), and fructose-1,6-bisphosphatase (FBPase) activities and glycogen content in the liver of experimental rats were assessed according to previously described methods (21).

RNA extraction and real-time polymerase chain reaction analysis

To identify gene expression, total RNA was extracted from snap-frozen tissue samples using an RNX-Plus kit (SinaClon, Iran), followed by DNase I digestion (Yekta Tajhiz Azma, Iran). Agarose gel electrophoresis was employed to confirm the integrity of isolated RNA. A NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA) was utilized to quantify RNA concentration in all experimental groups. The purity of RNA samples was determined using an A260/A280 ratio of 1.8-2.0. Next, isolated total RNA (1 μg) was reverse-transcribed to obtain complementary DNA (cDNA) using a PrimeScript RT Reagent Kit according to the manufacturer's recommendations (TaKaRa, Japan). Real-time quantitative PCR (qPCR) was carried out using YTA SYBR Green qPCR Master Mix (Yekta Tajhiz Azma, Iran) on a StepOnePlus Real-Time PCR system (Applied Biosystems, USA) at optimized cycles. The cDNA was amplified with gene-specific primer pairs (SinaClon, Iran), as listed in Table S1 (See Supplementary Online Information at www.celljounal.org). Melting curve analysis was applied to verify primer specificity. Each reaction was run in triplicate. Transcription of the β-actin (*Actb*) housekeeping gene was employed as the internal control for normalization. The relative target mRNA expression was determined via the $2^{-\Delta\Delta Ct}$ method (22).

MicroRNA extraction and real-time polymerase chain reaction for miR-27b

To evaluate the effect of VAE administration on miR-27b expression levels, the homogenized liver suspension was applied to extract total miRNA using a miRNA isolation kit following the manufacturer's protocol (Favorgen, Taiwan). Polyadenylation and reverse transcription were performed according to the manufacturer's recommendations using a miRCURY LNA Universal cDNA Synthesis Kit II (Exiqon, Denmark). Tissue-derived miR-27b expression levels (mature sequence, 5′-UUCACAGUGGCUAAGUUCUGC-3′) were analyzed by qPCR using ExiLENT SYBR Green master mix (Exiqon, Denmark). PCR assays were performed in triplicate for each sample utilizing the StepOnePlus Real-Time PCR system. Hepatic expression of miR-27b was normalized to that of U6 small nuclear RNA (U6 snRNA) and calculated using the $2^{-\Delta\Delta CT}$ method.

Histological analysis

The effects of VAE on the histopathological alterations in the liver were evaluated. The excised liver tissues were washed with normal saline, immediately fixed in a 10% neutral formalin solution for 48 hours, and routinely processed for histological examination using the paraffin method. Paraffin-embedded tissues were cut into serial sections with 5 μm thickness and separately stained with Hematoxylin and Eosin (H&E) dye. The slides were examined under a light microscope (Olympus Corp., Japan), and images captured using a digital camera (23).

Statistical analysis

All data are presented as mean ± standard deviation (SD). To conduct statistical analysis, data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism software version 8.4.3 (La Jolla, CA, USA). Statistical significance was set at $P<0.05$.

Results

Effect of VAE on the clinical parameters

Alterations in food and water intake, body mass gain, and blood glucose levels were monitored in all experimental rats during the 42-day treatment period. Food, water intake and blood glucose levels in diabetic animals were considerably increased compared to the NC group (P=0.00, Figs.S1, S2, S3, See Supplementary Online Information at www.celljounal.org). Compared to the NC groups, untreated diabetic rats exhibited marked loss of body weight (P=0.00, Fig.S4, See Supplementary Online Information at www.celljounal.org). Treatment of diabetic animals with VAE remarkably ameliorated these parameters.

Effect of VAE on the levels of serum insulin, free fatty acid, and reactive oxygen species

The levels of serum insulin, FFA, and ROS of control and experimental rats are enumerated in Table 1. The ELISA test showed that the serum insulin level was dramatically diminished (P=0.000) in untreated diabetic rats compared to normal controls at the end of the 42-day study period. The insulin level of VAE-treated diabetic rats was elevated notably in comparison to the corresponding controls (P=0.04).

As shown, DC rats exhibited elevated levels of serum FFA compared to normal rats (P=0.02). A marked decline in the serum FFA levels was observed in diabetic rats following VAE administration (P=0.03).

ROS levels were drastically raised in control diabetic animals compared to NC rats (P=0.000). However, in animals receiving VAE, the serum levels of ROS were significantly diminished compared to diabetic controls (P=0.006).

Effect of VAE administration on serum and hepatic TNF-α levels

To elucidate whether TNF-α levels were affected by VAE administration, the serum and hepatic levels of TNF-α were evaluated in all experimental groups. Figure 1A, B represent that serum and hepatic levels of TNF-α in diabetic animals were considerably higher than those in the NC group (P=0.000). Nevertheless, oral administration of VAE remarkably decreased serum and hepatic TNF-α levels (P=0.009 and P=0.046, respectively).

Effect of VAE administration on serum adiponectin levels

Figure 1C shows the effect of VAE on serum adiponectin levels in all experimental groups. Diabetic rats demonstrated a significant diminution in adiponectin concentration in comparison to the NC group (P=0.001). Administration of VAE to diabetic rats remarkably increased serum adiponectin level (P=0.012).

Effect of VAE on activities of hepatic carbohydrate metabolic enzymes and glycogen content

Table 2 exhibits the impact of VAE treatment on carbohydrate metabolic enzyme activities and glycogen levels in the liver of normal and alloxan-induced diabetic rats. DC rats demonstrated a prompt decline in the activity of GK (P=0.000) and G6PD (P=0.002) and a concomitantly significant elevation in the activity of G6Pase (P=0.04) and FBPase (P=0.005) compared to NC rats. The altered activities of these enzymes reverted to near-normal values after VAE administration.

Compared to the NC group, hepatic glycogen concentration was significantly diminished (P=0.002) in diabetic rats. The treatment of diabetic animals with VAE showed remarkable amelioration in hepatic glycogen storage (P=0.04).

Table 1: Effect of VAE on serum insulin levels and other variables

Groups	Normal control	Normal+VAE	Diabetic control	Diabetic+VAE
Serum insulin (μU/mL)	15.21 ± 0.98	14.84 ± 1.43	4.90 ± 0.82***	7.63 ± 1.25≠
Serum FFA (mg/mL)	70.76 ± 3.14	74.29 ± 5.66	151.14 ± 3.25*	92.88 ± 7.41≠≠
Serum ROS (U/ml)	3.34 ± 0.25	3.86 ± 0.81	31.62 ± 5.63***	11.27 ± 8.03≠≠

Each value represents the mean ± SD of eight rats per group. Evaluation was performed by one-way ANOVA followed by post hoc Tukey's test. *; P<0.05, ***; P<0.001, in comparison to the normal control group, ≠; P<0.05, ≠≠; P<0.01, in comparison to the diabetic control group, FFA; Free fatty acids, and ROS; Reactive oxygen species.

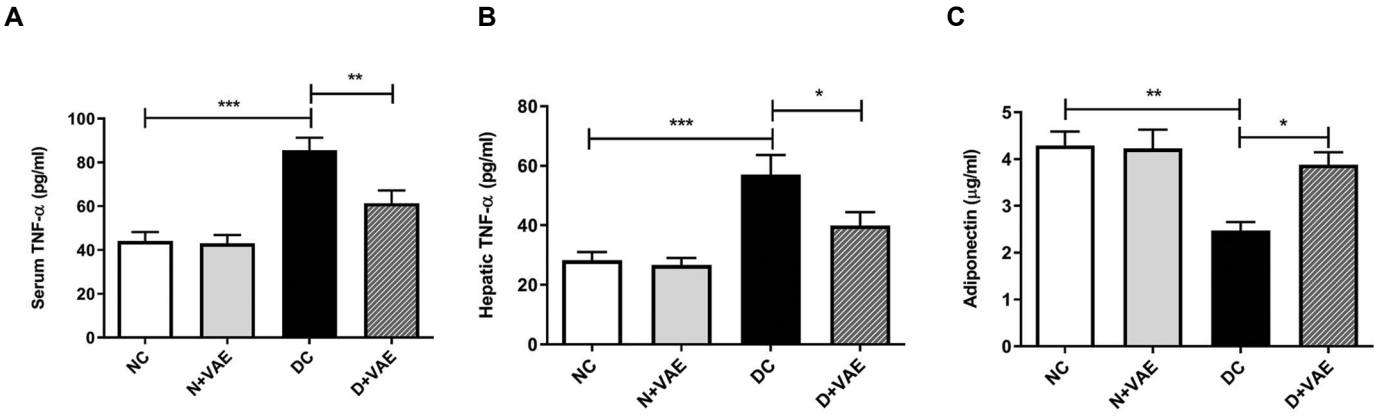

Fig.1: Effect of VAE on TNF-α and adiponectin levels in normal and experimental rats. **A.** Serum TNF-α levels, **B.** Hepatic TNF-α levels, and **C.** Serum adiponectin levels. Bars represent the mean ± SD of eight rats per group. Evaluation was carried out by one-way ANOVA followed by post hoc Tukey's test. *; P<0.05, **; P<0.01, ***; P<0.001: statistical differences in comparison to the diabetic control group, TNF-α; Tumor necrosis factor-α, NC; Normal control, N+VAE; Normal rats subjected to VAE treatment, DC; Diabetic control, and D+VAE; Diabetic rats subjected to VAE treatment.

Table 2: Effect of VAE on the activities of carbohydrate metabolic enzymes and glycogen content in the liver of all experimental rats

Groups	GK[a]	G6PD[b]	G6Pase[c]	FBPase[d]	Liver glycogen[e]
Normal control	0.19 ± 0.01	4.73 ± 0.12	0.17 ± 0.01	0.34 ± 0.02	45.24 ± 3.61
Normal+VAE	0.18 ± 0.04	4.77 ± 0.27	0.16 ± 0.01	0.32 ± 0.03	47.16 ± 4.05
Diabetic control	0.09 ± 0.01***	2.51 ± 0.23**	0.28 ± 0.01*	0.57 ± 0.04**	21.17 ± 2.18**
Diabetic+VAE	0.14 ± 0.02≠	3.01 ± 0.36≠	0.21 ± 0.02≠≠	0.39 ± 0.03≠	34.28 ± 3.23≠

Each value represents the mean ± SD of eight rats per group. Evaluation was carried out by one-way ANOVA followed by post hoc Tukey's test.*; P<0.05, **; P<0.01, ***; P<0.001, in comparison to the normal control group, ≠; P<0.05, ≠≠; P<0.01, in comparison to the diabetic control group, GK; Glucokinase, G6PD; Glucose-6-phosphate dehydrogenase, G6Pase; Glucose-6-phosphatase, FBPase; Fructose-1,6-bisphosphatase, [a]; µmol of glucose phosphorylated/hour/mg protein, [b]; nmol of NADPH formed/minute/mg protein, [c]; µmol of Pi liberated/minute/mg protein, [d]; µmol of Pi liberated/hour/mg protein, and [e]; mg/g tissue.

Effect of VAE on expression of key genes associated with glucose and lipid metabolism

To investigate the effect of VAE administration on the expression of genes involved in hepatic glucose and lipid metabolism, qPCR was performed in all experimental groups. These results revealed a dramatically reduced amount of insulin receptor substrate 1 (*Irs1*) expression in DC rats compared to that in NC animals (P=0.000). However, the expression levels of *Irs1* significantly enhanced in VAE-treated diabetic rats in contrast to the DC groups that did not receive treatment (P=0.025, Fig.2A). Glucose transporter 2 (GLUT2) plays an essential role in liver glucose transport (24). The transcription level of *Glut2* was notably downregulated in diabetes-induced rats in comparison to normal controls (P=0.002). On the other hand, the administration of VAE to diabetic group of rats led to a marked increase in *Glut2* expression levels (P=0.029, Fig.2B).

The expression of peroxisome proliferator-activated receptor gamma (*Pparg*) was found to be significantly upregulated in the liver of diabetic rats compared to that in normal controls (P=0.003). The treatment of diabetic rats with VAE considerably downregulated *Pparg* expression (P=0.032, Fig.2C). Diabetic rats displayed a drastic increase in the transcription level of sterol regulatory element-binding protein 1c (*Srebp1c*) compared to NC rats (P=0.000). VAE administration ameliorated the alloxan-induced increase in *Srebp1c* gene expression (P=0.03, Fig.2D).

Effect of VAE on expression levels of hepatic miR-27b

Next, we identified the potential effect of VAE on the expression levels of hepatic miR-27b. qPCR analysis was performed to examine the modulatory impact of VAE on the expression of miR-27b in all experimental groups. The hepatic expression of miR-27b was significantly lower in DC rats than in normal controls (P=0.0018). Meanwhile, VAE-treated diabetic rats showed a significant upregulation in miR-27b expression levels compared to untreated diabetic group (P=0.045, Fig.3).

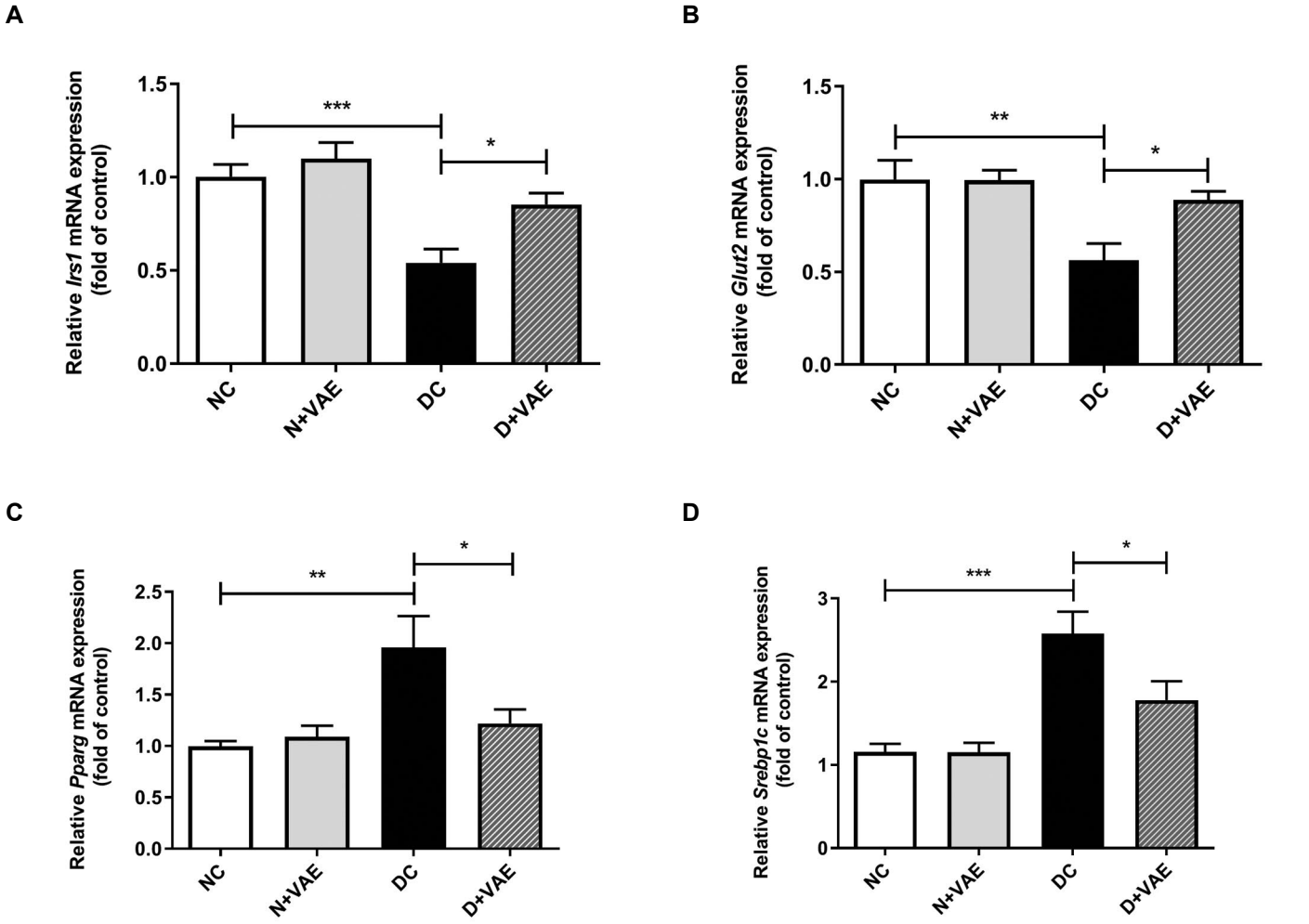

Fig.2: Effect of VAE on hepatic expression of key glucose and lipid metabolism-associated genes. **A.** *Irs1*, **B.** *Glut2*, **C.** *Pparg*, and **D.** *Srebp1c* expression levels. Bars represent the mean ± SD of eight rats per group. Evaluation was performed by one-way ANOVA followed by post hoc Tukey's test. *; P<0.05, **; P<0.01, ***; P<0.001: statistical differences in comparison to the diabetic controls, NC; Normal control, N+VAE; Normal rats subjected to VAE treatment, DC; Diabetic control, and D+VAE; Diabetic rats subjected to VAE treatment.

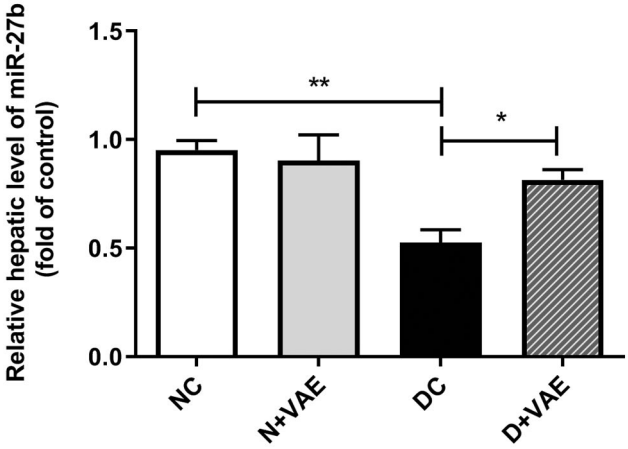

Fig.3: Effect of VAE on hepatic expression levels of miR-27b in normal and experimental rats. Bars represent the mean ± SD of eight rats per group. Evaluation was accomplished by one-way ANOVA followed by post hoc Tukey's test. *; P<0.05, **; P<0.01: statistical differences in comparison to the diabetic control group, NC; Normal control, N+VAE; Normal rats subjected to VAE treatment, DC; Diabetic control, and D+VAE; Diabetic rats subjected to VAE treatment.

Effect of VAE on histopathology of liver

To elucidate whether hepatic biochemical and molecular alterations lead to structural modifications at the microscopic level, a histological assessment was performed. Figure 4 illustrates the morphological features of the liver tissues of control and experimental rats. Photomicrographs of hepatic tissues from NC and normal rats treated with VAE displayed a well-preserved cellular architecture with normal nuclei and cytoplasm (Fig.4A, B). In comparison to the NC groups, histological abnormalities were observed in the liver tissues of DC rats characterized by disintegrated hepatic cells, degeneration, and mild inflammation accompanied by moderate fatty changes (Fig.4C). Treatment with VAE resulted in a near-normal hepatocyte arrangement with minimal inflammatory damage and fatty degeneration (Fig.4D). These findings imply that VAE treatment can ameliorate abnormal morphology in diabetic rats.

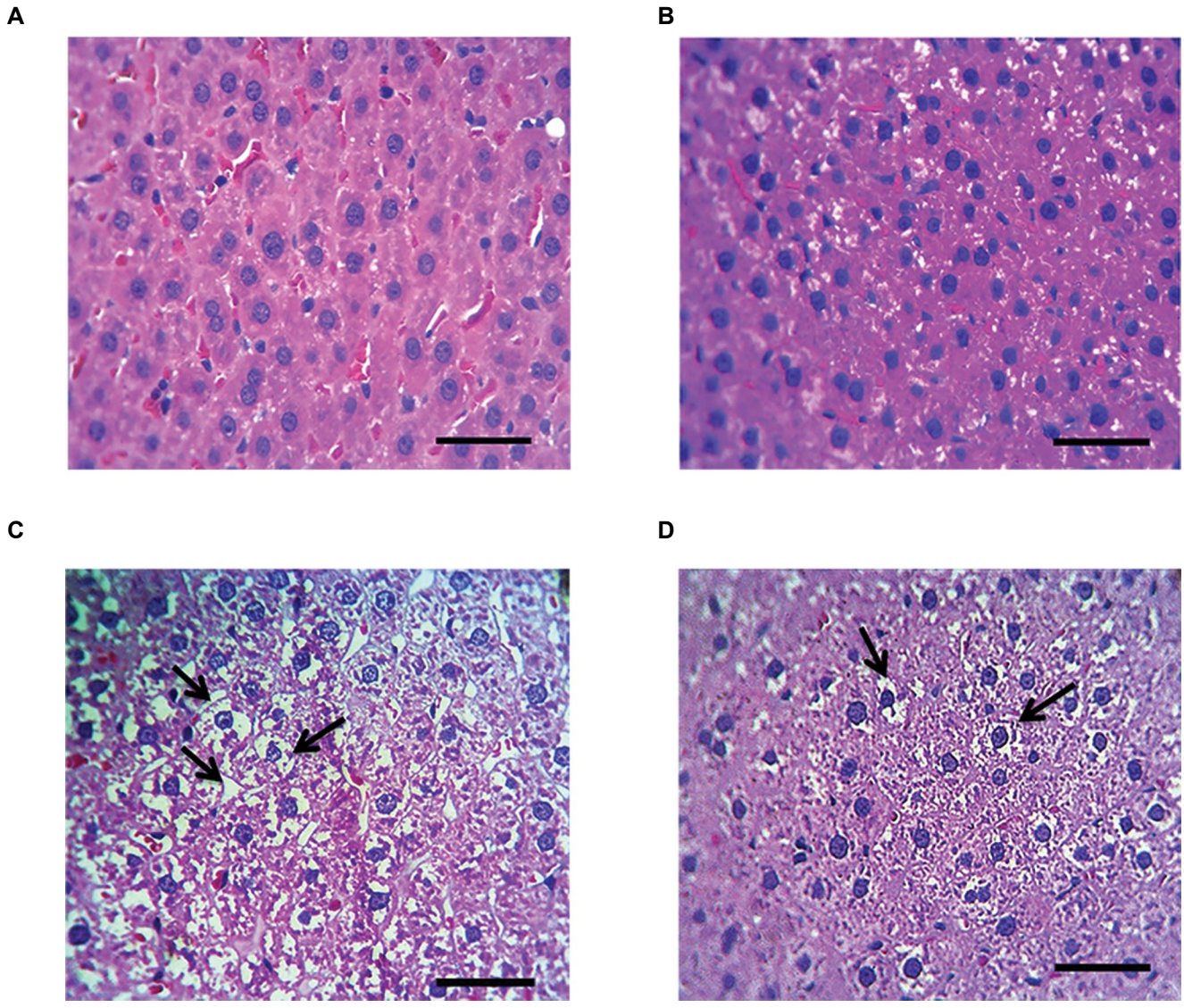

Fig.4: Histopathological observations of H&E-stained liver sections from normal and experimental rats (×40, scale bar: 150 µm). **A.** Normal control rats, **B.** Normal rats subjected to VAE treatment, **C.** Diabetic control rats, and **D.** Diabetic rats subjected to VAE treatment. Arrows indicate fatty changes.

Discussion

Diabetes is a heterogeneous metabolic disorder characterized by hyperglycemia arising from impaired carbohydrate metabolism and insulin secretion (1). The ultimate goal of all diabetes remedies is to normalize glycemia and improve health status. There is a rising trend toward the use of traditional medicinal plants for the diabetes treatment.

The antidiabetic therapeutic properties of *V. arctostaphylos* fruit have been tested in several clinical trials (12, 13). Administration of the hydroalcoholic extract of *V. arctostaphylos* berries to type 2 diabetic patients reduced hyperglycemic conditions through decreasing fasting blood sugar and HbA1c levels (13). The previous research conducted by this group showed that ethanolic fruit extract of *V. arctostaphylos* improved blood sugar levels, lipid profiles, and antioxidant enzymes activities in Allegan-induced diabetic rats (16). In light of these findings, the present investigation aimed to provide new insights into understanding other antidiabetic properties of *V. arctostaphylos*. This study examined the effects of *V. arctostaphylos* fruit on the mechanism of actions involved in hepatic glucose regulation and lipid metabolism. Moreover, to ascertain the molecular mechanisms mediating VAE antidiabetic properties, the expression of key metabolic genes as long as miR-27b were investigated at both transcriptional and posttranscriptional levels.

Liver dysfunction is among the foremost complications of Diabetes mellitus. The activities of key regulatory enzymes relevant to hepatic glycolysis, gluconeogenesis, and glycogenesis are altered in diabetes. GK is the first rate-limiting enzyme in the glycolytic pathway that transfers a phosphoryl group from ATP to glucose, resulting in glucose-6-phosphate. It is well-established that restored GK activity correlates with an overall improvement in glucose homeostasis (21). Our findings

indicated that hepatic GK activity was remarkably lower in diabetic rats than in normal controls. Decreased activity of GK is attributed to insulin deficiency as a repercussion of alloxan-mediated β-cell cytotoxicity and degeneration. However, the administration of VAE to diabetic rats improved the activity of this enzyme.

G6PD is the rate-limiting enzyme of the hexose monophosphate shunt that maintains anabolic biosynthesis and redox homeostasis. In diabetic conditions, the decrease in G6PD activity and subsequent NADPH levels makes the cells more susceptible to oxidative stress (25). According to our data, G6PD activity increased significantly in diabetic rats upon treatment with VAE.

G6Pase controls the last step of gluconeogenesis and dephosphorylates glucose-6-phosphate to glucose and free phosphate. Insufficient insulin levels elevate G6Pase activity and endogenous glucose production, thereby giving rise to diabetic complications (21). In the present work, diabetic rats exhibited high activity of G6Pase, while oral administration of VAE restored the enzyme activity close to normal levels. FBPase is responsible for the second irreversible step in gluconeogenesis and catalyzes the hydrolytic dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate, representing a promising target for glycemic control. Diabetes impairs the suppression of FBPase, leading to excessive hepatic glucose output and hyperglycemia (26). Our data revealed VAE decreased the activity of this gluconeogenic enzyme in diabetic rats.

The current study showed that the concentration of hepatic glycogen, the principal storage form of carbohydrates, was declined in diabetic animals compared to normal controls. The administration of VAE considerably augmented glycogen concentration in the livers of control diabetic rats. Overall, our aforementioned findings were consistent with those of studies reporting the beneficial effects of herbal supplements on hepatic glycogen and carbohydrate metabolizing enzymes in diabetic models (27).

Adiponectin is the most common adipokine synthesized by adipose tissue and possesses antidiabetic, anti-inflammatory, and anti-atherogenic properties. Low adiponectin levels correlate with insulin resistance, obesity, and fatty liver disease. The antidiabetic effects of adiponectin are thought to be partially mediated by abrogating the expression of phosphoenolpyruvate carboxylase (PEPCK) and G6PD which reduce the synthesis and release of endogenous glucose from liver cells (28). Chemically-induced diabetic rats showed reduced levels of circulating adiponectin (29), and these findings corroborate our observations in the diabetes-induced group. However, a dramatic increase was observed in diabetic rats treated with VAE compared to corresponding controls. Similarly, in a study by Dadgar et al. (30), the potential effect of herbal extracts was reported in elevating adiponectin levels in diabetic rodents. Adiponectin and TNF-α exhibit an inverse correlation (31). This notion could explain our findings, where we observed increased levels of TNF-α in both the serum and hepatic tissues of the untreated diabetic group. Supporting these findings, Ingaramo et al. (32) demonstrated elevated levels of TNF-α and its receptor in the livers of diabetic rats. Results of this study imply that supplementation with *V. arctostaphylos* fruit extract reduced TNF-α levels, thus highlighting its potent anti-inflammatory properties.

From a molecular point of view, we examined the expression levels of *Irs1* (upstream of insulin signaling) and *Glut2* in the livers of experimental animals to determine how VAE activates insulin signaling and glucose uptake. IRS1 plays a vital role in insulin-mediated responses, and decreased mRNA levels of *Irs* could potentially lead to the progression of insulin resistance and diabetes (11). Our data revealed a significant decrease in hepatic *Irs1* expression after toxic alloxan treatment. However, VAE administration increased the mRNA expression of *Irs1*, thereby promoting downstream signaling. It might be speculated that VAE may partially exert its antidiabetic effects by upregulating *Irs1* levels.

As evidenced, circulating glucose is taken up by GLUT2 in the liver. Reduced expression of hepatic *Glut2* impairs glucose uptake (24). We observed a diminution in hepatic *Glut2* mRNA levels in control diabetic animals. However, VAE administration significantly upregulated *Glut2* expression in the livers of diabetic rats compared to that in the untreated diabetic group. Collectively, VAE upregulated *Irs1* and *Glut2* and promoted liver function for glucose uptake.

Lipid perturbation can be a risk factor for diabetes. In this study, we investigated the expression levels of *Pparg* and *Srebp1c* to comprehend the hepatic lipid metabolism in diabetes-induced rats.

PPARγ is required for metabolic balance and has garnered considerable interest owing to its diverse functions in glucose and lipid metabolism. Studies using rodent models have demonstrated that PPARγ activation is critically associated with the development of hepatic steatosis and lipotoxicity (33). In this study, the DC group exhibited overexpression of *Pparg* compared to the normal controls. These results are in accordance with those of previous in vivo experiments (34). Modulation of *Pparg* expression by herbal compounds (35) supports the VAE-induced depletion of *Pparg* in diabetic rats.

SREBP1c is a crucial transcription factor that is involved in fatty acid biosynthesis. It has been reported that overexpression of *Srebp1c* increases the expression of lipogenic-associated genes, including fatty acid synthase (*Fasn*) and acetyl-CoA carboxylase (*Accca*), accumulating intracellular triglycerides within lipid droplets in hepatocytes (36). The findings of this research indicated the upregulation of hepatic *Srebp1c* in the diabetic state. This is consistent with earlier reports on diabetic rodent models (34). In this study, VAE administration effectively attenuated *Srebp1c* expression close to normal values, reducing lipid synthesis and droplet formation, as

supported by our histological findings.

The crucial role of miR-27b as a key metabolic regulator in hepatic lipid metabolism has been well-established in animal and human research (9). Ji et al. (37) reported that *in vitro* inhibition of miR-27b reestablished cytoplasmic lipid droplet formation in rat hepatic stellate cells. Bioinformatic and experimental target gene analyses demonstrated that miR-27b directly and indirectly regulates key genes involved in hepatic lipid metabolism [e.g., *Pparg*, *Srebp1c* (38)], and insulin signaling in hepatocytes (4). Notably, miR-27b attenuates hepatic glucose output by modulating the expression of gluconeogenic enzymes such as G6Pase. Our data revealed that miR-27b abundance was depleted in the livers of diabetic rats. Imperatively, the results of the current research indicated that VAE treatment stimulate miR-27b expression in diabetic animals.

Qin et al. found that a flavonoid derivative (Fla-CN) regulated miR-27 expression in the liver and adipose tissues of high-fat diet-induced obese mice (39). Therefore, it is plausible to speculate that VAE exerts its hepatoprotective effects by enhancing miR-27b expression and downregulating the expression of its target genes.

In view of the above findings, it is hypothesized that hepatic gene expression changes elicited by alloxan during the 42-day study period could slightly shift the metabolic status of liver cells toward fatty acid storage and dyslipidemia, as reinforced by our histological observations. DC rats in this study showed histopathological alterations, such as hepatocellular necrosis, inflammation, and fat droplets, compared to normal liver sections. Similar results were obtained in a previous experiment (40). In contrast, VAE administration significantly reduced these abnormalities and improved the liver histoarchitecture, suggesting the tissue-protective nature of VAE.

Conclusions

Taken together, the results of the current biochemical and expression-based study add to the picture of the antidiabetic properties of *V. arctostaphylos* fruit. These findings shed light on the mechanisms of action underlying the hepatoprotective effects of VAE. VAE efficiently ameliorates hyperglycemia, dyslipidemia, oxidative stress, and inflammatory states.

This study provides further scope to evaluate the effects of VAE at the molecular level. Apart from improving hepatic carbohydrate metabolizing enzymes activity and glycogen content, VAE also governs the expression of genes involved in glucose and lipid homeostasis in liver. According to our findings, it can be asserted that VAE may alleviate diabetes-induced hepatic lipotoxicity by upregulating miR-27b and modulating the expression of its target genes, which are correlated with lipid metabolism. These results are consistent with our hepatic histopathological assessment.

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Authors' Contributions

Sh.M.K.; Conceptualization, Validation, Writing, Review, Editing, Supervision, and Project administration. N.S.; Methodology, Software, Formal Analysis, Investigation, Data curation, Writing, Original draft preparation, and Visualization. M.I.; Data Curation, Writing, Review, and Editing. All authors read and approved the final version of the article.

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