Tropical Journal of Pharmaceutical Research February 2023; 22 (2): 289-295
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i2.10

Original Research Article

Vitis vinifera seed extract ameliorates streptozotocin-induced pancreatic dysfunction by reducing interleukin-1β and up-regulation of omentin-1 mRNA

Karima Bel Hadj Salah[1,2], Diana AH Al-Quwaie[1], Najat Binothman[3], Majidah Aljadani[3], Manal MS Mansoury[4], Nouf H Alsubhi[1], Ghadeer I Alrefaei[5], Ammar A Bayoumi[6], Maha Ibrahim[6], Hala AH Khattab[7]*

[1]Department of Biological Sciences, College of Science and Arts, Rabigh Campus, King Abdulaziz University, Jeddah, Saudi Arabia, [2]Laboratoire des Maladies Transmissibles et Substances Biologiquement Actives (LR99ES27), Faculté de Pharmacie, Université de Monastir, Tunisia, [3]Department of Chemistry, College of Sciences and Arts, King Abdulaziz University, Rabigh, [4]Department of Food and Nutrition, Faculty of Human Sciences and Design, King Abdulaziz University, Jeddah, Saudi Arabia, [5]Department of Biological Science, Faculty of Sciences, Jeddah University, Jeddah, [6]Department of Hematology, Medical Research, King Abdulaziz University, Saudi Arabia, [7]Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt

For correspondence:* ***Email:* *haya_khattab@hotmail.com;* ***Tel:*** *00201005248923*

Sent for review: 1 September 2022 *Revised accepted: 28 January 2023*

Abstract

Purpose: *To investigate the effect of Vitis vinifera ethanol seed extract (SE) on pancreatic function markers associated with streptozotocin-induced diabetes in rats.*

Methods: *Non-diabetic rats (n = 12) were divided into 2 groups (GRPs); GRP1 served as the control, and GRP2 was administered orally with V. vinifera SE (500 mg/kg). Diabetic rats were divided into 2 GRPs; GRP3 served as the streptozotocin-induced diabetic rats, and GRP4 diabetic rats received V. vinifera SE (500 mg/kg). Blood was collected at the end of 4 weeks, and serum glucose and lipid profile were measured using colorimetric enzymatic kits. Serum insulin, and interleukin-1 beta (IL-1β) levels were quantified using enzyme-linked immunosorbent assay (ELISA) kits. Histopathological alterations in pancreas were determined, while omentin-1 mRNA expression was investigated using quantitative real time-polymerase chain reaction (qRT-PCR).*

Results: *V. vinifera SE protected pancreatic function in diabetic rats. V. vinifera SE significantly increased serum insulin and HDL ($p < 0.001$), besides significantly decreasing serum glucose, cholesterol, triglyceride, and LDL ($p < 0.001$). Pathological studies also showed that V. vinifera SE preserved the pancreatic β cells' shape, size, and cell population in the diabetic GRP. The extract significantly decreased serum IL-1β levels in diabetic rats ($p < 0.001$). Besides, the data demonstrated a significant up-regulation in omentin-1 mRNA expression in diabetic rats treated with V. vinifera SE.*

Conclusion: *V. vinifera SE has anti-diabetic and anti-hyperlipidemic properties in streptozotocin-induced rat model. The underlying mechanism of action might be a decrease in the pro-inflammatory cytokine (IL-1β) and increase in omentin mRNA. Clinical trials are required to validate these findings.*

Keywords: *Diabetes mellitus, Omentin-1, Interleukin-1β, Vitis vinifera, Hyperlipidemia*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Globally, diabetes mellitus (DM) has emerged as an epidemic [1]. It is associated with either autoimmune pancreatic beta (β)-cells destruction that leads to insufficient insulin production or the body's resistance to insulin action [2]. People with diabetes are 2.5 times more likely to experience significant organ failure or damage to the eyes, kidneys, liver, testicles, and heart [3]. The prevalence of DM in Saudi Arabia (SA) is increasing dramatically, ranking second in the Middle East and seventh worldwide [4]. There are over seven million diabetic patients and three million prediabetics in SA, according to recent data. As a result, DM has emerged as one of the most challenging long-term health issues in patients' lives and medical costs in SA [5].

Oxidative stress and inflammation induced by excessive hyperglycemia are associated with DM complications [6]. Conventional hypoglycemic drugs are effective while bearing inevitable adverse effects. On the other hand, natural sources of antioxidant treatment were discovered to restore the glycemic index, prevent oxidative stress, and reduce the risk of DM complications [7].

Vitis vinifera (*V. vinifera*) (Linn. Vitaceae, family) is mentioned in the Holy Quran eleven times [8]. *V. vinifera* seeds contain several phytochemicals such as proanthocyanidins, flavonoids, catechin, phenolic acids, procyanidins, gallate, epicatechin, flavan-3-ols, organic acid, stilbenes, tannins, minerals, and vitamins [8]. Seeds extract (SE) of *V. vinifera* proved to have antihyperglycemic action, where catechin hinders intestinal glucose absorption, epicatechin aids in pancreatic β-cell restoration, and epicatechin gallate increases hepatic glycogen synthesis. Furthermore, phenolic compounds such as oligomeric proanthocyanidins have antioxidant properties, and anthocyanidins and procyanidins enhance the GLUT 4 translocation via stimulating AMPK pathways [9]. Furthermore, several pharmacological actions have been reported for *V. vinifera* SE, including antineoplastic, antimicrobial, and antioxidant [10].

Omentin is an adipokine secreted *via* the visceral adipose tissue. Numerous research supports omentin-1 anti-inflammatory benefits, macrophage aggregation, necrosis, and foam cell formation, as well as controlling the body's response to insulin, upregulating glycogen kinase-3 beta pathway, and diminishing atherosclerotic lesions, thus could ameliorate diabetes and cardiovascular dysfunction [11].

Therefore, this research investigates the potential of *V. vinifera* SE modulation of biochemical parameters associated with streptozotocin-induced pancreatic dysfunction as well as the omentin-1 mRNA expression in adipose tissue of diabetic rats.

EXPERIMENTAL

Preparation of *V. vinifera* SE

Ripened *V. vinifera* fruits were purchased from the local market in Jeddah, SA. The seeds were separated from the grapes' pulp after removing extraneous materials, dried at room temperature, and finely powdered. Seeds were extracted with 80 % ethanol then the filtrate was evaporated through a rotary evaporator under vacuum. The extract (ethanol-free semi-solid) obtained was kept at 4 °C.

Animal grouping

Male rats (n = 24, 180 - 200 g) were used in this study after acclimatization for one week at an optimal condition (temperature 22 - 25 °C, and 12 h dark/light cycle). During this period, the animals were maintained on a regular diet with free access to water.

Ethical approval

This design was certified by the College of Medicine Ethical Committee, KAU, Jeddah, SA (approval no. 563-21). The ethical guidelines of the International standard and Institutional Animal Care and Use committee (IACUC) were followed [12].

Induction of diabetes

Following overnight fasting, rats were divided into two sets. The first set of rats was injected intraperitoneal (IP) with citrate buffer (n = 12). The second set of rats was IP administered with streptozotocin dissolved in 0.1 M citrate buffer (pH 4.5), at a 55 mg/kg dose (n = 12). To overcome hypoglycemia, rats were allowed to drink a 5 % glucose solution for 24 h. A glucometer measured fasting blood glucose (FBG) levels after 72 h. Rats with FBG ≥ 300 mg/dL were considered diabetic and used subsequently in the experiment.

Study design

The first set of rats (n = 12) was divided into 2 groups (GRPs) of 6 rats each; GRP1 served as the control, while GRP2 was administered with an oral dose of *V. vinifera* SE (500 mg/kg). The

second set of rats was also divided into 2 GRPs; GRP3 included untreated diabetic rats, and GRP4 included diabetic rats that received oral *V. vinifera* SE (500 mg/kg) daily for 28 consecutive days [13].

After 4 weeks, rats were fasted overnight and euthanized after diethyl ether anesthesia. Blood was collected and serum was separated while removing adipose tissue. Serum and adipose tissue were frozen at -80 °C until use.

Evaluation of glucose, lipid profile markers, insulin, and interleukin-1 beta (IL-1β)

Serum levels of insulin and IL-1β were quantified using ELISA kits of Abcam, UK according to the manufacture's procedure. Serum glucose, lipid profiles markers (total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)) were measured using colorimetric enzymatic kits (BioVision, USA) as the manufacturer's instructed.

Quantitative real time-polymerase chain reaction (qRT-PCR)

Column method (Bio Basic, Canada) was followed for total RNA extraction from the adipose tissues. A cDNA synthesis kit (TaKaRa Inc., Japan) was used for RNA transcription. Table 1 shows the primer nucleotide sequences used. The qRT-PCR assay was carried out using SYBR Premix Ex Taq Π (TaKaRa Inc., Japan). Pre-denaturation at 95 °C for 5 min. Next, 40 cycles at 95 °C for 10 s, 55 °C (for omentin), and 57 °C (for beta-2 microglobulin (B2M)) for 20 s. Finally, 62 °C for 30 s followed by extension. The process was terminated by incubation and cooling to 4 °C.

Analysis of the melting curve was done. The outcomes were validated using the relative quantification approach (2CT). In comparison to the average of B2M, the average of three runs was computed.

Table 1: Primer nucleotide sequences used in PCR

Primer	Sequence (5′-3′)
B2M	Forward: CTTCAGCAAGGACTGGTC
	Reverse: TCTCGATCCCAGTAGACG
Omentin	Forward: GCTGAAGAGAACCTGGAC
	Reverse: AATAGAGACCATCTTGTGC

Statistical analysis

Data are reported as mean ± standard error of the mean (SEM). Comparisons between groups were made using GraphPad Prism 5 software, and $p \leq 0.05$ was considered significant.

RESULTS

Effect of *V. vinifera* extract on serum level of glucose

The results showed that serum glucose level of GRP3 (diabetes) was significantly elevated ($p < 0.001$) relative to GRP1 (control). On the other hand, serum glucose level of GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) was significantly decreased ($p < 0.001$) relative to GRP3 (diabetes) (Figure 1).

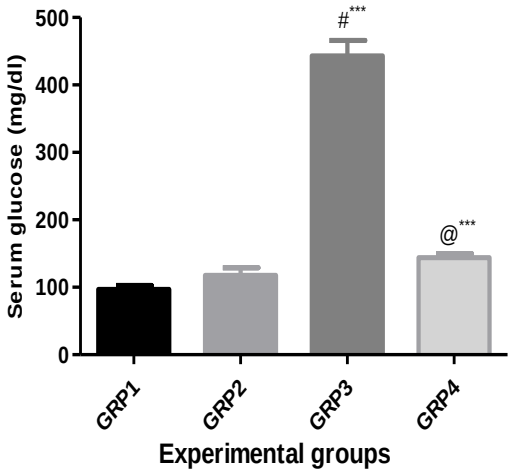

Figure 1: Effect of *V. vinifera* SE on serum level of glucose. GRP1, control, GRP2, *V. vinifera* SE (500 mg/kg); GRP3, diabetes; GRP4, diabetes + *V. vinifera* SE. Data are mean ± SE (n = 6). #significantly difference from GRP1, @significantly difference from GRP3. ***$P < 0.001$

Effect of *V. vinifera* SE on serum levels of cholesterol, triglyceride, LDL, and HDL

The results showed that serum cholesterol, triglyceride, and LDL levels of GRP3 (diabetes) were significantly increased ($p < 0.001$) relative to GRP1 (control). On the other hand, serum cholesterol, triglyceride, and LDL levels of GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) were significantly decreased ($p < 0.001$) relative to GRP3 (diabetes) (Table 2). The results also showed that serum HDL level of GRP3 (diabetes) was significantly decreased ($p < 0.001$) relative to GRP1 (control). On the other hand, the serum HDL level of GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) was significantly increased ($p < 0.001$) relative to GRP3 (diabetes) (Table 2).

Table 2: Effect of *V. vinifera* SE on serum levels of cholesterol, triglyceride, LDL, and HDL

Group	Cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
GRP1	118.5±2.3	75.7±1.6	71.7±3.0	46.8±1.5
GRP2	125.2±6.9	74.8±2.8	84.0±7.3	41.2±1.2
GRP3	289.3±17.8^{a***}	160.3±12.7^{a***}	265.8±18.6^{a***}	23.5±2.3^{a***}
GRP4	182.0±7.2^{b***}	90.5±3.8^{b***}	142.0±7.8^{b***}	40.0±2.9^{b***}

GRP1, control; GRP2, *V. vinifera* SE (500 mg/kg); GRP3, diabetes; GRP4, diabetes + *V. vinifera* SE. Data are mean ± SEM (n = 6). ^{a}significantly different from GRP1, ^{b}significantly different from GRP3. ***p < 0.001

Effect of *V. vinifera* SE on serum level of insulin

The results showed that serum insulin level of GRP3 (diabetes) was significantly decreased (p < 0.001) relative to GRP1 (control). On the other hand, serum insulin level of GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) was significantly increased (p < 0.001) relative to GRP3 (diabetes) (Figure 2).

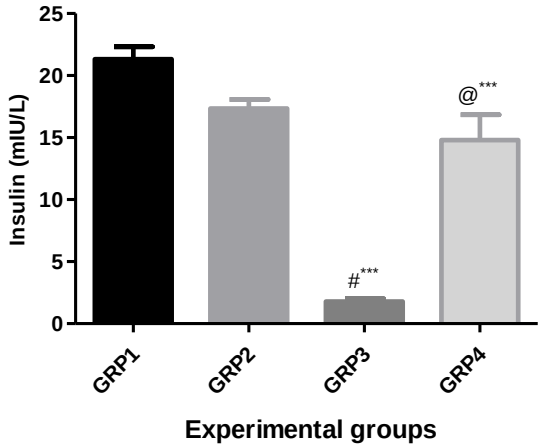

Figure 2: Effect of *V. vinifera* SE on serum level of insulin. GRP1, control; GRP2, *V. vinifera* SE (500 mg/dl); GRP3, diabetes; GRP4, diabetes + *V. vinifera* SE. Data are mean ± SE (n = 6). $^{\#}$significantly difference from GRP1, $^{@}$significantly difference from GRP3. ***P < 0.001

Effect of *V. vinifera* SE on the pancreatic β islets histopathological alteration

Photos of GRP1 (control) show normal size islet with normal population of islets cells. GRP2 (*V. vinifera* SE, 500 mg/kg) photos show apparently normal size islets with normal population of islets cells. GRP3 (diabetes) photos show mild decrease in islets size with numerous cells looked unstained and vacuolated. GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) show potential improvement with still central cells showing lightly stained cytoplasm and few vacuolation (Figure 3).

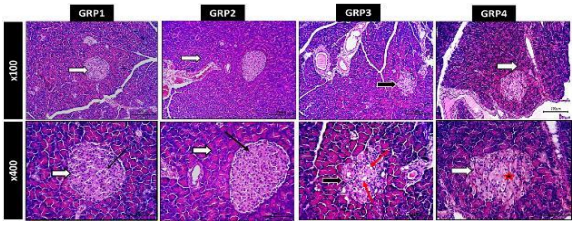

Figure 3: Effect of *V. vinifera* SE on the pancreatic β islets histopathological alteration (H & E stain, bars 200 and 100 µm). GRP1 (control) photos show normal size islet (white arrows) with normal population of islets cells (black arrows). GRP2 (*V. vinifera* SE, 500 mg/kg) photos show apparently normal size islets (white arrows) with normal population of islets cells (black arrows). GRP3 (diabetes) photos show mild decrease in islets size (black arrows) with numerous cells looked unstained and vacuolated (red arrows). GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) photos show potential improvement with still central cells showing lightly stained cytoplasm and few vacuolation (red star)

Effect of *V. vinifera* SE on serum level of IL-1β

The results showed that serum IL-1β level of GRP3 (diabetes) was significantly elevated (p < 0.001) relative to GRP1 (control). On the other hand, serum IL-1β level of GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) was significantly decreased (p < 0.001) relative to GRP3 (diabetes) (Figure 4).

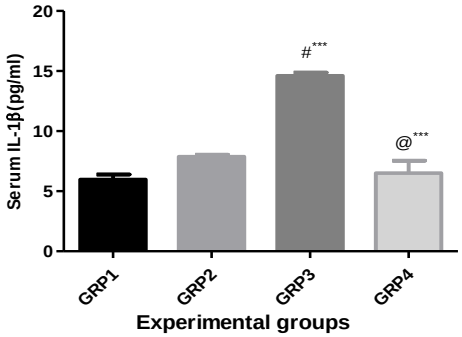

Figure 4: Effect of *V. vinifera* SE on serum level of IL-1β. GRP1, control; GRP2, *V. vinifera* SE (500 mg/kg); GRP3, diabetes; GRP4, diabetes + *V. vinifera* SE. Data are mean ± SE (n = 6). $^{\#}$significantly difference from GRP1, $^{@}$significantly difference from GRP3. ***P < 0.001

Effect of *V. vinifera* SE on adipose tissues mRNA relative expression

The results showed that adipose tissues omentin mRNA relative expression of GRP3 (diabetes) was significantly decreased ($p < 0.001$) relative to GRP1 (control). On the other hand, adipose tissues omentin mRNA relative expression of GRP4 (diabetes + *V. vinifera* SE (500 mg/kg)) was significantly increased ($p < 0.001$) relative to GRP3 (diabetes) (Figure 5).

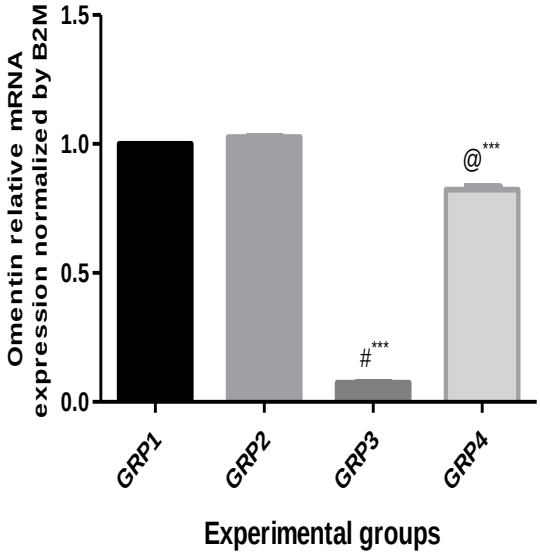

Figure 5: Effect of *V. vinifera* SE on adipose tissues mRNA relative expression. GRP1, control; GRP2, *V. vinifera* SE (500 mg/kg); GRP3, diabetes; GRP4, diabetes + *V. vinifera* SE. Data are mean ± SEM (n = 6). #significantly difference from GRP1, @significantly difference from GRP3. ****P* < 0.001

DISCUSSION

In this study, *V. vinifera* ethanolic SE reserved pancreatic function in diabetic rats. This was confirmed by increasing serum insulin, decreasing serum glucose, and improving lipid profile (decreasing serum cholesterol, triglyceride, and LDL while increasing serum HDL). The pathological study also showed that *V. vinifera* SE protected the pancreatic β-cells' shape, size, and cell population. In agreement with the current study results, it has previously been observed that the aqueous SE of *V. vinifera* protects against pancreatic dysfunction in adult male rats with diabetes mellitus [14]. Treatment with *V. vinifera* SE maintained nearly normal expression levels of GLUT2, which is involved in glucose-stimulated insulin secretion, according to Adama *et al* [14]. The availability of sufficient GLUT 2 might assist in boosting glucose inflow into the β-cells, which is required to evoke insulin release.

The standardized *V. vinifera* SE contains 92 – 95 % oligomeric proanthocyanidins. In type 2 DM, proanthocyanidins, particularly the oligomeric form, were found to control hyperlipidemia [15]. In addition to proanthocyanidins, *V. vinifera* SE is rich in catechins, epicatechins, and epicatechin-3-O-gallate. The antihyperglycemic actions of epicatechin and gallic acid may be related to the stimulatory effects of pancreatic secretion of insulin and C peptide and accelerated transport of blood glucose to peripheral tissue. Other pathways for their glucose-lowering effect include suppressing endogenous glucose synthesis by inhibiting gluconeogenesis in the liver and muscle [16].

The present results showed that *V. vinifera* ethanolic SE significantly decreased serum IL-1β levels in diabetic rats. The ability of *V. vinifera* SE to reduce inflammation in diabetic rats could explain the pancreas β-cells' apparently normal histopathological appearance [14]. The anti-inflammatory properties of *V. vinifera* SE can be linked to the extract's main chemical components, which include benzene 3-methyl cyclopentyl and 2,5-bis (1,1-dimethyl ethyl) [14].

In agreement with these earlier findings, the present data demonstrated a significant down-regulation in omentin-1 mRNA expression in the adipose tissues of diabetic rats compared to the control rats [17]. The current results showed for the first time that the expression of the omentin-1 gene was increased in adipose tissues of diabetes-induced rats administered *V. vinifera* SE. According to the findings of an *in vitro* investigation, omentin can improve insulin-triggered glucose absorption by enhancing protein kinase Akt or protein kinase B [18]. Omentin may play a probable potential role as an insulin sensitizer [17].

CONCLUSION

V. vinifera SE exhibits anti-hyperlipidemic and antidiabetic properties in a streptozotocin-induced rat model. The underlying mechanism of action may involve a reduction in the pro-inflammatory cytokine, IL-1β, and up-regulation of omentin mRNA. *V. vinifera* seed extract is a promising anti-hyperlipidemic and antidiabetic agent but clinical trials are necessary to verify these findings.

DECLARATIONS

Acknowledgements

The authors gratefully thank King Fahd Medical

Research for helping in conducting the experimental and practical part of this study.

Funding

None provided.

Ethical approval

This design was certified by the College of Medicine Ethical Committee, KAU, Jeddah, SA (approval no. 563-21).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

REFERENCES

1. *Rajput SA, Ashraff S, Siddiqui M. Diet and management of type ii diabetes mellitus in the United Kingdom: A narrative review. Diabetol 2022; 3: 72–78.*
2. *American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care 2014; 37(Suppl1): S81–S90.*
3. *Diabetes UK. Tackling the Crisis: Transforming Diabetes Care for a Better Future. 2020. Available online: 0524 Diabetes UK Tackling the Crisis Document_P6.indd.*
4. *Alanazi FK, Alotaibi JS, Paliadelis P, Alqarawi N, Alsharari A, Albagawi B. Knowledge and awareness of diabetes mellitus and its risk factors in Saudi Arabia. Saudi Med J 2018; 39: 981–989.*
5. *Itumalla R, Kumar R, Tharwat Elabbasy M, Perera B, Torabi MR. Structural factors and quality of diabetes health services in hail, Saudi Arabia: a cross-sectional study. Healthcare 2021; 9: 1691.*
6. *Rato L, Oliveira PF, Sousa M, Silva BM, Alves MG. Role of reactive oxygen species in diabetes-induced male reproductive dysfunction. in oxidants, antioxidants, and impact of the oxidative status in male reproduction; Elsevier: Amsterdam, The Netherlands, 2019; 135–147.*
7. *Mohammed FS, Pehlivan M, Sevindik E, Akgul H, Sevindik M, Bozgeyik I, Yumrutas O. Pharmacological properties of edible Asparagus acutifolius and Asparagus officinalis collected from North Iraq and Turkey (Hatay). Acta Alimentaria 2021; 50(1): 136–143.*
8. *Urbi Z, Hossain MS, Rahman KH, Zayed TM. Grape: a medicinal fruit species in the holy Qur'an and its ethnomedicinal importance. World Appl Sci J 2014; 30(3): 253–265.*
9. *Golovinskaia O, Wang C. The hypoglycemic potential of phenolics from functional foods and their mechanisms. Food Sci Human Wellness 2023; 12(4): 986-1007.*
10. *Karami S, Rahimi M, Babaei A. An overview on the antioxidant, anti-inflammatory, antimicrobial, and anti-cancer activity of grape extract. Biomed Res Clin Pra 2018; 3(2): 1–4.*
11. *Lin X, Sun Y, Yang S, Yu M, Pan L, Yang J, Yang J, Shao Q, Liu J, Liu Y, et al. Omentin-1 modulates macrophage function via integrin receptors αvβ3 and αvβ5 and reverses plaque vulnerability in animal models of atherosclerosis. Front Cardiovasc Med 2021; 8: 757926.*
12. *National Research Council, Guide for the Care and Use of Laboratory Animals, 7th ed. National Academy Press, Washington DC, 1996.*
13. *Giribabu N, Kumar KE, Rekha SS, Muniandy S, Salleh N. Vitis vinifera (muscat variety) seed ethanolic extract preserves activity levels of enzymes and histology of the liver in adult male rats with diabetes. Evid Based Complement Alternat Med 2015; 2015: 542026.*
14. *Adama SH, Giribabua N, Kassimb N, Kumarc KE, Brahmayyad M, Aryae A, Salleha N. Protective effect of aqueous SE of Vitis vinifera against oxidative stress, inflammation and apoptosis in the pancreas of adult male rats with diabetes mellitus. Biomed Pharmacother 2016; 81: 439–452.*
15. *Pourghassem-Gargari B, Abedini S, Babaei H, Aliasgarzadeh A, Pourabdollahi P. Effect of supplementation with grape seed (Vitis vinifera) extract on antioxidant status and lipid peroxidation in patient with type II diabetes. J Med Plant Res 2011; 5(10): 2029-2034.*
16. *Rui L. Energy metabolism in the liver. Comprehensive Physiol 2014; 4(1): 177-197.*
17. *Goodarzi G, Shirgir A, Alavi S, Khoshi A. Effect of insulin–glucose metabolism compared with obesity on adipose omentin gene expression in different models of diabetic C57BL/6 mice. Diabetol Metab Syndr. 2019; 11: 65.*

18. Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC, Shuldiner AR, Fried SK, McLenithan JC, Gong D. Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. Am J Physiol Endocrinol Metab 2006; 290(6): E1253–E1261.