

Research Paper:

The Extract of *Viola Odorata* Flowers Improves the Biochemical, Pancreas Histological, and Insulin Resistance Parameters in an Animal Model of Diabetes



Rouhollah Gazor¹, Fatemeh Niknezhad², Fatemeh Yousefbeyk³, Monireh Aghajany-Nasab⁴, Fahimeh Mohammadghasemi^{5*}

1. Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
2. Student Research Center, Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
3. Department of Pharmacognosy, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran.
4. Cellular & Molecular Research Center, Department of Biochemistry, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
5. Cellular & Molecular Research Center, Department of Anatomy, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.



How to cite this paper Gazor R, Niknezhad F, Yousefbeyk F, Aghajany-Nasab M, Mohammadghasemi F. The Extract of *Viola odorata* Flowers Improves the Biochemical, Pancreas Histological, and Insulin Resistance Parameters in an Animal Model of Diabetes. Iranian Journal of Toxicology. 2022; 16(1):43-50. <http://dx.doi.org/10.32598/IJT.16.1.869.1>

doi <http://dx.doi.org/10.32598/IJT.16.1.869.1>



Article info:

Received: 14 Aug 2021

Accepted: 17 Nov 2021

Online Published: 01 Jan 2022

*Corresponding author:

**Fahimeh Mohammadghasemi,
PhD.**

Address: Cellular & Molecular
Research Center, Department of
Anatomy, School of Medicine, Guilan
University of Medical Sciences,
Rasht, Iran.

E-mail: parsahistolab@gmail.com

ABSTRACT

Background: The antioxidant defense in the pancreas is low because they are exposed to toxic substances daily. This study aimed to evaluate the ameliorating effect of *Viola odorata* flowers Extract (VOE) on the pancreas histology and function in Streptozotocin (STZ)-induced diabetic rats.

Methods: Forty male rats were divided into five groups, consisting of controls; STZ; and STZ plus various doses of VOE (100, 200 or 400 mg/kg). The amylase, lipase, insulin and total antioxidant capacity levels were measured in the sera. The homeostatic model assessment of insulin resistance was also measured. The histopathological alterations of the rats' pancreases were examined microscopically.

Results: The serum amylase and total antioxidant activities were reduced in diabetic rats ($P=0.001$). Varying doses of VOE reduced the serum amylase and glucose levels, and increased the total antioxidant activities compared to that of the diabetic rats ($P<0.05$). There were no significant differences in the serum lipase and insulin levels among the groups. Treatment with VOE at all doses significantly lowered the insulin resistance compared to that of the diabetic group ($P=0.001$). Significant reductions were observed in the areas of the pancreatic Langerhans islets and the number of beta cells in the STZ group ($P=0.001$).

Conclusion: This study demonstrated that VOE ameliorated the adverse effects induced by STZ in the rat's pancreas in the short-term. These effects are likely to be due to the reduced insulin resistance and amylase activity, and increased total antioxidant activity along with the histopathological alterations in the pancreas.

Keywords: Amylases, Diabetes mellitus, Lipases, *Viola odorata*, Pancreas

Introduction

T

ype 2 Diabetes Mellitus (T2DM) is a metabolic and multifactorial disorder. Various conditions, such as lifestyle, family inheritance, obesity, hypertension, aging, and

other risk factors lead to diabetes, in which insulin secretion or action is deficient [1]. Following diabetes, hyperglycemia occurs and the metabolism of electrolytes, lipids and proteins are disturbed [1]. If left untreated, diabetes may lead to cardiovascular, kidney and neurological complications and may ultimately result in death

[2]. The prevalence of T2DM is rising globally and the number of adult diabetes may probably reach 592 million in 2035 [1]. This disorder can be successfully managed with proper diet, exercise and therapeutic drugs [3]. Diabetes is associated with oxidative stress, a prevalent cause of which is glucose autoxidation [4]. Persistent hyperglycemia disturbs the antioxidants and leads to oxidative stress and inflammation, which may cause the death of the pancreas β -cells [4]. The antioxidant defense in pancreas is usually low if the cells are exposed to toxic substances daily or frequently. Therefore, the cells are damaged due to the oxidative stress.

In addition to the endocrine secretions by the Langerhans islands, pancreas has exocrine secretions from its acinar cells. These cells secrete digestive enzymes, including proteases, lipase and amylase [5]. Since the acinar and Langerhans island cells are in close proximity to each other, defect in insulin secretion may have adverse effects on the acinar cells' function [6]. Both insulin deficiency in diabetes type 1 and 2, and insulin resistance in obesity and metabolic syndrome lead to low serum amylase [6, 7]. The serum amylase and lipase levels are important for determining the pathologic mechanisms in pancreas [7]. In pancreatitis, pancreatic cancer and pancreatic duct obstruction, the lipase and amylase secretions increase [6]. Treatment with STZ in neonate rats reduces the pancreatic amylase without affecting its lipase and trypsin secretions after 16 days [8].

In experimental animal models, Streptozotocin (STZ), is usually used to induce diabetes. This compound interacts with the pancreatic beta-cells via glucose transporter-2 receptors and causes the production of Reactive Oxygen Species (ROS) [9]. Currently, the use of drugs such as insulin or its sensitizers or insulin secretory drugs are the main treatment for diabetes. However, hypoglycemia and gastrointestinal discomforts are known to be the side effects of these drugs. Therefore, there is a need for safer and more effective drugs for the management of diabetes [1]. Nowadays, the application of traditional medicine is growing in the management of many

chronic conditions, including diabetes. Traditional medicine has been used widely in developing countries and about two-thirds of the people use traditional medicine for health care purposes globally [10]. Traditional medicines are cheaper, more accessible and patients have better tolerance using them [3]. They may have better and useful effects on diabetes due to their antioxidant properties [4].

Various substances with antioxidant properties have been identified in various parts of plants such as fruits,

stem, root, leaves, flowers, and seeds [4]. *Viola odorata* L. or sweet violet from the Violaceae family of plants, has traditionally been used for the management of hypertension, anxiety, mouth infection, sleep disorders and fever in children [11]. This plant product has anti-inflammatory, antibacterial, antioxidant and anticancer properties [12, 13]. In addition, this product protects against conditions that adversely affect the liver, vasculature, and the antioxidative systems [14]. The *Viola* blossoms powder protects both liver and kidney functions in rats treated with carbon tetrachloride (CCl₄) by reduction of malondialdehyde [15]. The *Viola* flowers also reduce lipid peroxidation and have protective effects against rat's kidney damages induced by STZ [11]. Studies on the effects of *Viola odorata* on the endocrine and exocrine functions of the pancreas in diabetic animal models are lacking. Thus, the present study was conducted to evaluate the therapeutic and ameliorative effects of the extract of *Viola odorata* flowers on the pancreas histology and function in streptozotocin-induced diabetes in rats.

Materials and Methods

Extraction procedure: *Viola odorata* (VO) flowers were collected from the plant in Mazandaran province, Iran. The plant was identified and given herbarium specimen number (HGUM-302) by a botanist at Pharmacology Department, Guilan University of Medical Sciences. The flowers were powdered, using an electric mill, and dissolved in 70% ethanol for 72hr in darkness. Then, the solution was filtered through a sterile paper filter and dried by evaporating the alcohol. The prepared powder was then stored in a refrigerator at 4°C [11, 17].

Animal study: The study protocol was reviewed and approved by the Laboratory Animals Ethics Committee of Guilan University of Medical Sciences (Ethics Code:1395.226). A total of forty adult Wistar male rats, weighing 250±10 g each, were used in this study. They were divided equally into five groups of eight rats each, and were kept under 12hr of light and dark cycles at 23°C temperature. They had free access to food pellets and tap water *ad libitum*.

Animals were divided in groups as follows: A: the control group was treated with normal saline; B: the diabetic group treated with STZ; C, D, and E: the experimental groups received STZ (45 mg/kg) and the following doses of VOE at 100, 200, and 400 mg/kg, respectively. To induce diabetes in rats, a single dose of STZ (45 mg/kg) was injected Intraperitoneally (IP). On the 3rd day after the STZ injection, all animals were checked for their serum glucose levels. Blood samples were collected from

their tails and the rats with the glucose levels higher than 250 mg/dl were considered as diabetic [18, 19]. Three days after the induction of diabetes, the VOE was administered IP to the rats daily, for the next 30 days. On day 31, the animals were anesthetized with 50 mg/kg ketamine and 22 mg/kg xylazine. The pancreas tissue was removed surgically from each animal for the subsequent histopathological examinations. Blood samples were also collected from the rats' inferior vena cava.

Biochemical assays: The sera were obtained by centrifuging the blood samples at 4000 rpm for 10 min and stored at -20°C for further analysis. The serum glucose, amylase and lipase levels were measured using the respective kits (Parse Azmun, Tehran, Iran) for spectrophotometric assays. The kits' sensitivity levels were 5mg/dl (glucose) and 3 IU/L for lipase and amylase. Insulin was measured by a rat ELISA kit (Demeditec, Germany) with a sensitivity of 0.1ng/ml. The Total Antioxidant Capacity (TAC) was measured by the specific kit (Zell-Bio, Germany) at 0.1 mM sensitivity. All procedures were performed by adhering to the kits' instructions.

The homeostatic model of assessing insulin resistance: The Homeostatic Model of Assessing Insulin Resistance (HOMA-IR) was determined based on the following formula: $HOMA-IR = \text{Insulin} \times \text{Glucose} / 22.5$ [20].

Histopathological analyses: The pancreas tissue samples were fixed in 10% formaldehyde for 72 hours, then dehydrated using graded concentration of ethanol. Xylene was also used as a transitional solution. The histology samples were embedded in melted paraffin and sectioned in 5-micrometer thick tissue slices, using a rotary microtome (Leitz, Germany). The slides were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus, Japan). The morphology of the Langerhans islets and the acinar cells

were examined microscopically at 400 X magnification. The islet cells were counted from 50 microscopic fields in each group. The areas of islets were measured using a computer software (Digimizer Version 11). To determine the beta cell numbers, slides were stained with aldehyde fuchsin. For each rat, 10 Langerhans islets were examined and the beta cell numbers were counted and divided by the sum of the beta cells versus none beta cell. The answer in each case was multiplied by 100 and recorded as the percentage.

Statistical analyses: The statistical analysis of the data was performed on SPSS version 16 and Graph Pad Prism version 7. Analysis of Variance (ANOVA) and Tuckey's tests were used to compare the data among the groups. The data were reported as the means and standard error. The value of $P < 0.05$ was considered as a statistically significant difference.

Results

Biochemical parameters: As reflected by the data in Table 1, the administration of VOE at 100, 200 and 400mg/kg reduced the serum glucose and amylase levels significantly compared to that of the STZ and control groups, respectively ($P = 0.001$). Doses of VOE reduced the serum amylase compared to that of the diabetic rats ($P < 0.05$), (Table 1). The serum lipase was insignificantly lower in STZ group than the controls, and there were no significant differences in the serum lipase levels among all groups. Also, an insignificant reduction the serum insulin was detected in the STZ group compared to that of the controls. There were no significant differences in the insulin levels among all of the experimental groups (Table 1). A lower serum TAC was present in the STZ group compared to that of the controls. The admin-

Table 1. Biochemical parameters in different groups

Groups	Mean±SE					
	Glucose (mg/dl)	Insulin (ng/ml)	HOMA-IR	Amylase (U/L)	Lipase (U/L)	TAC (mM)
Control	105.85±9.35 ^b	0.810±0.02	5.9±0.75 ^b	1593.01±73	53.2±6.2	0.49±0.01
STZ	325.00±32.0 ^a	0.796±0.0	25.24±1.9 ^a	989.0±83 ^a	41.4±8.3	0.12±0.08 ^a
STZ+100mg/kg VOE	152.2±17.1 ^{ab}	0.794±0.01	20.3±3.1 ^{ab}	894.2±34 ^{ab}	44.4±6.8	0.32±0.05 ^b
STZ+200mg/kg VOE	156.50±43.2 ^{ab}	0.797±0.08	19.6±2.8 ^{ab}	789.1±41 ^{ab}	45.68±7.4	0.36±0.06 ^b
STZ+400mg/kg VOE	140.00±4.14 ^{ab}	0.795±0.02	18.5±1.4 ^{ab}	723.1±37 ^{ab}	48.5±12.4	0.34±0.03 ^b

^aSignificant values compared to the control group; $P = 0.001$; ^b significant values compared to the STZ treated group; $P < 0.05$. Streptozotocin (STZ), *Viola odorata* flower Extract (VOE); Total Antioxidant Capacity (TAC); Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).

Table 2. Histologic findings of pancreas in different groups

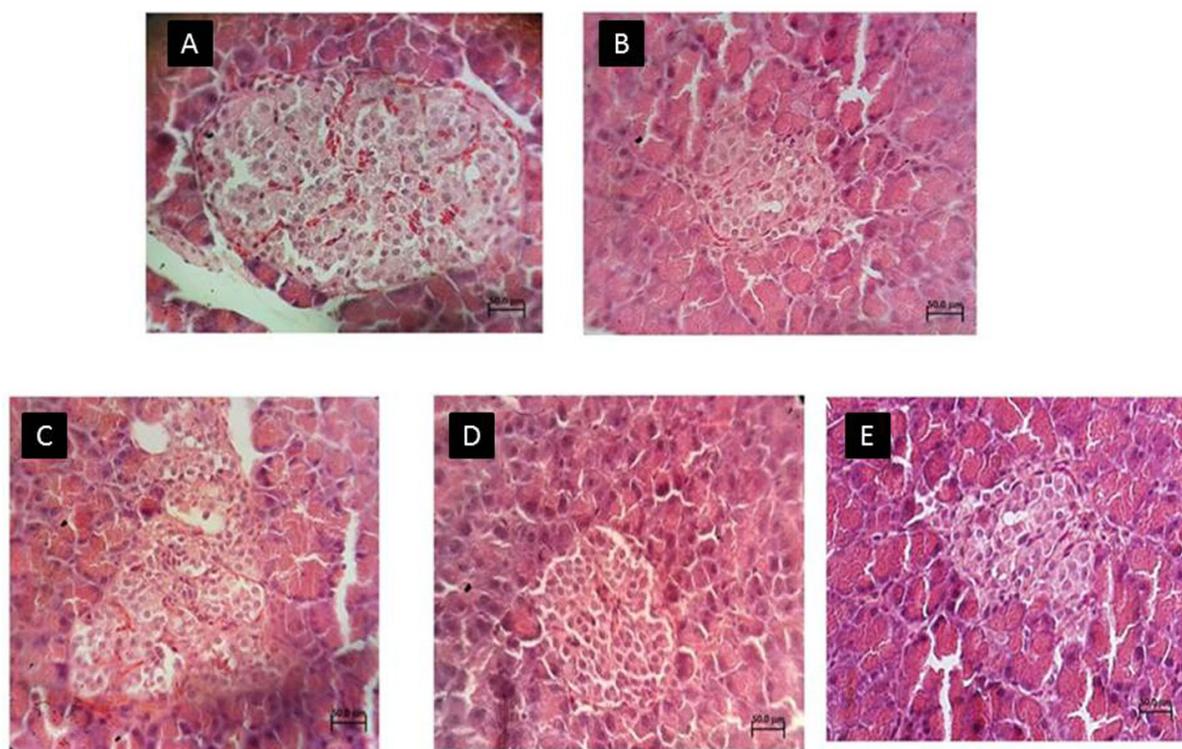
Groups	Mean±SE		
	Beta Cell Numbers (%)	Islet Size (μm^2)	Islet Numbers/50 fields
Control	56.2±3.1	113566±642	14.2±3.1
STZ	18.61±1.5 ^a	40355±5886 ^a	8.4±0.6
STZ+100mg/kg VOE	16.68±0.68 ^a	40964±1536 ^a	10.2±0.6
STZ+200mg/kg VOE	16.28±0.9 ^a	61345±182 ^{ab}	11.4±1.8
STZ+400mg/kg VOE	16.03±1.4 ^a	63241±3481 ^{ab}	12.2±2.3

^aSignificant values compared to the control group; P=0.001; ^bSignificant values compared to the STZ-treated group; P=0.01; Streptozotocin (STZ).

istration of VOE increased the TAC compared to that of the STZ groups (P=0.001) (Table 1).

HOMA-IR: Within the STZ group, the HOMA-IR was significantly higher compared to that of the controls (P=0.001). However, the treatment with VOE at all doses significantly lowered HOMA IR compared to that of the STZ group (P=0.001).

Histological findings: The histologic findings revealed a severe atrophy in both the islets and non-islets of the pancreas samples. There were no clear morphological differences among the groups with respect to the exocrine tissues. A significant reduction in the pancreatic islet areas was observed in the STZ group compared to that of the controls (P=0.001). Treatment with doses of VOE increased the pancreatic islet areas significantly (P=0.01). The mean number of beta cells in the pancreatic islets

**Figure 1.** Histology micrographs of the pancreas in rats

A: control; B: STZ; C: STZ+100mg/kg VOE; D: STZ+200mg/kg VOE; E: STZ+400 mg/kg VOE. Streptozotocin (STZ), *Viola odorata* flower Extract (VOE). Note the regression of Langerhans Island in STZ-treated rat and alteration of the sizes in 200 and 400 mg/kg (VOE)-treated-rats. Stained with Hematoxylin and Eosin. Magnification: 400x.

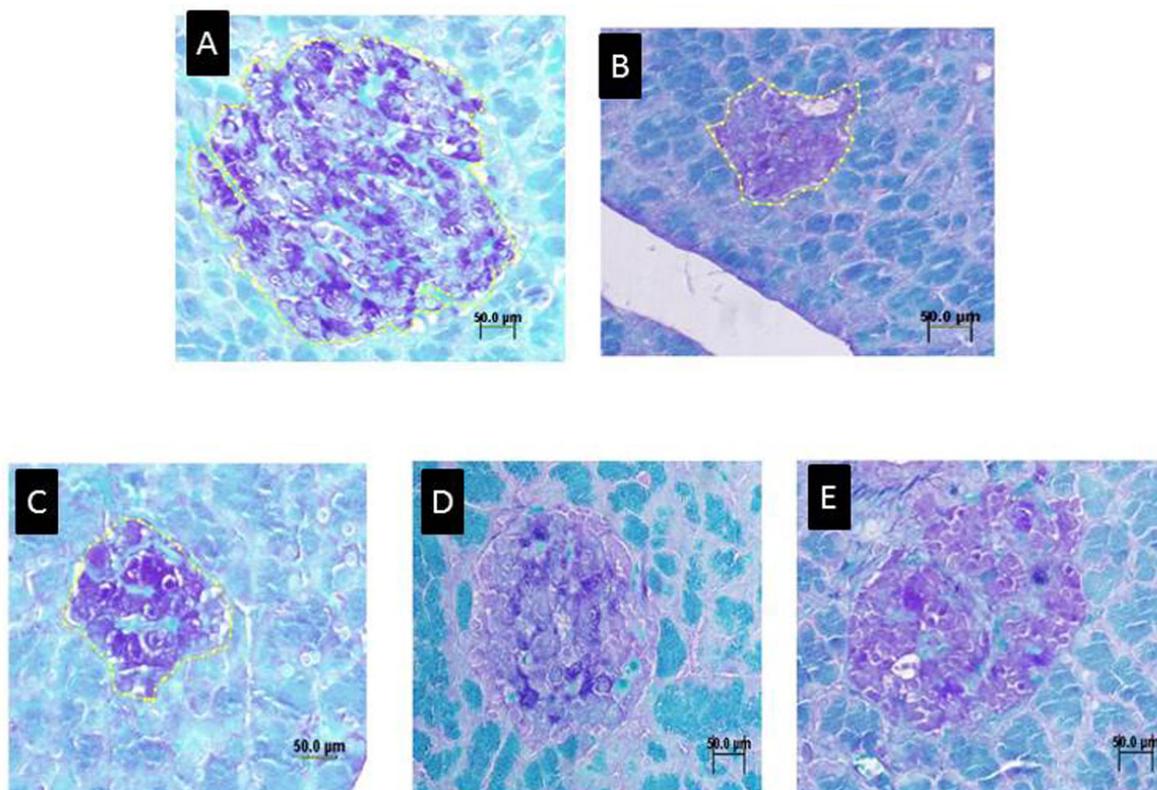


Figure 2. Histology micrographs of pancreas in rats

A: Control; B: STZ; C: STZ+100 mg/kg VOE; D: STZ+200 mg/kg VOE; E: STZ+400 mg/kg VOE. Streptozotocin (STZ), *Viola odorata* flower Extract (VOE). Dark blue cells are beta cells in Langerhans islands. Stained with aldehyde fuchsin. Magnification: 400x.

reduced significantly in the STZ group compared to that of the controls ($P=0.001$). There were no significant differences in the number of beta cells between the STZ group and VOE-treated groups. An insignificant reduction in the Islets' numbers was observed within the STZ group compared to that of the controls. The VOE at varying doses increased the Islets numbers insignificantly. See details in Figures 1 and 2, and Table 2.

Discussion

This study demonstrated that the VOE prevented the hyperglycemia and ameliorated the side effects induced by STZ treatment in rat pancreas. The administration of VOE at 100, 200 and 400mg/kg reduced the serum glucose and amylase in rats. The findings also confirmed that the VOE reduced the HOMA-IR, and that STZ reduced the numbers of beta cells in the pancreatic islets.

Toxic mechanisms: The exact mechanisms by which STZ leads to a reduction in the pancreatic islet cells is not fully clear. One possibility may be the reduction in the Nicotinamide Adenine Dinucleotide (NAD), which plays multiple roles in cellular metabolism. It may also

be due to the methylation of DNA in the pancreatic islet cells [21, 22]. It is also known that STZ increases the generation of free radicals in cells. In this context, the adverse effects of STZ on glucose and HOMA-IR supports its toxicity against the beta cells. In this respect, it has been demonstrated that no correlation exists between the glycemic control and ROS generation in diabetes [4]. Indeed, the generation of ROS in diabetes is multifactorial [4]. Multiple variables are involved in the generation of ROS [4]. They include glucose autoxidation, increased level of ferritin and homocysteine as pro-oxidants, glycation of protein and increased level of Advanced Glycation End products (AGEs), and imbalance in free radicals and other antioxidants [4].

Antioxidant effects: This study showed that the Total Antioxidant Capacity (TAC) declined in STZ groups and the VOE at varying doses increased that capacity, confirming the anti-oxidative properties of the extract. The anti-oxidant activity of both hydroalcoholic and water extract of VOE has been demonstrated in previous studies [12, 23]. In this context, it has been shown that VOE possesses cyclotides, violacein A [24], which gives it various metabolites, with anti-bacterial, anti-cancer and

immune-stimulant properties [24]. About 30 cyclotides have extracted from the roots of *V. odorata* plant. Phenolic and flavonoid compounds have been isolated from the *V. odorata* leaves [12, 23]. The antioxidant property arises from its constituents, such as alkaloids, steroids, tannins, phenolics, flavonoids glycoside, flavonoids, saponins, methyl salicylate, mucilage and vitamin C [13]. The current study demonstrated a significant decrease in serum amylase and along with an insignificant decrease in lipase compared to those measured for the STZ-treated group. This finding may be due to the altered cell function in the exocrine parts of the damaged pancreatic islets.

Effect of phenolics: Dietary phenolic compounds have inhibitory effects on α -amylase. Therefore, they can be potential candidates for the management of Type 2 diabetes. Alpha-amylase enzyme catalyzes the breakdown of starch to malto-oligosaccharides, which are degraded to glucose and lead to hyperglycemia and insulinemic changes [25]. Both α -amylase and α -glucosidase enzymes are inhibited by polyphenols of plants origin [26]. The reduced amylase level in rats treated with VOE may be due to the inhibitory activities of polyphenols present in the extract. As demonstrated by the current study, following amylase reduction, glucose was also reduced due to the hypoglycemic effect of the VOE. Acute pancreatitis, pancreas tumors, diabetic ketoacidosis and renal dysfunction may be associated with a rise in serum amylase [7]. Insulin deficiency in type-1 and some cases of type 2 diabetes, and insulin resistance may be associated with low serum amylase [7]. Low serum amylase and lipase is associated with diabetes and metabolic syndrome [6].

HOMA-IR: Our findings showed that a rise in HOMA-IR occurs in STZ-treated rats, and the VO extract was able to reduce the HOMA-IR compared to that of the STZ-treated group. Probably, low amylase in rats treated with the VOE in this study is due to a low HOMA-IR and glucose levels that we achieved. Insulin, regulates amylase secretion through its receptors on exocrine cells. However, similar mechanism of action about lipase is not known in details in diabetic patients [22]. Clinical studies have shown low amylase and lipase can occur in type 1 or type 2 diabetes [6, 7, 27]. Similarly, a recent experimental study has reported that 66% and 43% reductions, respectively, occur in amylase and lipase secretion by the pancreas in diabetic rats [28]. However, there are conflicting reports about the serum amylase and lipase levels in diabetic patients [29, 30]. Other factors responsible for the exocrine insufficiency of pancreas may be the overexpression of TGF β -1 in pancreas, aging and reduction of free calcium in the cytosol of the pancreatic cells [6]. The

exact mechanism of action of the VOE on pancreas tissue is not fully understood and needs further investigations.

Conclusions

This study provided evidence regarding the beneficial effects of VOE on the pancreas tissue in STZ-treated rats. The VOE at doses of 200 and 400 mg/kg improved both the histologic structures and function of the pancreas. These effects are likely due to reductions in HOMA-IR and/or amylase activity and the increase in total antioxidant capacity that may be linked to the phenolic characteristics of the VOE.

Limitations of the study: One of the limitations was using the rat model. Although there are many physiological similarities between rats and humans, obviously, they are not identical in all respects. Another limitation was the STZ treatment, which may have toxic effects on other organs. Lack of laboratory resources to quantify the VOE in the blood and pancreas tissues of the treated rats were other limitations.

Recommendations for future research: The evaluation of cellular and molecular mechanisms and histological examination of the pancreatic also ultrastructure following treatment with VOE and STZ are recommended in future studies.

Ethical Considerations

Compliance with ethical guidelines

Sampling and protocols were approved by the ethics committee of Guilan University of Medical Sciences (Code: IR.GUMS.REC.1395.226).

Funding

This study is supported by the Vice-Chancellor and Research Technology Guilan University of Medical Sciences (No: 95071709).

Authors' contributions

Conceptualization and supervision: Rouhollah Gazor and Fahimeh Mohammadghasemi; Methodology: Fate-meh Niknezhad; Investigation, writing - original draft, and writing - review & editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

We wish to thank the vice-chancellor and research technology of the Guilan University of Medical Sciences for their supports.

Reference

- [1] Zhao XQ, Guo S, Lu YY, Hua Y, Zhang F, Yan H, et al. *Lycium barbarum* L. leaves ameliorate type 2 diabetes in rats by modulating metabolic profiles and gut microbiota composition. *Biomed Pharmacother.* 2020; 121:109559. [DOI:10.1016/j.biopha.2019.109559] [PMID]
- [2] Kouamé NGM, Koffi C, N'Zoué KS, Yao NAR, Doukouré B, Kamagaté M. Comparative antidiabetic activity of aqueous, ethanol, and methanol leaf extracts of *Persea Americana* and their effectiveness in Type 2 diabetic rats. *Evid Based Complement Alternat Med.* 2019; 2019:5984570. [DOI:10.1155/2019/5984570] [PMID] [PMCID]
- [3] Mei H, Xu J, He Y, Yang X, Liu W, Tian W, et al. Protein-rich extract of *Musca domestica* larvae alleviated metabolic disorder in STZ-induced type 2 diabetic rat model via hepatoprotective and pancreatic β -cell protective activities. *J Biosci.* 2018; 43(5):969-83. [DOI:10.1007/s12038-018-9804-z] [PMID]
- [4] Rahimi R, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother.* 2005; 59(7):365-73. [DOI:10.1016/j.biopha.2005.07.002] [PMID]
- [5] Atkinson MA, Campbell-Thompson M, Kusmartseva I, Kaestner KH. Organisation of the human pancreas in health and in diabetes. *Diabetologia.* 2020; 63(10):1966-73. [DOI:10.1007/s00125-020-05203-7] [PMID] [PMCID]
- [6] Madole MB, Iyer CM, Madivalar MT, Wadde SK, Howale DS. Evaluation of biochemical markers serum amylase and serum lipase for the assessment of pancreatic exocrine function in diabetes mellitus. *J Clin Diagn Res.* 2016; 10(11):BC01-4. [DOI:10.7860/JCDR/2016/23787.8900] [PMID] [PMCID]
- [7] Nakajima K, Nemoto T, Muneyuki T, Kakei M, Fuchigami H, Munakata H. Low serum amylase in association with metabolic syndrome and diabetes: A community-based study. *Cardiovasc Diabetol.* 2011; 10:34. [DOI:10.1186/1475-2840-10-34] [PMID] [PMCID]
- [8] Lee PC, Brooks S, Leberthal E. Streptozotocin effect on the development of the exocrine pancreas in neonate rats. *Diabetes Res Clin Pract.* 1986; 2(5):269-75. [DOI:10.1016/S0168-8227(86)80003-1] [PMID]
- [9] Rashid K, Sil PC. Curcumin enhances recovery of pancreatic islets from cellular stress induced inflammation and apoptosis in diabetic rats. *Toxicol Appl Pharmacol.* 2015; 282(3):297-310. [DOI:10.1016/j.taap.2014.12.003] [PMID]
- [10] Alves RR, Rosa IM. Biodiversity, traditional medicine and public health: Where do they meet? *J Ethnobiol Ethnomed.* 2007; 3:14. [DOI:10.1186/1746-4269-3-14] [PMID] [PMCID]
- [11] Tafazoli V, Shahriari M, Heydari M, Nikbakht HA, Zarshenas MM, Nimrouzi M. The effect of *Viola odorata* L. Oil for fever in children: A randomized triple-blinded placebo-controlled clinical trial. *Curr Drug Discov Technol.* 2020; 17(5):696-703. [DOI:10.2174/1570163816666190620142256] [PMID]
- [12] Tayarani-Najaran Z, Yazdian-Robati R, Amini E, Salek F, Arasteh F, Emami SA. The mechanism of neuroprotective effect of *Viola odorata* against serum/glucose deprivation-induced PC12 cell death. *Avicenna J Phytomed.* 2019; 9(6):491-8. [DOI:10.22038/AJP.2019.13098] [PMID] [PMCID]
- [13] Zeinoddini S, Nabiuni M, Jalali H. The synergistic cytotoxic effects of doxorubicin and *Viola odorata* extract on human breast cancer cell line T47-D. *J Cancer Res Ther.* 2019; 15(5):1073-9. [DOI:10.4103/jcrt.JCRT_990_17] [PMID]
- [14] Jamshed H, Siddiqi HS, Gilani AuH, Arslan J, Qasim M, Gul B. Studies on antioxidant, hepatoprotective, and vasculoprotective potential of *Viola odorata* and *Wrightia tinctoria*. *Phytother Res.* 2019; 33(9):2310-8. [DOI:10.1002/ptr.6411] [PMID]
- [15] Elhassaneen Y, Sabry S, Musalum T, El-Eskafy A, El-Fatah AA. Effect of sweet violet (*Viola odorata* L.) blossoms powder on liver and kidney functions as well as serum lipid peroxidation of rats treated with carbon tetrachloride. *J Am Sci.* 2013; 9(5):88-95. https://www.academia.edu/40052858/Effect_of_Treated_with_Carbon_Tetrachloride
- [16] Gazor R, Ashouri A, Aghajany-nasab M, Yousefbeyk F, Khakpour Taleghani B, Mohammadghasemi F. Co-administration of hydroalcoholic *Viola odorata*'s extract and streptozotocin on histopathological and biochemical changes in rat kidney. *Pharm Sci Asia.* 2021; 48(4):339-45. [DOI:10.29090/psa.2021.04.20.061]
- [17] Muhammad N, Saeed M. Biological screening of *Viola betonicifolia* Smith whole plant. *Afr J Pharm Pharmacol.* 2011; 5(20):2323-9. [DOI:10.5897/AJPP11.641]
- [18] Furman BL. Streptozotocin-induced diabetic models in mice and rats. *Curr Protoc Pharmacol.* 2015; 70:5.47.1-5.47.20. [DOI:10.1002/0471141755.ph0547s70] [PMID]
- [19] Kesari AN, Gupta RK, Singh SK, Diwakar S, Watal G. Hypoglycemic and antihyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. *J Ethnopharmacol.* 2006; 107(3):374-9. [DOI:10.1016/j.jep.2006.03.042] [PMID]
- [20] Mohammadghasemi F, Abbasi M, Rudkhaneei K, Aghajany-Nasab M. Beneficial effect of apple vinegar on reproductive parameters in male rat model of nonalcoholic fatty liver disease. *Andrologia.* 2018; 50(8):e13065. [DOI:10.1111/and.13065] [PMID]
- [21] Li XM. Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats. *Int J Biol Macromol.* 2007; 40(5):461-5. [DOI:10.1016/j.ijbiomac.2006.11.002] [PMID]
- [22] Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β -cell damage in rat pancreas. *Pharmacol Res.* 2005; 51(2):117-23. [DOI:10.1016/j.phrs.2004.06.002] [PMID]
- [23] Stojković D, Glamočlija J, Ćirić A, Šiljegović J, Nikolić M, Soković M. Free radical scavenging activity of *Viola odorata* water extracts. *J Herbs Spices Med Plants.* 2011; 17(3):285-90. [DOI:10.1080/10496475.2011.603588]

- [24] Tammineni R, Gulati P, Kumar S, Mohanty A. An overview of acyclotides: Past, present and future. *Phytochem.* 2020; 170:112215. [DOI:10.1016/j.phytochem.2019.112215] [PMID]
- [25] Sun L, Gidley MJ, Warren FJ. The mechanism of interactions between tea polyphenols and porcine pancreatic alpha-amylase: Analysis by inhibition kinetics, fluorescence quenching, differential scanning calorimetry and isothermal titration calorimetry. *Mol Nutr Food Res.* 2017; 61(10):1700324. [DOI:10.1002/mnfr.201700324] [PMID] [PMCID]
- [26] Jemaa HB, Jemia AB, Khlifi S, Ben Ahmed H, Ben Slama F, Benzarti A, et al. Antioxidant activity and α -amylase inhibitory potential of *Rosa canina* L. *Afr Netw Ethnomed.* 2017; 14(2):1-8. [DOI:10.21010/ajtcam.v14i2.1] [PMID] [PMCID]
- [27] Swislocki A, Noth R, Hallstone A, Kyger E, Triadafilopoulos G. Secretin-stimulated amylase release into blood is impaired in type 1 diabetes mellitus. *Horm Metab Res.* 2005; 37(5):326-30. [DOI:10.1055/s-2005-861478] [PMID]
- [28] Aughsteeen AA, Mohammed FI. Insulin enhances amylase and lipase activity in the pancreas of streptozotocin-diabetic rats. An in vivo study. *Saudi Med J.* 2002; 23(7):838-44. [PMID]
- [29] Farhood HB, Ra'id M, Radhi MN. Clinical studies to evaluate pancreatic functions in the patients of type 2 diabetes mellitus. *Int J Innov Appl Stud.* 2014; 7(1):413-20. <https://www.proquest.com/docview/1556059541>
- [30] Vantyghem M, Haye S, Balduyck M, Hober C, Degand PM, Lefebvre J. Changes in serum amylase, lipase and leukocyte elastase during diabetic ketoacidosis and poorly controlled diabetes. *Acta Diabetol.* 1999; 36(1-2):39-44. [DOI:10.1007/s005920050143] [PMID]