Anti-Diabetic Effect of a Polysaccharide Fraction From *Momordica Charantia*: Modulation of Notch Signaling

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Abstract

Given the impact of notch signaling in the modulation of metabolic diseases and normal tissue homeostasis, this study aimed to evaluate whether notch signaling has a role in anti-diabetic and islet regenerative effects of isolated polysaccharide from *Memordica charantia* in diabetic rats. The polysaccharide was isolated from *Memordica charantia* (MCP) and characterized using FTIR and LC-MS/MS. Diabetic model was established by intrapritoneal administration of STZ in male Wistar rats. The levels of Hes1, Notch 1, DLL4, Jagged1, Pdx1, CD34, CD31 and VEGF were analyzed by using immunohistochemistry and real-time PCR. Structural analyses have revealed the carbohydrate structure of fraction. Blood glucose was halted by treatment with fraction. MCP scaled up the mRNA levels of Ins1, jagged1, Pdx1 and Hes1 while scaled down the levels of Notch1, Dll4 and the ratio of Bax/Bcl2 in diabetic rats. Furthermore, the immunohistochemistry staining of hes1, cyclin d1 and VEGF proteins was increased in the pancreas of MCP-treated diabetic rats compared to the diabetic group. These findings provide insights into the anti-diabetic potential of MCP through modulation of islets regeneration and suggest that modulation of notch and angiogenesis pathways may play the pivotal role in the restoration of islets to relieve diabetes.

Introduction

Diabetes mellitus (DM) is an ongoing and pervasive metabolic ailment characterized by chronic hyperglycemia and insulin deregulation and primarily leads to impairment in the metabolism of carbohydrate, lipid and protein [1]. In fact, DM is considered as one of the oldest diseases which is commonly accompanied by defects in function and/or secretion of insulin. Globally, the International Diabetes Federation (FDA) is calculated that the number of patients with diabetes is more than 380 million and proposed to reach 600 million cases by 2035 [2]. Among them, 60% of them are correlated to Asia and about 30% related to China [3]. According to recent studies, DM is commonly classified into insulin-dependent or type 1, insulin-independent or type 2 and several other specific types such as gestational diabetes. A number of molecular mechanisms underlying diabetes pathogenesis encompass epigenetic and genetic alterations as well as signaling pathways' dysregulation. Therefore, targeting of them may shed light on offering novel therapeutic strategies in treatment of diabetes. What is more, seeing that β-cells are lost in the progression of diabetes, it is required to develop therapeutic interventions by transplantation of β-cells and/or activation of regenerative mechanisms [4]. In adults, new pancreatic β-cells appear to be produced from the pre-existing islet cells which may be induced by the loss of residual β-cells [5, 6]. In the regeneration process, β-cells are able to be derived from adult and embryonic stem/progenitor cells, from reprogramming of cells as well as delivery and/or activation of pro-differentiation signaling pathways [4]. It seems that modulation of cells toward β-cells through induction of growth factors and/or other signaling pathways shed light on the new pharmaceutical strategies to treat diabetes [7].

It has been shown that the notch pathway is substantially involved in the pancreas development and diabetes pathogenesis [8]. Notch pathway is intimately promoted by the intercellular short-range

communications to induce embryonic and post-embryonic growth and homeostasis through modulation of cell fate decision, proliferation, differentiation, apoptosis and cell cycle in both physiologic and pathologic conditions [9–12]. The activation of notch pathway is commenced by interaction of ligands including Delta1, 3, 4 and Jagged1, 2 on the cell with notch receptors (Notch 1–4) expressed on the adjacent cells leading to cleavage of the receptor by γ-secretase and formation of notch intracellular domain (NICD). NICD can enter the nucleus and binds to the transcription factor RBPJj/CBF-1, converting it from a repressor to an activator of gene transcription. Ultimately, the expression of primary downstream targets including the hairy/enhancer of split (Hes) and Hey family proteins was activated [13]. It has been shown that re-activation of the notch1 signaling seems to be involved in dysfunction of β-cells in diabetes [13].

Natural products are known to contribute in development of pharmaceutical biology [14]. The alternative therapeutic system by using medicinal plants and their isolated active ingredients has aroused considerable interest in medicine [15]. *Mommordia charantia* (bitter gourd or bitter melon) belongs to the family of Cucurbitaceae is traditionally used in folk medicine to manage diabetes throughout the world [16, 17]. The anti-diabetic effect of *M. charantia* powder was recently established in STZ-diabetic rats [18]. *M. charantia* have been reported to include saponins, polysaccharides, proteins, triterpenoids, alkaloids, flavonoids, quinine, amino acids, fatty acids and trace elements [19–21]. It has been shown that the isolated polysaccharide from *M. charantia* may be the active component to relieve hyperglycemia in STZ-diabetic mice [22]. In another study, triterpenoids isolated from the fruits of *M. charantia* were shown to be the active agents in the management of hyperglycemia [23]. In a recent study, a novel bioactive peptide from the seeds of *M. charantia* called BG-4 has been indicated to exert anti-inflammatory potential [24]. Notably, no adverse effects of bitter melon were observed in the studies [25]. Owing to the promising effect in the management of diabetes, it is required to isolate the active component from *M. charantia* and characterize its molecular mechanism. Taken together, the study aimed to isolate an ingredient from *M. charantia* with a polysaccharide structure. Then, the involvement of notch pathway in anti-diabetic effect of the isolated polysaccharide was investigated in diabetic rat.

Materials And Methods

Isolation and purification of polysaccharide

Ripe fruits of *Mommordia charantia* were supplied from Kermanshah province in western Iran during Nov–Dec. The extract was filtered and further subjected to a column of silica gel of G-60 (5 cm×40 cm). Then, the collected eluent was partitioned two times against a triple volume of ethanol. After filtration, the filtrate was fractioned using a column of silica gel of G-60 (5 cm×40 cm) and conditioned by ethanol-water mixture. The first fraction was passed over a flash column chromatography (2.5 cm×25 cm) with a sephadex LH20 resin (Fluka from Switzerland) and then further purified on a column (2.5 cm×25 cm) filled with the silica gel RP-18 resin (25–40 μm, MERCK from Germany) to provide an polysaccharide fraction. The purified fraction was collected, lyophilized and monitored at 200 nm–400 nm for detection of proteins and other impurities. It was further structurally analyzed using a high performance liquid

chromatography diode array detector tandem mass spectroscopy (HPLC-DAD MS/MS), and infrared radiation (IR).

Structural analysis of isolated polysaccharide

FTIR (Fourier Transform Infrared) Spectroscopy

FT-IR of a compound and the chemical interactions of different functional groups were revealed by using FT-IR spectrophotometer (IR prestige-21, Shimadzo Co., Japan) through potassium bromide disk method. The scanning range was 400–4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. 5–6 mg of samples were mixed and triturated with potassium bromide (100 mg) and placed in the sample holder.

LC–MS/MS system

An Agilent 1200 series LC system on a column (150×4.6 mm) consisting of a quaternary delivery pump, a thermostated column compartment, a degasser (Agilent Technologies, Germany) and a Rheodyne 7725i manual injector valve with a 20 µL sample loop (Cotati, CA, USA) was used. The mass analysis was performed with an Agilent 6410 Triple Quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) which was run by Agilent MassHunter Workstation data acquisition system B.01.03. Ionization was achieved using electrospray ionization (ESI) in the negative mode with the capillary voltage 4000 V. Nitrogen was used as a nebulizer gas with a nebulizer pressure of 40 psi and a source temperature of 100°C. Drying gas (nitrogen) was heated to 300°C and delivered at a flow rate of 10 L/min.

Laboratory animals and experimental design

At least thirty-two adult male Wistar rats were purchased from Pasteur Institute, Iran. They were kept under free access to food and water at a room temperature of 22 ^{0}C with 12:12 h light: dark cycle and 45–50% relative humidity. All animals were treated under the principles of laboratory animal care (National Institutes of Health). The rats were segregated randomly into four groups, 8 Rats in each group, including control, diabetic, metformin and treatment groups. The treatment group was diabetic rats who were taken 10 mg/kg/day polysaccharide fraction of *Momordica charantia*. The control group was given orally water. Diabetes was induced by using an intraperitoneal injection of Streptozotocin with a dose of 50 mg/kg body weight. After 72 h, blood sampling was done and blood sugar of more than 250 mg/dl was considered positive.

RNA extraction, cDNA synthesis and real time RT-PCR

Total RNA was extracted from the isolated pancreas of each rat, using a guanidinium thiocyanate/phenol buffer according to the protocol provided by the manufacturer (TRIzol reagent; ThermoFisher, USA). Total RNA at a concentration of 3 mg, free conditions of any DNase and RNase, using oligo primers and reverse transcription enzyme was reverse-transcribed according to the protocol provided by the RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, USA). The supplement cDNA was synthesized and cDNA generated was stored at -20°C until use. Quantitative real-time PCR was used to monitor the expression of mRNA according to the protocol provided by the maxima SYBR Green/ROX qPCR Master Mix kit (Thermo

Scientific, USA). The results were calculated by the software of the device or using the formula ($\Delta\Delta C_T$ method). The C_T value of each gene was compared with the internal control gene (Housekeeping) for normalization of the results. The internal control gene expression in different conditions of cell treatment was fixed and used as an internal control. The studied genes are DLL4, Notch1, Hes1, Glut1, GCK, Ins1, Pdx1, and β-actin as internal control gene which primers are shown in Table 1. Real-time RT-PCR data from the control samples and test will be analyzed by reference gene using relative expression method and software Relative Expression Software Tool (REST) and $2^{-\Delta\Delta Ct}$ formula [26].

Immunohistochemistry

Immunohistological staining was performed on formalin-fixed paraffin-embedded tissue sections using antibodies against insulin, Hes1 and cyclin d1. For this aim, 4 µm tissue sections were deparaffinized and heated in a water bath for 20 min. Then, slides were immersed in a solution of 3% Hydrogen peroxide in methanol for 10 min. After washing with PBS, slides were incubated with primary and secondary antibodies in a humid and dark place at room temperature. The slides were stained with the substrate-chromogen solution known as 3,3-diaminobenzidinetetra-hydrochloride (DAB) for 5 min. The counterstaining was performed with hematoxylin for 30 sec and washed in water. Then, slides were mounted to study under a microscope. Negative controls were exposed to antibody diluent replacing primary antibody [27].

Statistical analysis

Statistical analyses were carried out in SPSS16 software (SPSS/PC-25.SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± SEM. The one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for analysis of data. The $p < 0.05$ was considered statistically significant.

Results

Characterization of the isolated fraction

To isolate the final fraction, the extract from *M. charantia* fruit was filtered and chromatographed according to our previous method [28]. The FT-IR spectrum of the polysaccharide is shown in Figure 1A. The spectrum of the polysaccharide indicated the absorption peaks of C-H symmetric and asymmetric stretching vibrations at 2926 and 2854 cm^{-1}, respectively. The peak at 1745 cm^{-1} was characteristic to the C=O stretching vibration of the ester and the peaks at 1143 and 1051 cm^{-1} were related to C-O stretching vibration of the ester. Also, the peak at 1665 cm^{-1} was a feature of C=O stretching vibration and the peaks at 1103, 1051 and 1018 cm^{-1} were a feature of C-O stretching vibration of the acid carboxylic. The broad peak at about 2500-3600 cm^{-1} has corresponded to OH stretching vibration. The presence of these peaks in the FT-IR spectrum confirmed the stretchers of polysaccharide. As indicated in the Fig. 1B, the mass spectrum of isolated fraction shows that the percentage of fragments was about 936.0 (3.5), 772.3 (7.1), 745.3 (16.6), 710.7 (8.2), 597.1 (7.1), 555.0 (11.7), 460.3 (11.7), 408.1 (11.7), 366.1 (47.1), 275.0 (29.4), 255.1 (30.5), 193.0 (17.6), 175.1 (25.9), 171.1 (100.0), 113.0 (97.6). As shown

in Fig. 1C, the isolated fraction has a carbohydrate pattern which is very similar to the structure of an isolated polysaccharide from *Rosa canina*.

Isolated fraction improved the function of pancreas

As indicated in table 2, the level of blood glucose was increased in diabetic rats compared to healthy ones and fraction treatment significantly decreased the blood sugar in diabetic rats ($P \leq 0.05$). In addition, loss of weight was obvious in diabetic rats ($P \leq 0.05$) compared to normal animals. However, diabetic rats treated with fraction gained weight over the course of 6 weeks ($P \leq 0.05$). Owing to the causative role of insulin in normal function of the islets, the protein level of insulin was evaluated in the pancreas tissue. In animals treated with fraction, the expression level of insulin was remarkably increased compared to untreated diabetic ones (Fig. 2A). In addition, measurement of mRNA expression showed that insulin and Pdx-1 gene expressions were upregulated in fraction treated rats with respect to diabetic ones ($P \leq 0.05$) (Fig. 2B).

Effect of isolated polysaccharide on the mRNA expression of Notch signaling pathway

The mRNA expression of Notch 1 was increased to about 2.24 fold in untreated and metformin-treated diabetic rats and increased to above more than 1.64 fold in MCP-treated diabetic rats compared to normal group (Fig. 3A). However, the expression of hes1 as a downstream signaling factor of notch signaling pathway was not altered in diabetic group (1.07) while increased about 1.22 and 1.38 fold in metformin and MCP-diabetic rats. Considering the disconcertion between Notch1 and hes1, the expression of two ligands, DLL4 and Jagged1, were studied. As shown in Fig. 3A, the mRNA expression of Jagged1 was increased to about 2.05, 3.7 and 2.4, respectively, in non-treated, MCP- treated and metformin-treated diabetic rats compared to normal rats. Albeit, the expression of DLL4 was increased to nearly 1.32 fold in diabetic group while it was decreased to 0.72 and 0.88 fold, respectively, in MCP and metformin groups in comparison with healthy rats. In follow, the protein expression of hes1 and cyclin d1 was examined in the pancreas of rats. The expression levels of insulin, Hes1, cyclin d1 were evaluated by IHC in the pancreas tissues of treated and untreated diabetic rats. As shown in Fig. 3B, the expression of cyclin d1 was about 20%, 15%, 30% and 55%, respectively, in normal, diabetic, metformin and MC-treated diabetic rats. The expression of Hes1 was cytoplasmic in acinar cells of diabetic rats while it was positive in the cytoplasm and nucleus of acinar and islet cells from metformin and MCP-treated diabetic and normal rats. The ratio of Bax/Bcl2 is considered as the indicator of cell death. Owing to the significant change in the expression of cyclin d1, it was prompted to measure the expression of Bax and Bcl2 as two factors involved in cellular survival and death. As shown in Fig. 3C, the levels of Bax and also Bax/Bcl2 ratio was enhanced in diabetic rats compared to the healthy group. However, MC and metformin treatment reduced the expression level of Bax and also the ration of Bax/Bcl2 relative to diabetic rats.

Effect of isolated polysaccharide on the mRNA expression of VEGF, CD31 and CD34

Owing to the prominent role of angiogenesis and vascular system in diabetes, the expression of VEGF, CD31 and CD34 were assessed in the pancreas from diabetic rats. The expression of CD31 was negative

in normal and diabetic rats and was positive in the vascular walls in metformin and MC-treated diabetic ones. The CD34 in vascular walls was strongly stained in normal, metformin and MC-treated diabetic rats and was poorly stained in diabetic rats. The VEGF expression was 3+ in acinar cells of normal rats. In untreated, and metformin-treated diabetic rats, the level of VEGF was 2+ and 3+ in islet and acinar cells, respectively. The expression of VEGF in MC-treated diabetic rats was 2+, 1+ and 3+ in islet, stromal and acinar cells, respectively (Fig. 4).

Discussion

The study presented here provides insights into the evaluation of the underlying mechanism of an anti-diabetic polysaccharide isolated from *M. charantia* through modulation of Notch signaling pathway. According to our previous study, *M. charantia* alleviates diabetes through induction of the regeneration of pancreatic islet cells [18]. As an important feature of the adult pancreas, cellular plasticity confers dedifferentiation, transdifferentiation and/or development of specific lineages such as acinar, endocrine and ductal cells in response to stress, injury and also recovery. For example, β-cells are given rise to undifferentiated and/or α-cells during FOXO1 depletion after induction of stress. On the other hand, β-cells can proliferate to compensate for lost β-cells and producing new ones which is further supported by recent evidence [29]. What is more, exocrine pancreatic cells such as ductal and acinar cells with overexpression of Ngn-3, Pdx-1 and MafA are able to undergo reprogramming towards β-cells [30]. In this line, several embryonic signaling pathways including Notch, Wnt and Hh are activated during regeneration and dedifferentiation [31–33]. Among them, Notch signaling purportedly seems to hold great promise for differentiation, proliferation and regeneration of pancreatic endocrine and exocrine cells [11]. Amid the course of the experiments, notch signaling as an evolutionarily conserved cell-cell communication pathway is involved in the metabolic regulation and progression of related diseases [34]. During both type 1 and 2 diabetes, ample evidence supports the impetus effect of Notch signaling in the modulation of β-cell function and regeneration [8].

An interestingly recent report unravel the functional impact of notch signaling in development of pancreatic beta cells [35]. Owing to the increased level of notch signaling factors in diabetic mice compared to normal ones, DLL4 antibody could attenuate blood glucose and infiltrate inflammatory cells as well as increase insulin activity in islets [36]. In comparison, administration of dibenzazepin as one of the γ-secretase inhibitors of whole factors of notch signaling pathway indicated no effect on β-cells indicating that specific inhibition of DLL4-Notch signaling pathway is crucial in the treatment of diabetes. Consistent with this evidence, our results indicated that hes1 as the main downstream factor of the notch pathway is downregulated in STZ-diabetic rats which is upregulated with MC treatment. In addition, the expression of cyclin d1 as another downstream factor of notch signaling is reduced in diabetic rats (15%) which is reached to 50% upon treatment of diabetic rats with MCP.

Among diverse lineage in the pancreas, β-cells are the most sensitive cells to nutrients. Namely, pancreatic tissue expansion during pregnancy and obesity is a growing need to compensate for glucose-stimulated insulin secretion. Streptozotocin (STZ)-induced diabetes is mediated by induction of β-cells

death in the pancreas. Mounting evidence raise the possibility that regeneration of β-cells can be achieved from the proliferation of residual β-cells, transdifferentiation of α-cells, and reprogramming of acinar and/or ductal cells during reversal of diabetes in STZ-induced diabetic animal models. In this context, a regenerative gene product (Reg) as a regenerative factor in the autocrine/paracrine pancreas acts through activation of Cyclin D1 [37]. In the cell cycle, G1 to S progression is activated by cyclin D1 mediated CDK4 activation. In fact, stimulation of Akt/ATF-2/cyclin D1 signaling pathway by Reg factor is required to induce regeneration of pancreatic β-cells [37]. It has been established that wnt3a signaling through activation of CDK4, cyclin D1 and cyclin D2 escalates the proliferation of β-cells [38, 39]. Surprisingly, the potential involvement of cyclin d1 in proliferation of β-cells has been demonstrated in vitro culture of pancreatic cell while it is not induced tumorigenesis [40].

Given the strict interplay between notch signaling and angiogenesis pathway as well as the causal role of angiogenesis in diabetes, we proposed that the isolated polysaccharide may affect the factors involved in angiogenesis. To testify to our hypothesis, the expression of VEGF, CD31 and CD34 was evaluated in treated and untreated diabetic rats. As indicated, the levels of VEGF and CD34 were found to be increased in polysaccharide-treated diabetic rats compared to the diabetic group. DLL4, as one of the Notch ligand, has been considered as the main factor in the remodeling of vascular tissue [41–43]. At the onset of angiogenesis, VEGF induces the expression of DLL4 in endothelial cells which by increasing DLL4, the expression of VEGF is downregulated via feedback inhibiting effect of DLL4 [44]. The integration of Notch in diabetes was reported in diabetic nephropathy in which upregulation of jagged-1 induced by diabetes is associated with suppression of Notch signaling pathway in endothelial cells implying the key role of Notch pathway in diabetic microvacular pathologies [45, 46]. This finding thus offers insights into this proposal that the polysaccharide through modulation of notch signaling pathway can raise the expression of angiogenic factors in diabetic rats and in follow, can relieve damaged pancreas and treat diabetes. In this line, further analyses are required to scrutinize the regulatory effects of polysaccharide and also the interplay between notch and angiogenesis in diabetes.

Conclusion

Overall, our study has explored the possible functional consequence of diabetes treatment through an isolated polysaccharide by activation of regenerative mechanisms. In this context, modulation of notch signaling and angiogenesis pathways seems to be considered as the causal reagents in the regeneration of pancreatic islet cells and treatment of diabetes.

Abbreviations

DM, Diabetes mellitus; **FTIR,** Fourier Transform Infrared; **Ins1,** Insulin1; **H&E,** Hematoxylin and eosin; **Hes:** hairy/enhancer of split; **Herp;** HES-related repressor protein; **HPLC-DAD MS/MS,** high performance liquid chromatography diode array detector tandem mass spectroscopy; **IHC,** Immunohistochemistry; **IR,** infra-red radiation; **MCP,** Polysaccharide was isolated from *Memordica charantia*;**NICD,** Notch intracellular

domain; **NMR,** nuclear magnetic resonance; **Pdx1,** Pancreatic and duodenal homeobox1; **RT-PCR,** Real time-polymerase chain reaction; **VEGF,** Vascular endothelial factor; **STZ,** Streptozotocin.

Declarations

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Conflicts of interest/Competing interests

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contribution

Soraya Sajadimajd wrote the paper draft. Soraya Sajadimajd and Gholamreza Bahrami designed the experiments. Soraya Sajadimajd, Bahareh Mohammadi, Razieh Hatami, Seyed Hamid Madani, and Seyed shahram Miraghaee conducted the experiments and analyzed the results. Gholamreza Bahrami revised the manuscript. All authors read and approved the final manuscript.

Ethics approval

The Research Ethics Committee of "Ministry of Health and Medical Education" and "National Institute for Medical Research Development (NIMAD)" approved the protocol (Protocol Number: 977454 and ethical Number: IR.NIMAD.REC.1397.374)

Consent to participate

Not applicable

Consent for publication

Not applicable

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Tables

Table 1. qRT-PCR primer sequences

Gene	Forward	Reverse
DLL4	CACACCAACGTGGTCTTCAAGC	TAGACAATGGAGCCACGGATGT
Notch1	GGCAAACTGCAGAACCACACA	GACATGCCGGCTTTTCACTGT
Hes1	GTCATCAAAGCCTATCATGGAG	GTGCGCCTGCCCGGGGTAGGTC
Glut2	TAGTCAGATTGCTGGCCTCAGCTT	TTGCCCTGACTTCCTCTTCCAACT
GCK	CTATGAAGACCGCCAATGTG	CAGCTCCACATTCTGCATTTT
Ins1	CTACAGTCGGAAACCATCAGCA	GCCGTCATGCTCACATAACTCA
Pdx1	TGGAAAAGCCAGTGGGCAGG	GATGTGTCTCTCAGTCAAGT
β-actin	GCGTCCACCCGCGAGTACAA	ACATGCCGGAGCCGTTGTCG

Table 2. Effect of MCP on the body weight and the blood glucose.

Groups	Body Weight (g)		Blood Glucose (mg/dl)		
	0 h	6 weeks	72 h	3 weeks	6 weeks
Control	256.71±6.75	299.85±28.38	109.14±6.62	107.43±4.31	106.57±1.32
Diabetic	257.43±14.6	210.43±8.34	356.43.±36.91	409.14±33.69	495.43±37.06
MCP	244.29±18.67	320.28±19.80	329.28±42.73	189.57±36.35	166.28±31.33
Metformin	262.14±14.15	272.00±29.81	369.00±37.86	366.57±37.78	328.28±45.55
Significance between groups (P-value)	0.134	0.086	0.171	0.069	0.122

Figures

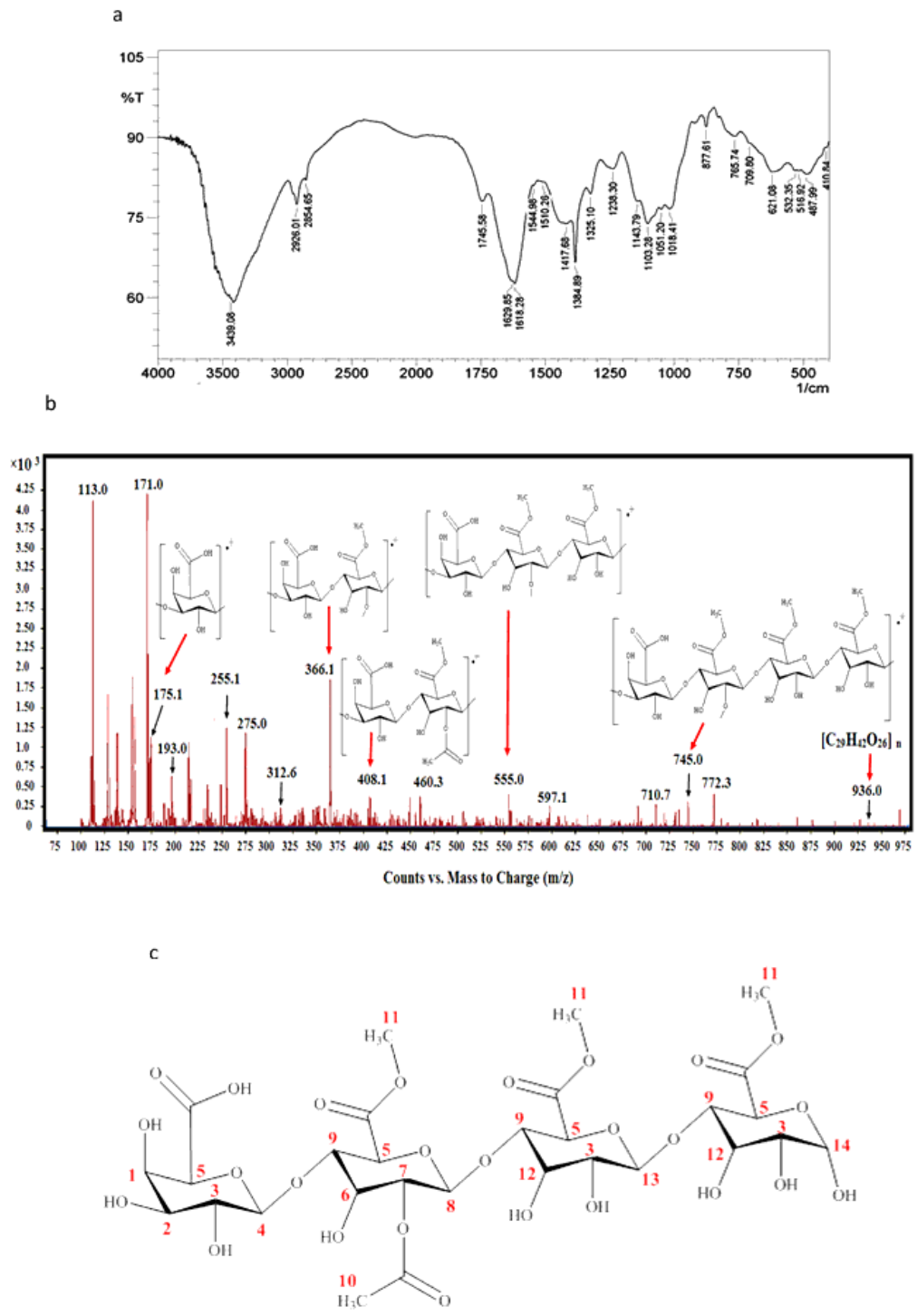

Figure 1

Structural analysis of isolated polysaccharide from M. charantia. (A) FTIR spectrum of isolated polysaccharide. (B) Mass spectrum of isolated polysaccharide with LC-MS/MS. (C) The proposed structure of isolated polysaccharide with repeated units of the presented tetrasaccharide

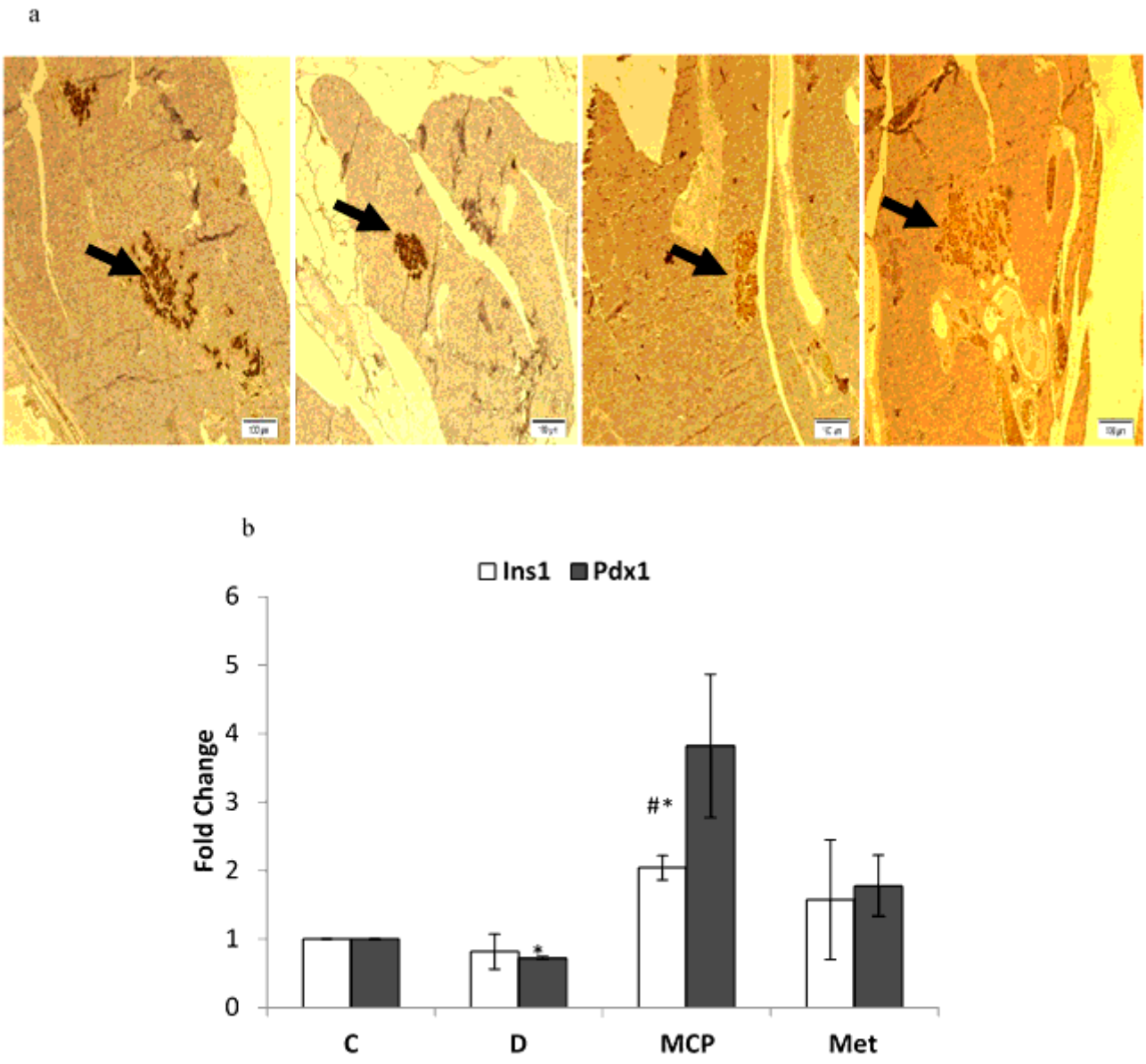

Figure 2

Isolated fraction from M. charantia (MCP) improved the function of pancreas. (A) The insulin immunohistochemical staining in pancreas from MCP-treated and untreated rats. Straight arrow indicates the pancreas islets (C) The mRNA levels of insulin (Ins1) and Pdx1 in the pancreas from treated and untreated diabetic rats. Asterisks indicate the statistical difference from control cells: * $p < 0.05$. ≠ indicates the statistical difference from diabetic cells

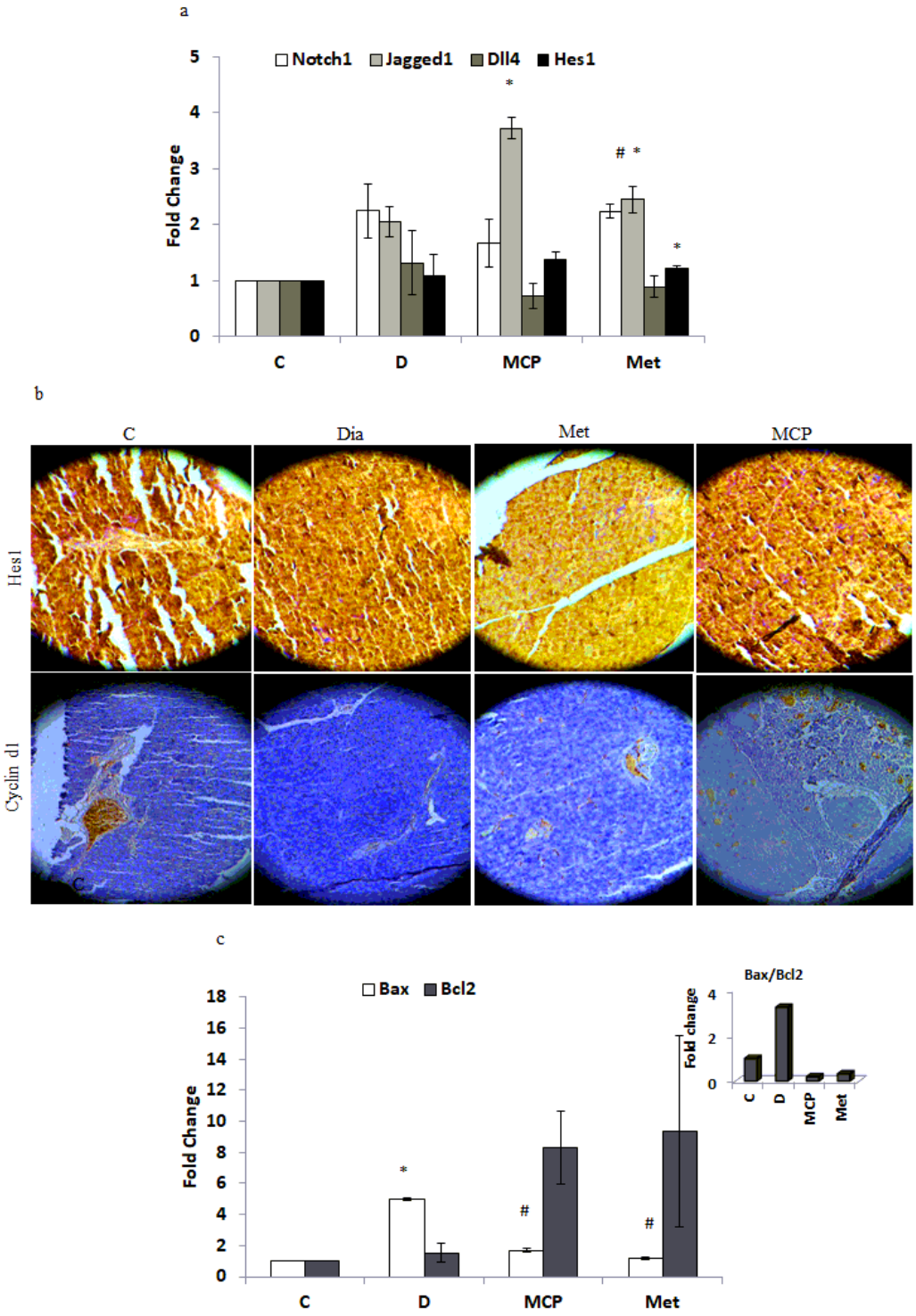

Figure 3

The expression of involved signaling factors in diabetic rats. The mRNA levels of Notch1, Jagged 1, Dll4 and Hes1 in treated and untreated STZ-induced diabetic rats (a). The expression of hes1 and cyclin d1 proteins in the pancreas tissue from treated and untreated diabetic rats (b). The expression of Bax and Bcl2 in diabetic rats treated with MCP and metformin (c). %. Asterisks indicate the statistical difference from control cells: * $p < 0.05$. ≠ indicates the statistical difference from diabetic cells

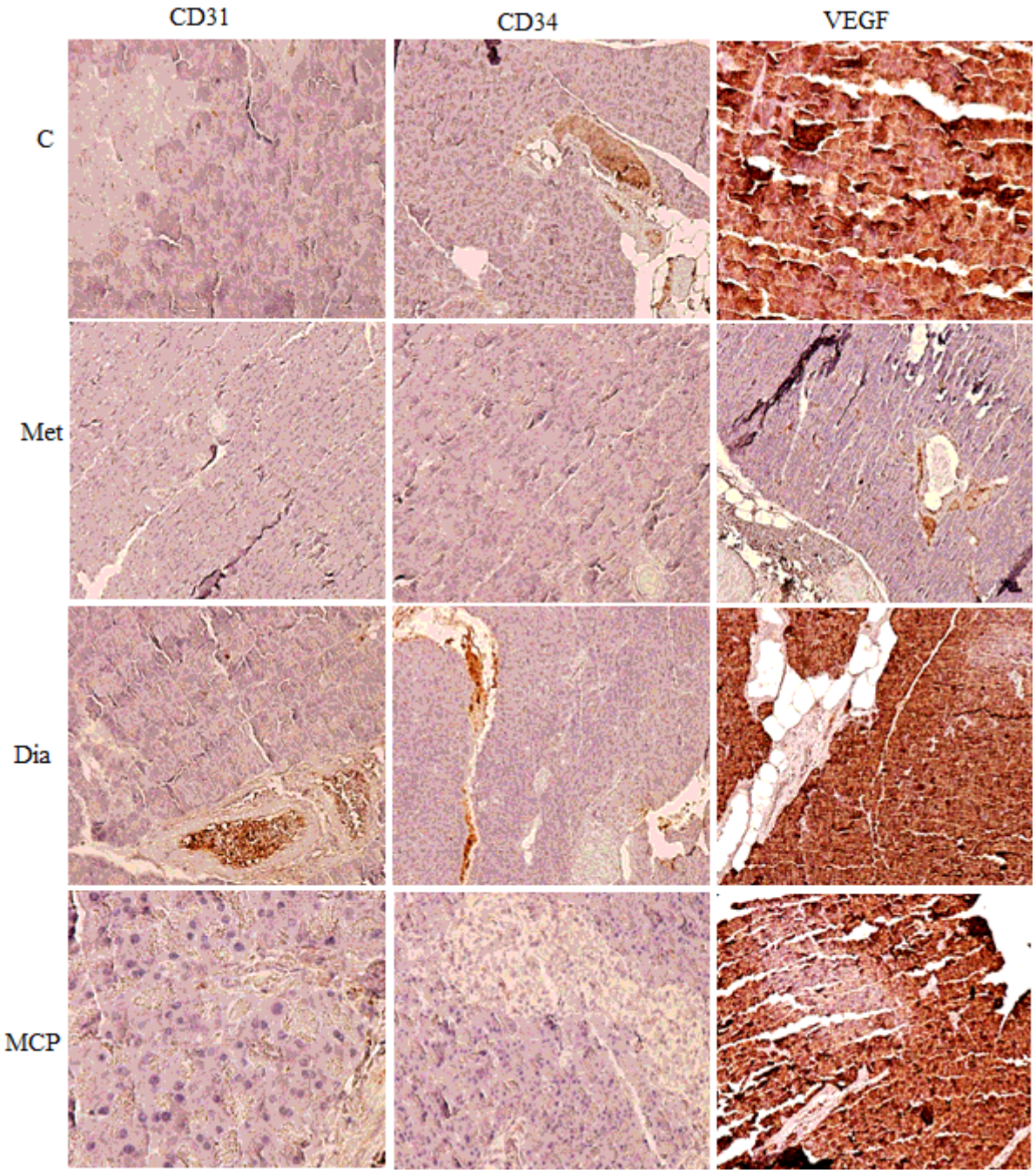

Figure 4

The expression of VEGF, CD31 and CD34 in the pancreas tissue from treated and un-treated diabetic rats. After the treatment course, pancreatic tissues were cut, paraffinized and stained using VEGF, CD31 and CD34 antibodies according to IHC procedure

Supplementary Files

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- graphic.tif