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# *Passiflora incarnata* extract-induced VEGF and TGF-β1 mRNA expression in the cardiovascular system using the rat model of type 2 diabetes

Anna Wesołowska
Poznan University of Medical Sciences, Department of Pharmacology, Poznań, Poland
https://orcid.org/0000-0001-5029-2265
Corresponding author: anna.wesolowska@ump.edu.pl

Marcin Ożarowski
Institute of Natural Fibres and Medicinal Plants – National Research Institute, Department of Biotechnology, Poznań, Poland
https://orcid.org/0000-0001-5881-2966

Przemysław Ł. Mikołajczak
Poznan University of Medical Sciences, Department of Pharmacology, Poznań, Poland
https://orcid.org/0000-0002-1252-6523

Agnieszka Stelmaszyk
Poznan University of Medical Sciences, Department of Pharmacology, Poznań, Poland
https://orcid.org/0000-0001-6091-8751

Anna Maliszewska-Dworacka
Collegium Da Vinci, Poznań, Poland
https://orcid.org/0009-0004-2044-399X

Saule Iskakova
Kazakh National Medical University, Department of Pharmacology, Almaty, Kazakhstan
https://orcid.org/0000-0001-7624-1570

Bartosz A. Frycz
Poznan University of Medical Sciences, Department of Biochemistry and Molecular Biology, Poznań, Poland
https://orcid.org/0000-0001-6729-1832

Paweł P. Jagodziński
Poznan University of Medical Sciences, Department of Biochemistry and Molecular Biology, Poznań, Poland
https://orcid.org/0000-0002-9046-6802

Marzena Dworacka
Poznan University of Medical Sciences, Department of Pharmacology, Poznań, Poland
https://orcid.org/0000-0002-4277-0615

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## ABSTRACT

**Aim**. To investigate the possible effects of *Passiflora* extract on VEGF and TGF-β1 mRNA expression – growth factors closely related to the development of diabetes in the cardiovascular system.
**Material and methods.** The animal model of type 2 diabetes, rich-fat/STZ Wistar rats, was used. Animals were randomised into four groups: *Passiflora*-treated type 2 diabetes mellitus model group; metformin-treated type 2 diabetes mellitus model group; placebo-treated type 2 diabetes mellitus model group; and placebo-treated non-diabetic control group. *Passiflora incarnata* leaf extract was administered orally once daily

for eight consecutive weeks. mRNA VEGF, VEGF-R1, VEGF-R2 and TGF-β1 expression were measured in the myocardium and the aorta.
**Results.** *Passiflora incarnata* extract increases VEGF and VEGFR2 mRNA expression in the myocardium of rats with type 2 diabetes and decreases expression in the aortal wall. The expression of TGF-β1 mRNA in both the myocardium and the aorta is reduced in *Passiflora incarnata*-treated rats with diabetes. Most observed effects are independent of the animals' current metabolic status.
**Conclusions.** The current data provide novel findings on the beneficial effects of *Passiflora* extract on the myocardium in the context of type 2 diabetes, potentially through mechanisms involving VEGF and TGF-β1. However, the significance of the impact of *Passiflora* extract on the aorta wall via VEGF and TGF-β1 is uncertain.

## Introduction

It is undeniable that diabetes mellitus and its cardiovascular complications constitute a serious health problem due to the high frequency of occurrence and mortality rates [1]. Disturbed angiogenesis leads to the progression of atherosclerosis. It is widely believed that neovascularisation of the atherosclerotic plaque increases its susceptibility to damage, potentially leading to rupture [2]. On the other hand, collateral or compensatory arteriogenesis in response to occlusive ischaemia appears to be beneficial for patients with diabetes [3]. Overexpressed fibrosis is another process that is closely linked to cardiovascular dysfunction in diabetes [4]. In these two processes, growth factors and their receptors play a crucial role. This particularly concerns vascular endothelial growth factor (VEGF) and its receptors, as well as transforming growth factor-β1 (TGF-β1).

VEGF is a proangiogenic factor acting directly on vascular endothelial cells. VEGF exerts its effects primarily through binding to appropriate receptors: VEGF receptor 1 (VEGFR-1; fms-related tyrosine kinase 1 – Flt1) and VEGF receptor 2 (VEGFR-2; kinase insert domain receptor – KDR; foetal liver kinase 1 – Flk1). VEGFR-1 has approximately ten times higher affinity for VEGF than VEGFR-2, but its tyrosine kinase activity is relatively weaker, and thus the primary signal transducer in the endothelium is VEGFR-2 [5]. TGF-β1 activates a matrix-preserving phenotype in cardiac fibroblasts and is the central effector of myocardial fibrosis [6]. The role of TGF-β1 as a regulator of the vascular endothelium is complex; among other effects, it strongly induces VEGF production, thereby promoting angiogenesis and vascular remodelling [7]. Moreover, TGF-β1 affects atherosclerotic plaque formation by stimulating multiple pro-inflammatory factors that are important in the early phase of atherosclerotic lesion formation [6].

*Passiflora incarnata* possesses numerous pharmacological properties and contains flavonoids, maltol, cyanogenic glycosides and indole alkaloids [8]. In several experiments, *Passiflora incarnata* extracts exhibited potential effects for the treatment of anxiety, insomnia, hypertension, and epilepsy [9]. The positive influence of *Passiflora* species on the glycaemic profile has also been demonstrated in numerous experimental studies, including *Passiflora incarnata* leaves [10]. Some studies have focused on the anti-inflammatory and antioxidant activities of *Passiflora incarnata* [8], which may have cardioprotective effects. It's necessary to underline that glucose control is not the only therapeutic goal for patients with diabetes. Very essential, but highly complex to achieve, is to delay the onset of chronic diabetic complications, especially those affecting the cardiovascular system.

The current study aimed to investigate the possible effects of *Passiflora* extract on the mRNA expression of VEGF and TGF-β1– key growth factors that are closely related to the development of diabetic complications within the cardiovascular system.

## Materials and methods

### Animals

The animal experiment was performed in accordance with Directive 2010/63/EU of the European

Parliament and of the European Council and with Polish governmental regulations (2005.01.21). It was approved by the Local Ethics Committee on the Use of Laboratory Animals in Poznan, Poland (No. 54-55/2013).

The experiment was carried out on 51 8-week-old male Wistar rats. The animals were housed in individual cages in an environmentally controlled room at 22°C under a reversed 12:12 h light: dark cycle to acclimatise for 1 week. The animals were randomly assigned to one of four groups: *Passiflora*-treated type 2 diabetes mellitus model group (P-DM), n = 13; a metformin-treated type 2 diabetes mellitus model group (M-DM), n = 14; a placebo-treated type 2 diabetes mellitus model group (DM), n = 12; and a placebo-treated control group without diabetes mellitus (nonDM), n = 12.

## Developing a model of type 2 diabetes mellitus

The type 2 diabetes model (low-dose-STZ-treated/fat-fed rats) was induced by an intraperitoneal injection of 20 mg/kg streptozotocin (STZ) (streptozotocin – Sigma-Aldrich, Saint Louis, USA) dissolved in a citrate buffer, followed by 4 weeks of feeding with a rich-fat diet (Labofeed B, 61% of fat) (groups P-DM, M-DM, DM). Animals in the non-diabetic control group (nonDM) received citrate buffer as a vehicle and commercial standard chow (Labofeed B standard, 2.8% fat) for the same duration. All animals received water *ad libitum*. Such a model was previously described to replicate the natural history and metabolic characteristics of type 2 diabetes mellitus in humans, including obesity, insulin resistance, and mildly impaired insulin secretion [11].

To confirm or exclude the development of diabetes, an intragastric glucose tolerance test (GTT) was performed. After 12 h of overnight fasting, the rats were given 1 g/kg b.w. of glucose dissolved in sterile water via intragastric gavage. The glucose concentration in the blood was measured using a glucometer at 0 and 1 h in samples obtained from superficial capillary vessels at the tip of the rats' tails. Rats showing a fasting glucose of 126 mg/dL (7.0 mmol/L) and/or a glucose concentration measured one hour after glucose solution administration of 140 mg/dL (7.8 mmol/L) were designated for the study. Rats with glucose levels > 200 mg/dL (11.1 mmol/L) and/or with clinical symptoms of hyperglycaemia were excluded from the study.

## Study design

The main experiment lasted for 8 weeks. During this period, rats in the P-DM group received *Passiflora* extract (200 mg/kg) [12] via intragastric gavage, and rats in the M-DM group received 300 mg/kg of metformin (Siofor®, Berlin Chemie) suspended in 1 % methylcellulose solution. Participants in the DM and non-DM groups were administered an equivalent volume of 1% methylcellulose as a placebo. All rats were treated similarly with respect to daily handling. At the end of the experiment, the animals were euthanised by decapitation. Samples of blood and selected tissues, including heart muscle and aortic wall, were collected and stored for further analysis.

## Plant material

Leaves of *Passiflora incarnata L*. were obtained from plants grown in the greenhouse of the Botanical Garden, Poznan University of Medical Sciences (Poland). The material was identified by the Department of Medicinal and Cosmetic Natural Products, Faculty of Pharmacy, Poznan University of Medical Sciences. The following conditions for cultivated plants were: temperature (25–40 °C), humidity (60–70%), and a peat substrate in pots. The voucher specimen has been deposited in the Herbarium of the Institute of Natural Fibres and Medicinal Plants in Poznan, Poland.

## Preparation of the extracts

Healthy leaves after drying, comminuting (0.5kg), and portioning (5 portions, 100g each) were extracted with pure methanol (Sigma-Aldrich, Poznan, Poland) (1:10, m/V) three times (each part) for one hour by reflux using a rotavapor. All collected extracts were combined and concentrated under vacuum to remove methanol. The yield of the dry extract was 22% for *P. incarnata*. The extraction conditions and efficiency were the same as in our previous studies [13]. The analysis performed at that time identified 52 chemical compounds (C-glycosides, O-glycosides, phenolic acids, and others). The most representative metabolites were glycosides of apigenin, luteolin, vitexin, isovitexin, isoorientin, chrysin and quercetin [13].

## Analysis

### *Metabolic parameters and angiogenic factors*

The glucose concentration in the blood samples was measured using a glucometer (Diagnostic GOLD Strip/System; Diagnosis S. A.; Poland). The concentration of 1,5-anhydro-D-glucitol (1,5-AG), a retrospective marker of acute hyperglycaemia, was analysed using the enzymatic colourimetric method [14]. C-peptide in the serum was measured with a rat-specific ELISA (C-peptide rat ELISA, R&D Systems, Minneapolis, USA).

### *mRNA expression*

Relative mRNA expression of VEGF, VEGF-R1, VEGF-R2, and TGF-β1 in the myocardium and aortic wall was determined by reverse transcription and real-time quantitative polymerase chain reaction (RQ-PCR). The tissue samples derived from the rats' heart muscles and aortas were stored at -80 °C until homogenisation in liquid nitrogen. Total RNA was isolated with Trizol reagent (Thermo Fisher Scientific, Waltham, USA) [15] and treated with DNase I (Roche, Mannheim, Germany). RNA quantity was assessed using a Nano-100 spectrophotometer (Hangzhou Allsheng, Zhejiang, China), and RNA integrity was evaluated by gel electrophoresis. Reverse transcription of 1 µg of RNA isolated from each rat tissue sample into cDNA was performed according to the manufacturer's protocol (Life Technologies, Carlsbad, USA). RQ-PCR was carried out in a Light Cycler480 II Real-time PCR detection system (Roche Diagnostics GmbH, Mannheim, Germany). SYBR® Green I was used as the detection dye. Transcript levels in tissues were expressed as the multiplicity of the cDNA concentration relative to the calibrator. The calibrator was prepared as a cDNA mix from all the rat samples. Successive calibrator dilutions were used to construct a standard curve and to estimate reaction efficiency, according to the manufacturer's instructions. For amplification, one µL of the cDNA solution was added to 9 µL of qPCR Master Mix (EURx, Gdansk, Poland) containing the following primer sequences: VEGF, 5′-CTCCACCATGCCAAGTGGTC-3′ and 5′-AATAGCTGCGCTGGTAGACG-3′; VEGFR-1, 5′-GCAGAGCCAGGAACATATAC-3′ and 5′-GAGGTTTTGAAGCAGGAGTG-3′; VEGFR-2, 5′-CGCGTTTTCAGAGTTGGTGG-3′ and 5′-TGAGGTAGGCAGGGAGAGTC-3′; TGF-β1 5′-CCATCTGTTGTTGTGCCTC-3′ and 5′-CAGTATGTGGGTTCAATTCC-3′. Primers for VEGF amplification were previously published [16,17], whereas the remaining primers were designed using OLIGO Primer Analysis Software (version 5.0; Molecular Biology Insights, Inc., Colorado Springs, USA). The number of transcripts for the analysed genes in each sample was standardised to the β-actin reference gene level.

### Statistical analysis

Statistical calculations were performed with STATISTICA v. 13 on the licence owned by Poznan University of Medical Sciences. All data is shown as mean ± standard deviation and/or median. ANOVA or Kruskal-Wallis tests were used to compare the four groups with regular or non-normal distribution, respectively. Analysis of covariance (ANCOVA) was performed to adjust for the influence of metabolic factors on group differences. The correlation between variables was analysed using Spearman's rank correlation test. A p-value ≤ 0.05 was considered to be significant.

## Results

### Characteristics of the studied groups

There were no differences in initial and final body weight or serum C-peptide levels across groups. Fasting glycemia, glucose levels at 1 hour in the GTT and HbA1c blood concentrations were higher in all groups with diabetes than in the control group. In contrast, the plasma 1,5-AG level was lower in all diabetic groups than in the control group (see **Table 1**).

### The effect of *Passiflora incarnata* extract on VEGF expression

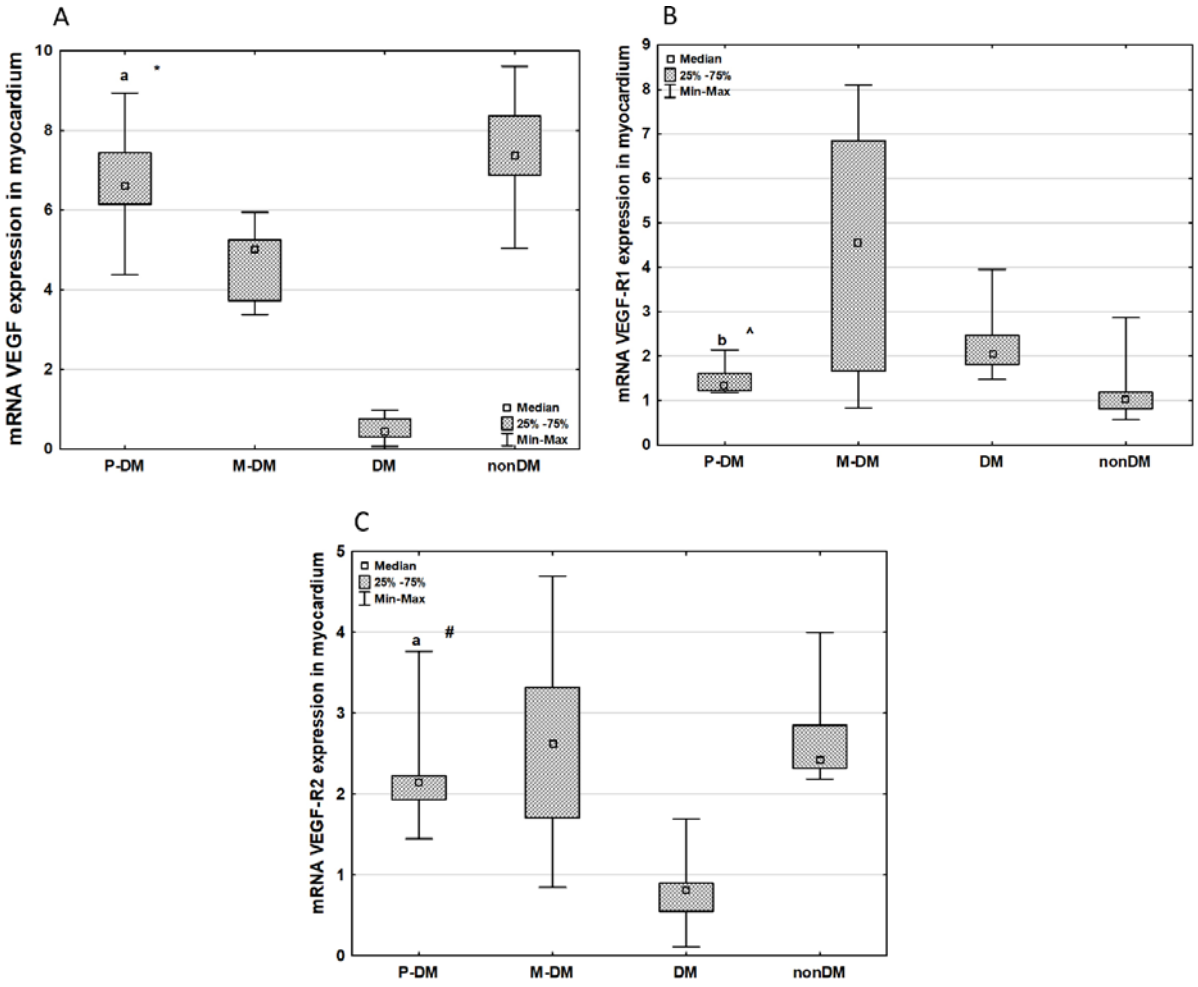
VEGF mRNA and VEGFR2 mRNA expression in the myocardium were higher in the P-DM group than in the DM group, while there were no differences in these two factors between the P-DM group and all the other groups (see **Figure 1A** and **1C**; tags for ANOVA or ANOVA Kruskal-Wallis test). VEGFR1 mRNA expression in the myocardium in the P-DM group was lower than in the M-DM group and similar to the other groups (see **Figure 1B**; tags for ANOVA or ANOVA Kruskal-Wallis test).

VEGF mRNA expression in the aorta wall in the P-DM group was higher than in the non-DM group,

**Table 1.** The general characteristics of the examined groups.

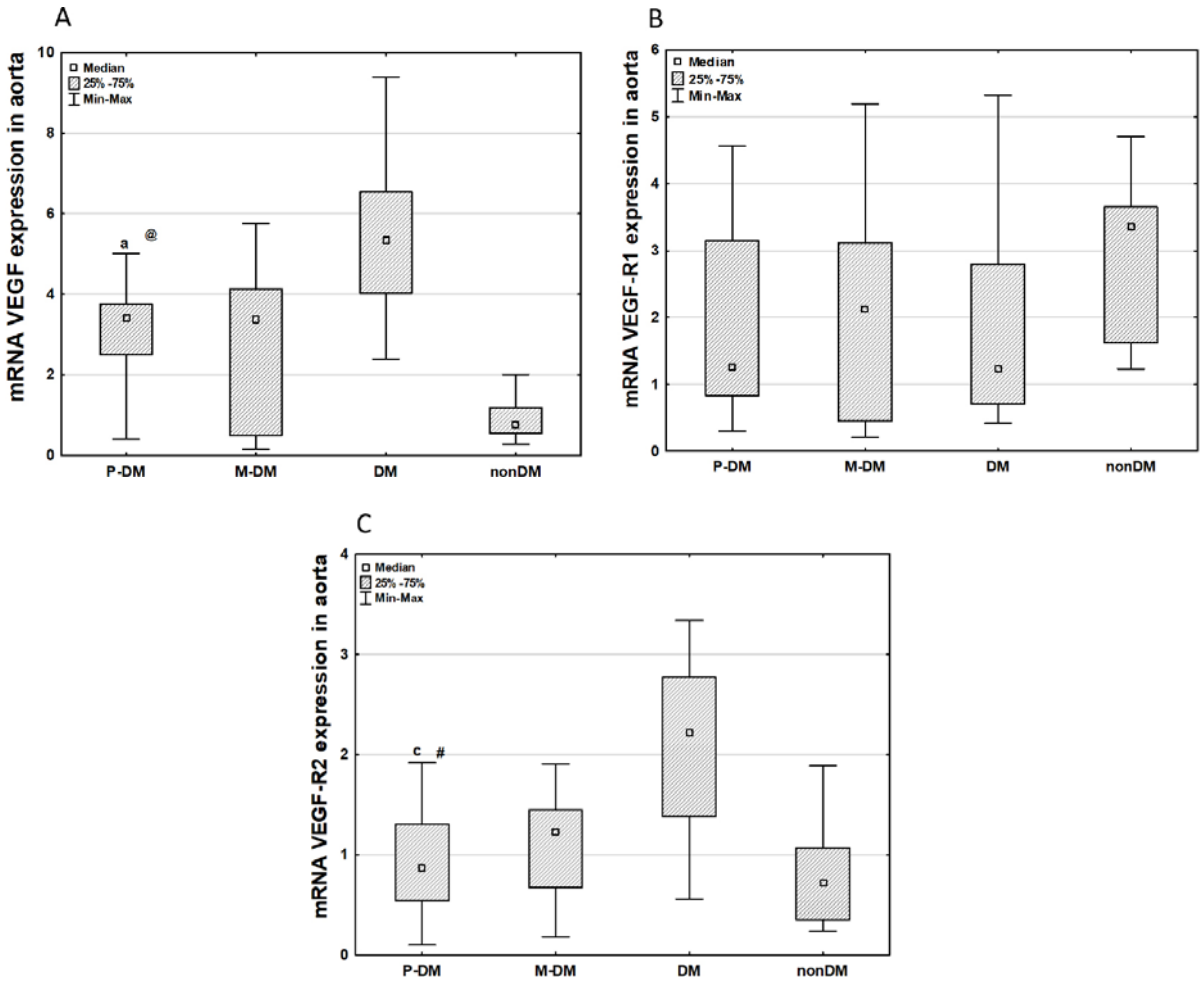
| | P-DM<br>n = 13 | M-DM<br>n = 14 | DM<br>n = 12 | nonDM<br>n = 12 |
|---|---|---|---|---|
| Initial body weight<br>[g] | 230.00 ± 14.14<br>(230.00) | 232.14 ± 17.62<br>(230.00) | 238.33 ± 13.37<br>(235.00) | 245.83 ± 11.65<br>(250.00) |
| Final body weight<br>[g] | 553.85 ± 50.75<br>(530.00) | 541.43 ± 70.59<br>(569.00) | 568.33 ± 44.48<br>(555.00) | 569.17 ± 32.88<br>(570.00) |
| GTT 0h<br>[mg/dL] | 131.23 ± 19.28[a]<br>(129.00) | 136.21 ± 9.70[a]<br>(135.00) | 160.83 ± 34.34[a]<br>(159.50) | 94.00 ± 8.46<br>(93.50) |
| GTT 1h<br>[mg/dL] | 220.54 ± 94.18[a]<br>(192.00) | 230.29 ± 74.72[a]<br>(206.50) | 286.08 ± 98.13[a]<br>(279.50) | 116.67 ± 30.31<br>(110.50) |
| $HbA_1c$<br>[%] | 4.82 ± 0.84<br>(4.50) | 4.51 ± 0.77<br>(4.60) | 6.58 ± 0.56[b]<br>(6,70) | 3.47 ± 0.38[b]<br>(3.65) |
| 1,5-AG<br>[µg/mL] | 8.54 ± 0.55[a]<br>(8.63) | 9.02 ± 2.07[a]<br>(8.42) | 9.43 ± 2.02[a]<br>(9.15) | 15.68 ± 0.56<br>(15.79) |
| C-peptide<br>[pmol/L] | 511.65 ± 219.88<br>(507.79) | 554.74 ± 208.22<br>(603.29) | 452.04 ± 174.41<br>(425.90) | 693.97 ± 479.55<br>(562.85) |

Values are expressed as Mean ± SD (Median); a – statistically significant against the nonDM group; $p \leq 0.05$ (ANOVA Kruskal-Wallis test); b – statistically significant against the other groups; $p \leq 0.05$ (ANOVA Kruskal-Wallis test).




a – statistically significant against the DM group; b – statistically significant against the M-DM group; $p \leq 0.05$; ANOVA or ANOVA Kruskal-Wallis test for data with normal or non-normal distribution, respectively; * – statistically significant against the M-DM and DM groups; # – statistically significant against the DM group; ^ – statistically significant against the M-DM group; @ – statistically significant against the nonDM and DM groups; $p \leq 0.05$; ANCOVA, Scheffe test.

**Figure 1.** VEGF, VEGFR1, and VEGFR2 mRNA expression in the myocardium of the examined groups.




**a – statistically** significant against the DM group; c – statistically significant against the nonDM group; $p \leq 0.05$; ANOVA or ANOVA Kruskal-Wallis test for data with normal or non-normal distribution, respectively; * – statistically significant against the M-DM and DM groups; # – statistically significant against the DM group; ^ – statistically significant against the M-DM group; @ – statistically significant against the nonDM and DM groups; $p \leq 0.05$; ANCOVA, Scheffe test.

**Figure 2.** VEGF, VEGFR1, and VEGFR2 mRNA expression in the aorta of the examined groups.

and there were no differences between the P-DM, M-DM and DM groups (see **Figure 2A**; tags ANOVA or ANOVA Kruskal-Wallis test). No differences were found among the studied groups considering VEGFR1 mRNA expression in the aorta wall, while VEGFR2 expression in the P-DM group was lower compared to the DM group and similar to the M-DM and non-DM groups (see **Figure 2B** and **2C**; tags ANOVA or ANOVA Kruskal-Wallis test).

Taking into consideration the possible influence of some metabolic factors, such as $HbA_1c$, 1,5-AG or C-peptide, on VEGF, VEGFR1, and VEGFR2 expression, the analysis of covariance (ANCOVA) was performed. The ANCOVA results revealed that the therapeutic model (group) determined mRNA expression for all examined parameters, except for VEGFR1 mRNA in the aorta and VEGFR2 mRNA in the myocardium (see **Table 2**). However, the ANCOVA post hoc test results indicate that VEGF mRNA expression in the myocardium of the P-DM group is not only higher than in the DM group but also higher than in the M-DM group (see **Figure 1A**, **1B**, **1C**; ANCOVA tags). Simultaneously, VEGF mRNA expression in the aorta wall was not only lower in the P-DM group than in the DM group, but also higher in comparison to the non-DM group (see **Figure 2A**, **2B**, **2C**; tags for ANCOVA).

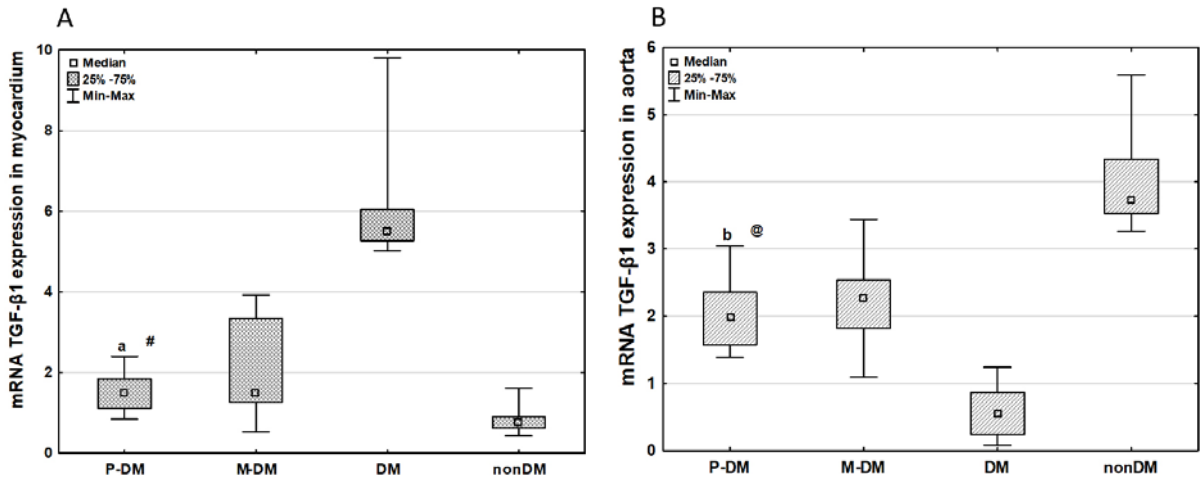
**Table 2.** Analysis of covariance (ANCOVA) results – the model with C-peptide,1,5-AG and $HbA_1c$ as independent variables and VEGEF, VEGFR1, VEGFR2 and TGF-β1 mRNA expression In the myocardium or in the aorta wall as dependent variables.

| F value for the model | $R^2$ value for the model | p for the model | independent variable which determines intergroup differences in the dependent variable |
|---|---|---|---|
| **dependent variable: VEGF mRNA in myocardium** | | | |
| 46.38 | 0.84 | 0.001* | Group |
| **dependent variable: VEGF mRNA in aorta** | | | |
| 8.26 | 0.47 | 0.000005* | Group |
| **dependent variable: VEGFR1 mRNA in myocardium** | | | |
| 8.85 | 0.49 | 0.000002* | Group |
| **dependent variable: VEGFR1 mRNA in aorta** | | | |
| 0.88 | -0.01 | 0.51 | None |
| **dependent variable: VEGFR2 mRNA in myocardium** | | | |
| 9.74 | 0.52 | 0.000001* | $HbA_1c$ |
| **dependent variable: VEGFR2 mRNA in aorta** | | | |
| 5.07 | 0.33 | 0.0005* | Group |
| **dependent variable: TGF-β1 mRNA in myocardium** | | | |
| 34.78 | 0.80 | 0.000001* | Group |
| **dependent variable: TGF-β1 mRNA in aorta** | | | |
| 35.50 | 0.80 | 0.000001* | Group |

*statistically significant

## The effect of *Passiflora incarnata* extract on mRNA expression of TGF-β1 in the aorta wall and in the myocardium

TGF-β1 mRNA expression in the myocardium of the P-DM group was lower than in the DM group and similar to the other groups, while expression of TGF-β1 mRNA in the aorta wall of the P-DM group was also higher compared to the DM group and lower than observed in the non-DM group (see **Figure 3A**, **3B**; tags for ANOVA or ANOVA Kruskal-Wallis test). For both TGF-β1 mRNA expression in the myocardium and in the aorta wall, ANCOVA confirmed that the differences among the groups are determined only by the examined group, and the post-hoc tests proved the same differences among the groups as the ANOVA tests (see **Figure 3A**, **3B**; tags for ANCOVA).




**a – statistically** significant against the DM group; b – statistically significant against the DM and non-DM groups; $p \leq 0.05$; ANOVA or ANOVA Kruskal-Wallis test for data with normal or non-normal distribution, respectively; * – statistically significant against the M-DM and DM groups; # – statistically significant against the DM group; ^ – statistically significant against the M-DM group; @ – statistically significant against the non-DM and DM groups; $p \leq 0.05$; ANCOVA, Scheffe test.

**Figure 3.** TGF-β1 mRNA expression in the myocardium and the aorta wall of the examined groups.

### The relationship between metabolic control in the course of type 2 diabetes and angiogenic factors

$HbA_1c$ was correlated with TGF-β1 mRNA expression in the myocardium [r = 0.7] and in the aorta [(-0.76)], with VEGF mRNA expression in the myocardium [(-0.64)] and with VEGFR2 mRNA expression in the myocardium [(-0.67)]. There were no correlations between plasma 1,5-AG levels or serum C-peptide levels and the analysed growth factors.

## Discussion

In recent years, there has been growing interest in plant-derived substances for the treatment of type 2 diabetes mellitus, as these compounds may offer additional benefits beyond standard therapeutic regimens with a relatively low risk of adverse effects.

It is also well established that effective management of diabetes should go beyond metabolic control, aiming not only to regulate glycaemia but also to provide additional benefits that help reduce the risk of long-term cardiovascular complications [18].

Both angiogenesis and fibrosis are processes closely related to atherosclerosis and can modify cardiovascular function [19,20]. However, the relationship between pharmacological treatment for patients with diabetes and these two processes remains poorly established, particularly for plant-derived substances. A promising source of new medicinal drugs may be species from the *Passifloraceae* family [21].

It is well established that diabetes interferes with angiogenesis in the heart muscle, increasing its susceptibility to hypoxia, creating conditions favourable for the development of ischaemic heart disease and raising the risk of myocardial infarction [22]. In the *Passiflora* extract-treated animals with type 2 diabetes, VEGF mRNA expression in the myocardium was higher than in the placebo-treated rats with diabetes; moreover, VEGF mRNA expression in the myocardium in the *Passiflora* extract-treated animals did not differ from that of the non-diabetic control group. These observations suggest that *Passiflora* extract may stimulate angiogenesis, which plays a protective role for the heart muscle affected by metabolic derangement. Interestingly, in *Passiflora* extract-treated rats, VEGF mRNA expression in the myocardium was even higher than in metformin-treated animals with type 2 diabetes. The metformin-treated group in our study served as an additional control to compare the pharmacological effects of *Passiflora* extract with those induced by the standard antidiabetic agent. The same conclusion supports the observation of VEGFR2 mRNA expression in the myocardium of *Passiflora* extract-treated animals because the main effect of VEGFR2 stimulation by VEGF is the induction of angiogenesis [23].

Angiogenesis in the course of diabetes involves two opposing angiogenic tissue-specific conditions. In the myocardium under diabetic conditions, angiogenesis plays a crucial role in collateral vessel growth [24,25]. It is worth emphasising that a very similar observation was made by Sasso et al. [26], who also revealed an increase in VEGF mRNA expression in the myocardium of animals with induced type 2 diabetes. Our results are noteworthy, given that TGF-β1 mRNA expression in the myocardium of animals treated with *Passiflora* extract was lower than in placebo-treated diabetic rats. Liu et al. [27] and Jesmin et al. [28] also observed an increase in myocardial TGF-β1 mRNA expression in the course of diabetes, just as it was found in our experiment. Increased expression of TGF-β1 in the myocardium is considered to initiate the process of fibrosis and, consequently, the loss of contractile function of the heart muscle. It increases extracellular matrix synthesis and reduces its degradation by inhibiting proteinases. Consequently, excessive TGF-β1 activity in the heart muscle appears unfavourable [7]. It cannot be overlooked, however, that TGF-β1 in the cardiovascular system may promote both pro- and anti-angiogenic effects, and these effects are highly context-dependent [29].

VEGF activity has been confirmed in aortic endothelial cells, foam cells, and smooth muscle cells that cover the plaque, demonstrating a crucial role for this molecule in the progression of atherosclerotic lesions [30]. VEGF functions as a pro-angiogenic and pro-inflammatory factor within the vascular wall, and newly formed vessels constitute the primary route for inflammatory cells to reach the atherosclerotic plaque [31]. The ingrowth of vessels within the atherosclerot-

ic plaque may rupture it and, consequently, accelerate the clinical progression of coronary artery disease [32]. VEGF mRNA expression in the aorta wall of the rats with type 2 diabetes was significantly lower than in the placebo-treated animals with diabetes. Simultaneously, it was higher than in the placebo-treated non-diabetic rats. At the same time, the expression of VEGFR2 mRNA in the *Passiflora* extract-treated group was significantly lower in comparison to the placebo-treated diabetic animals.

The role of TGF-β1 in the development of atherosclerotic lesions in the aortic wall appears complex. According to previous research, TGF-β1 expression in the aorta was reduced in the early stages of atherosclerotic lesions (stages I–II according to the American Heart Association). In contrast, in advanced stages (III and VI), TGF-β1 expression was increased to levels higher than in healthy vessels [30]. TGF-β signalling may contribute to the development of atherosclerosis. Still, it promotes a stable lesion phenotype by stimulating smooth muscle cell differentiation and preventing the switch from contractile to proliferative smooth muscle cells [29].

On the other hand, TGF-β1 modulates the inflammatory components of the early atherosclerotic lesion [29,33]. In our study, groups of animals with early-phase type 2 diabetes do not develop atherosclerosis; therefore, the lower TGF-β1 mRNA expression in the aortic wall than in placebo-treated rats with diabetes is not associated with limited fibrosis, which does not play a significant role at this stage of diabetes-induced vasculature impairment. Instead, it appears to be linked to anti-inflammatory effects in the aortic wall, potentially playing an important role in protecting against the progression of atherosclerosis in diabetes.

It should be emphasised that most of the effects of *Passiflora* extract on the analysed growth factors were independent of the current metabolic status (ANCOVA test) expressed by $HbA_1c$, 1,5-AG and C-peptide concentrations in the serum. The only exception was VEGR2 mRNA expression in the myocardium, as HbA1c values accounted for the observed differences among groups. This observation appears particularly important because it is well established that the expression of VEGF and TGF-β1 is modified by hyperglycaemia [5,6].

In summary, the current data provide novel findings on the beneficial effects of *Passiflora* extract on the myocardium in the context of type 2 diabetes, potentially mediated by VEGF and TGF-β1. However, the precise significance of *Passiflora* extract's influence on the aortic wall mediated by these factors remains uncertain. To our knowledge, *Passiflora incarnata* extract has been first documented to be beneficial in inhibiting or delaying cardiovascular fibrosis and promoting angiogenesis in the heart muscle, which, in turn, may positively affect chronic cardiovascular complications associated with diabetes.

## Declarations

### Statement on the welfare of animals

All procedures and protocols adhered to the Guide for the Care and Use of Laboratory Animals from the Institute for Laboratory Animal Research, National Research Council, Washington, D.C., National Academy Press, 2011. Procedures and protocols were in accordance with Polish governmental regulations (2005.01.21) and were approved by the Local Ethics Committee on the Use of Laboratory Animals in Poznan, Poland (permissions 54-55/2013).

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### Conflicts of interest

None.

### Statement of individual author contributions

**Anna Wesołowska**: study design, laboratory experiments, data analysis and interpretation, preparation of the first draft of the manuscript; **Marcin Ożarowski**: acquisition of research material, laboratory experiments, manuscript revision; **Przemysław Ł. Mikołajczak**: scientific supervision, expert consultation, manuscript revision; **Agnieszka Stelmaszyk**: laboratory experiments, molecular analysis, participation in data analysis and interpretation; **Anna Maliszewska-Dworacka**: data analysis and interpretation; **Saule Iskakova**:

study design, participation in results interpretation; **Bartosz A. Frycz**: laboratory experiments, molecular analysis; **Paweł P. Jagodziński**: scientific and expert consultation; **Marzena Dworacka**: study conception and design, data analysis and interpretation, scientific supervision, expert consultation, revision and final approval of the manuscript.

### Final approval

All authors confirm that they have read and approved the final version of the manuscript before its submission to the journal.

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