Journal of Traditional and Complementary Medicine 12 (2022) 250–259

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Stellaria media tea protects against diabetes-induced cardiac dysfunction in rats without affecting glucose tolerance

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ARTICLE INFO

Article history:
Received 29 April 2021
Received in revised form
4 August 2021
Accepted 5 August 2021
Available online 10 August 2021

Keywords:
Diabetic co-morbidity
Prevention
Medicinal herb
Flavonoid
Signal transducer and activator of transcription 3

ABSTRACT

Background and aim: Common chickweed (*Stellaria media*) tea has traditionally been applied for treatment of various metabolic diseases including diabetes in folk medicine; however, experimental evidence to support this practice is lacking. Therefore, we aimed to assess the effect of *Stellaria media* tea on glucose homeostasis and cardiac performance in a rat model of diabetes.
Experimental procedure: Hot water extract of *Stellaria media* herb were analyzed and used in this study, where diabetes was induced by fructose-enriched diet supplemented with a single injection of streptozotocin. Half of the animals received *Stellaria media* tea (100 mg/kg) by oral gavage. At the end of the 20-week experimental period, blood samples were collected and isolated working heart perfusions were performed.
Results and conclusion: Compared to the animals receiving standard chow, serum fasting glucose level was increased and glucose tolerance was diminished in diabetic rats. *Stellaria media* tea did not affect significantly fasting hyperglycemia and glucose intolerance; however, it attenuated diabetes-induced deterioration of cardiac output and cardiac work. Analysis of the chemical composition of *Stellaria media* tea suggested the presence of rutin and various apigenin glycosides which have been reported to alleviate diabetic cardiomyopathy. Moreover, *Stellaria media* prevented diabetes-induced increase in cardiac STAT3 phosphorylation. We demonstrated for the first time that *Stellaria media* tea may beneficially affect cardiac dysfunction induced by diabetes without improvement of glucose homeostasis.

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

https://doi.org/10.1016/j.jtcme.2021.08.003

Rutin and/or apigenin glycosides as well as modulation of STAT3 signaling may be implicated in the protection of *Stellaria media* tea against diabetic cardiomyopathy.

List of abbreviations

Akt	protein kinase B
ALP	alkaline phosphatase
ALAT	alanine aminotransferase
ASAT	aspartate aminotransferase
AUC	area under the curve
CK	creatine kinase
CK-MB	creatine kinase – myocardial band
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
ELISA	enzyme-linked immunosorbent assay
Erk	extracellular signal-regulated kinase
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein cholesterol
HPLC	high performance liquid chromatography
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LDH	lactate dehydrogenase
LVDP	left ventricular developed pressure
LVEDP	left ventricular end-diastolic pressure
OGTT	oral glucose tolerance test
ORAC	oxygen radical absorbance capacity
QE	quercetin equivalent
Stellaria m.	*Stellaria media*
STAT3	signal transducer and activator of transcription 3
STZ	streptozotocin
UPLC	ultra-performance liquid chromatography

1. Introduction

Diabetes is a common metabolic disease characterized by elevated blood glucose level and impaired glucose tolerance. The prevalence of diabetes for adults was approximately 171 million patients worldwide in 2000 and according to predictions, this number is expected to be doubled by 2030.[1] Due to the continuously increasing trend in diabetes morbidity, investigation of harmful consequences of the disease and potential therapeutic interventions is a relevant current research area.[1] In the absence of proper therapy diabetes may lead to development of various complications including cardiomyopathy, cataract, kidney failure, as well as neuronal damage. Diabetic cardiomyopathy is characterized by diastolic and/or systolic dysfunction which can lead to heart failure without the presence of classic risk factors such as dyslipidaemia, hypertension, or coronary artery disease.[2,3]

Nowadays, medicinal plants are gaining popularity in prevention and treatment of various diseases including diabetes. There are some herbs with a well-described and scientifically proven *anti*-hyperglycaemic properties, for instance aqueous leaf extract of stinging nettle (*Urtica dioica*)[4,5] or powdered fenugreek seeds (*Trigonella foenum graecum*).[6,7] In folk medicine, *Stellaria media* is believed to be a remedy to lose weight[8] and it is suggested to be used for its believed beneficial effects on blood lipid profile.[9] According to a popular Hungarian traditional healer, *Stellaria media* tea improves general metabolism, lowers blood glucose level, making it an adjuvant therapy for diabetic patients.[10] Moreover, consumption of chickweed tea for lowering blood glucose level is recommended by some websites dealing with medicinal plants and health issues.[11,12] Although, an animal experiment proposed an *anti*-hyperglycaemic effect of intraperitoneally administered alcoholic *Stellaria media* extract,[13] the clinical translation of this design is limited since *Stellaria media* is mostly consumed as tea. Nevertheless, potential effects of *Stellaria media* tea on diabetes-induced cardiac dysfunction has never been investigated.

Taken together, there is no firm experimental or clinical evidence supporting the anti-diabetic effect of *Stellaria media* tea. Therefore, the aim of the present study was to investigate the potential therapeutic efficacy of *Stellaria media* tea on the severity of diabetes and on harmful cardiac consequences induced by diabetes.

2. Materials and methods

2.1. Animals

The experiment conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health [National Institutes of Health publication 85–23, revised 1996] and the regulations of the Hungarian Act No. XXVIII of the year 1998 on protection and care of animals were strictly followed. The study was approved by the local animal ethics committee of the University of Szeged (XV.1181/2013).

Altogether 30 adult (9-week old) male Wistar rats were used in this study (purchased from Charles River Laboratories, Göttingen, Germany), weighing 292–420 g at the onset of the experiments. Rats were kept under standard climatic conditions (22 ± 2 °C room temperature, 12h light/dark cycles) in pairs in individually ventilated cages (Sealsafe IVC system, Buguggiate, Italy) and had *ad libitum* access to tap water and rat chow.

2.2. Experimental setup

After one week of acclimatization, the rats were randomized into three groups: Control, Diabetes, Diabetes + *Stellaria media* treatment. Rats in the control group (n = 10) received standard laboratory rat chow for 20 weeks. The other 20 rats were fed a special fructose-enriched diet, i.e. a standard laboratory rat chow (Innovo Ltd., Isaszeg, Hungary) supplemented with 60% (w/w) fructose (Floravita Ltd., Soltvadkert, Hungary) for 20 weeks[14] and were treated with a low-dose streptozotocin (STZ) injection (20 mg/kg body weight, ip.) on the 17th week. Combination of fructose-enriched diet/drinking water and low-dose STZ treatment has been reported to be an alternative approach to achieve experimental type 2 diabetes[15–17] (Fig. 1A). In order to prevent STZ-induced hypoglycaemia, drinking water containing 10% glucose

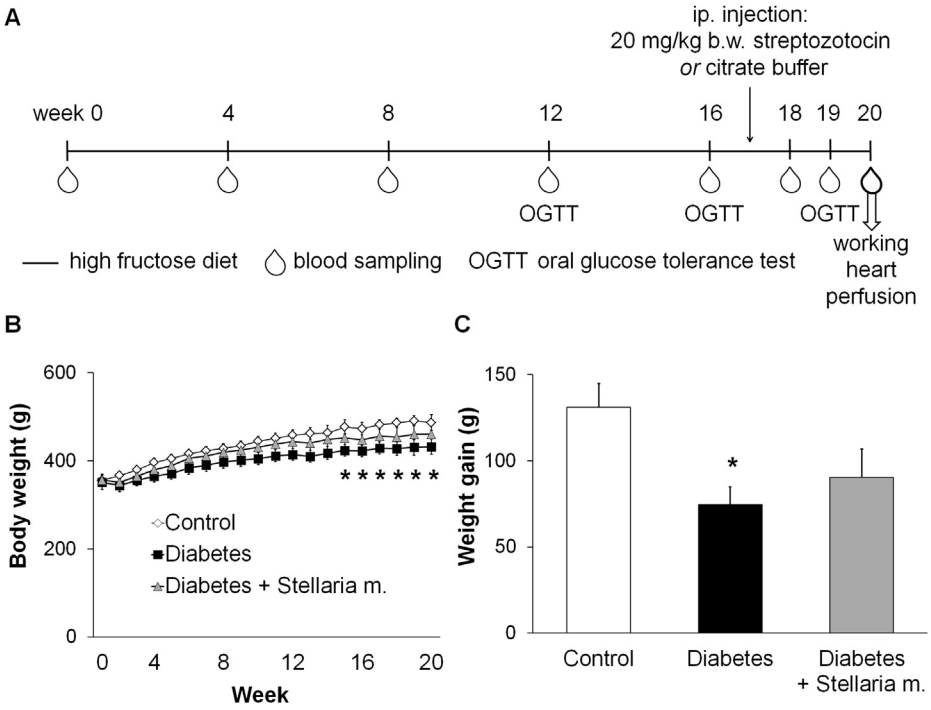

Fig. 1. Experimental protocol: rats were divided into Control, Diabetes and Diabetes + *Stellaria media* groups receiving either a standard chow or a chow supplemented with 60% fructose, respectively, for 20 weeks (A). Fasting blood glucose measurement was performed every four weeks until week 16. Oral glucose tolerance test (OGTT) was performed on week 12, 16 and 19. At week 17, rats in the Diabetes and Diabetes + *Stellaria media* groups were injected with a low-dose streptozotocin (20 mg/kg body weight) intraperitoneally. Fasting blood glucose was measured on week 18 and 19 to monitor the effect of streptozotocin injection. On week 20, the animals were anaesthetised and sacrificed. Blood samples were collected from the abdominal aorta, hearts were isolated and subjected to working heart perfusion according to Neely (A). Body weight (B) and weight gain (C) during 20 weeks in the Control group (circles), Diabetes group (squares) and Diabetes + *Stellaria media* group (triangles). Results are means ± SEM ($n = 8–10$), analyzed by repeated measures two-way ANOVA or one-way ANOVA with Holm-Sidak *post hoc* test,*$p < 0.05$ Diabetes vs. Control.

was given to the animals after STZ injection. The diet of 10 animals receiving fructose-enriched chow was further supplemented with *Stellaria media* tea administered by oral gavage (Diabetes + *Stellaria media*) in order to examine the potential effects of *Stellaria media* tea on the glucose homeostasis and cardiac function, while the other rats received equal amount of distilled water. One animal in the Control group and two animals in the Diabetes group were excluded due to technical problems occurring during the treatments.

Fasting blood glucose level measurements were performed every 4 weeks, accompanied by oral glucose tolerance (OGTT) tests at weeks 12, 16 and 19 (Fig. 1A). On the 20th week, rats were anaesthetised with sodium pentobarbital (Euthasol, 50 mg/kg body weight, ip., Produlab Pharma b.v., Raamsdonksveer, The Netherlands), the abdominal cavity was opened, and blood samples were taken from the abdominal aorta. Collected blood was allowed to clot and was centrifuged (2000 g, 20 min, 4 °C), then serum was separated for analysis of various serum parameters. Then the rats were given 500 U kg^{-1} heparin intravenously into the *vena cava inferior*. Isolated hearts were subjected to working heart perfusion according to Neely in order to evaluate cardiac function.

2.3. Preparation of Stellaria media tea

Stellaria media was harvested in Algyő (Hungary) by 'Ezerjófű' Association in 2017. Voucher specimen (No: 882) was deposited in Herbarium of the University of Szeged, Faculty of Pharmacy, Department of Pharmacognosy. The drug was dried and stored at room temperature. The dried and grounded drug was extracted with boiling water (1:10 w/v ratio) for 15 min by ultrasonication. The highly dense extract was separated from solid particles by mechanical press. The aqueous extract was dry-freezed. 1.5 g of dark-green lyophilized powder was equal to 10 g of dried drug. The lyophilized powder was dissolved in distilled water to achieve a final concentration of 100 mg/ml. The extract and the vehicle (distilled water) were then stored at 4 °C and were brought to room temperature before administration.

2.4. Stellaria media administration

Rats in the Diabetes + *Stellaria media* group received 100 mg/kg body weight *Stellaria media* tea with traditional oral gavage technique once a day since the onset of the experiment. The Control and Diabetes groups received equal amount of distilled water. The treatment was carried out at the same 2-h time range every day to minimalize the possible influence of circadian rhythm. The 100 mg/kg body weight dose of *Stellaria media* tea was considered as equal to the recommended human daily dose, that is 2 cups of tea, prepared with 3 g dried herb per cup, calculated according to Nair and Jacob considering the differences in body surface area.[18]

2.5. Working heart perfusion

Cardiac performance and function was assessed in isolated Neely working hearts, as described earlier.[19,20] Cardiac functional parameters including heart rate, coronary flow, aortic flow, cardiac output, left ventricular developed pressure (LVDP) and its first derivatives (dp/dt max and dp/dt min), and left ventricular end-diastolic pressure (LVEDP) were measured. Cardiac work was calculated by multiplying cardiac output and maximum pressure. For more information, see Supplementary Material.

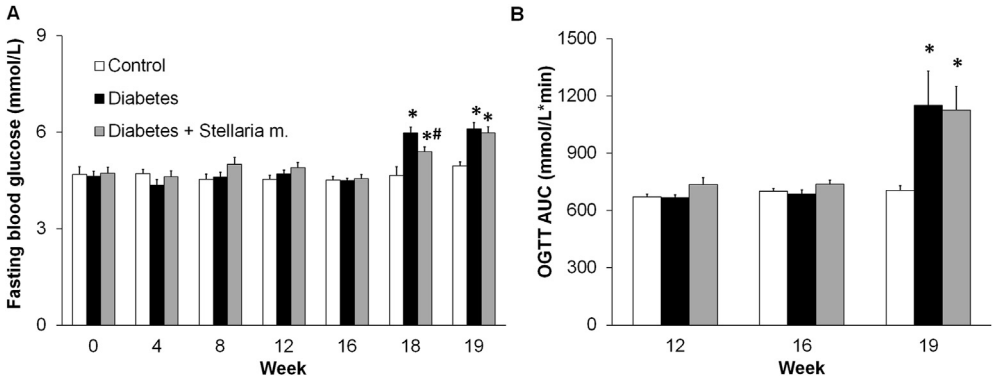

Fig. 2. Fasting blood glucose levels (A) and area under the curve (AUC) (B) values of oral glucose tolerance tests (OGTT) measurements. Results are means ± SEM (n = 8–10), analyzed by one-way ANOVA followed by Holm-Sidak *post hoc* test,*p < 0.05 vs. Control, #p < 0.05 vs. Diabetes.

2.6. Measurements of serum parameters

Several serum parameters describing pancreas, liver and kidney function, cardiac markers, lipid panel and electrolytes were analyzed by Roche Cobas 8000 Analyzer System using enzymatic colorimetric assays from Roche (Mannheim, Germany). For further details, please see Supplementary Material. Haemoglobin A1c (HbA1c) levels were analyzed by DCA Vantage Analyzer System (Siemens) provided by Diagnosticum Ltd. (Budapest, Hungary).

2.7. Measurement of serum insulin levels

Serum insulin levels were determined by enzyme-linked immunosorbent assay (ELISA) technique (Mercodia, Ultrasensitive Rat Insulin ELISA) according to the instructions of the manufacturer.[21]

2.8. Total flavonoid content and screening for flavonoids

The total flavonoid content was determined as quercetin equivalent (QE) using the aluminum chloride colorimetric method. The lyophilizate powder was dissolved in methanol to get a solution with a concentration of 1 mg/mL. For calibration curve 5, 10, 25, 50 μg/mL methanolic solutions of quercetin were prepared. As reagent, 2% aluminum chloride methanolic solution was used. Reaction mixtures were prepared by mixing 2 mL of solution and 2 mL of aluminum chloride, respectively. After 60 min of incubation at room temperature, the absorbance was measured against blank by applying UV-VIS spectrophotometer (Helios β ThermoSpectronic) at 420 nm. The total flavonoid concentration was calculated using a calibration plot ($R^2 = 0.9999$). The measurements were carried out in triplicate.

The flavonoid content of the lyophilized *Stellaria media* aqueous extract was screened by ultra-performance liquid chromatography (UPLC). For this experiment 1 mg/mL methanolic solution was prepared from the lyophilizate. The presence of ubiquitous flavonoids in plants, namely apigenin, apigenin-7-glucoside, kaempferol, luteolin, quercetin, and rutin was screened by UPLC. Experiments were carried out on a Shimadzu Nexera X2 UHPLC liquid chromatography system. For more information, see Supplementary Material.

2.9. Liquid chromatography-tandem mass spectrometry analysis

In order to gain more information about the chemical composition, the aqueous extract was filtered through a 0.22 μm membrane filter and diluted tenfold with 0.1% formic acid and analyzed by data-dependent liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis using a Waters MClass nanoUPLC system online coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer. MS2 data were subjected to a spectral library search against the MzCloud database using the Compound Discovery software. For more information, see Supplementary Material.

2.10. Measurement of the antioxidant activity

The measurement of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging activity was carried out according to the method of Fukumoto et al. with some modifications.[22] The Oxygen Radical Absorbance Capacity (ORAC) assay was carried out according to the method of Mielnik et al.[23] For further details, please see Supplementary Material.

2.11. Western blot analysis

Phosphorylation of key proteins of well-known cardioprotective signaling pathways, signal transducer and activator of transcription 3 (STAT3), protein kinase B (Akt) and extracellular signal-regulated kinase (Erk) proteins and activation of apoptosis-related Bax and Bcl-XL proteins and GLUT4 glucose transporter were detected by Western blot technique as described earlier.[24] For more information, see Supplementary Material.

2.12. Statistical analysis

All values are presented as mean ± SEM. Repeated measures two-way ANOVA was applied for analysis of time-dependent body weight change. One-way ANOVA was used to determine the differences among the three experimental groups and $p < 0.05$ was accepted as statistically significant difference, using Holm-Sidak *post hoc* test. In the analysis of the working heart perfusion and Western blot results, those data which were out of mean ± 2SD range were excluded in order to minimalize the effect of extremities.

3. Results

3.1. Time course of body weight, fasting blood glucose and OGTT values

Body weight was measured every 3 days in order to calculate *Stellaria media* tea dosage and to monitor weight gain. It showed a continuous increase from 356 ± 7 g at the onset of the experiment to 482 ± 16 g at week 20 in the Control group fed with standard

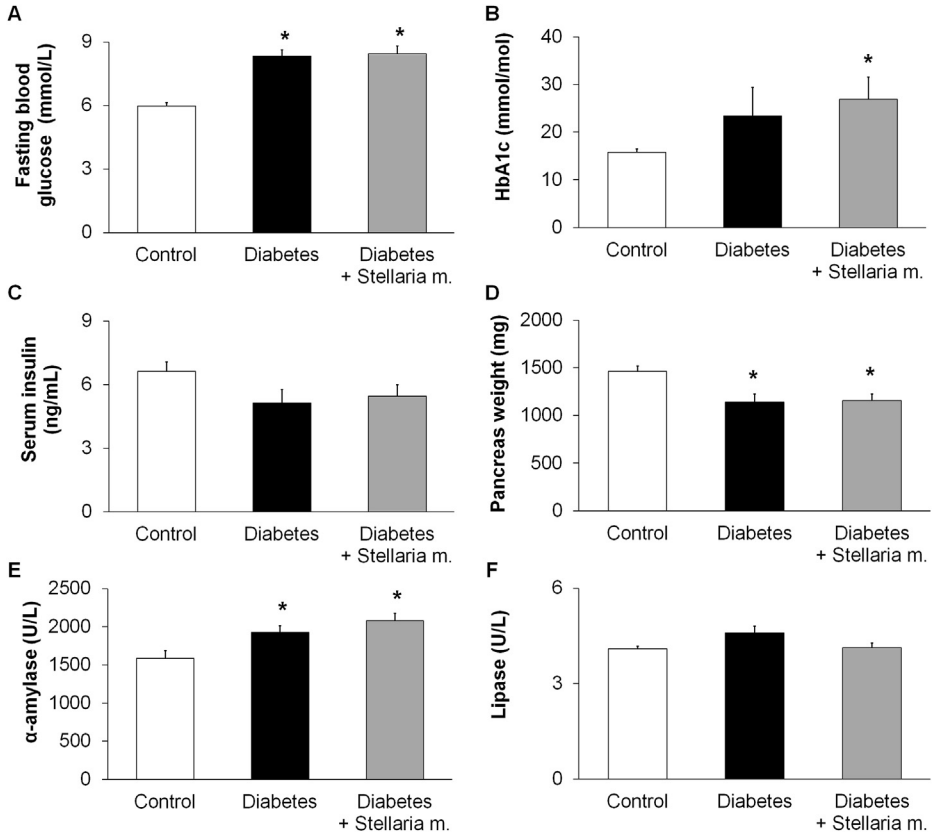

Fig. 3. Parameters representing pancreatic function at week 20: fasting blood glucose at termination (A), HbA1c levels (B), serum insulin levels (C), pancreas weight (D), enzyme activities of α-amylase (E) and lipase (F). Results are means ± SEM (n = 8–10 except for serum insulin measurement where *n* = 6–8), analyzed by one-way ANOVA followed by Holm-Sidak *post hoc* test,*p < 0.05 vs. Control.

laboratory rat chow (Fig. 1B). Compared to the controls, fructose-enriched diet plus STZ injection reduced body weight increase in the last 6 weeks in the Diabetes group, however, this effect was not significant in the Diabetes + *Stellaria media* group (Fig. 1B). Weight gain during the feeding protocol was significantly lower in the Diabetes group compared to the Control group (Fig. 1C). Similarly, there was a tendency of decrease in the Diabetes + *Stellaria media* group compared to the controls; however, it did not reach the level of statistical significance (Fig. 1C).

Fasting blood glucose levels were measured every 4 weeks since the onset of the experiment, accompanied by OGTT at weeks 12, 16 and 19. In fasting blood glucose levels and glucose tolerance, there was no significant difference among the three groups until week 16 (Fig. 2A and B). On the 17th week, the rats in the Diabetes and Diabetes + *Stellaria media* groups were injected with a low dose of STZ injection (20 mg/kg body weight, ip.), while the Control group was treated with equal amount of vehicle (citrate buffer). Following the STZ injection a significant elevation in fasting blood glucose levels could be observed in the Diabetes group compared to the Control group, achieving an experimental diabetes state (Fig. 2A). Although on week 18 the blood glucose elevation in the Diabetes + *Stellaria media* group was significantly lower compared to the Diabetes group, this difference was faded away by week 19 (Fig. 2A). According to our OGTT results, there was a significant elevation in the area under the curve (AUC) levels in the Diabetes group compared to the Control group on week 19 (Fig. 2B) representing an impairment of glucose tolerance. *Stellaria media* treatment did not affect glucose intolerance (Fig. 2B).

Table 1
Serum parameters representing liver and kidney function, cardiac markers, lipid panel and electrolytes.

	Control	Diabetes	Diabetes + *Stellaria m.*	*p*
Liver function:				
ALAT (U/L)	34.7 ± 2.0	23.9 ± 2.2*	29.1 ± 2.5	<0.05
ASAT (U/L)	65.1 ± 2.5	50.4 ± 1.8*	55.6 ± 1.9*	<0.05
ALP (U/L)	46.9 ± 1.9	86.5 ± 12.7*	101.4 ± 10.4*	<0.05
Albumin (g/L)	40.8 ± 0.8	41.8 ± 0.6	42.0 ± 0.4	ns
Total protein (g/L)	54.0 ± 1.5	55.9 ± 0.4	57.3 ± 0.7*	<0.05
Kidney function:				
Urea (mmol/L)	6.3 ± 0.3	3.8 ± 0.7*	3.8 ± 0.3*	<0.05
Creatinine (μmol/L)	32.1 ± 1.3	35.3 ± 1.8	35.2 ± 1.6	ns
Cardiac markers:				
CK (U/L)	276.9 ± 25.7	235.1 ± 19.4	289.7 ± 29.8	ns
CK-MB (U/L)	438.6 ± 29.1	433.8 ± 37.9	524.8 ± 55.6	ns
LDH (U/L)	281.7 ± 26.0	298.4 ± 23.3	359.3 ± 42.9	ns
Lipid panel:				
Cholesterol (mmol/L)	1.58 ± 0.05	1.74 ± 0.16	1.78 ± 0.11	ns
HDL-Cholesterol (mmol/L)	0.95 ± 0.04	1.03 ± 0.08	1.08 ± 0.10	ns
Electrolytes:				
Sodium (mmol/L)	142.1 ± 0.9	141.5 ± 0.9	140.2 ± 1.1	ns
Potassium (mmol/L)	4.8 ± 0.1	5.1 ± 0.1	5.1 ± 0.2	ns
Chloride (mmol/L)	101.9 ± 0.5	101.3 ± 0.8	101.0 ± 0.5	ns

Results are means ± SEM (n = 8–10), analyzed by one-way ANOVA followed by Holm-Sidak *post hoc* test,*p < 0.05 vs. Control. ALP alkaline phosphatase, ALAT alanine aminotransferase, ASAT aspartate aminotransferase, CK creatine kinase, CK-MB creatine kinase – myocardial band, HDL high-density lipoprotein cholesterol, LDH lactate dehydrogenase, *ns* non-significant.

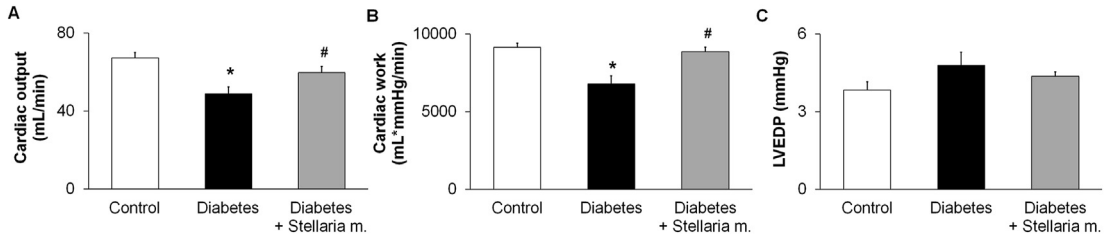

Fig. 4. Cardiac function in isolated hearts subjected to working perfusion according to Neely: cardiac output (A), cardiac work (B), left ventricular end diastolic pressure (LVEDP) (C). Results are means ± SEM (n = 8–10), analyzed by one-way ANOVA followed by Holm-Sidak *post hoc* test,*p < 0.05 vs. Control, #p < 0.05 vs. Diabetes.

Table 2
Parameters measured by working heart perfusion according to Neely.

	Control	Diabetes	Diabetes + *Stellaria m.*	*p*
Aortic flow (mL)	44.4 ± 2.6	27.3 ± 2.4*	34.4 ± 2.3*	p < 0.05
Coronary flow (mL)	22.8 ± 0.7	21.8 ± 1.5	23.7 ± 1.0	ns
Max dp/dt (mmHg/s)	6323 ± 282	6260 ± 439	6431 ± 487	ns
Min dp/dt (mmHg/s)	− 4520 ± 188	− 4512 ± 397	− 4496 ± 374	ns
Aortic diastolic pressure (mmHg)	37.6 ± 0.5	37.9 ± 0.8	37.8 ± 1.1	ns
Aortic systolic pressure (mmHg)	114.8 ± 2.5	110.4 ± 3.3	114.9 ± 3.1	ns
LVDP (mmHg)	136.2 ± 4.6	130.0 ± 4.5	131.3 ± 5.2	ns
Heart rate (1/min)	240 ± 10	211 ± 16	231 ± 11	ns

Results are means ± SEM (n = 8–10), analyzed by one-way ANOVA with Holm-Sidak *post hoc* test,*p < 0.05 vs. Control. LVDP left ventricular developed pressure, *ns* non-significant.

3.2. Parameters reflecting endocrine and exocrine function of the pancreas

At the end of the 20-week experiment, elevated fasting blood glucose, non-significantly increased HbA1c (3.6 ± 0.1% in Control and 4.3 ± 0.5% in Diabetes, respectively) and serum insulin levels decreased by approximately 20% indicated impaired endocrine pancreatic function (Fig. 3A–C) in the Diabetes group. *Stellaria media* tea failed to improve these parameters (Fig. 3A–C), and HbA1c was 4.6 ± 0.4% in this group. Pancreas weight was significantly lower in the Diabetes group compared to the Control group (Fig. 3D), showing that there might have been pancreatic damage due to the fructose-enriched diet plus STZ injection. *Stellaria media* did not affect this alteration. The serum activity of α-amylase was significantly elevated both in the Diabetes and Diabetes + *Stellaria media* groups (Fig. 3E), while there was no difference among the three groups in the activity of lipase enzyme (Fig. 3F).

3.3. Serum parameters of liver and kidney function, cardiac markers, lipid panel and electrolytes

At termination of the animals, blood samples were collected from the abdominal aorta and several serum parameters were measured. Markers of liver function, such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) enzyme activities, as well as albumin and total protein concentrations were measured. Diabetes caused a significant decrease in ALAT and ASAT enzyme activities and an elevation in ALP enzyme activity without affecting albumin or total protein concentration indicating lack of considerable liver damage (Table 1). Serum parameters describing kidney function were also measured. Urea levels were significantly lower in the Diabetes group compared to the Control group (Table 1). There was no difference in the creatinine levels. *Stellaria media* tea did not affect liver and kidney function compared to the Diabetes group (Table 1). There was no significant difference among the three groups in creatine kinase (CK), Creatine kinase – myocardial band (CK-MB) and lactate dehydrogenase (LDH) activities suggesting the lack of significant cellular injury in the heart and other tissues (Table 1). Cholesterol, high-density lipoprotein (HDL) cholesterol, as well as relevant electrolytes, such as sodium, potassium and chloride levels were not changed significantly among the groups (Table 1).

3.4. Ex vivo working heart perfusion

Cardiac dysfunction is a frequent consequence of diabetes, therefore we assessed cardiac performance in hearts subjected to working heart perfusion. Aortic flow, cardiac output and cardiac work, reflecting systolic heart function, were significantly impaired in the Diabetes group compared to the Control group (Fig. 4A and B, Table 2), indicating diabetic adverse effects on the heart. *Stellaria media* treatment significantly improved cardiac output and cardiac work suggesting that *Stellaria media* tea may have beneficial effects on the heart in a diabetic state (Fig. 4A and B). The diastolic function of the hearts was assessed by measurements of LVEDP, which inversely correlates with the function. LVEDP showed a tendency of elevation in the Diabetes group compared to the Control group, however, *Stellaria media* had no prominent effect on this parameter (Fig. 4C). There were no significant alterations in the other analyzed cardiac functional parameters among the three experimental groups (Table 2).

3.5. Total flavonoid content, LC-MS/MS analysis and antioxidant activity of Stellaria media tea

Flavonoids are active compounds in several medicinal herbs and are related to anti-oxidative properties, therefore we analyzed the flavonoid content of the *Stellaria media* tea. The total flavonoid content determined by means of UV-VIS absorbance was 9.88 ± 0.10 mg quercetin equivalent/gram (QE/g). The flavonoid screening by means of UPLC indicated rutin being a possible component, based on comparison of its retention time and UV spectrum with a reference standard.

In the LC-MS/MS analysis, several components have been

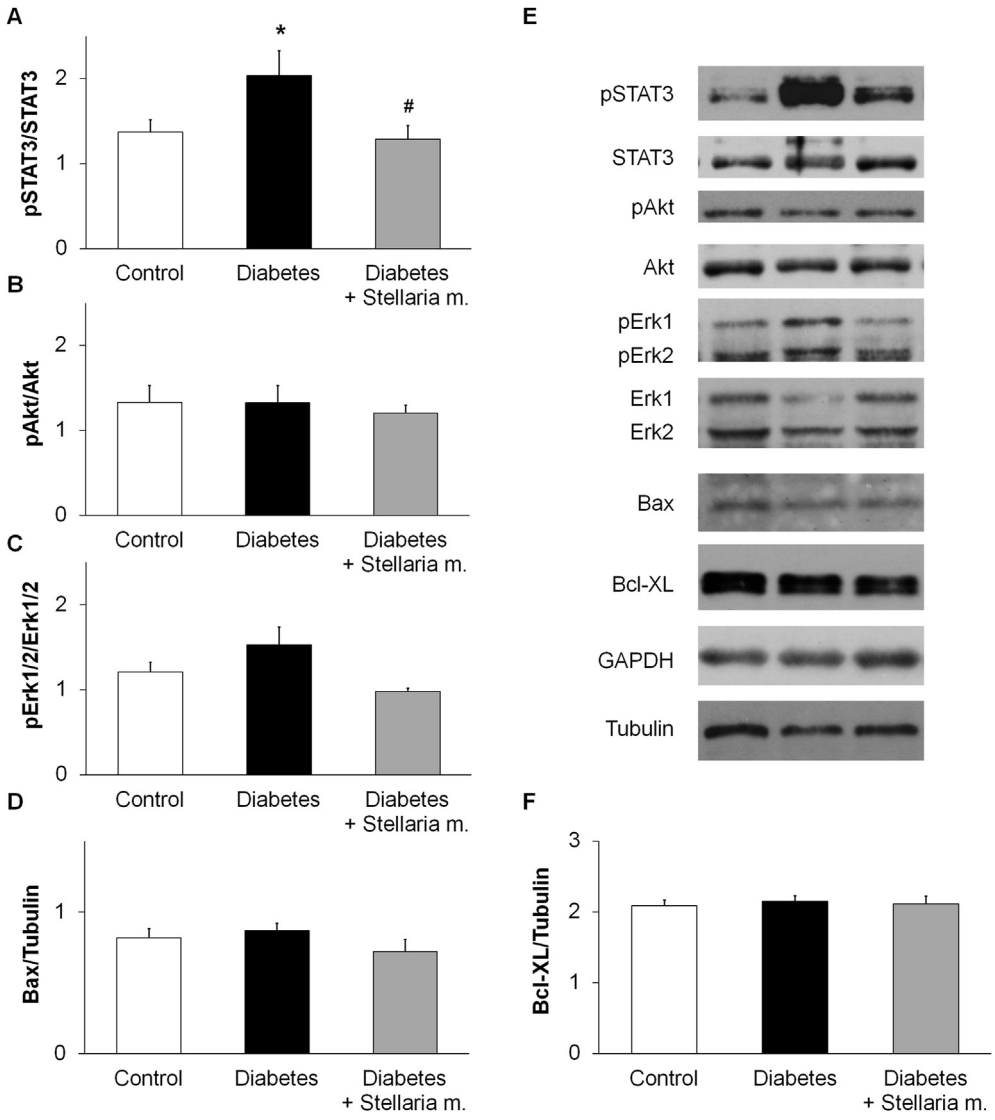

Fig. 5. Western blot analysis of phosphorylation of proteins: signal transducer and activator of transcription 3 (STAT3) (A), protein kinase B (Akt) (B), extracellular signal-regulated kinase (Erk) (C), proapoptotic Bax (D), representative bands (E), antiapoptotic Bcl-XL (F). Results are means ± SEM (n = 7), analyzed by one-way ANOVA followed by Fisher LSD *post hoc* test,*p < 0.05 vs. Control, #p < 0.05 vs. Diabetes.

detected. These comparisons revealed altogether five possible components out of the ten most intense sample components, which appeared to be various glycosylated apigenin-derivatives. MS2 spectrum of *m*/*z* 595.1660 displayed reasonable resemblance (MzCloud best match: 62.9) to MzCloud spectral library entries to two isomeric compounds, 6-arabinosyl-8-galactosylapigenin (corymboside) and 6-β-D-glucopyranosyl-8-β-D-ribopyranosyla pigenin (schaftoside), while MS2 spectrum of *m*/*z* 595.1660 showed good agreement (MzCloud best match: 82.8) with apigenin-6,8-di-*C*-glucoside (vicenin). Two further components, *m*/*z* 757.2195 and 933.2657 showed similarity to MS2 data acquired on 2″-*O*-α-L-rhamnopyranosyl-isovitexin, a compound with the same apigenin base structure. The molecular mass of these two components were 178.1 and 354.1 Da higher compared to the database entry. Six further components (*m*/*z* 274.2737, 535.1448, 679.2974, 677.2817, 611.1608 and 381.0793) did not show any resemblance to MzCloud entries indicating that these components were not included with an MS2 spectrum in the spectral library.

Moreover, the lyophilized powder was evaluated for antioxidant activity using DPPH and ORAC assays. The lyophilized aqueous extract of *Stellaria media* exerted low direct antioxidant capacity in both assays: EC_{50} 168.30 ± 11.06 μg/L in the DPPH assay and 0.97 ± 0.16 Trolox Equivalent mmol/g in the ORAC study.

3.6. Cardiac signaling pathways

Activation of STAT3 is proposed to play a role in diabetes-induced cardiac dysfunction. The phosphorylation of STAT3 was significantly elevated in the Diabetes group which was attenuated by *Stellaria media* treatment (Fig. 5A), suggesting an association with the beneficial cardiac effect of *Stellaria media*. There were no significant differences among the three groups in the phosphorylation of Akt and Erk proteins (Fig. 5B and C). Since increased cardiac apoptosis represents greater risk for the development of diabetic cardiomyopathy,[25–27] we examined apoptosis related proteins in our study. Diabetes and *Stellaria media* treatment had no effects on the proapoptotic Bax and antiapoptotic Bcl-XL proteins (Fig. 5D and F). We also investigated GLUT4 transporter expression

as GLUT4-mediated glucose uptake may play a relevant role in diabetes. Diabetes and *Stellaria media* treatment did not influence GLUT4 protein expression (data not shown).

4. Discussion

In the present study, we tested the effects of *Stellaria media* tea on the severity of diabetes as well as on diabetes-induced cardiac consequences in an *in vivo* rat model. Based on our findings, *Stellaria media* tea appears to have beneficial effects on cardiac dysfunction induced by diabetes, since the treatment ameliorated the impaired cardiac output and cardiac work. However, this effect seems to be independent of the modulation of diabetes severity as the application of the tea treatment did not influence fasting hyperglycaemia or glucose intolerance.

In folk medicine *Stellaria media* is consumed mainly as tea. To the best of our knowledge, this is the first study investigating the hot water extract of chickweed in the settings of diabetes. In our study, *Stellaria media* was not effective in lowering blood glucose level or improving glucose tolerance in diabetic rats. In the literature only one other study examined specifically the effect of *Stellaria media* on the severity of diabetes. Ethanolic leaf extract of *Stellaria media* in a dose of 100–400 mg/kg/day administered by intraperitoneal injection has been shown to attenuate hyperglycaemia in a 21-day alloxan-induced diabetic rat model.[13] In that study, *Stellaria media* treatment attenuated fasting blood glucose levels, decreased haemoglobin A1c levels and inhibited pancreatic α-amylase and β-glucosidase enzyme activities.[13] There are also some studies using various models where glucose homeostasis was estimated among other metabolic parameters. For instance, ethanolic radix extract of another member of the *Stellaria* genus (*Stellaria dichotoma*) improved glucose homeostasis in high-fat-diet fed mice.[28] In contrast, Chidrawar et al. found that ethanolic extract of *Stellaria media* was ineffective to decrease hyperglycaemia in both cafeteria-diet- and progesterone-induced obesity models.[29,30] However, they also found that 200 and 400 mg/kg methanolic extract significantly attenuated serum glucose levels in these models.[29,30] These controversial results may be explained by the differences in (i) the composition of the extracts, (ii) delivery time, administration and dose of *Stellaria media,* (iii) the applied animal models and strains. It should be also noted that intraperitoneal application of ethanolic and methanolic extracts of *Stellaria media* has limited translational value in the view of the human consumption of this medicinal plant.

Diabetic cardiomyopathy is one of the major consequences of diabetes. In our study, aortic flow, cardiac output and cardiac work were significantly decreased in the Diabetes group in comparison to the Control group, showing that this experimental diabetes model has some adverse effects on the heart. *Stellaria media* tea treatment significantly improved cardiac output and cardiac work, suggesting that *Stellaria media* tea may have beneficial effects on the heart in a diabetic state. To the best of our knowledge, in the literature currently there is no other experimental data concerning the effects of *Stellaria media* on cardiac function or cardiomyopathy. Our research group tested the effects of *Stellaria media* tea lyophilizate in another chronic metabolic disease, i.e. hypercholesterolaemia, where we demonstrated that the treatment has no blood cholesterol lowering effect in diet-induced hypercholesterolaemia in rats.[31] In the same study, we investigated some safety issues of the *Stellaria media* treatment on the heart. Our transthoracic echocardiographic measurements showed that *Stellaria media* treatment did not affect cardiac morphology and parameters related to cardiac function in diet-induced hypercholesterolaemia.[31]

Various mechanisms may play a role in the development of cardiac dysfunction in diabetes, e.g. oxidative stress, diffuse apoptosis of cardiomyocytes, dysregulation of cardiac signaling pathways, mitochondrial dysfunction, fibrosis or hypertrophy.[26,32] Interestingly, there are more than 50 medicinal herbs, such as sesame,[33] which have beneficial effects on experimental diabetic cardiomyopathy (for review please see Refs. 32,34). These plants have been suggested to exert antioxidant properties that may attenuate oxidative stress or inflammation, and to reduce apoptosis, and cardiac remodelling.[32] In the literature, there are some experimental data suggesting similar antioxidant and anti-inflammatory properties of *Stellaria media*, which can be associated with the improvement of certain cardiac parameters in our present study.[35] Therefore, we tested the *in vitro* antioxidant capacity of *Stellaria media* tea, and we found a low antioxidant activity. This phenomenon was observed by another study too, where the antioxidant activity of the aqueous and methanolic extract was weak.[36] *Stellaria media* has been reported to contain active metabolites e.g. phenolic compounds, flavonoids or steroid saponins, that may play a role in pharmacological activities.[35] We determined the total flavonoid content of *Stellaria media* tea and it was 9.88 ± 0.10 mg QE/g. The flavonoid content of *Stellaria media* has been discussed by several papers.[36–38] In a phytochemical study, the flavonoid content was determined not less than 1.2% in raw plant material.[38] In an experiment, the total flavonoid content (determined by HPLC) of a lyophilized juice was 25.6 mg/g, and in an ethanolic extract 63.9 mg/g[36]. Our extract was prepared with hot water (in accordance with the human use) and this might explain the lower flavonoid content. UPLC analysis of flavonoid screening indicated rutin being a possible component in the *Stellaria media* tea in our present study. The beneficial cardiovascular effect of rutin in diabetes has been proposed. Some studies demonstrated that rutin alleviates diabetic cardiomyopathy and improve left ventricular dysfunction in STZ-induced diabetes[39–42] and in high-carbohydrate, high-fat diet models.[43] Rutin was also shown to exert neuroprotective effects which may be beneficial in diabetes as well.[44] LC-MS/MS analysis of the extract we used in our study afforded identification of various glycosylated apigenin-derivatives, which finding is in accordance with literature data.[36] The glycosylated flavonoid derivatives can be poorly absorbed in the intestines directly, therefore during digestion there is a deglycosylation step, and after that only the aglycone part of the molecule will be absorbed,[45] therefore being responsible for the biological effects. The aglycone part of these components is apigenin, which has already been reported to alleviate STZ-induced diabetic cardiomyopathy[46] and to exert protective effects against cardiac dysfunction in myocardial infarction in diabetic rats,[47] suggesting that apigenin-derivatives may contribute to the beneficial cardiac effects of *Stellaria media* tea.

The limited antioxidant capacity of the water extract raised the question whether signaling pathways are involved in the cardiac effects. Based on literature data, STAT3 is proposed as a key mediator of diabetes-induced cardiac dysfunction.[48–50] In conjunction with other studies[51–53] (for specific review please see Ref. 54), we showed that diabetes increased cardiac STAT3 phosphorylation. Moreover, *Stellaria media* tea treatment prevented diabetes-induced cardiac STAT3 dysregulation, suggesting a protective role. Indeed, some studies demonstrated that attenuation of the enhanced cardiac STAT3 activation may be beneficial in diabetes. In a high-glucose, high-fat diet and STZ injection-induced diabetic rat model, losartan attenuated the cardiac STAT3 phosphorylation and improved heart function.[53] In STZ-induced diabetic mice hearts, inhibition of diabetes-induced activation of EGFR-STAT3 signaling was associated with restoring cardiac fibrosis and hypertrophy related factors, and prevented diabetes-induced cardiac dysfunction.[50] Interestingly, the possible presence of rutin in *Stellaria media*

tea may be a feasible explanation for the observed effects in our study, as rutin was suggested to exert cytoprotection by inhibiting STAT3 phosphorylation.[55,56] Similarly, apigenin and its derivatives has been shown to attenuate STAT3 activation in tumor cells,[57,58] which is in agreement with our present findings observed in cardiac tissue and may be related to the beneficial effect of *Stellaria media* tea on diabetic cardiomyopathy.

Nevertheless, a better understanding of the molecular mechanism underlying the proposed beneficial cardiac effects of *Stellaria media* tea would require additional research in the future. Further investigation of STAT3 signaling pathway could identify downstream targets or direct cause-effect mechanisms. Applying other doses of the tea, using a different extraction method or combination with standard therapies may also contribute to a deeper knowledge. Testing the effects of the tea in female rats could reveal sex differences in the effectiveness of the extract. It would be worth studying the effects of *Stellaria media* tea not only in a rat model, but in humans as well whether we can observe functional cardiac improvement in diabetic patients who drink chickweed tea regularly.

Although we provide interesting data on the effects of *Stellaria media* in diabetes, as always, there may be some possible limitations of our present study. The animals received *Stellaria media* tea from the onset of the experiments, together with the fructose-enriched diet. It would be worthwhile testing its effects in a developed diabetic state, which may have greater clinical relevance. Moreover, elucidation of the causal relation between the tea's beneficial effect and STAT3 phosphorylation with state-of-the-art experiments or further analysis of downstream targets in diabetic cardiomyopathy would be straightforward in the future. We tested the effects in a rat model, which might differ from the human metabolism. Moreover, the lack of a group receiving only herbal treatment without fructose-enriched diet can also be considered as a possible limitation.

In conclusion, the tea made of *Stellaria media* (i.e. common chickweed) may protect against diabetes-induced cardiac dysfunction; however, this effect seems to be independent of the modulation of fasting hyperglycemia or glucose tolerance in rats. *Stellaria media* prevented diabetes induced STAT3 phosphorylation in the heart, which may play a role in the beneficial cardiac effect. Nevertheless, further studies are needed to reveal the exact molecular mechanisms underlying the proposed cardioprotective effect of *Stellaria media* in diabetic conditions.

Declaration of competing interest

None.

Acknowledgements

We would like to thank Dr. Márta Sárközy and Enikő Páger for their help in the *Stellaria media* administration, Ágota Berek and Tibor Kiss for providing the plant material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2021.08.003.

Funding

This work was supported by the Economic Development and Innovation Operative Programme [GINOP-2.3.2-15-2016-00012 and GINOP-2.3.2-15-2016-00006] and EFOP-3.6.2-16-2017-00006, and by grants from the National Research, Development and Innovation Office [K115990, K115796 and PD128271] the Ministry of Human Capacities, Hungary [20391–3/2018/FEKUSTRAT] and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. HCEMM has received funding from the EU's Horizon 2020 research and innovation program under grant agreement No. 739593. V.D., A.F. and F.D.G. were supported by the New National Excellence Program of the Ministry of Human Capacities, respectively [UNKP-19-3-SZTE-47, UNKP-20-2–SZTE-60, UNKP-20-2–SZTE-61].

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. May 2004;27(5):1047–1053. https://doi.org/10.2337/diacare.27.5.1047.
2. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia*. Jan 2018;61(1):21–28. https://doi.org/10.1007/s00125-017-4390-4.
3. Devereux RB, Roman MJ, Paranicas M, et al. Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation*. May 16 2000;101(19):2271–2276. https://doi.org/10.1161/01.cir.101.19.2271.
4. Bnouham M, Merhfour FZ, Ziyyat A, Mekhfi H, Aziz M, Legssyer A. Antihyperglycemic activity of the aqueous extract of *Urtica dioica*. *Fitoterapia*. Dec 2003;74(7-8):677–681. https://doi.org/10.1016/s0367-326x(03)00182-5.
5. Das M, Sarma B, Khan A, et al. The antidiabetic and antilipidemic activity of aqueous extract of *Urtica dioica* L. on type2 diabetic model rats. *J Bio Sci*. 2009;17:1–6. https://doi.org/10.3329/jbs.v17i0.7092.
6. Fallah Huseini H, Fakhrzadeh H, Larijani B, Shikh Samani AH. Review of antidiabetic medicinal plant used in traditional medicine. مروری بر گیاهان دارویی مورد استفاده در بیماری دیابت. *jmpir*. 2006;5(17):1–8.
7. Moradi B, Abbaszadeh S, Shahsavari S, Alizadeh M, Beyranvand F. The most useful medicinal herbs to treat diabetes. *Biomedical Research and Therapy*. 2018;5(8):2538–2551. https://doi.org/10.15419/bmrat.v5i8.463.
8. Traditional MH. *Folk Remedies: A Comprehensive Herbal*. Century Publications; 1987.
9. S G, Z L-S. *A Bükki Füvesember Gyógynövényei [The Medicinal Plants Used by a Traditional Healer in the Bükk Mountains]*. Szabó György Magánkiadás [György Szabó Private Edition; 2008.
10. https://gyorgytea.hu/gyogynovenyek/tyukhur-stellariae-herba/. Accessed November 13, 2020. https://gyorgytea.hu/gyogynovenyek/tyukhur-stellariae-herba/.
11. https://www.herbahaz.hu/mecsek-tea-tyukhurfu-50g/?maganszemely=reg0. Accessed November 13, 2020. https://www.herbahaz.hu/mecsek-tea-tyukhurfu-50g/?maganszemely=reg0.
12. https://www.greencityshop.com/chickweed-for-diabetes-and-more/. Accessed November 13, 2020. https://www.greencityshop.com/chickweed-for-diabetes-and-more/.
13. Ahmad W, Ahmad M, Khan RA. *Stellaria media* attenuates the hyperglycemia and hyperlipidemia in alloxan-induced diabetic rat. *Bangladesh J Pharmacol*. 2019;14(2):80–86. https://doi.org/10.3329/bjp.v14i2.39847.
14. Szucs G, Soja A, Peter M, et al. Prediabetes induced by fructose-enriched diet influences cardiac lipidome and proteome and leads to deterioration of cardiac function prior to the development of excessive oxidative stress and cell damage. *Oxid Med Cell Longev*. 2019;2019:3218275. https://doi.org/10.1155/2019/3218275.
15. Wilson RD, Islam MS. Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. *Pharmacol Rep*. 2012;64(1):129–139. https://doi.org/10.1016/s1734-1140(12)70739-9.
16. Gomaa AA, Makboul RM, Al-Mokhtar MA, Nicola MA. Polyphenol-rich *Boswellia serrata* gum prevents cognitive impairment and insulin resistance of diabetic rats through inhibition of GSK3beta activity, oxidative stress and pro-inflammatory cytokines. *Biomed Pharmacother*. Jan 2019;109:281–292. https://doi.org/10.1016/j.biopha.2018.10.056.
17. Xiang X, Wang Z, Zhu Y, Bian L, Yang Y. [Dosage of streptozocin in inducing rat model of type 2 diabetes mellitus]. *Wei Sheng Yan Jiu*. Mar 2010;39(2):138–142.
18. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm. Mar*. 2016;7(2):27–31. https://doi.org/10.4103/0976-0105.177703.
19. Csont T, Viappiani S, Sawicka J, et al. The involvement of superoxide and iNOS-derived NO in cardiac dysfunction induced by pro-inflammatory cytokines. *J Mol Cell Cardiol*. Nov 2005;39(5):833–840. https://doi.org/10.1016/j.yjmcc.2005.07.010.
20. Pipicz M, Kocsis GF, Sarvary-Arantes L, et al. Low-dose endotoxin induces late preconditioning, increases peroxynitrite formation, and activates STAT3 in the rat heart. *Molecules*. Mar 8 2017;22(3). https://doi.org/10.3390/molecules22030433.
21. Csont T. Determination of serum insulin level by ELISA. *Practical Course: Basic Biochemical Methods and Ischemic Heart Models Hungary-Romania cross Border co-operation programme 2007-2013*. 2013:3–4, 01/01.
22. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of

phenolic compounds. *J Agric Food Chem*. Aug 2000;48(8):3597–3604. https://doi.org/10.1021/jf000220w.
23. Mielnik MB, Rzeszutek A, Triumf EC, Egelandsdal B. Antioxidant and other quality properties of reindeer muscle from two different Norwegian regions. *Meat Sci*. Dec 2011;89(4):526–532. https://doi.org/10.1016/j.meatsci.2011.05.021.
24. Fekete V, Murlasits Z, Aypar E, et al. Myocardial postconditioning is lost in vascular nitrate tolerance. *J Cardiovasc Pharmacol. Sep*. 2013;62(3):298–303. https://doi.org/10.1097/FJC.0b013e3182993ae0.
25. Gurusamy N, Watanabe K, Ma M, et al. Dominant negative 14-3-3 promotes cardiomyocyte apoptosis in early stage of type I diabetes mellitus through activation of JNK. *Biochem Biophys Res Commun*. Jul 30 2004;320(3):773–780. https://doi.org/10.1016/j.bbrc.2004.06.023.
26. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res*. Feb 16 2018;122(4):624–638. https://doi.org/10.1161/CIRCRESAHA.117.311586.
27. Kuethe F, Sigusch HH, Bornstein SR, Hilbig K, Kamvissi V, Figulla HR. Apoptosis in patients with dilated cardiomyopathy and diabetes: a feature of diabetic cardiomyopathy? *Horm Metab Res*. Sep 2007;39(9):672–676. https://doi.org/10.1055/s-2007-985823.
28. Li Y, Liu X, Fan Y, Yang B, Huang C. *Radix Stellariae* extract prevents high-fat-diet-induced obesity in C57BL/6 mice by accelerating energy metabolism. *PeerJ*. 2017;5, e3305. https://doi.org/10.7717/peerj.3305.
29. Chidrawar V, Patel K, Bothra S, Shiromwar S, Koli A, Kalyankar G. Anti-obesity effect of *Stellaria media* methanolic extract in the murine model of cafeteria diet induced obesity. Original Article. *International Journal of Nutrition, Pharmacology, Neurological Diseases*. May 1, 2012;2(2):121–131. https://doi.org/10.4103/2231-0738.95963, 2012.
30. Chidrawar VR, Patel KN, Sheth NR, Shiromwar SS, Trivedi P. Antiobesity effect of Stellaria media against drug induced obesity in Swiss albino mice. *Ayu*. 2011;32(4):576–584. https://doi.org/10.4103/0974-8520.96137.
31. Demjan V, Kiss T, Siska A, et al. Effect of *Stellaria media* tea on lipid profile in rats. *Evid Based Complement Alternat Med*. 2020;2020:5109328. https://doi.org/10.1155/2020/5109328.
32. Shabab S, Gholamnezhad Z, Mahmoudabady M. Protective effects of medicinal plant against diabetes induced cardiac disorder: a review. *J Ethnopharmacol*. 2021;265:113328. https://doi.org/10.1016/j.jep.2020.113328. Jan 30.
33. Lin YC, Thuy TD, Wang SY, Huang PL. Type 1 diabetes, cardiovascular complications and sesame (zhi ma). *J Tradit Complement Med. Jan*. 2014;4(1):36–41. https://doi.org/10.4103/2225-4110.124817.
34. Choudhury H, Pandey M, Hua CK, et al. An update on natural compounds in the remedy of diabetes mellitus: a systematic review. *J Tradit Complement Med*. Jul 2018;8(3):361–376. https://doi.org/10.1016/j.jtcme.2017.08.012.
35. Oladeji OS, Oyebamiji AK. *Stellaria media* (L.) Vill.- A plant with immense therapeutic potentials: phytochemistry and pharmacology. *Heliyon*. Jun 2020;6(6), e04150. https://doi.org/10.1016/j.heliyon.2020.e04150.
36. Rogowska M, Lenart M, Srečec S, Ziaja M, Parzonko A, Bazylko A. Chemical composition, antioxidative and enzyme inhibition activities of chickweed herb (*Stellaria media* L., Vill.) ethanolic and aqueous extracts. *Ind Crop Prod*. 2017;97:448–454. https://doi.org/10.1016/j.indcrop.2016.12.058, 2017/03/01.
37. Budzianowski J, Pakulski GC. O- and C-Glycosylflavones from *Stellaria media*. *Planta Med*. Dec 1988;54(6):576. https://doi.org/10.1055/s-2006-962585.
38. Vodoslavskyi VM. The quantitative content of the phenolic compounds in the *Stellaria media* herb. *The Pharma Innovation Journal*. 2017;6:174–175.
39. Guimaraes JF, Muzio BP, Rosa CM, et al. Rutin administration attenuates myocardial dysfunction in diabetic rats. *Cardiovasc Diabetol*. Jul 17 2015;14:90. https://doi.org/10.1186/s12933-015-0255-7.
40. Huang R, Shi Z, Chen L, Zhang Y, Li J, An Y. Rutin alleviates diabetic cardiomyopathy and improves cardiac function in diabetic ApoEknockout mice. *Eur J Pharmacol*. Nov 5 2017;814:151–160. https://doi.org/10.1016/j.ejphar.2017.08.023.
41. Krishna KM, Annapurna A, Gopal GS, et al. Partial reversal by rutin and quercetin of impaired cardiac function in streptozotocin-induced diabetic rats. *Can J Physiol Pharmacol*. Apr 2005;83(4):343–355. https://doi.org/10.1139/y05-009.
42. Wang YB, Ge ZM, Kang WQ, Lian ZX, Yao J, Zhou CY. Rutin alleviates diabetic cardiomyopathy in a rat model of type 2 diabetes. *Exp Ther Med*. Feb 2015;9(2):451–455. https://doi.org/10.3892/etm.2014.2090.
43. Panchal SK, Poudyal H, Arumugam TV, Brown L. Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. *J Nutr*. Jun 2011;141(6):1062–1069. https://doi.org/10.3945/jn.111.137877.
44. Colucci-D'Amato L, Cimaglia G. *Ruta graveolens* as a potential source of neuroactive compounds to promote and restore neural functions. *J Tradit Complement Med*. May 2020;10(3):309–314. https://doi.org/10.1016/j.jtcme.2020.05.002.
45. Nemeth K, Plumb GW, Berrin JG, et al. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*. Jan 2003;42(1):29–42. https://doi.org/10.1007/s00394-003-0397-3.
46. Liu HJ, Fan YL, Liao HH, et al. Apigenin alleviates STZ-induced diabetic cardiomyopathy. *Mol Cell Biochem*. Apr 2017;428(1-2):9–21. https://doi.org/10.1007/s11010-016-2913-9.
47. Mahajan UB, Chandrayan G, Patil CR, et al. The protective effect of apigenin on myocardial injury in diabetic rats mediating activation of the PPAR-gamma pathway. *Int J Mol Sci*. 2017;(4):18. https://doi.org/10.3390/ijms18040756. Apr 4.
48. Dai B, Cui M, Zhu M, Su WL, Qiu MC, Zhang H. STAT1/3 and ERK1/2 synergistically regulate cardiac fibrosis induced by high glucose. *Cell Physiol Biochem*. 2013;32(4):960–971. https://doi.org/10.1159/000354499.
49. Haghikia A, Ricke-Hoch M, Stapel B, Gorst I, Hilfiker-Kleiner D. STAT3, a key regulator of cell-to-cell communication in the heart. *Cardiovasc Res*. May 1 2014;102(2):281–289. https://doi.org/10.1093/cvr/cvu034.
50. Luo W, Huang L, Wang J, et al. Inhibition of EGFR-STAT3 attenuates cardiomyopathy in streptozotocin-induced type 1 diabetes. *J Endocrinol*. Sep 1 2019;242(3):199–210. https://doi.org/10.1530/JOE-19-0058.
51. Lo SH, Hsu CT, Niu HS, Niu CS, Cheng JT, Chen ZC. Ginsenoside Rh2 improves cardiac fibrosis via PPARdelta-STAT3 signaling in type 1-like diabetic rats. *Int J Mol Sci*. Jun 26 2017;18(7). https://doi.org/10.3390/ijms18071364.
52. Sun X, Chen RC, Yang ZH, et al. Taxifolin prevents diabetic cardiomyopathy in vivo and in vitro by inhibition of oxidative stress and cell apoptosis. *Food Chem Toxicol*. Jan 2014;63:221–232. https://doi.org/10.1016/j.fct.2013.11.013.
53. Wang L, Li J, Li D. Losartan reduces myocardial interstitial fibrosis in diabetic cardiomyopathy rats by inhibiting JAK/STAT signaling pathway. *Int J Clin Exp Pathol*. 2015;8(1):466–473.
54. Pipicz M, Demjan V, Sarkozy M, Csont T. Effects of cardiovascular risk factors on cardiac STAT3. *Int J Mol Sci*. Nov 12 2018;(11):19. https://doi.org/10.3390/ijms19113572.
55. Choi JS, Kang SW, Li J, et al. Blockade of oxidized LDL-triggered endothelial apoptosis by quercetin and rutin through differential signaling pathways involving JAK2. *J Agric Food Chem*. Mar 11 2009;57(5):2079–2086. https://doi.org/10.1021/jf803390m.
56. Choi KS, Kundu JK, Chun KS, Na HK, Surh YJ. Rutin inhibits UVB radiation-induced expression of COX-2 and iNOS in hairless mouse skin: p38 MAP kinase and JNK as potential targets. *Arch Biochem Biophys*. Oct 1 2014;559:38–45. https://doi.org/10.1016/j.abb.2014.05.016.
57. Ai XY, Qin Y, Liu HJ, et al. Apigenin inhibits colonic inflammation and tumorigenesis by suppressing STAT3-NF-kappaB signaling. *Oncotarget*. Nov 21 2017;8(59):100216–100226. https://doi.org/10.18632/oncotarget.22145.
58. Cao HH, Chu JH, Kwan HY, et al. Inhibition of the STAT3 signaling pathway contributes to apigenin-mediated anti-metastatic effect in melanoma. *Sci Rep*. Feb 25 2016;6:21731. https://doi.org/10.1038/srep21731.