

STUDY REGARDING THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF *SYRINGAE VULGARIS FLOS F. VIOLÁCEA* TINCTURE IN EXPERIMENTAL DIABETES

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Abstract

Recently, the interest in possible health benefits of medicinal plants as antioxidant and hypoglycaemic supplements has increased, due to their phytochemical compounds exhibiting potent antioxidant properties.

The aim of this study was to assess the *in vivo* antioxidant activity and antidiabetic potential of a tincture of *Syringae vulgaris flos f. violácea* (SV), properties less investigated for this plant. We evaluated its influence on glycemia and blood antioxidant enzymes activities in streptozotocin-induced diabetic in rats, compared to an ethanolic extract of *Myrtilli folium* (MF), already accepted for its antidiabetic and antioxidant properties.

Daily administration of ethanolic extracts of SV to diabetic rats reduced blood glucose levels compared to untreated diabetic animals. Blood activity for all antioxidant enzymes was improved in diabetic rats treated with SV extract and we didn't reported differences between the two tinctures tested.

The study provides a scientific validation for the use of *Syringae vulgaris flos f. violácea* tincture as an adjuvant in the early stages of diabetes in order to prevent its oxidative induced complications.

Rezumat

În ultimii ani a crescut interesul pentru posibilele beneficii terapeutice ale plantelor medicinale ca suplimente alimentare cu acțiune antioxidantă și hipoglicemiantă datorită constituenților lor fitochimici cu puternice proprietăți antioxidante.

Obiectivul studiului a fost evaluarea activității antioxidante *in vivo* și a potențialului antidiabetic ale tincturii de *Syringae vulgaris flos f. violácea* (SV), proprietăți mai puțin investigate pentru această plantă. A fost analizată influența acestei tincturi asupra glicemiei și activității enzimelor antioxidante asupra șobolanilor cu diabet indus cu

streptozotocină, în comparație cu un extract de *Myrtilli folium* (MF), plantă recunoscută ca având proprietăți antidiabetice și antioxidante.

Administrarea zilnică la șobolanii diabetici a tincturii de SV a determinat scăderea glicemiei în comparație cu animalele diabetice. Activitatea tuturor enzimelor antioxidante a fost îmbunătățită la șobolanii diabetici tratați cu tinctura de SV și nu au fost înregistrate diferențe semnificative între cele două tipuri de tincturi testate.

Acest studiu aduce dovezi științifice privind utilizarea tincturii de *Syringae vulgaris flos* f. *violacea* ca adjuvant în stadiile incipiente ale diabetului pentru a preveni complicațiile induse de stresul oxidativ.

Keywords: *Syringa vulgaris*, streptozotocin-induced diabetes, antioxidant activity, antidiabetic potential

Introduction

Diabetes mellitus, the most important metabolic disorder, is characterized by insufficiency of insulin secretion or action. These deficiencies determine an alteration of metabolic parameters such as hyperglycaemia and hyperlipaemia. Glucose oxidation is believed to be the main source of free radicals so diabetes is accompanied by increased production of reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide, or impaired antioxidant systems [1].

High levels of free radicals determine damage to proteins, fatty acids from membrane lipids and lipoproteins, and nucleic acids leading finally to cell death [2]. Increased oxidative stress is now accepted as an important factor in the development of diabetes and its complications [3]. Many authors indicate changes in oxidative stress markers such as superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione, catalase, lipid peroxides in diabetes.

In the last years it was registered an increase of interest in the therapeutic potential of medicinal plants as antioxidant and hypoglycaemic supplements, because these seem to be less toxic and to have negligible side effects [4-9]. Different plant organs such as leaves, flowers, roots, fruits can all be constituents of herbal medicines [10].

Syringa vulgaris L. (lilac) belongs to the *Oleaceae* family, different parts of this plant being edible for medicinal purposes. As an herbal remedy, literature states that lilac has been used in folk medicine to treat rheumatoid arthritis and gout due to its antiinflammatory and immunomodulatory properties, but it also holds other therapeutic virtues [11].

Therefore this study was designed to assess the antidiabetic and *in vivo* antioxidant potential of an ethanolic extract of *Syringae vulgaris flos* f. *violacea*, properties less investigated for this plant.

Materials and Methods

Chemicals. Solvents and reagents used in this study were all of analytical grade. Streptozotocin (STZ) and 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich GmbH (prod. no. S0130 and T5500). Evaluation of the antioxidant enzymes was made with kits purchased by Randox Labs., UK: Ransod (cat. no. SD125), Ransel (cat. no. RS504) and Glut Red (cat. no. GR2368). Haemoglobin was determined with a Randox kit, HG 1539. The absorbance of all samples was measured using a Beckman UV/VIS DU-65 spectrophotometer equipped with a peltier temperature controller (Beckman Instruments, Fullerton, USA). Blood glucose was measured using an Accu-Chek[®] InstantPlus glucometer (Roche Diagnostics).

Plant materials. *Syringae vulgaris flos f. violácea* and *Myrtilli folium* were harvested from the Botanical Garden of the University of Craiova, Romania. The collected plant material was shade dried at room temperature. The species were identified and the voucher specimens were deposited at the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, Romania. Dried flowers and leaves were powdered with an electric grinder. Tinctures were obtained by simple percolation in a ratio plant material/solvent (ethanol 70⁰) of 1:5 (FR X). Tinctures were divided into brown glass bottles, maintained tightly closed, protected from light, at room temperature.

Test animals. Experiments were performed on adult male Wistar rats (180-250g) provided with standard chow and water *ad libitum*. All studies were performed in accordance with the rules of scientific research bioethics of our University Committee for Research and Ethical Issues and in concordance with the guidelines of the IASP Committee for Research and Ethical Issues and with the Directive 86/609/EEC/24.11.1986, regarding the protection of animals for the maintenance and use of animals.

Toxicity study. Acute toxicity of *Syringae vulgaris flos f. violácea* was evaluated using a recognized protocol [12] by administration of different concentrations of tincture and examination of animals for any signs of behavioural changes or mortality for 48h after a single dose, respectively for two weeks of daily administration. These experiments did not reveal any toxic or lethal effects for our tinctures so these could be considered as non-toxics.

Study design. Diabetes was induced using STZ at a dose of 70 mg/kg b.w., injected i.p. to overnight fasted rats. Rats with fasting glucose levels >150 mg/dL were considered diabetics. Control rats were injected with physiologic saline solution 0.153 M. Rats were randomized into four groups

of five rats each as follows: Ist group (G1) – untreated control rats; IInd group (G2) – untreated diabetic rats; IIIrd group (G3) – diabetic rats treated daily with 150 mg/kg b.w., p.o., from a 20% tincture of *Syringae vulgaris flos f. violácea* (SV) for six weeks; IVth group (G4) – diabetic rats treated daily with 150 mg/kg b.w., p.o., from a 20% tincture of *Myrtilli folium* (MF) for six weeks. Blood glucose level and changes of body weight were evaluated each morning at the same hour. After six weeks animals were sacrificed, blood was collected and processed for plasma separation and erythrocytes hemolysis in order to assess some oxidative stress biomarkers (superoxide dismutase, glutathione peroxidase, glutathione reductase, lipid peroxides).

Assay of blood superoxide dismutase (SOD) activity. 0.5 mL of whole blood collected in vacutainers containing lithium heparinate was centrifuged for 10 minutes at 3000 rpm, plasma was aspirated and the cells were washed four times with physiologic saline solution and centrifuged after each wash. Erythrocytes were lysed by adding up to 2 mL cold redistilled water, vigorously vortex-mixed and left at 4°C for 15 minutes. The lysate was then diluted with 0.01M phosphate buffer solution pH 7 and used for the assay. SOD activity was measured by the rate of inhibition of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium transformation in a red formazan dye by the superoxide generated from the oxidation of xanthine with xanthine oxidase at 505 nm.

Assay of glutathione peroxidase (GPX) activity. 0.05 mL whole blood collected in heparinized vacutainers was diluted with 1 mL diluting agent and 1 mL haemoglobin reagent. Within 20 minutes of adding haemoglobin reagent, GPx activity was measured by the degree of absorbance at 340 nm after the oxidation of reduced glutathione (GSH) by cumene hydroperoxide catalysed by GPx and the reduction of oxidized glutathione (GSSG) with glutathione reductase and NADPH,H⁺. GPx activity was expressed as U/g Hb.

Assay of glutathione reductase (GR) activity. 0.5 mL of whole blood collected in heparinized vacutainers was centrifuged at 2000 rpm, plasma and buffy coat were aspirated and the cells were washed three times with physiologic saline solution with centrifugation after each wash. Cells were lysed by adding up to 0.5 ml cold redistilled water, vigorously vortex-mixed and left at 4°C for 10 minutes. The lysate was centrifuged and diluted with physiologic saline solution in a ratio 1:20 for GR assay. GR activity was measured from the decrease in absorbance at 340 nm after the oxidation of NADPH,H⁺ when GSSG is reduced to GSH. GR activity was expressed as U/g Hb.

Assay of malondialdehyde (MDA). Lipid peroxides were estimated in terms of thiobarbituric acid reactive substances (TBARS) using malondialdehyde as standard according to Buege and Aust method [13]. Plasma was treated with a mixture of TBA-TCA-HCl (thiobarbituric acid-trichloroacetic acid-chlorhydric acid) solution, boiled for 15 minutes and then centrifuged at 10000 g. The absorbance was read at 535 nm and MDA concentration was calculated using its extinction coefficient and expressed as mmol/L.

Statistical analysis. Values are presented as mean \pm SEM. Results obtained were compared with the t-test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Effect on blood glucose levels. As one can observe in Table I, blood glucose levels were significantly increased in diabetic rats compared to control and groups treated daily with tinctures for six weeks. Regarding the hypoglycaemic effect of SV tincture, this was comparable with that of MF tincture, a vegetal product well known for its beneficial effects on diabetes mellitus.

Table I.

Effect of tinctures on blood glucose level after one week and respectively six weeks of treatment

Group	Blood glucose level [mg/dL]	
	1 st week	After 6 weeks
Control untreated – G1	95.2 \pm 8.8	98.3 \pm 7.5
Control diabetic – G2	204.5 \pm 35.3*	278.8 \pm 51.3*
Diabetic treated SV – G3	196.3 \pm 31.2*	231.4 \pm 12.8* [#]
Diabetic treated MF – G4	212.6 \pm 26.1*	118.2 \pm 23.1** [#]

* $p < 0.001$; ** $p < 0.05$ compared with the untreated control group; [#] $p < 0.001$ compared with the untreated diabetic group.

The effects on blood levels of oxidative stress biomarkers in different groups at the end of the experiment are summarized in Table II.

Table II.

Blood levels of oxidative stress biomarkers after six weeks of treatment

Group	SOD [U/mL]	GPx [U/g Hb]	GR [U/g Hb]	MDA [mmol/L]
Control untreated – G1	153.2 \pm 10.7	39.5 \pm 2.54	12.8 \pm 1.02	1.30 \pm 0.15
Control diabetic – G2	117.1 \pm 15.1*	14.07 \pm 2.15*	9.2 \pm 1.4*	3.50 \pm 0.39*
Diabetic treated SV – G3	130.2 \pm 10.5* [#]	35.5 \pm 3.83** ^Δ	10.5 \pm 1.1* [#]	2.72 \pm 0.20* [#]
Diabetic treated MF – G4	135.7 \pm 12.1*** [#]	36.2 \pm 2.34** ^Δ	10.8 \pm 1.72*** [#]	1.90 \pm 0.17* ^Δ

* $p < 0.001$; ** $p < 0.05$; *** $p < 0.005$ compared with the untreated control group; ^Δ $p < 0.001$; [#] $p < 0.05$ compared with the untreated diabetic group.

We noted a significant increase of blood SOD activity level in diabetic rats treated with SV and MF tinctures ($p < 0.05$) compared with the untreated diabetic animals (Figure 1).

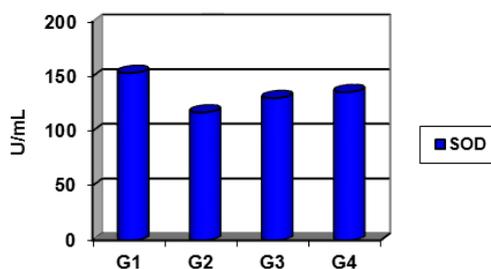


Figure 1.

Level of superoxide dismutase (SOD) activity after six weeks of treatment (G1-control untreated; G2-control diabetic; G3-diabetic treated with SV tincture; G4-diabetic treated with MF tincture)

The treatment of diabetic rats with SV and MF tinctures had a very high significant influence on GPx activity ($p < 0.001$) compared with untreated diabetic rats (Figure 2). Regarding GR, we noted that SV and MF tinctures significantly increased its activity in diabetic rats treated ($p < 0.05$), compared with untreated diabetic controls (Figure 2).

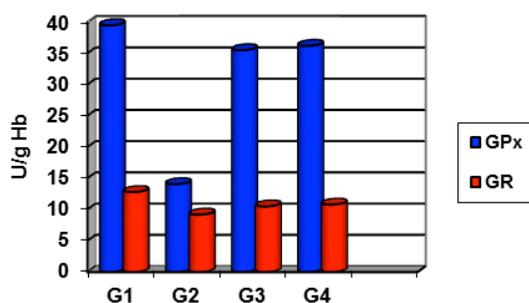


Figure 2.

Levels of glutathione peroxidase (GPx) and glutathione reductase (GR) activities after six weeks of treatment (G1-control untreated; G2-control diabetic; G3-diabetic treated with SV tincture; G4-diabetic treated with MF tincture)

Plasma MDA levels (used to evaluate lipid peroxidation) decreased significantly in diabetic rats treated with SV and MF tinctures compared with untreated diabetic rats ($p < 0.001$) (Figure 3).

Comparing the effects of the two tinctures tested, we noted that the differences between the levels of all blood oxidative stress biomarkers were not statistically significant.

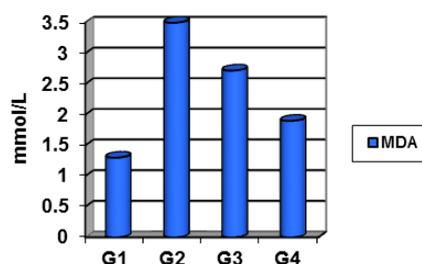


Figure 3.

Plasma malondialdehyde (MDA) concentration after six weeks of treatment (G1-control untreated; G2-control diabetic; G3-diabetic treated with SV tincture; G4-diabetic treated with MF tincture)

This study was designed to assess the hypoglycaemic and antioxidant effects of SV extracts in STZ-induced diabetes.

In this context, a plethora of plants have been screened by several research teams [4, 5, 14, 15]. Researches on diabetogenic response to STZ after single intraperitoneal injection revealed that STZ causes in adult Wistar rats degeneration of pancreatic β -cells inducing experimental diabetes in several days confirmed by measuring blood glucose levels [16, 17].

Studies carried out on STZ-induced diabetes in rats demonstrated that treatment with *Vaccinium myrtillus* concentrate had a regenerative effect on pancreatic β -cells and a hypoglycaemic effect [18]. In our study, the daily administration of the ethanolic extracts of SV and MF to diabetic rats reduced blood glucose levels compared to untreated diabetic animals. Lower blood glucose levels may be due to a regenerative effect that certain compounds from the extracts could have on pancreatic β -cells. The therapeutic efficiency of bilberry is attributed to the various flavonoids with antioxidant activities [19, 20]. Similar studies proved the antidiabetic effect of various vegetal extracts by the improvement of C-peptide and insulin levels or the rejuvenation of pancreatic cells from Langerhans islets [6, 18, 21].

Glucose oxidation is considered the main source of oxygen free radicals in diabetes: first, it is oxidized to reactive ketoaldehydes and superoxide anion radicals; then the superoxide anion undergoes dismutation to hydrogen peroxide and, if not destroyed by antioxidant systems, it could generate extremely reactive hydroxyl radicals [22]. *In vitro* and *in vivo*

studies revealed stimulation of hydrogen peroxide production in pancreatic β -cells in diabetes mellitus.

Maintenance of the antioxidant status has a major role in pancreatic β -cells survival and in preservation of islet secretory capacity. Antioxidant enzymes are the main scavengers of free radicals and under oxidative stress they may act as compensatory mechanisms by increasing their activity [1].

SOD and GPx are able to neutralize ROS and they are playing a significant role in body's antioxidant defence systems. SOD accelerates superoxide anion dismutation to hydrogen peroxide that is removed by GPx and CAT. GR catalyses the reduction of GSSG to GSH in the presence of NADPH/H⁺. GSH has a major role in neutralizing free radicals of oxygen, this action being of great importance, especially for the pancreatic β -cells.

These antioxidant enzymes are interconnected and a decrease of their activity causes lipid peroxides accumulation and oxidative stress intensification [1].

For the activities of SOD, GPx and GR there were reported significant decreases in diabetic rats compared to untreated controls. Decreased activity of these enzymes could be due to excessive production of oxygen free radicals which consume the antioxidant potential.

The increase of lipid peroxidation revealed by higher levels of MDA after STZ-induced diabetes sustain either an intensive production of ROS or a decrease in the activity of antioxidant defence systems.

The research revealed that oral administration of SV tincture in diabetic rats increased SOD, GPx and GR activities and alleviated lipid peroxidation as confirmed by diminished MDA levels. These findings suggest the protective role of SV tincture which may be due to the antioxidant action of flavonoids and polyphenols from plant products that act by neutralizing ROS [19, 20].

The ability of SV tincture to improve the imbalance between ROS generation and antioxidant enzymes scavenging ability was comparable to MF tincture.

The antioxidant activity was already proven for *Vaccinium myrtillus* [19, 23]. *Vaccinium myrtillus* (bilberry) is part of the *Ericaceae* family; both leaves and fruits are used for medicinal purposes. The hypoglycaemic action of bilberry is assigned to myrtillines; it seems to cause slight β -cells hypergenesis correlated with decreased blood glucose levels [24].

Pharmacological properties of lilac extracts (obtained from leaves, fruits or bark) that make them useful in the treatment of inflammatory (rheumatoid arthritis, gout, rheumatism) and cardiovascular disorders (coronary atherosclerosis, pectoris angina, cardiac arrhythmias) could be

related to several phytopharmaceutical compounds such as flavonosides, iridosides, lignans, polyphenol acids, etc. [25-29]. The medical use of *Syringae vulgaris extracts* tended to be limited by the idea of a potential toxicity, but our preliminary studies carried out in female rats showed their non-toxic nature.

Conclusions

The present research revealed a significant antioxidant effect of SV tincture by increasing SOD, GPx and GR activities and alleviating the lipid peroxides level.

This study provides a scientific validation for the use of *Syringae vulgaris flos f. violácea* tincture as an adjuvant in the early stages of diabetes in order to prevent its complications. Moreover, it could be taken into consideration the isolation of active compounds to obtain potent antidiabetic drugs, eventually in combination with other herbal remedies with hypoglycaemic and antioxidant effects.

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