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Dose-dependent alterations in the biomarkers of lipid and protein oxidation in the blood of patients with type 2 diabetes mellitus after *in vitro* incubation with extracts of *Chelidonium majus* L.

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The role of oxidative stress in the occurrence and development of diabetes mellitus is both critical and pivotal. Several molecular event cascades in different metabolic pathways such as glycolytic, hexosamine, protein kinase C, polyol and advanced glycation end-product pathways have been identified as pro-oxidative processes and are usually up-regulated in diabetics. Consistent with our previous studies, we continue to evaluate the antioxidant potential of great celandine (*Chelidonium majus* L., CM), a representative of the Papaveraceae family, collected from northern parts of Poland using the blood samples of patients with type 2 diabetes mellitus. Therefore, in the present study, oxidative stress biomarkers (2-thiobarbituric acid reactive substances (TBARS), aldehydic and ketonic derivatives of oxidative modification of proteins (OMP)) were used to evaluate the antioxidant properties of the stalk and root extracts of CM in final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1} and 0.63 mg.mL^{-1}. Plant materials were collected from natural habitats on the territory of the Kartuzy district in the Pomeranian province (northern part of Poland). The use of extracts derived from both roots and stalks of CM collected from both urban and rural agglomerations in final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} resulted in a significant enhancement of lipid peroxidation in the blood samples. On the contrary, only incubation of blood samples with stalk extracts of CM collected from urban areas at a final dose of 0.63 mg.mL^{-1} resulted in a no-significant decrease in TBARS levels contributing to the protection of lipid structures in membranes. Similar results were obtained by analyzing levels of aldehydic derivatives of oxidatively modified proteins in the blood samples after *in vitro* incubation with the extracts, where final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} significantly increased the oxidation process in protein structures. Analysis of levels of ketonic derivatives of oxidatively modified proteins showed that the use of root extracts of CM collected from urban agglomerations in final doses of 2.5 and 1.25 mg.mL^{-1} reduced levels of oxidatively modified proteins, while the use of stalk extracts of CM harvested from urban agglomerations in a final dose of 0.63 mg.mL^{-1} statistically significantly reduced levels of ketonic derivatives of oxidatively modified proteins compared to the control samples. These *in vitro* studies indicate that extracts derived from this plant are a significant source of natural metabolites that could be cytotoxic in final doses of 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, and 1.25 mg.mL^{-1} to the blood of patients with T2DM. Only a final dose of 0.63 mg.mL^{-1} no significantly changed levels of lipid and protein oxidation in the blood samples.

Keywords: great celandine, root and stalk extracts, blood samples, lipid peroxidation, oxidatively modified proteins, type 2 diabetes mellitus

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Introduction

Diabetes mellitus (DM) is a chronic endocrine and metabolic disorder which is underlined by insulin deficiency or insulin insensitivity or both, and characterized by hyperglycemia and vascular complications (micro and macro). Several pathogenic processes are involved in the development of DM. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is the deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of hyperglycemia. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or nonketotic hyperosmolar syndrome. Diabetic complications result in considerable morbidity and mortality leading to major healthcare delivery costs (*Diabetes Care*, 2011). Although there are several studies to elucidate the molecular mechanisms underlying the development of DM complications, their precise pathophysiology is not completely understood (Forbes and Cooper, 2013).

Similar to several other health conditions such as cancer and neurodegenerative disorders, oxidative stress has been widely linked with the incidence of DM. Several studies have shown that oxidative stress is a key element in the development and progression of DM and its associated complications. Free radicals are active biomolecules that are physiologically generated during metabolic pathways and/or by immune cells (Yaribeygi et al., 2019, 2020). Free radicals have physiological roles in many molecular pathways including those of cellular signalling, synaptic plasticity, memory formation, defence against invader pathogens, cell-cell interactions, cell growth, autophagy, apoptotic processes, and ageing. When free radical generation increases above the physiological range, it overcomes the antioxidant mechanisms of cells and results in oxidative stress. Free radicals are active derivatives of either the oxygen molecule such as reactive oxygen species (ROS: hydroperoxyl, superoxide, hydrogen peroxide, and hydroxyl radicals) and nitrogen molecules such as the reactive nitrogen species (RNS) such as peroxynitrite. Oxidative stress occurs when there is a distortion in the redox balance of the cell, causing damage to membranes and vital biomolecules such as DNA, proteins, and lipids. Oxidative stress has been shown to compromise the two major mechanisms failing during DM which are insulin secretion and insulin action (Giacco and Brownlee, 2010; Angelova et al., 2018).

Antioxidants can act as chain breakers, scavenging chain-initiating radicals like hydroxyl, alkoxyl, or peroxyl, quenching singlet oxygen, decomposing hydroperoxides, and chelating prooxidative metal ions (Scalbert et al., 2005). Epidemiological studies confirm that the incidence of oxidative stress-related conditions is lowered by the use of medical plants rich in compounds possessing high antioxidant activity (Pisoschi et al., 2016). Plants containing antioxidants and antioxidant nutrients play an important role in the prevention of many disorders and diseases. Recent scientific reports show that plants of the Papaveraceae family contain some metabolites possessing antioxidative properties such as alkaloids, polyphenols, and tannins. *Chelidonium majus* L. (CM) (Papaveraceae family), or greater celandine, is an important plant in western phytotherapy and traditional Chinese medicine (Nawrot et al., 2021). Crude extracts of CM as well as purified compounds derived from it exhibit a broad spectrum of biological activities (antioxidant, anti-inflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective, etc.) that support some of the traditional uses of CM. However, herbal medicine also claims that this plant has several important properties which have not yet been scientifically studied. This species is known to produce a broad range of secondary metabolites, ensuring its therapeutic properties (Zielinska et al., 2018). The main constituents of CM responsible for biological properties are isoquinoline alkaloids such as chelidonine, chelerythrine, sanguinarine, coptisine, berberine, allocryptopine, and protopine. They are reported to have anti-inflammatory, antimicrobial, antibacterial, antiviral, immunomodulatory, anticancer, choleretic, hepatoprotective, and analgesic properties (Zielinska et al., 2018). Celandine raw materials exhibited significant differences in the composition of alkaloids and other antioxidant substances in different parts of plants (Seidler-Łożykowska et al., 2016; Krizhanovska et al., 2021).

This study is a continuation of our previous investigations aimed at the assessment of the antioxidative properties of CM using different

cell models. The aim of this study was evaluation the changes in the biomarkers of oxidative stress (2-thiobarbituric acid reactive substances, carbonyl derivatives of oxidative modification of proteins) in the blood samples collected from T2DM patients *in vitro* exposed to different doses of extracts derived from roots and stalks of CM. These plants were collected in urban and rural agglomerations of the Kartuzy district in the Pomeranian province (northern part of Poland).

Material and methodology

Collection of plant materials

Plant materials (*Chelidonium majus* L.) were harvested from natural habitats on the territory of the Kartuzy district (54° 20′ N 18° 12′ E) in the Pomeranian province (northern part of Poland) (Figure 1). The plant collection covered the period from June to July 2020. For our studies, we collected CM plants in phases beginning with flowering (flower buds visible) and full flowering (yellow flowers blooming, young fruits small and developing). Kartuzy is located about 32 kilometres (20 miles) west of Gdańsk and 35 km (22 miles) south-east of the town of Lębork on a plateau at an altitude of approximately 200 meters (656 feet) above sea level on average. The plateau, which is divided by the Radaune lake, comprises the highest parts of the Baltic Sea Plate (http://www.kartuzy.pl/). Plants were collected from urban (n = 5) and rural agglomerations (n = 15) on the territory of the Kartuzy district.

Preparation of plant extracts

Freshly collected roots and stalks were washed, weighed, crushed, and homogenized in 0.1M phosphate buffer (pH 7.4) (in proportion 1 : 19, w/w) at room temperature. The extracts were then filtered and used for analysis. The obtained extracts were stored at -20 °C until use.

Patients with diabetes mellitus type 2 (T2DM) and collection of blood samples

A total of 7 patients with T2DM between 42 and 68 years old were studied. The participants of the study were recruited among patients of non-public Health Care Center U & O Zdrowie – Home-based long-term care (Lębork, Poland). A detailed medical history was taken, and a physical examination was performed on all participants. The Research Ethics Committee of the Regional Medical Commission in Gdańsk (Poland) approved the current study (KB-31/18; KB-21/19). All patients provided written informed consent before the start of the study procedures. Participants included in the current study were selected according to the following criteria: first, they were diagnosed with type 2 diabetes mellitus patients; second, they were free of

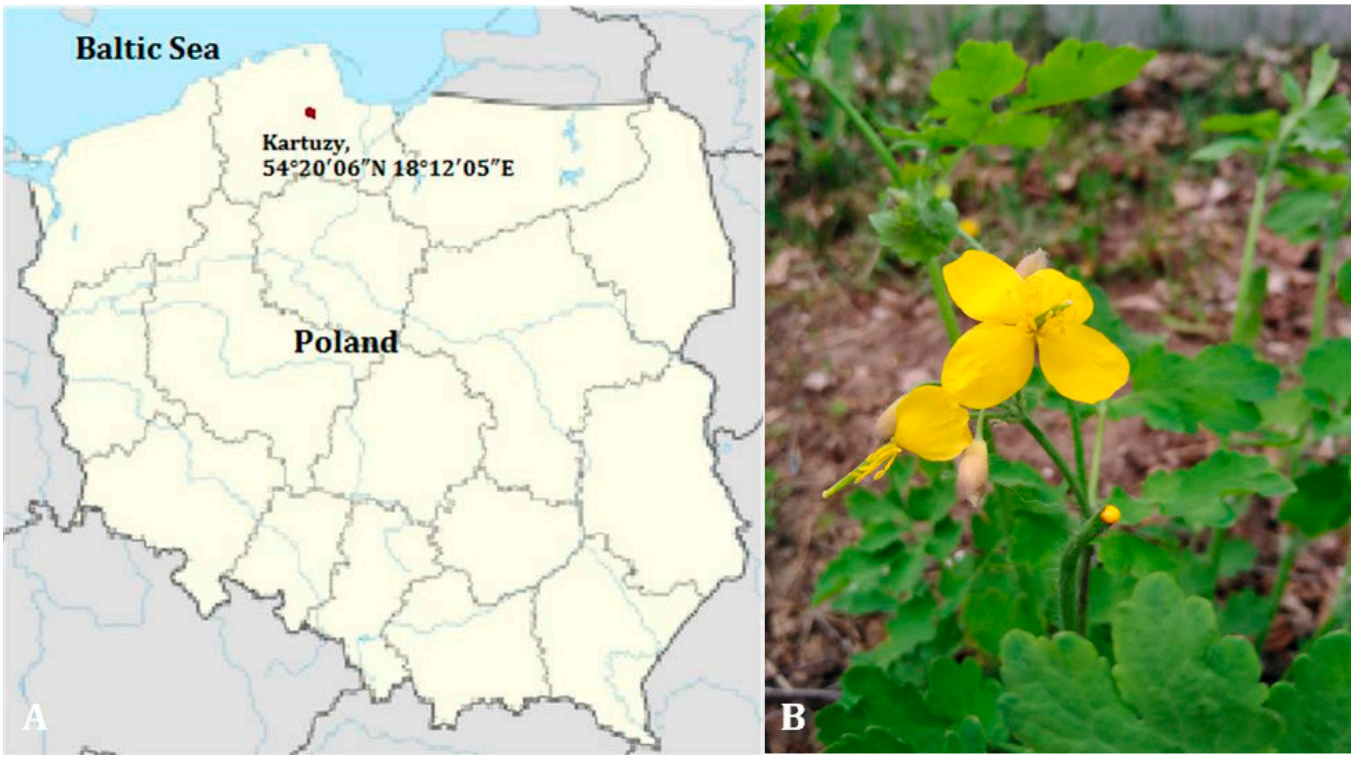

Figure 1 Location of Kartuzy in the map of Poland (A), where the greater celandine (B) was collected

any ailment which could affect the parameters under study. Hemolytic anaemia, haemoglobin variants, hepatic disease, and infectious diseases, such as tuberculosis and sarcoidosis, were excluded from the study.

Blood samples were collected into commercial tubes after overnight fasting for the analysis of laboratory parameters. Venous blood samples (25 ml) were obtained from the capital vein of each participant using sterile disposable plastic syringes. Specimens were collected at the same standardized time to minimize any effect of diurnal variation. The blood samples in the tubes were left to clot and the serum was separated by centrifugation. The clear, non-hemolyzed supernatant sera were separated using clean, dry disposable plastic syringes. Blood samples were stored at +4 °C and used within 2 days for the analysis of biomarkers of oxidative stress.

The 2-Thiobarbituric acid reactive substances (TBARS) assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration. This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a coloured adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives of protein oxidative modification (OMP) assay

To evaluate the protective effects of extracts derived from roots and stalks of CM collected in urban and rural agglomerations against free radical-induced protein damage in blood samples, a content of carbonyl derivatives of protein oxidative modification (OMP) based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the blood was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine et al. (1990) and as modified by Dubinina et al. (1995). DNFH was used for determining the contents of carbonyl groups in soluble and insoluble proteins. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehyde derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Statistical analysis

The mean ± S.E.M. values were calculated for each group to determine the significance of the intergroup difference. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the levels of oxidative stress biomarkers (significance level, $p < 0.05$) was examined using the Kruskal–Wallis test by ranks (Zar, 1999). All statistical calculation was performed on separate data from each individual with Statistica 13.3 software (TIBCO Software Inc., Krakow, Poland).

Results and discussion

Figure 2 presents the values of TBARS levels obtained by incubating blood samples collected from patients with T2DM in the presence of aqueous extracts derived from roots and stalks of CM collected from rural and urban agglomerations. The final concentrations of extracts in the blood samples were 5 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, 1.25 $mg.mL^{-1}$, and 0.63 $mg.mL^{-1}$.

Analyzing the final dose of CM extracts at 5 $mg.mL^{-1}$, we observed a statistically significant increase in TBARS level by 18.7% ($p < 0.05$) for root extracts of CM collected from urban areas (52.97 ±5.14 $nmol.mL^{-1}$) and by 35.1% ($p < 0.05$) for stalk extracts of CM collected from urban areas (60.31 ±3.77 $nmol.mL^{-1}$) compared to the untreated control samples (44.63 ±0.91 $nmol.mL^{-1}$). We obtained similar results after *in vitro* incubation of the blood samples with stalk extracts of CM collected from rural agglomerations at a dose of 5 $mg.mL^{-1}$, where we also recorded a statistically significant increase in TBARS levels by 17.2% ($p < 0.05$) compared to the control samples (52.31 ±3.98 $nmol.mL^{-1}$ vs. 44.63 ±0.91 $nmol.mL^{-1}$). We noted different trends after *in vitro* incubation of blood samples with root extracts of CM (at a final dose of 5 $mg.mL^{-1}$) collected from rural areas, where there was a non-statistically significant reduction (by 10%, $p > 0.05$) in the concentration of TBARS compared to the control samples (40.15 ±4.42 $nmol.mL^{-1}$ vs. 44.63 ±0.91 $nmol.mL^{-1}$).

By lowering the final dose of extracts to 2.5 $mg.mL^{-1}$, we observed the highest statistically significant increase in TBARS levels by 73.5% ($p < 0.05$) compared to the control samples using extracts derived from the roots of CM collected from urban areas (77.44 ±3.98 $nmol.mL^{-1}$ vs. 44.63 ±0.91 $nmol.mL^{-1}$). Using stalk extracts of CM

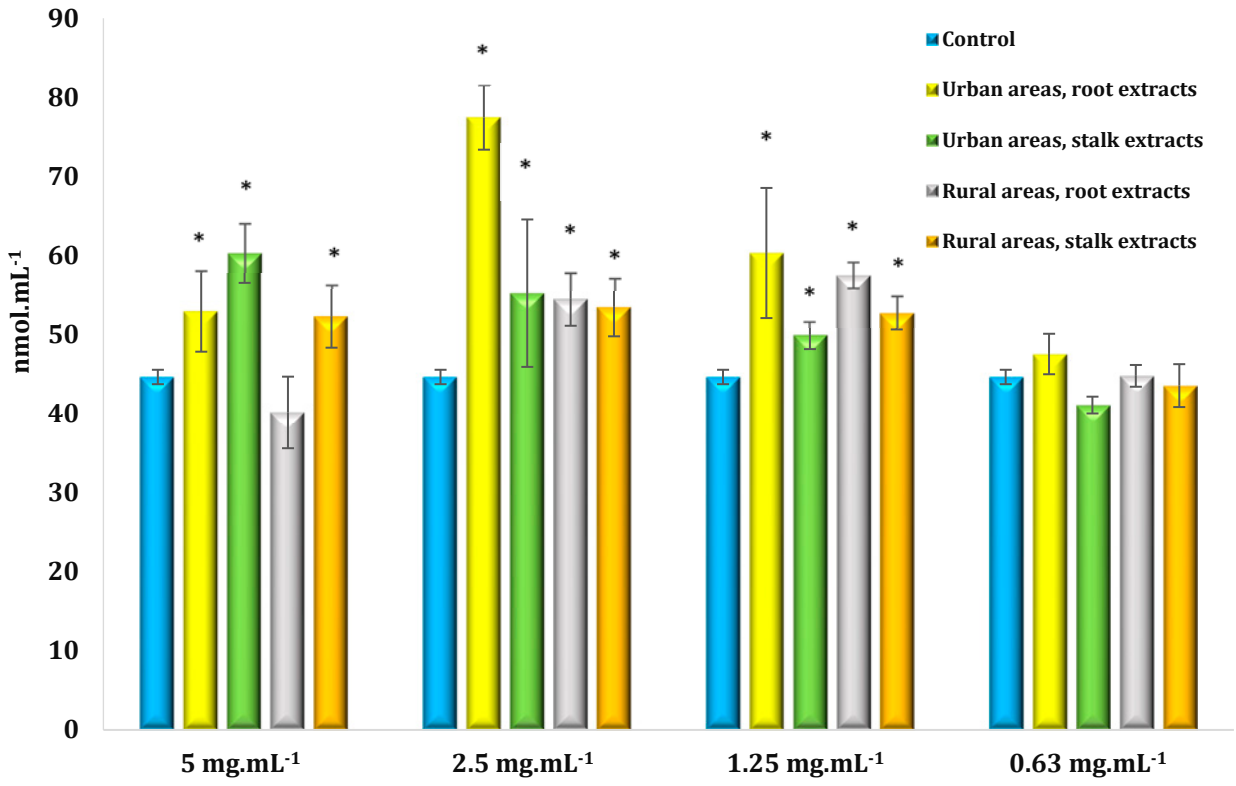

Figure 2 The TBARS content (nmol.mL^{-1}) as a biomarker of lipid peroxidation in the blood samples obtained from patients with type 2 diabetes mellitus after *in vitro* incubation with root and stalk extracts derived from *Chelidonium majus* L. collected from rural and urban areas of the Pomeranian region (M ±m, n = 8). The final concentrations of extracts in the blood samples were 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, and 0.63 mg.mL^{-1}
*– statistically significant differences ($p < 0.05$) compared to the control samples

collected from urban areas (at a final dose of 2.5 mg.mL^{-1}) also resulted in a statistically significant increase in TBARS content by 23.8% ($p < 0.05$) compared to the control samples (55.23 ±9.23 nmol.mL^{-1} vs. 44.63 ±0.91 nmol.mL^{-1}). Similar results were obtained after incubating blood samples with extracts (at a final dose of 2.5 mg.mL^{-1}) derived from both roots (54.51 ±3.39 nmol.mL^{-1}) and stalks (53.49 ±3.72 nmol.mL^{-1}) of CM harvested from rural areas, where there was also a statistically significant increase in TBARS levels (by 22.1%, $p < 0.05$ and 19.9%, $p < 0.05$, respectively) compared to the control samples (44.63 ±0.91 nmol.mL^{-1}).

Similar results were obtained after lowering the final dose of the extracts used *in vitro* to 1.25 mg.mL^{-1}, where we also recorded a statistically significant increase in TBARS levels in the blood samples of diabetic subjects by 35.1% ($p < 0.05$) for root extracts of CM collected from urban areas (60.31 ±8.23 nmol.mL^{-1}) and by 11.9% ($p < 0.05$) for stalk extracts of CM collected from urban areas (49.95 ±1.79 nmol.mL^{-1}) compared to the control samples (44.63 ±0.91 nmol.mL^{-1}). After incubating the blood samples with extracts (at a final dose of 1.25 mg.mL^{-1}) derived from both roots (57.59 ±1.6 nmol.mL^{-1}) and stalks (52.82 ±2.17 nmol.mL^{-1}) of CM harvested from rural areas, we recorded statistically significant increases in TBARS levels by 29% ($p < 0.05$) and 18.4% ($p < 0.05$), respectively.

Similar but statistically no-significant increases in TBARS levels were obtained after incubating *in vitro* the blood samples with extracts (at a final dose of 0.63 mg.mL^{-1}) derived from roots of CM collected from both urban (47.54 ±2.56 nmol.mL^{-1} vs. 44.77 ±1.38 nmol.mL^{-1}) and rural areas (by 6.5%, $p > 0.05$ and 0.3% $p > 0.05$, respectively), compared to control samples. We noted different trends after using stalk extracts (at a final dose of 0.63 mg.mL^{-1}) of CM collected from urban (41.08 ±1.06 nmol.mL^{-1}) and rural (43.54 ±2.71 nmol.mL^{-1}) areas, where we recorded a reduction in TBARS levels compared to the control samples (44.63 ±0.91 nmol.mL^{-1}) (by 8% $p > 0.05$ and 2.4%, $p > 0.05$, respectively) (Figure 2).

The aldehydic and ketonic derivatives of oxidatively modified proteins in the blood samples of patients with T2DM after *in vitro* incubation with root and stalk

extracts derived from CM collected from rural and urban areas of the Pomeranian region were present in Figure 3.

Analyzing the results of levels of protein oxidation, we observed a statistically significant increase in the concentration of aldehydic derivatives of OMP after incubating the blood samples with extracts (at a final dose of 5 mg.mL^{-1}) derived from both roots (17.67 ±2.05 nmol.mL^{-1}) and stems (17.6 ±1.26 nmol.mL^{-1}) of CM collected from an urban agglomeration compared to the control samples (14.68 ±0.4 nmol.mL^{-1}). There was a statistically significant increase in levels of aldehydic derivatives of OMP by 20% ($p < 0.05$) and 19.9% ($p < 0.05$), respectively. We obtained similar results after *in vitro* incubation of the blood samples with root extracts of CM collected from rural areas, where we also observed a statistically significant increase in the levels of aldehydic derivatives of OMP by 56% ($p < 0.05$) compared to the control samples

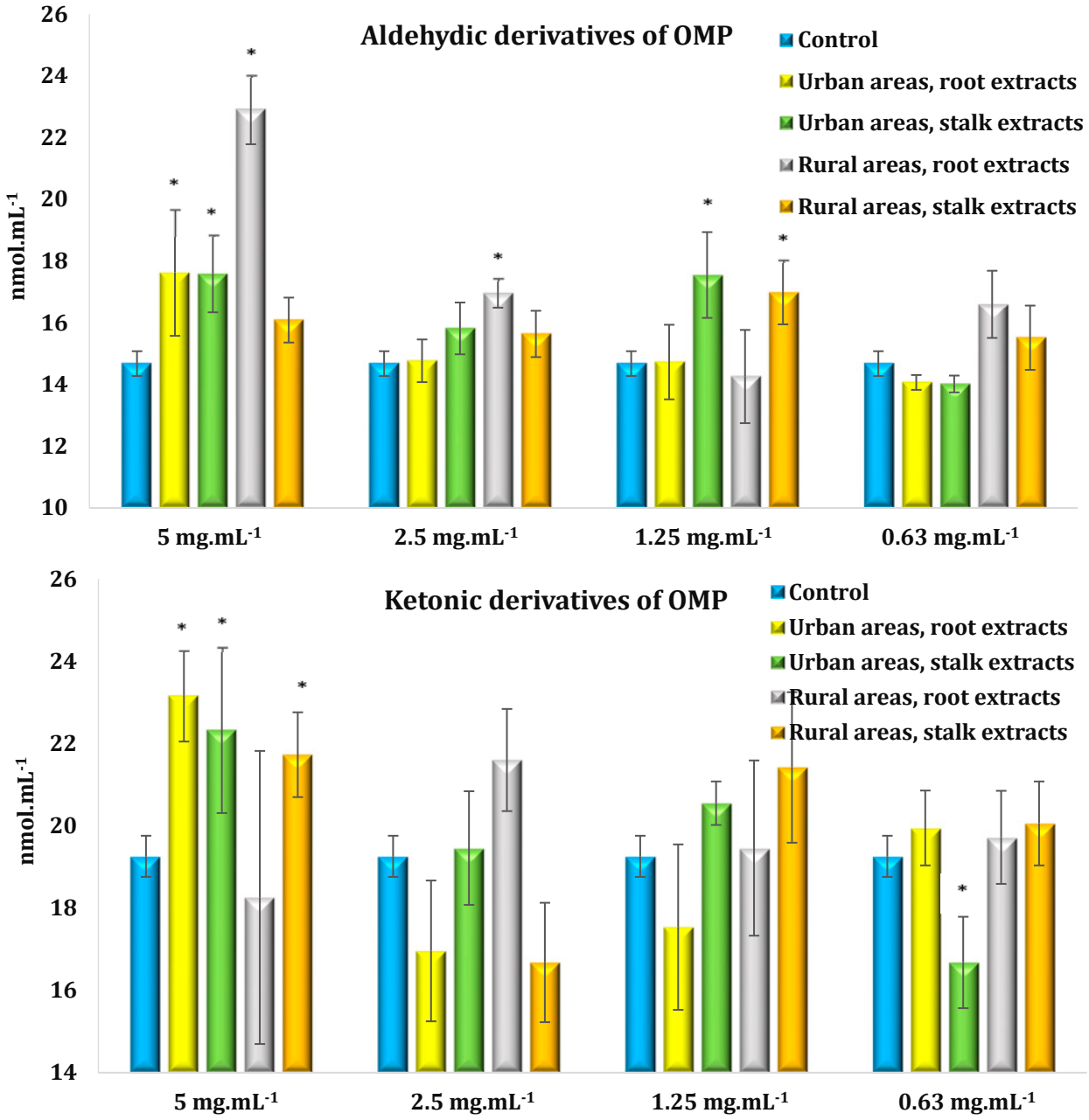

Figure 3 The aldehydic and ketonic derivatives of oxidatively modified proteins (nmol.mL^{-1}) in the blood samples of patients with type 2 diabetes mellitus after *in vitro* incubation with root and stalk extracts derived from *Chelidonium majus* L. collected from rural and urban areas of Pomeranian region (M ±m, n = 8). The final concentrations of extracts in the blood samples were 5 mg.mL^{-1}, 2.5 mg.mL^{-1}, 1.25 mg.mL^{-1}, and 0.63 mg.mL^{-1}
*– statistically significant differences ($p < 0.05$) compared to the control samples

(22.92 ±1.13 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}). On the other hand, no statistically significant increase in the levels of aldehydic derivatives of OMP (by 9.6%, p >0.05) was observed after incubating the blood samples with stalk extracts of CM collected from rural areas compared to the control samples (16.09 ±0.73 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}).

By lowering the dose of both root and stalk extracts from CM collected from urban areas to a value of 2.5 mg.mL^{-1} and incubating them with the blood samples, we also observed an increase in the level of aldehydic derivatives of oxidatively modified proteins compared to the control (14.77 ±0.69 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for root extracts; 15.82 ±0.84 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for stalk extracts). There was a statistically insignificant increase of 0.6% (p >0.05) and 7.8% (p >0.05), respectively. *In vitro* application of stalk extracts of CM collected from rural agglomerations to blood samples also resulted in a 6.5% (p >0.05) increase in levels of aldehydic derivatives compared to the control samples (15.64 ±0.75 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}). Only incubation of root extracts of CM collected from rural areas (at a final dose of 2.5 mg.mL^{-1}) with blood samples resulted in a statistically significant elevation in levels of aldehydic derivatives of OMP by 15.6% (p <0.05) compared to the control samples (16.97 ±0.48 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}).

We observed a statistically significant elevation in levels of aldehydic derivatives of OMP after *in vitro* incubation with stalk extracts of CM collected from both urban and rural areas with the blood samples (at a final dose of 1.25 mg.mL^{-1}) compared to the control samples (17.75 ±1.39 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} after using stalk extracts of CM collected from urban areas; 17 ±1.05 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} after using stalk extracts of CM collected from rural areas). This increase was by 19.6% (p <0.05) and 15.8% (p <0.05), respectively. After incubating the blood samples with root extracts of CM collected from urban areas (at a final dose of 1.25 mg.mL^{-1}), we observed a statistically no-significant increase in levels of aldehydic derivatives of OMP by 0.3% (p >0.05) compared to the control samples (14.73 ±1.21 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}). We observed different trends after *in vitro* incubation of blood samples with root extracts of CM collected from rural areas (at a final dose of 1.25 mg.mL^{-1}), where there was a statistically no-significant decrease in levels of aldehydic derivatives of OMP by 2.9% (p >0.05) compared to the control samples (14.26 ±1.51 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1}).

The application of CM extracts at a final dose of 0.63 mg.mL^{-1} resulted in statistically no-significant changes in levels of aldehydic derivatives of OMP compared to the untreated control samples. We observed a decrease in levels of aldehydic derivatives of OMP after *in vitro* incubation of blood samples with extracts derived from both roots and stems of CM harvested from urban areas compared to the control samples (14.07 ±0.24 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for root extracts; 14.02 ±0.27 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for stalk extracts). This decrease was 4.2% (p >0.05) and 4.5% (p >0.05), respectively. Other results were obtained after incubation of blood samples with extracts derived from both roots and stalks of CM collected from rural agglomerations, where there was an increase in levels of aldehydic derivatives of OMP (by 13.1%, p >0.05 and 5.7%, p >0.05) compared to the control samples (16.6 ±1.09 nmol.mL^{-1} vs. 14.68 ±0.4 nmol.mL^{-1} for root extracts; 15.52 ±1.04 nmol.mL^{-1} vs 14.68 ±0.4 nmol.mL^{-1} for stalk extracts) (Figure 3).

We observed a statistically significant increase in levels of ketonic derivatives of OMP after *in vitro* incubation with the blood samples of diabetic patients with extracts (at a final dose of 5 mg.mL^{-1}) derived from both roots and stalks of CM harvested from urban agglomerations compared to the control samples (23.16 ±1.11 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for root extracts; 22.33 ±2.02 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for stalk extracts). There was a statistically significant increase of 20.2% (p <0.05) for root extracts and 15.9% (p <0.05) for stalk extracts, respectively. We obtained similar results after incubating blood samples with stalk extracts of CM collected from rural areas, where there was also a statistically significant increase in levels of ketonic derivatives of OMP by 12.8% (p <0.05) compared to the control samples (21.73 ±1.03 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). Other results were obtained after incubating blood samples with root extracts of CM collected from rural areas (at a final dose of 5 mg.mL^{-1}), where we noted a statistically no-significant decrease in levels of ketonic derivatives of OMP (by 5.2%, p <0.05) compared to the control samples (18.26 ±3.56 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}).

After incubating the blood samples with root extracts of CM collected from urban areas and stalk extracts of CM collected from rural areas (at a final dose of 2.5 mg.mL^{-1}), we observed a statistically non-significant decrease in levels of ketonic derivatives of OMP compared to control

samples (16.96 ±1.71 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for root extracts of CM collected from urban areas; 16.68 ±1.45 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for stalk extracts of CM collected from rural areas). There was a statistically significant increase of 11.9% ($p < 0.05$) for root extracts of CM collected from urban areas and 13.4% ($p < 0.05$) for stalk extracts of CM collected from rural areas, respectively. We obtained different results when we applied stalk extracts (at a final dose of 2.5 mg.mL^{-1}) of CM collected from urban areas (19.46 ±1.38 nmol.mL^{-1}) and root extracts of CM collected from rural areas (21.6 ±1.24 nmol.mL^{-1}) after incubation with the blood samples, where there was a statistically no-significant increase in levels of ketonic derivatives of OMP compared to the control samples (19.26 ±0.5 nmol.mL^{-1}). This increase was 1% ($p > 0.05$) for stalk extracts of CM collected from urban areas and 12.1% ($p > 0.05$) for root extracts of CM collected from rural areas, respectively.

After *in vitro* incubation of blood samples with root extracts of CM collected from urban areas (at a final dose of 1.25 mg.mL^{-1}), we observed a statistically no-significant decrease in levels of ketonic derivatives of OMP by 8.9% ($p > 0.05$) compared to the control samples (17.54 ±2.1 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). Other results were obtained after incubating blood samples with stalk extracts of CM collected from urban areas, where there was a no-significant increase in levels of ketonic derivatives of OMP by 6.7% ($p > 0.05$) compared to the control samples (20.55 ±0.53 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). We recorded similar results after *in vitro* incubation of blood samples with extracts derived from both roots (19.46 ±2.13 nmol.mL^{-1}) and stalks (21.42 ±1.83 nmol.mL^{-1}) of CM collected from rural agglomerations (at a final dose of 1.25 mg.mL^{-1}), where there was a statistically no-significant increase in levels of ketonic derivatives of OMP compared to the control samples (19.46 ±1.38 nmol.mL^{-1}). This increase was 1% ($p > 0.05$) for root extracts and 11.2% ($p > 0.05$) for stalk extracts, respectively.

Only at a final dose of 0.63 mg.mL^{-1} after incubation of blood samples with stalk extracts of CM collected from urban areas we observed a statistically significant decrease in levels of ketonic derivatives of OMP by 13.4% ($p < 0.05$) compared to the control samples (19.95 ±0.91 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). We obtained different results after *in vitro* incubation of blood samples with root extracts of CM collected from urban areas, where there was a statistical no-significant increase in levels of ketonic derivatives of OMP by 3.6% ($p > 0.05$) compared to control samples (16.68 ±1.11 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1}). We observed similar trends after incubating blood samples with extracts derived from both roots and stalks of CM collected from rural agglomerations, where there was a statistically no-significant increase in levels of ketonic derivatives of OMP compared to the control samples (19.72 ±1.13 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for root extracts; 20.06 ±1.02 nmol.mL^{-1} vs. 19.26 ±0.5 nmol.mL^{-1} for stalk extracts). This increase was 2.4% ($p > 0.05$) and 4.2% ($p > 0.05$), respectively (Figure 3).

The current study is the continuation of our investigation aimed to assess the antioxidant and antibacterial properties of root and stalk extracts of CM collected from rural and urban areas of Pomeranian regions. In our previous study (Stefanowski et al., 2021d, e) on muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbum), we also demonstrated the antioxidant activity of CM extracts. Our results showed that extracts of CM collected from both urban and rural areas statistically significantly reduced the level of aldehyde derivatives of OMB by 18.8% ($p < 0.05$). The analysis of the levels of ketonic derivatives of OMP showed that extracts of CM collected from both urban and rural areas statistically significantly decreased the level of ketonic derivatives of OMP by 20.6 and 21.5%, respectively (for urban areas), as well as 26.7 and 12.5% (for rural areas). Lower levels of lipid peroxidation were observed after incubation with stalk extracts, while those collected from rural areas showed the lowest result (by 11%). Root extracts of CM collected from urban and rural areas increased TBARS levels. Analysis of oxidatively modified protein levels in the blood of rainbow trout after *in vitro* incubation with root and stem extracts shows that extracts can inhibit the production of oxidative carbonyls by scavenging free radicals.

Also, in another of our studies (Stefanowski et al., 2021a, b) on equine plasma, we demonstrated the antioxidant activity of CM extracts. Our results demonstrated that statistically significant reductions in lipid peroxidation byproducts were noted after incubation with extracts derived from roots of CM collected from both urban (by 35%, $p < 0.05$) and rural (by 34%, $p < 0.05$) agglomerations compared to the control samples. Stem extracts derived from CM also reduced TBARS levels, but only extracts derived from CM were collected from the rural areas; a statistically significant decrease (by 21%, $p < 0.05$) was observed compared to the control samples. The lowest values in the content of the aldehydic derivatives of OMP were observed after incubation with extracts derived from roots of CM collected from both rural and urban areas. On the other hand, levels of ketonic derivatives

of OMP were significantly increased after incubation with extracts derived from stems of CM collected from both rural and urban areas compared to the control samples, in contrast to extracts derived from roots of CM collected from urban areas, where there was a statistically significant reduction in ketonic derivatives of OMP (by 15%, $p < 0.05$) compared to the control samples.

We also demonstrated the antioxidant properties of CM extracts in the blood model of clinically healthy subjects (Stefanowski et al., 2021c). Our results showed, that the level of total antioxidant capacity (TAC) was statistically significantly changed in the human blood incubated with extracts derived from the stalks of CM collected from rural agglomerations (49.67 ±1.88%) compared to the untreated samples (63.18 ±5.07%). We observed different results after incubation of extracts derived from roots of CM collected from rural areas with human blood (62.12 ±1.88%) compared with the control samples (63.18 ±5.07%). Noting the results after incubation of human blood with root and stalk extracts of CM collected from urban agglomerations, we observed a decrease in TAC levels (61.67 ±3.2% for stalk extracts; 56.07 ±4.06% for root extracts) compared with the control samples (63.18 ±5.07%).

Studies by other researchers have shown that CM cell cultures are rich in polyphenolic compounds and isoquinoline alkaloids with confirmed antimicrobial, antioxidant, and anti-inflammatory properties. Chelerythrine, a natural benzo-phenanthridine alkaloid of CM, inhibited inflammatory and pain reactions in several *in vivo* and cell models employed by Lanfeld et al. (1981). *In vivo*, i.p. administration of the alkaloid (1–5 $mg.kg^{-1}$) alleviated mouse ear oedema, rat paw oedema, and abdominal constriction (pain reaction). Also, the isolated peritoneal macrophages upon treatment with 0.0001–1 $\mu g.ml^{-1}$ chelerythrine had dose-dependently reduced prostaglandin E2 and cyclooxygenase-2 expression. Alkaloid fraction and sanguinarine were efficient against carrageenan-induced rat paw oedema but chelerythrine showed lower activity (Lanfeld et al., 1981). However, in the later study by Mikołajczak et al. (2015), was demonstrated that various fractions of water extract at relatively high doses of 200 $mg.kg^{-1}$ body weight failed to alleviate the inflammation in a similar model. The crude water extract treatment aggravated the paw inflammation. Conversely, the extracts containing mainly coptisine and chelidonine were effective in the hot plate test for antinociceptive properties that suggests a supramedullary way of action (Mikołajczak et al., 2015).

Chelidonine is an isoquinoline alkaloid and the main alkaloid of CM. It has been also reported to have anti-cancer properties in a variety of tumour systems. Chelidonine and CM alkaloid extract were shown to overcome drug resistance by inhibiting the expression of p-glycoprotein (MDR-1) and several enzymes of the cytochrome P450 system, involved in xenobiotic metabolism in leukaemia and colon cancer cells and the induction of caspase-dependent apoptosis. However, published results concerning the effectivity and cancer-selectivity of chelidonine are still controversial. However, until now CM alkaloids have not been tested as possible therapeutic agents in cell lines and corresponding non-malignant primary cells of the mucosa of the upper respiratory tract (El-Readi et al., 2013; Herrmann et al., 2018).

Chelidonine exhibits effects on the central nervous system similar to those of morphine but weaker, and spasmolytic effects on smooth muscles similar to those of papaverine but also weaker. It is also a spindle poison. Chelerythrine strongly irritates the skin and mucous membranes, benumbs the central nervous system, and acts as a local anaesthetic (Perez Gultierrez, 2011; Isolani et al., 2012). Sanguinarine exhibits a vasorelaxant effect and an inhibitory effect on smooth muscle contractions. It is also an inhibitor of acetylcholinesterase and 5-lipoxygenase and it presents antimicrobial activity (Jagiełło-Wójtowicz et al., 1989; Miao et al., 2011). Protopine can act as an analgesic and inhibits histamine H1 receptors and thrombocyte aggregation. Coptisine was found to present neuroprotective, cytotoxic, and inhibitory effects on monoamine oxidase, and cardioprotective bioactivities (Wang et al., 2013).

In a study by Jang et al. (2021), it was found that chelidonine inhibited the proliferation of BxPC-3 and MIA PaCa-2 human pancreatic cancer cells in a dose- and time-dependent manner, confirming its apoptotic potential. In addition, flow cytometry analysis revealed that over 50% of BxPC-3 and MIA PaCa-2 cells exhibit early- and late-phase apoptosis after exposure to chelidonine (1 μM) for 24 h. These changes in expression levels following chelidonine treatment were re-confirmed through the analysis of transcription factor activity in both pancreatic cancer cell lines (Jang et al., 2021).

Nawrot et al. (2021) described the isolation and identification of a novel major latex protein (CmMLP1) composed of 147 amino acids and present a model of its structure containing a conserved hydrophobic cavity with high affinity to berberine, 8-hydroxycheleritrine,

and dihydroberberine. CmMLP1 and the accompanying three alkaloids were present in the eluted chromatographic fractions of latex. They decreased *in vitro* viability of human cervical cancer cells (HPV-negative and HPV-positive). The authors combined, for the first time, research on macromolecular and low-molecular-weight compounds of latex-bearing plants in contrast to other studies that investigated proteins and alkaloids separately. The observed interaction between latex protein and alkaloids may influence our knowledge of plant defense (Nawrot et al., 2021).

In the study of Noureini et al. (2017), the authors focused on the mechanism of telomerase inhibition by stabilization of telomeric G-quadruplex structures by berberine, chelerythrine, chelidonine, sanguinarine, and papaverine. Authors estimated telomerase activity and mRNA levels of hTERT using quantitative telomere repeat amplification protocol (q-TRAP) and qPCR, in MCF-7 cells treated with different groups of alkaloids. The results highlight the strong inhibitory effects of chelerythrine, sanguinarine, and berberine on telomerase activity, most likely through substrate sequestration. These isoquinoline alkaloids interacted strongly with the telomeric sequence G-quadruplex. In comparison, chelidonine and papaverine had no significant interaction with the telomeric quadruplex, while they strongly inhibited telomerase at the transcription level of hTERT (Noureini et al., 2017).

Sanguinarine (SNG), a natural compound of the Papaveraceae family, possesses favorable therapeutic potential against a variety of cancers. Prabhu et al. (2021) examined the underlying molecular mechanisms of SNG in non-small cell lung cancer (NSCLC) cells. SNG suppressed cell growth and induced apoptosis *via* downregulation of the constitutively active JAK/STAT pathway in all the NSCLC cell lines. siRNA silencing of STAT3 in NSCLC cells further confirmed the involvement of the JAK/STAT signaling cascade. SNG treatment increased Bax/Bcl-2 ratio, which contributed to a leaky mitochondrial membrane leading to cytochrome c release accompanied by caspase activation. In addition, the authors established the antitumor effects of SNG through reactive oxygen species (ROS) production, as inhibiting ROS production prevented the apoptosis-inducing potential of SNG. An *in vivo* tumor xenograft model further confirmed they're *in vitro* results. The study of Prabhu et al. (2021) investigated the molecular mechanisms by which SNG induces apoptosis in NSCLC, providing avenues for developing novel natural compound-based cancer therapies.

Conclusions

In the current study, we investigated the *in vitro* effects of CM extracts on lipid peroxidation and biomarkers of oxidatively modified proteins in the blood of patients with T2DM. The use of extracts derived from both roots and stalks of CM collected from both urban and rural agglomerations at final doses of 5.0 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ resulted in a significant enhancement of lipid peroxidation in the blood samples. On the contrary, only incubation of blood samples with extracts of stalks of CM collected from urban areas at a final dose of 0.63 $mg.mL^{-1}$ resulted in a no-significant decrease in TBARS level contributing to the protection of lipid structures in the membranes. Similar results were obtained by analyzing levels of aldehydic derivatives of oxidatively modified proteins in the blood of patients with T2DM after *in vitro* incubation with the extracts, where doses of 5.0 $mg.mL^{-1}$, 2.5 $mg.mL^{-1}$, and 1.25 $mg.mL^{-1}$ significantly increased the oxidation process of protein structures. Analysis of levels of ketonic derivatives of oxidatively modified proteins showed that the use of root extracts of CM collected from urban agglomeration at final doses of 2.5 $mg.mL^{-1}$ and 1.25 $mg.mL^{-1}$ reduced levels of ketonic derivatives, while the use of stalk extracts of CM harvested in urban agglomerations at a final dose of 0.63 $mg.mL^{-1}$ statistically significantly reduced levels of ketonic derivatives of oxidatively modified proteins compared to the control samples. The comparison of these results showed that CM extracts can effectively inhibit the formation of protein carbonyls by the elimination of free radicals. This phenomenon may explain the use of CM in medicine through its destructive effect on the membrane structures of cancer cells, due to the presence of a wide range of active compounds and other secondary metabolites throughout the plant. Our results may suggest that CM is a rich source of biomolecules that exhibit cytotoxic properties.

Conflict of interests

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

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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