

RESEARCH ARTICLE

# Characterization of compounds in Cornelian cherry (*Cornus mas* L.) and its effect on interior milieu in ZDF

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## ABSTRACT

The risk of type 2 *Diabetes mellitus* is constantly increasing and therefore it is necessary to seek effective and new therapies. Cornelian cherry (*Cornus mas* L.) presents an opportunity in an alternative for treatment of type 2 *Diabetes mellitus* due its high antioxidant effects. The objective of the study was to assess the effect of Cornelian cherry pulp and stone on ZDF rats, which are suitable biological model for type 2 diabetes. ZDF rats received Cornelian cherry in three doses (1500, 2000 mg kg<sup>-1</sup> body weight of the pulp and 250 mg kg<sup>-1</sup> body weight of the stone) using the sterile oral rodent gavage for 4 months. Blood glucose, insulin, antioxidant activity and compounds in Cornelian cherry were determined to investigate the effects. Cornelian cherry stone significantly decreased affected blood glucose levels in ZDF rats when compared to the untreated control group. Total polyphenols and phenolic acids had significantly higher values in Cornelian cherry stone against pulp. Also, high concentrations of the anthocyanins were determined in the pulp. The results suggest that Cornelian cherry stone would potentially provide promising source of natural antioxidants and use in the management of *Diabetes mellitus*.

**Keywords:** Antioxidants; Cornelian cherry; *Diabetes mellitus*; Hyperglycaemia; ZDF rats

## INTRODUCTION

It is well known that many plants which we used today have a lot of medicinal properties. They may help with serious diseases like a cancer, cardiovascular, neurological disorders and are useful in treatment of chronic diseases, *Diabetes mellitus* and gastrointestinal disorders. The diet containing fruits and vegetables has a numerous positive effect on animal's health (Francik et al., 2014). The researches have explored possibilities of plants use instead of synthetic drugs for treatment of different diseases. The beneficial effect of plants can be associated with the antioxidant capacity of non-nutritive compounds and nutritive compounds which are contained in plants and they play an important role in protection against cellular oxidation processes (Hosseinpour et al., 2017). There are enzymatic and non-enzymatic antioxidants, which are protecting cells

against reactive oxygen species. Enzymatic antioxidants include superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase etc. Non-enzymatic antioxidants consist of vitamins (A, C and E), polyphenolic compounds, the transition metals (selenium, copper and zinc), melatonin and polyamides (Matteo and Esposito, 2003). One of medicinal plants used in studies due to its antioxidant effect is Cornelian cherry (*Cornus mas* L.) (Hosseinpour et al., 2017). Cornelian cherry is delicious, sweet-sour in taste fruit of the attractive ornamental plant. Modern studies have shown that Cornelian cherry fruits are valued as a rich source of organic acids, sugars, vitamin C, polyphenolics and tannins. Several beneficial effects are attributed to anthocyanins, which are important bioactive compounds found in Cornelian cherry. All these compounds have antioxidant, antidiabetic, anticancer, lipid-lowering, anti-inflammatory, antibacterial effects

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and protective effect on cardiovascular system (Moldovan et al., 2016; Zhang et al., 2017). Fruit like this one, can be used as a natural food ingredient, because antioxidants and other bioactive compounds it contains can help to improve the overall quality of the final food product (Espinoza et al., 2017). *Diabetes mellitus* (DM) is a metabolic syndrome with typical symptoms as relative or complete insulin deficiency or chronic hyperglycaemia (American Diabetes Association 2009). The number of people with DM is increasing worldwide. According to estimates, more than 382 million people suffered from DM in the last years and it will reach 592 million by 2035 worldwide (Sen and Chakraborty 2015). The most important causes of this disease are heredity, consumption of diets rich in energy, obesity, aging, modern world sedentary lifestyle and physical inactivity (Hosseinpour et al., 2017). According to the American Diabetes Association (ADA) DM is divided into type 1 (DMT1), type 2 (DMT2), gestational *Diabetes mellitus* and other types (American Diabetes Association, 2014). DMT2 is the most prevalent type in the world (90–95%) compared to the other types of DM (Dabelea et al., 2014). DMT2 is characterized by insulin resistance because of dysfunction of pancreatic  $\beta$ -cells (Halban et al., 2014). The symptoms of insulin resistance are especially obesity, essential hypertension, non-alcoholic fatty liver disease, systemic inflammation, dyslipidaemia and nephropathy (Reaven, 2014). Main factors of pathogenesis of DMT2 are genetic, epigenetic and environmental. The mechanism of epigenetic factor includes transcriptional regulation of genes and genes silencing that are related to modulation of chromatin structure and availability to transcription factors. Activity of major non-physiological mediators of *diabetes* and its effects like oxidative stress, inflammation and hyperglycaemia can cause changes in gene expression (Reddy and Natarajan, 2011). Zucker diabetic fatty (ZDF) rats are widely used as a genetic model for obese DMT2. Male ZDF rats develop insulin resistance and obesity at a young age, and then while aging, they progressively develop hyperglycaemia. This hyperglycaemia is due to loss of pancreatic  $\beta$ -cell mass and decreased responsiveness of liver and extrahepatic tissues to the actions of glucose and insulin. It is common knowledge about similarities between male ZDF rats and humans with DMT2 associated with obesity (Shiota and Printz, 2012). Our previous study revealed promoting potential of Cornelian cherry with possibility to decrease the risk of DMT2 if taken regularly (Capcarova et al., 2019). In the other study of Capcarova et al. ZDF rats were given with consumption of bee bread. Treatment with bee bread showed beneficial effects on glucose metabolism and water intake in diabetic rats (Capcarova et al., 2019). Actually, there is still not enough information about Cornelian cherry and its beneficial effect on treatment of DMT2 using ZDF

rats. The objective of the study was to determine blood glucose, insulin parameters, antioxidant properties of ZDF rats after treatment of Cornelian cherry. In the present study the different doses of Cornelian cherry pulp and stone were administrated to examine beneficial effects on onset, development and complication of DMT2. We also determined bioactive substances such as total polyphenols, flavonoids, phenolic acids and anthocyanins in Cornelian cherry reportedly responsible for this effect.

## MATERIAL AND METHODS

### Plant material and sample preparation

The Cornelian cherry (*Cornus mas L.*) was obtained from the Institute of Biodiversity Conservation and Biosafety of Slovak University of Agriculture in Nitra, Slovak Republic. The fresh ripe fruits were washed, separated from the stones, crushed, mixed and stored frozen at  $-20\text{ }^{\circ}\text{C}$ . Stones were also crushed into small pieces with a sharp knife and were ground into a fine powder in the mortar (Fig. 1). During the experiment, aliquot doses were prepared in distilled water (Sigma Aldrich, Germany) and homogenised in order to be suitable for the administration by rodent gavage.

### Animals

The experiment was approved by the Ethical committee and the State Veterinary and Food Administration of the Slovak Republic under the number 2288/16–221. Zucker diabetic fatty (ZDF) rats were purchased from the Breeding Facility of the Institute of Experimental Pharmacology and Toxicology (Dobra Voda, Slovak Republic, SK CH 24016) and allowed to quarantine for at least two weeks after arrival (Fig. 2). In this experiment male ZDF rats were used at 12 weeks of age. All animals were kept in pairs under monitored conditions with 12:12 h light-dark cycles. The animals were divided to 5 groups as followed: lean ( $n=10$ , non-diabetic control group), the control ( $n=10$ , ZDF diabetic positive controls), S ( $n=10$ , ZDF diabetic group, dose of Cornelian cherry crushed stone  $250\text{ mg kg}^{-1}\text{ bw}$ ), P ( $n=10$ , ZDF diabetic group, dose of Cornelian cherry crushed pulp  $1500\text{ mg kg}^{-1}\text{ bw}$ ), PP ( $n=10$ , ZDF diabetic group, dose of Cornelian cherry crushed pulp  $2000\text{ mg kg}^{-1}\text{ bw}$ ). The groups S, P and PP received the accurate dose of Cornelian cherry (stone or pulp) every day directly to the stomach using the sterile oral rodent gavage (Instech, Plymouth, USA). Lean and the control group received distilled water by the same way. All groups of rats were fed with normal chow – KKZ-P/M (complete feed mixture for rats and mouse, register number 6147, Dobra Voda, Slovak Republic) on *ad libitum* basis. The composition of feed mixture is shown in Table 1. Experiment lasted for 4 months.

**Table 1: Complete feed composition for rats and mouse (KKZ-P/M)**

|                          |  |
|--------------------------|--|
| Analytical compounds (%) |  |
| Nitrogenous compounds    | 19.10  |
| Dietary fibre            | 3.60   |
| Oils and fats            | 5.10   |
| Ash                      | 5.85   |
| Humidity                 | 9.10   |
| Additives                |  |
| Vitamins                 | E672 Vitamin A–20,000 unit of measure,<br>E671 Vitamin D3–2000 unit of measure,<br>Vitamin E – 70 mg |
| Amino acids              | DL-Methionine 1.2 g,<br>L-Lysine 0.8 g   |

### Determination of glucose and insulin

Blood glucose was measured weekly with the FreeStyle Optimum Neo glucose system (Abbott Diabetes Care Ltd., UK) after overnight fasting using the test strips. A drop of blood was obtained from the tail of the rat in the morning between 8:00 to 10:00 a.m. for determination of glucose level. Diabetes was diagnosed if the blood glucose concentration exceeded 15 mmol L<sup>-1</sup>. At the end of the experiment, rats were euthanized by intraperitoneal injection of xylazine in combination with zoletil, and a blood sample was taken from their hearts. The obtained samples were centrifuged at 3000 rpm for 30 minutes. Insulin concentration in the blood serum was determined using ELISA and commercial kits (Biotech, SR).

### Determination of HOMA-IR index, QUICKI index and the fasting glucose:insulin ratio (G:I)

Serum insulin and glucose values were used to calculate the HOMA-IR (insulin sensitivity index), a mathematical model that involves the interactions between serum insulin and serum glucose values. The formula (Haffner et al., 1997) was used to calculate HOMA-IR:

$$\text{HOMA-IR} = [\text{serum insulin (mmol L}^{-1}) \text{ serum glucose (mmol L}^{-1})] / 22.5$$

To determine QUICKI (quantitative insulin sensitivity check index), serum insulin and glucose values were used according to the formula:

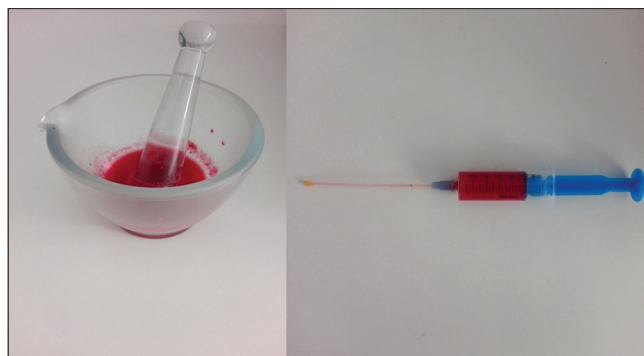
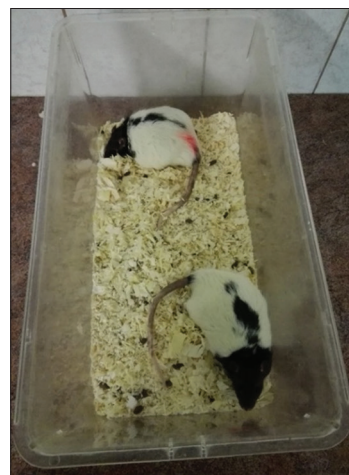
$$\text{QUICKI} = 1 / (\log I_0 + \log G_0),$$

where  $I_0$  means fasting insulin and  $G_0$  means fasting glucose.

At the end of the experiment, the glucose to insulin ratio (G:I) was calculated.

### Determination of superoxide dismutase activity

To determine the SOD activity, absorbance at 505 nm with a heated shield at 37 °C was measured using a Genesys 10

**Fig 1.** Preparation of Cornelian cherry.**Fig 2.** ZDF rats.

spectrophotometer (Thermo Fisher Scientific Inc., USA). The results were calculated based on the observed values using a standard curve made according to the Randox Laboratories kit manual (Randox Laboratories, UK).

### Determination of glutathione peroxidase activity

GPx activity was determined according to the Randox RANSEL commercial kit (Randox Laboratories). The rate of absorbance decrease was recorded using a Genesys 10 spectrophotometer (Thermo Fisher Scientific Inc., USA) and the results evaluated.

### Determination of compounds in Cornelian cherry

Cornelian cherry has an antioxidant activity. The presence of polyphenolic substances is responsible for this activity. For this reason, the total content of substances in pulp and stone was determined and the results were compared with each other. The total polyphenol content was monitored by using the Folin – Ciocalteu reagent method and expressed in GAE (gallic acid equivalent) using the method of Singleton and Rossi (Singleton and Rossi, 1965). The total content of flavonoids, phenolic acids and anthocyanins were determined, where flavonoids were expressed in QE (quercetin equivalent) using the procedure by Willett (Willett, 2002) and phenolic acids were expressed in CAE (caffeic

acid equivalent) as described by Polskie Towarzystwo Farmaceutyczne (Polskie Towarzystwo Farmaceutyczne, 1999). Anthocyanins were determined using the method of Fuleki and Francis (Fuleki and Francis, 1968). The content of substances was measured using a spectrophotometer (Jenway 6405 UV/Vis, UK) at different wavelengths and subsequently evaluated on the basis of calibration curves.

### Statistical analysis

Data are stated as mean  $\pm$  S.E. (standard error of mean). One-way ANOVA test was realized to calculate basic statistical characteristic and determine significant differences followed by Tukey test for multiple comparisons. T-test was used to compare the stone and the pulp compounds in Cornelian cherry. Statistical software GraphPad Prism 5 (GraphPad Prism 5 ISO, USA, 2009) was used. Differences were compared for statistical significance at the level  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ .

## RESULTS

### Effect of Cornelian cherry on glycaemia of ZDF rats

The results of Cornelian cherry on glycaemia are listed in Fig. 3. At the beginning of the study, the blood glucose levels stayed similar in all groups till 7th week of the experiment. The control group showed progress to the hyperglycaemia from 11th week of the experiment. Significant higher values ( $p < 0.001$ ) were found in the control from 13th week until end of the experiment when compared to the lean. The most important data were monitored in 14th and 15th week, because significant decrease ( $p < 0.001$ ) in blood glucose was found in S group when compared to the control. Generally, the blood glucose level in S group was more balanced and maintained below 10 mmol L<sup>-1</sup> during the whole 4-month experiment. Additionally, the glucose level was in S group significantly lower ( $p < 0.01$ ) in comparison to the P and PP groups at the end of the study.

### Effect of Cornelian cherry on insulin level of ZDF rats, HOMA-IR, G:I ratio and QUICKI

Insulin values of all experimental groups including the control group were lower than those found in the lean group (Fig. 4), significantly ( $p < 0.05$ ) in the control and P group. What is important, the diabetic groups treated stone and pulp (PP

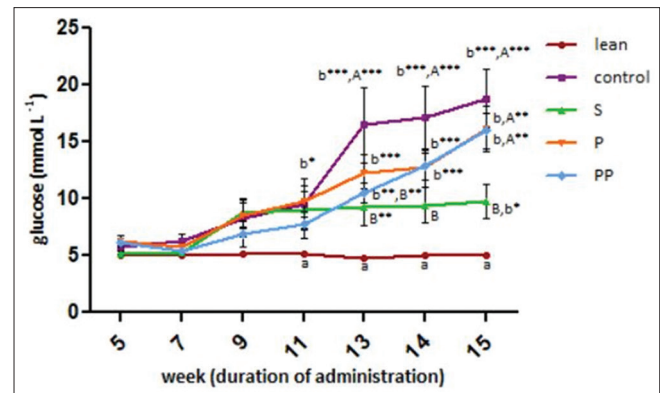
group) showed similar levels of insulin in comparison to the lean group. Values of HOMA-IR were significantly higher ( $p < 0.05$ ) in the groups P, PP and the control against the lean. Between the lean and S group no significant difference ( $p > 0.05$ ) was found (Table 2). In the case of G:I the lowest level was found in the lean group. Significantly lower ( $p < 0.05$ ) values against the lean were found in the control, P and PP groups. QUICKI index (Table 2) was significantly lower ( $p < 0.05$ ) in the control, P and PP groups against the lean. However, between S group and the lean group not significant difference ( $p > 0.05$ ) was found.

### Effect of Cornelian cherry on SOD level of ZDF rats

The activity of SOD (Fig. 5) in ZDF rats was similar in the all groups and the differences among the groups remained insignificant ( $p > 0.05$ ).

### Effect of Cornelian cherry on GPx level of ZDF rats

Significantly higher values ( $p < 0.001$ ) in GPx activity (Fig. 6) in the diabetic rats (control, S, P, PP) compared to the lean non-diabetic group were measured. Significantly higher ( $p < 0.01$ ) values were observed in the group P compared to the untreated ZDF rats from the control group. The higher dose of pulp (2000 mg kg<sup>-1</sup> bw) caused



**Fig 3.** Effect of Cornelian cherry on blood glucose level in ZDF rats lean – control non-diabetic group, control – untreated diabetic group, S – experimental diabetic group with dose of Cornelian cherry stone 250 mg kg<sup>-1</sup> bw, P – experimental diabetic group with dose of Cornelian cherry pulp 1500 mg kg<sup>-1</sup> bw, PP – experimental diabetic group with dose of Cornelian cherry pulp 2000 mg kg<sup>-1</sup> bw. Values are mean  $\pm$  S.E. a-b or A-B means significant difference in particular week of the experiment. \*means significant difference ( $p < 0.05$ ), \*\*means significant difference ( $p < 0.01$ ) and \*\*\*means significant difference ( $p < 0.001$ ).

**Table 2: HOMA-IR, G:I ratio and QUICKI of ZDF rats**

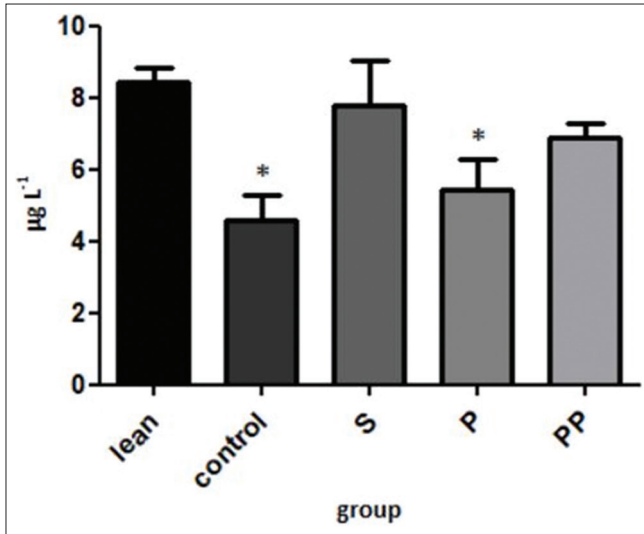
|           | Lean                          | Control                       | S                               | P                             | PP                            |
|-----------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|-------------------------------|
| HOMA-IR   | 1.37 $\pm$ 0.08 <sup>a</sup>  | 6.09 $\pm$ 1.15 <sup>b</sup>  | 4.14 $\pm$ 0.86 <sup>a,b</sup>  | 6.69 $\pm$ 0.69 <sup>b</sup>  | 6.27 $\pm$ 0.99 <sup>b</sup>  |
| G:I ratio | 0.64 $\pm$ 0.07 <sup>a</sup>  | 4.15 $\pm$ 1.12 <sup>b</sup>  | 3.72 $\pm$ 0.69 <sup>b</sup>    | 3.31 $\pm$ 0.29 <sup>b</sup>  | 4.63 $\pm$ 0.69 <sup>b</sup>  |
| QUICKI    | 0.37 $\pm$ 0.004 <sup>a</sup> | 0.35 $\pm$ 0.005 <sup>b</sup> | 0.36 $\pm$ 0.002 <sup>a,b</sup> | 0.31 $\pm$ 0.003 <sup>b</sup> | 0.32 $\pm$ 0.002 <sup>b</sup> |

lean – control non-diabetic group, control – untreated diabetic group, S – experimental diabetic group with dose of Cornelian cherry stone 250 mg kg<sup>-1</sup> bw, P – experimental diabetic group with dose of Cornelian cherry pulp 1500 mg kg<sup>-1</sup> bw, PP – experimental diabetic group with dose of Cornelian cherry pulp 2000 mg kg<sup>-1</sup> bw, HOMA-IR – homeostasis assessment of insulin resistance, G:I – fasting glucose: insulin ratio, QUICKI – quantitative insulin sensitivity check index, values are mean  $\pm$  S.E., a-b mean significant difference ( $p < 0.05$ ) in rows

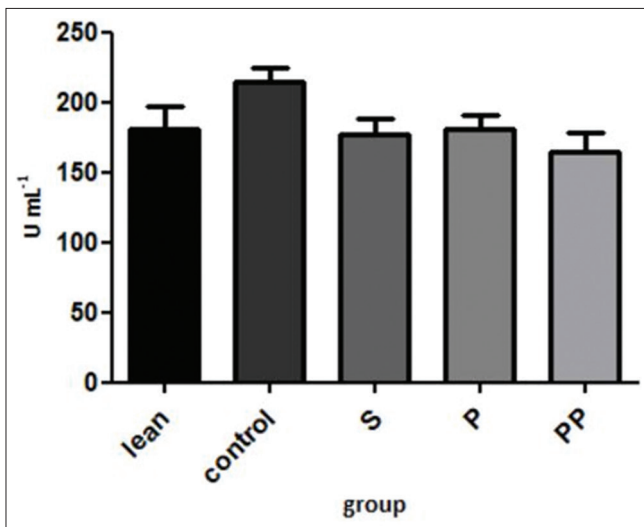
significantly lower values of GPx activity ( $p < 0.05$ ) against lower dose (1500 mg kg<sup>-1</sup> bw).

#### Determination of compounds in Cornelian cherry

Determination of the total polyphenols in the pulp (Fig. 7) showed significant ( $p < 0.001$ ) lower values when compared to the stone. Stone value was 29.608 mg GAE g<sup>-1</sup> while pulp contained only 10.204 mg GAE g<sup>-1</sup>.



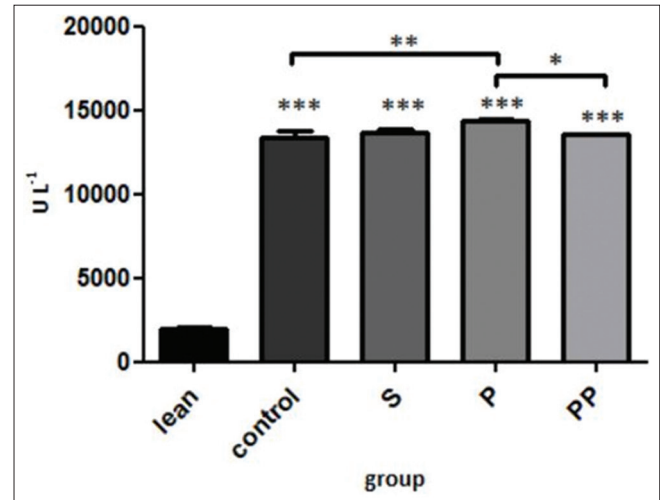
**Fig 4.** Effect of Cornelian cherry on insulin level in ZDF rats lean – control non-diabetic group, control – untreated diabetic group, S – experimental diabetic group with dose of Cornelian cherry stone 250 mg kg<sup>-1</sup> bw, P – experimental diabetic group with dose of Cornelian cherry pulp 1500 mg kg<sup>-1</sup> bw, PP – experimental diabetic group with dose of Cornelian cherry pulp 2000 mg kg<sup>-1</sup> bw. Values are mean±S.E. \*means significant differences ( $p < 0.05$ ) against the lean group.



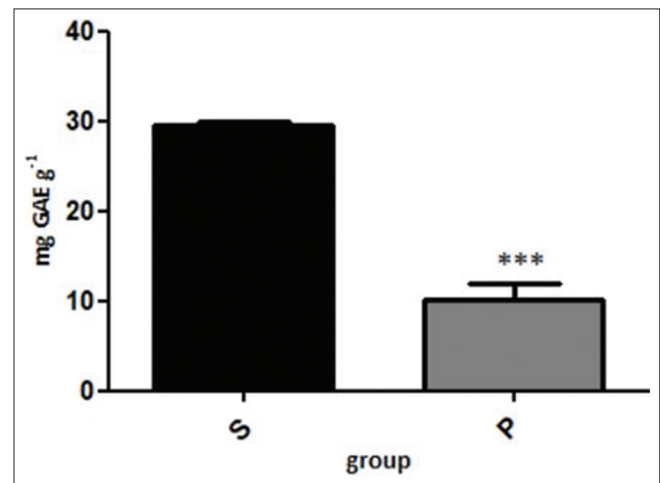
**Fig 5.** Effect of Cornelian cherry (*Cornus mas* L.) on superoxide dismutase activity in ZDF rats lean – control non-diabetic group, control - untreated diabetic group, S – experimental diabetic group with dose of Cornelian cherry stone 250 mg kg<sup>-1</sup> bw, P – experimental diabetic group with dose of Cornelian cherry pulp 1500 mg kg<sup>-1</sup> bw, PP – experimental diabetic group with dose of Cornelian cherry pulp 2000 mg kg<sup>-1</sup> bw. Values are mean± S.E.

Of the total polyphenols, flavonoids were examined (Fig. 8). The results revealed that differences between the groups stayed insignificant ( $p > 0.05$ ).

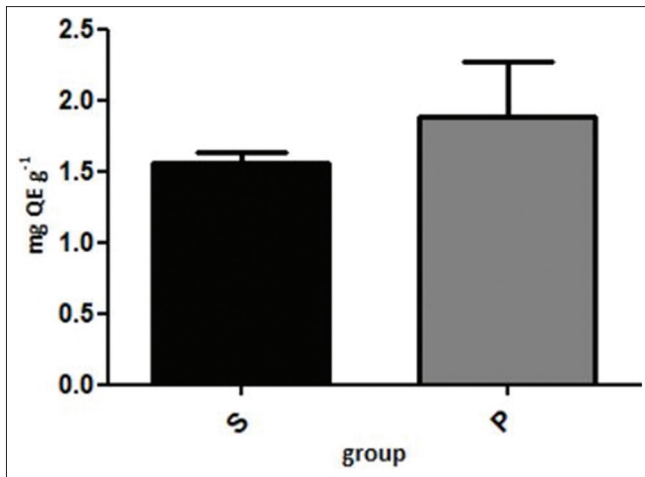
When comparing quantity of phenolic acids in the pulp and stone, there was a big difference as is shown in Fig. 9. The pulp contained significantly lower ( $p < 0.001$ ) values against the stone. Cornelian cherry pulp possessed 6.659 mg CAE g<sup>-1</sup> while pulp only 0.621 mg CAE g<sup>-1</sup>. The values of the anthocyanins determined in the pulp had an amount of 0.54 g from 2 g of total amount of Cornelian cherry.



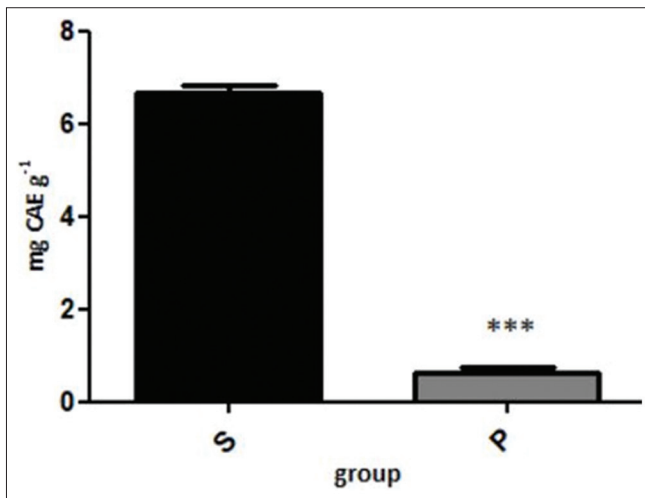
**Fig 6.** Effect of Cornelian cherry (*Cornus mas* L.) on glutathione peroxidase activity in ZDF rats lean – control non-diabetic group, control – untreated diabetic group, S – experimental diabetic group with dose of Cornelian cherry stone 250 mg kg<sup>-1</sup> bw, P – experimental diabetic group with dose of Cornelian cherry pulp 1500 mg kg<sup>-1</sup> bw, PP – experimental diabetic group with dose of Cornelian cherry pulp 2000 mg kg<sup>-1</sup> bw. Values are mean±S.E. \*means significant differences ( $p < 0.05$ ), \*\*means significant differences ( $p < 0.01$ ), \*\*\*means significant differences ( $p < 0.001$ ) against the lean group.



**Fig 7.** Determination of total polyphenols in Cornelian cherry S – Cornelian cherry stone, P – Cornelian cherry pulp. Values are mean±S.E. \*\*\*means significant difference ( $p < 0.001$ ) against S group.



**Fig 8.** Determination of flavonoids in Cornelian cherry S – Cornelian cherry stone, P – Cornelian cherry pulp. Values are mean  $\pm$  SEM.



**Fig 9.** Determination of phenolic acids in Cornelian cherry S – Cornelian cherry stone, P – Cornelian cherry pulp. Values are mean  $\pm$  S.E. \*\*\*means significant difference ( $p < 0.001$ ).

In the stone the level of anthocyanins was very low and below detection limit.

## DISCUSSION

In the present study we determined the effects of Cornelian cherry (*Cornus mas* L.) on internal milieu of ZDF rats. ZDF rats are valuable animal model when studying DM and diabetic complications. Possible remedies for the treatment and prevention of DM are presently being researched, as current DM drugs have certain limitations, such as adverse effects and high rates of secondary failure (Shapiro and Gong, 2002). Because of this situation, alternative therapies are discussing. Natural products with antioxidant compounds are good option because of low or no side effects and multi-target

actions (Sacan et al., 2004). The most surprising result in our study was the significant decrease of fasting glucose after the oral administration of Cornelian cherry stone in the dose of 250 mg kg<sup>-1</sup> bw in 13th, 14th and 15th week of the experiment against the diabetic control group. In our previous study (Capcarova et al., 2019; Dupak et al., 2020) we found significant decrease of glucose level in ZDF rats after oral gavage of Cornelian cherry fruit in the dose of 1000 mg kg<sup>-1</sup> bw against the diabetic control group from 5 to 7 week of the experiment. In this study we did not observe similar effect when using higher doses (1500 and 2000 mg kg<sup>-1</sup> bw) of Cornelian cherry fruit. It seems that the dose of Cornelian cherry pulp responsible for beneficial effect is 1000 mg kg<sup>-1</sup> bw and further increasing of the dose is not effective. On the other hand, Cornelian cherry stone was able to properly maintain glucose in normoglycemic level. In this group blood glucose did not exceed 15 mmol L<sup>-1</sup> during the whole experiment period. Decreased glucose levels and anti-diabetic activities are probably associated with the presence of polyphenolic compounds (flavonoids, anthocyanins) that inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase activity (Shishehbor et al., 2016; Mathivha et al., 2019) which is also confirmed by our results. Levels of blood glucose could be also affected by presence of ursolic acid due to its antidiabetic and antioxidant activity (Jang et al., 2014; Lee et al., 2014). Pulp in the fruit contains large quantity of water. On the other hand, the stone is dry and more concentrated. On the basis of this fact we used a lower dose of stone in comparison to the pulp. The dose of stone was a starting dose in our experiment and to our knowledge there are no relevant literature sources pointed at the use of Cornelian cherry stone in diabetic studies with ZDF rats. This dose is basis for our next studies with Cornelian cherry stone using in treatment of ZDF rats. The doses of pulp were calculated and determined with reference to our previous study with Cornelian cherry (Capcarova et al., 2019). Doses for ZDF rats in our study corresponded with doses in human studies. Several methods have been proposed for the measuring and evaluating the blood glucose and insulin. HOMA-IR index was higher in the diabetic ZDF rats ( $9.3 \pm 0.9$ ) against the lean rats ( $1.2 \pm 0.1$ ) in the study of Agil et al. (2012). In our study we observed similar results with the lean group, but the diabetic groups had lower HOMA-IR index compared to Agil et al. (2012). In our previous study (Capcarova et al., 2019; Dupak et al., 2020) G:I in ZDF rats was significantly higher in the experimental group treated with dose of 500 mg kg<sup>-1</sup> bw of Cornelian cherry against the lean group, but dose of 1000 mg kg<sup>-1</sup> bw stayed insignificant against the lean group. Our results showed that G:I ratio was significantly higher between the lean and all other groups. Cornelian cherry in our study did not affect this

parameter. We observed lower values of QUICKI in the experimental groups when compared to the lean group. However, the stone group had positive effect in this parameter, because this group was significantly higher against all other groups except the lean. Reactive oxygen species (ROS) and oxidative stress play a crucial role in diabetic pathophysiology (Dewanjee et al., 2009). Role of the antioxidant enzymes is elimination ROS in cells (Nagata et al., 1999). SOD effectively breaks down the superoxide radical and converts it into less toxic hydrogen peroxide. Hydrogen peroxide is further decomposed by other enzymes (GPx, CAT) into oxygen and water (Baudrimont et al., 1997; Reiter et al., 2000). We found significant increase of GPx activity in blood of the ZDF rats after treatment with Cornelian cherry pulp against the control group. The antioxidant activity of Cornelian cherry is caused by their bioactive compounds including phenolic substances (flavonoids, tannins, anthocyanins and phenolic acids), carotenoids and phytosterols (Somi et al., 2014; Slobodnikova et al., 2016; Dzedziński et al., 2020). In our study we determined and compared total polyphenols, flavonoids, phenolic acids and anthocyanins in Cornelian cherry stone and pulp. The values of total polyphenols and phenolic acids were surprisingly significantly higher in the stone than in the pulp. We believe that high levels of polyphenols are likely to cause a positive reduction in the glucose levels in Cornelian cherry stone group. The determination of polyphenols in the stones has assumed increasing importance with the recognition that this part of fruit is often a source of unique compounds in much higher concentration than in the pulp (Antolovich et al., 2000). However, the content of flavonoids was higher in the pulp than stone and anthocyanins were found only in the pulp. The study of Ivanisova et al. (2019) showed different contents of phenolic compounds of the fresh fruit and jellies including *Cornus mas*. Authors revealed that total polyphenol content (mg GAE g<sup>-1</sup>) of the fresh fruits and jellies made from one species ranged from 6.02 (*Actinidia chinensis*) to 12.30 (*Viburnum opulus*) and total flavonoid content (mg QE g<sup>-1</sup>) from 0.06 (*Cornus mas*) to 2.29 (*Lonicera caerulea*). It is known that fruit, especially less known species starting to be very popular nowadays in pharmacy, medicine and gastronomy, because of sufficient bioactive substances. They can be consumed fresh or be prepared a different kind of products. Gaštol et al. (2013) reported that Cornelian cherry juices have the highest levels of total polyphenols and organic acids compared to plum, pear and apple juices. Since oxidative stress is involved in diabetes, intake of antioxidants is desirable. Role of antioxidants is damaging reactive oxygen species, which is preventing their functions. Mostly exogenous antioxidants such as

flavonoids, vitamin C, vitamin E, vitamin A, β-carotene, selenium, zinc and coenzyme Q10 with combination of endogenous antioxidants (SOD, GPx, CAT, bilirubin and uric acid) are decreasing oxidative stress and insulin resistance (Esterbauer et al., 1991; Pitocco et al., 2013).

## CONCLUSIONS

To the authors' knowledge, this is the first report dealing with treatment of type 2 diabetes using Cornelian cherry stone in ZDF rats during 4-month experiment contributing to the possibility of treatment and prevention of DMT2. The application of 250 mg kg<sup>-1</sup> bw of Cornelian cherry stone was able to reduce hyperglycemia and improve insulinemia in ZDF. Cornelian cherry has a high content of bioactive substances such as polyphenols, most of which are phenolic acids and anthocyanins. Cornelian cherry stone could have more positive results in prevention of diabetic complications when received routinely. Our study reinforces the interest in using natural substances as is Cornelian cherry in treatment and prevention of type 2 *Diabetes mellitus* and supports the need for further research with different doses and duration of the experiment.

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## Authors' contributions

Rudolf Dupak contributed to the conception and data analyses of the study and wrote the manuscript. Klaudia Jaszczka, Anna Kalafova, Monika Schneidgenova, Eva Ivanisova, Katarina Tokarova participated in the coordination of experimental work and data collection greatly. Marcela Capcarova supervised the experiment, checked and approved the final manuscript.

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