https://doi.org/10.31925/farmacia.2022.4.5 **ORIGINAL ARTICLE**

TWO BIOACTIVE COMPOUNDS, ROSMARINIC ACID AND SINAPIC ACID, DO NOT AFFECT THE DEPLETED GLUTATHIONE LEVEL IN THE LENSES OF TYPE 2 DIABETIC FEMALE RATS

MARIA ZYCH [1]*, WERONIKA BORYMSKA [1], KATARZYNA URBISZ [1], MAREK KOSTRZEWSKI [2], ILONA KACZMARCZYK-ŻEBROWSKA [1]

[1]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland*
[2]*Society of Silesian Veterinary Polyclinics, Katowice, Poland*

**corresponding author: mzych@sum.edu.pl*

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Abstract

Oxidative stress arises during numerous diseases, such as diabetes. It affects various tissues and organs, including the eye lens leading eventually to cataract. Phenolic acids are known to reveal antioxidative properties. Thus, this study aimed to evaluate the effect of two bioactive compounds: rosmarinic acid (RA) and sinapic acid (SA) on the oxidative stress markers in the lenses of type 2 diabetic female rats. Both these phenolic acids were administered by gavage for 28 days to the rats, in which diabetes was induced by a high fat diet and streptozotocin. The following doses of phenolic acids were used: 10 and 50 mg/kg body weight (bw) for RA and 5 and 25 mg/kg bw for SA. Oxidative stress markers, including antioxidative enzymes, non-enzymatic antioxidants and oxidative damage markers, were evaluated in the lenses. Obtained results indicate that type 2 diabetes affected mainly the glutathione, as the levels of both reduced and oxidized glutathione were reduced in the lenses of diabetic female rats. Oral administration of the examined phenolic acids did not counteract these changes, regardless of the dose. Since glutathione plays a crucial role in lens transparency maintenance, neither RA nor SA can be considered promising agents in diabetic cataract prevention.

Rezumat

Stresul oxidativ este prezent în multiple patologii, diabetul zaharat fiind una dintre ele. Afectarea diabetică este prezentă la nivelul numeroselor țesuturi și organe, inclusiv lentilele cristalinului, ducând în timp la cataractă. Acizii fenolici sunt compuși cu acțiune antioxidantă bine cunoscută. Obiectivul studiului a fost evaluarea efectului a doi compuși bioactivi, acidul rosmarinic și acidul sinapic, asupra markerilor de stres oxidativ din lentilele oculare ale șoarecilor femele cu diabet de tip II. Ambii compuși fenolici au fost administrați prin gavaj, timp de 28 de zile. Dozele de polifenoli administrate au fost de: 5 și 10 mg/kg corp pentru acidul rosmarinic și de 5 și 25 mg/kg corp pentru acidul sinapic. Markerii de stres oxidativ, inclusiv enzimele antioxidante, markeri non-enzimatici și de deteriorare oxidativă au fost evaluați la nivelul lentilelor cristalinului. Rezultatele obținute indică faptul că diabetul zaharat de tip II afectează în principal glutationul, nivelurile de glutation-redus și oxidat fiind scăzute. Adminstrarea orală a polifenolilor analizați nu a contracarat stresul oxidativ, indiferent de doza administrată. Având în vedere rolul esențial pe care glutationul îl joacă în transparența lentilelor, niciunul dintre compușii investigați nu pot fi considerați molecule promițătoare în profilaxia cataractei diabetice.

Keywords: phenolic acids, lenses, glutathione, oxidative stress, diabetic rats

Introduction

Currently, diabetes is one of the most important health problems worldwide. It is a chronic and progressive disease, with a number of cases that, according to epidemiologists, might reach 700 million worldwide in 2045 [54]. Diabetes is a heterogeneous group of metabolic disorders, the main symptom of which is hyperglycaemia [27, 28, 40]. Depending on the cause, many types of diabetes are distinguished. The most important are: type 1 diabetes (autoimmune) and type 2 diabetes, latter accounts representing as much as 90 - 95% of the cases of this disease. The risk factors for type 2 diabetes are mostly obesity related to poor diet and lack of physical activity, medications and stimulants, hypertension, or cardiovascular diseases [40, 54]. Among these factors, many authors also mention the age of a patient [3, 25, 28, 40, 59]. Increased occurrence of type 2 diabetes is observed in patients over the age of 45, but people over 65 are even more exposed to the risk of developing this disease. More than half of patients with type 2 diabetes in developed countries are people over 65, while only 8% are under the age of 44 [3, 40]. Aging processes, described as a progressive loss of tissues and organs functions, disrupt the energy homeostasis of the body by adversely affecting carbohydrate metabolism, which leads to hyperglycaemia and increases the risk of developing or worsening the symptoms of type 2 diabetes [20, 25, 32]. Both hyperglycaemia and aging processes are associated with an increase in oxidative stress in the body [6, 63]. Oxidative

stress occurs when the concentration of reactive oxygen species is temporarily or chronically increased. It disturbs cellular metabolism, its regulation and damages cellular components [35]. Oxidative stress affects, among other things, various eye structures, including the lenses. Lenses are mainly made of proteins called crystallins, which play a crucial role in maintaining the transparency of this ocular structure [22, 51]. Reactive oxygen species cause posttranslational changes in crystallins, which lead to the formation of insoluble protein aggregates. These changes negatively affect the transparency of the lens and result in the appearance of the initial stage of cataract [52, 66]. Numerous studies in experimental models indicate that exogenous antioxidants delay the progress of cataract formation [2, 10, 12, 23, 43, 55, 64, 65]. They also have a positive effect on the parameters related to the antioxidant capacity, as well as reduce the amount of oxidative damage in the lenses [57, 74, 75]. It should also be mentioned that several studies involving humans revealed that some antioxidative substances indeed positively affected the lenses with regard to cataract formation. However, there are studies conducted on human subjects indicating that antioxidants have no effect on the lenses or even show a negative impact on this structure [7, 33].

Phenolic acids, including rosmarinic acid (RA) and sinapic acid (SA), exhibit antioxidative properties [1, 9, 26, 61]. SA occurs in many edible plants, especially in vegetables from the *Brassicaceae* family, citrus and berry fruits, oil plants, grains and spices [9, 45, 61]. RA has been proved to occur in about 240 plant species. It can be found mainly in plants belonging to the *Lamiaceae* family, such as spice herbs: *Rosmarinus officinalis* L., *Melissa officinalis* L., *Salvia officinalis* L., *Satureja thymbra* L., *Mentha spp.*, *Origanum spp.* or *Perilla spp.* [1, 29, 49]. There are scientific reports describing a positive effect of RA on the lenses: *ex vivo* using human lenses after phacoemulsification [8] and *in vivo* in rats [8, 67]. These *in vivo* studies were performed in a model of sodium selenite-induced cataract. RA was administered intraperitoneally or subcutaneously [8, 67]. As a matter of fact, numerous animal studies employ a route of drug administration other than oral [23]. Nevertheless, when a new drug or dietary supplement is being developed, the best route of administration of such a substance is the oral route, because it is the easiest and most convenient way for patients. After oral intake, the active substances undergo absorption, cross numerous barriers, or are metabolized. Therefore, oral administration, treatment by gavage, or giving an active substance in diet should also be chosen in designing an experiment using an animal model, as this would mimic the route of drug intake in humans [56, 60, 70, 71]. In our previous study, conducted on rats in the early period of the oestrogen deficiency, we administered both RA and SA orally (by gavage) and both these phenolic acids revealed a beneficial effect on the redox status in the lens of these animals [81]. However, the effect of RA and SA on the redox status in the lenses of animals with type 2 diabetes has not been investigated yet, and as mentioned above, type 2 diabetes accounts for the majority of the occurrence of this disease in the population. Therefore, the aim of the experiment was to analyse the effect of these two phenolic acids on the oxidative stress parameters in the lens of rats with diabetes induced by a high fat diet (HFD) and streptozotocin (STZ), where changes similar to type 2 diabetes are observed.

Materials and Methods

Drugs and Chemicals

Rosmarinic acid (RA), sinapic acid (SA), chloramine T and 1,1,3,3-tetraethoxypropane were purchased from Sigma-Aldrich (St. Louis, MO, USA). From Cayman Chemical Company (Ann Arbor, MI, USA) following kits were obtained: Superoxide Dismutase (SOD) assay kit, Catalase (CAT) assay kit, reduced Glutathione (GSH) assay kit, Glutathione Peroxidase (GPx) assay kit and Glutathione Reductase (GR) assay kit. Oxi-Select™ Advanced Glycation End Product (AGE) Competitive ELISA and OxiSelect™ Protein Carbonyl Spectrophotometric assay kits were provided by Cell Biolabs (San Diego, CA, USA) and Glucose 6 Phosphate Dehydrogenase assay kit by Pointe Scientific (Canton, MI, USA). BioSystems (Costa Brava, Barcelona, Spain) developed and provided kits for protein total and γ-glutamyl transpeptidase (GGT) assay kit assessment. Ketamine (drug name Ketamine 10%) and xylazine (drug name Xylapan) were manufactured by Biowet Puławy Sp. z o.o., Puławy, Poland and Vetoquinol Biowet, Gorzów Wlkp., Poland, respectively.

Animals and Experimental Design

The biological material used in the course of this research – the lenses of rats – were obtained from animals which were studied during the larger experiment, the results of which have been partly published [80]. In order to conduct the experiment, sexually mature female Wistar rats were purchased from the Centre of Experimental Medicine, Medical University of Silesia, Katowice, Poland. The experiment was carried out with the approval obtained from the Local Ethics Commission, Katowice, Poland (permission numbers: 38/2015, 148/2015 and 66/2016). The animals were kept in laboratory conditions consistent with the 2010/63/EU Directive.

The animals were assigned to the experimental groups, as follows: C – control, non-diabetic rats; DM2 – control rats with type 2 diabetes induced by HFD and STZ; RA10 – rats with type 2 diabetes, treated orally with RA at a dose of 10 mg/kg bw; RA50 – rats with type 2 diabetes, treated orally with RA at a dose of 50 mg/kg bw; SA5 – rats with type 2 diabetes, treated orally with SA at a dose of 5 mg/kg bw and SA25 – rats

with type 2 diabetes, treated orally with SA at a dose of 25 mg/kg bw.

As previously described [80], the number of rats at the beginning of the experiment was 10 in each group, but only rats classified as type 2 diabetic animals were included in further analyses. The final number of rats in the groups was as follows: C: n = 10; DM2: n = 7; RA10: n = 8; RA50: n = 6; SA5: n = 7 and SA25: n = 7. The rats were fed with adequate diets (C rats with a standard laboratory chow, while diabetic groups with HFD) *ad libitum* and had access to the drinking water without limitations. HFD in all diabetic groups was introduced two weeks before STZ injection and was administered until the end of the study. In these rats (i.e., DM2, RA10, RA50, SA5 and SA25 groups) diabetes was induced by an intraperitoneal injection of 40 mg/kg bw STZ dissolved in 0.1 M citrate buffer pH 4.5 after 8-hours fasting. In order to maintain the identical conditions, the control group of rats (group C) was also fasted and then received an injection with citrate buffer only. The standard chow (Labofeed B) as well as HFD (Labofeed B 32% fat) were purchased from Wytwórnia Pasz "Morawski", Kcynia, Poland. Seven days after STZ administration, the development of diabetes was checked by measuring non-fasting blood glucose using a MicroDot glucometer and adequate strips (Cambridge Sensor USA, Plainfield, IL, USA). Blood for measuring glucose concentration was drawn from the tip of the tail. Animals with glucose levels above 200 mg/100 mL were qualified for further research.

The duration and route of RA and SA administration, as well as the specifics of the experimental design including euthanasia with ketamine + xylazine was described in detail in our previous article [80].

After the euthanasia, the eyeballs were removed from which the lenses were extracted. They were homogenized in PBS buffer, pH 7.4 according to their weight in order to obtain 10% w/v homogenate. The total homogenate was portioned and part of it was frozen. Total homogenate was used to determine the content of thiobarbituric acid reactive substances (TBARS). In order to determine advanced glycation end products (AGEs) in the lenses, the defrosted total homogenate was centrifuged, and the obtained supernatant was used to conduct the assay. The remaining part of the homogenate was centrifuged at 10,000 × g at 4°C for 15 minutes prior to freezing. The supernatant obtained after centrifugation was frozen and used to determine the remaining biochemical parameters. The analyses were performed using a Tecan Infinite M200 PRO plate reader (Tecan Austria, Grödig, Austria) with Magellan 7.2 Software.

Biochemical assays performed in the lenses

Right after the isolation from the eyeballs, the lenses were weighed on an AS 310 R2 analytical balance (Radwag, Radom, Poland). The determination of the soluble protein was performed with the biuret method using reagents from the BioSystems kit in the supernatant obtained after centrifugation.

As described before [81], the concentration of total glutathione (TotGSH) and oxidized glutathione (GSSG) were determined in the lens homogenate using the glutathione assay kit. The GSH concentration was calculated according to the formula: GSH = TotGSH - 2 × GSSG (nmol/mL). Based on the GSH and GSSG content in the lenses, the GSH/GSSG ratio was determined.

In order to determine the activity of the enzymes related to glutathione metabolism, similar like in [81], the adequate kits for GPx, GR, G6PD (glucose-6-phosphate dehydrogenase) and GGT, mentioned above were utilized according to the manufacturer's instructions. And as far as the other antioxidative enzymes, SOD and CAT, are concerned, the appropriate kits were used.

Advanced oxidation protein products (AOPP) content in the lenses was assessed according to the protocol presented by Witko-Sarsat *et al*. [73], absorbance was measured at a wavelength of 340 nm, and standard curve was prepared with chloramine T. Protein carbonyl groups (PCG) in the lenses was assayed spectrophotometrically with a commercially available kit, described above. Determination of TBARS was carried out spectrophotometrically (wavelength 535 nm) using the colour reaction of lipid peroxidation products with thiobarbituric acid according to the Ohkawa *et al*. method [48] with 1,1,3,3-tetraethoxypropane used as a standard. AGEs were determined with the ELISA kit.

Statistical analysis

The results are presented as the arithmetic mean ± standard error of the mean (SEM). One-way ANOVA and Duncan's post-hoc test were used to assess the statistical significance of the results (Statistica 13.3, TIBCO Software Inc., USA). It was assumed that the results are statistically significant if $p < 0.05$. The results, for which $p \leq 0.05$ in the Student's T-test, were described as the tendency. Moreover, principal component analysis (PCA) was performed for all biochemical parameters in the lenses in the Past 3 software [21]. PC scores were then subjected to MANOVA analysis with Duncan's post-hoc test in the Statistica software.

Results and Discussion

In the DM2 rats, the mean mass of the lenses and soluble protein content did not change significantly when compared to the C rats. The use of RA at the doses of 10 and 50 mg/kg bw and SA at the doses of 5 and 25 mg/kg bw did not change these parameters in the lenses of phenolic acid-treated diabetic rats, compared to the DM2 animals (Table I).

Table I

The mean mass of the lens and soluble protein content in the lenses of the studied rats

Parameter/group	C	DM2	RA10	RA50	SA5	SA25
Lens mass (g)	0.060 ± 0.002	0.056 ± 0.002	0.056 ± 0.001	0.057 ± 0.002	0.056 ± 0.002	0.057 ± 0.002
Soluble protein (mg/g of the lens)	179.9 ± 13.4	190.7 ± 5.8	197.1 ± 6.8	198.0 ± 8.8	203.2 ± 15.3	200.9 ± 14.1

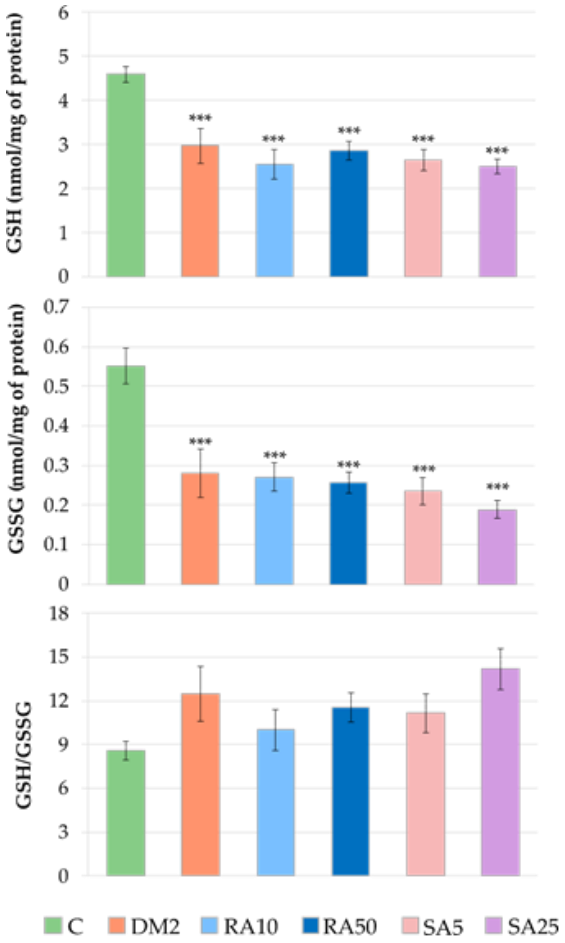

Figure 1.

GSH and GSSG content in the lenses of the studied rats and the calculated lens GSH/GSSG ratio

*** $p < 0.001$: significant differences with regard to the control rats

In the lenses of the DM2 rats, the content of GSH decreased significantly compared to the content of GSH in the lenses of the C rats. The administration of RA and SA to diabetic rats did not change the GSH content in the lenses. Similarly, the content of GSSG also decreased significantly in the lenses of the DM2 rats in relation to the GSSG content in the lenses of the C rats. The GSH/GSSG ratio in the lenses of diabetic and non-diabetic rats did not show statistically significant differences. The administration of both phenolic acids at both doses to the rats with type 2 diabetes did not significantly change the GSH/GSSG ratio of the lenses (Figure 1).

The activity of the glutathione metabolism-related enzymes: GPx, GR, G6PD and GGT in the lenses did not change significantly in the DM2 rats, compared to their activity in the lenses of the C rats. When the activity of these enzymes was examined in the lenses of the RA10, RA50, SA5 and SA25 groups of rats, it turned out that administration of RA and SA did not affect them, as compared to the lenses of the DM2 rats (Table II).

The SOD activity in the lenses of the DM2 rats did not differ significantly from the activity of SOD in the lenses of the C rats. There was no effect of RA and SA administration to the RA10, RA50, SA5 and SA25 rats with regard to the SOD activity in the lenses when compared to the DM2 rats. In the lenses of the DM2 rats, a statistically significant increase in the CAT activity was noted as compared to the C rats. In comparison to the C rats, increased CAT activity in the lenses was observed in the rats receiving RA at both doses. The CAT activity in the lenses of the SA5 and SA25 rats was not significantly different from that observed in the lenses of the DM2 and C rats (Figure 2).

Table II

The glutathione-related enzymes activity in the lenses of the studied rats

Parameter/group	C	DM2	RA10	RA50	SA5	SA25
GPx (nmol/min/mg of protein)	3.17 ± 0.24	3.17 ± 0.14	2.46 ± 0.33	2.81 ± 0.05	2.82 ± 0.19	2.93 ± 0.09
GR (nmol/min/mg of protein)	0.486 ± 0.057	0.399 ± 0.039	0.371 ± 0.090	0.279 ± 0.035	0.424 ± 0.054	0.409 ± 0.058
G6PD (nmol/min/mg of protein)	3.43 ± 0.51	2.60 ± 0.30	2.52 ± 0.25	2.27 ± 0.38	2.54 ± 0.21	2.41 ± 0.18
GGT (nmol/min/mg of protein)	0.094 ± 0.021	0.057 ± 0.010	0.074 ± 0.011	0.063 ± 0.012	0.064 ± 0.018	0.064 ± 0.009

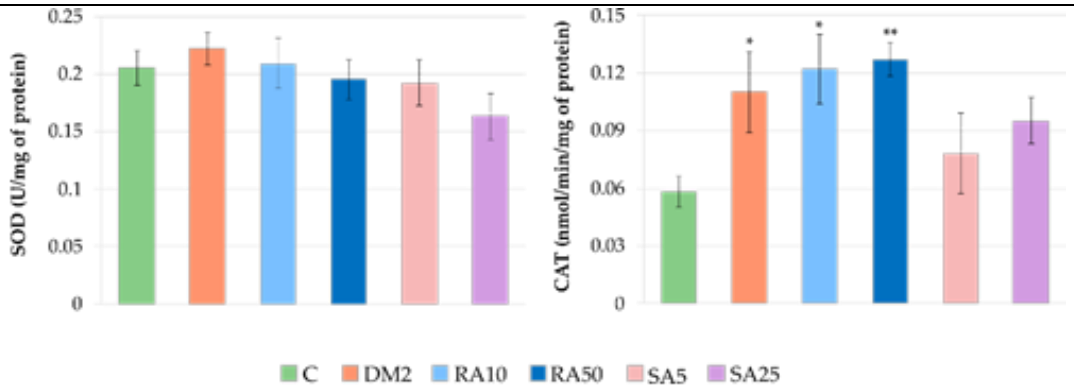

Figure 2.
SOD and CAT activities in the lenses of the studied rats
* $p \leq 0.05$, ** $p < 0.01$: significant differences with regard to the control rats

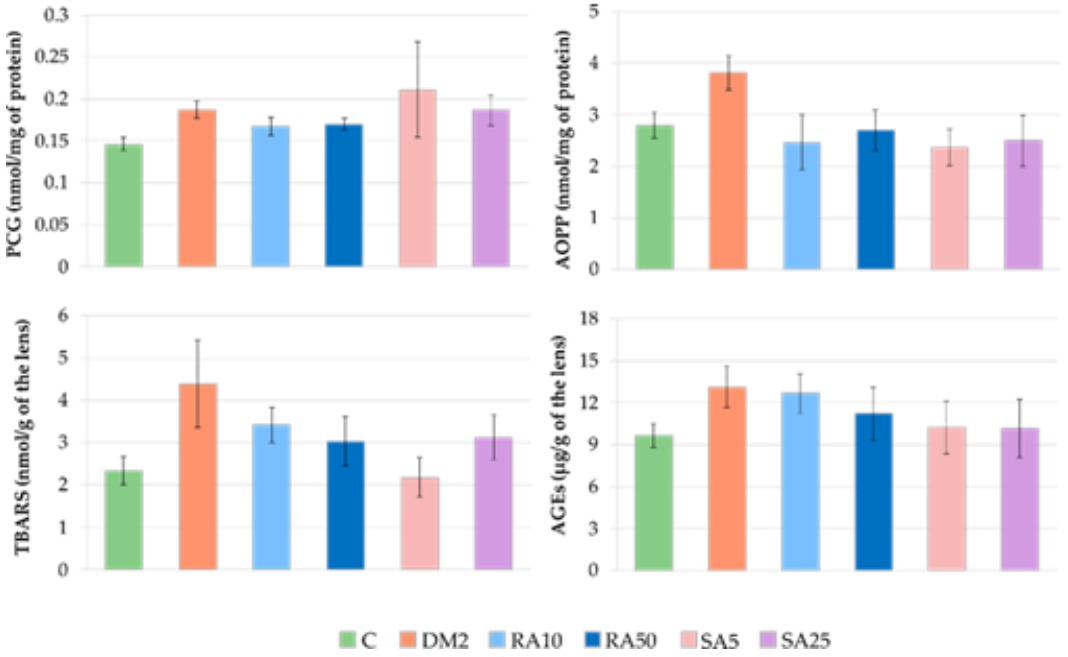

Figure 3.
PCG, AOPP, TBARS and AGEs content in the lenses of the studied rats

The content of AOPP, TBARS, PCG and AGEs in the lenses of the DM2 rats tended to increase compared to the content of these oxidative damage products in the lenses of the C rats. The "tendency" means that based on the Student's t-test, the obtained results from the DM2 rats were different from the values recorded in the C rats ($p \leq 0.05$), but in ANOVA no significant differences were revealed. After administration of RA at a dose of 50 mg/kg bw and SA in both doses (5 and 25 mg/kg bw), there was a tendency to decrease the AOPP content in the lenses when compared to the DM2 rats. The content of the PCG, TBARS and AGEs in the lenses of the RA10, RA50, SA5 and SA25 rats did not change, compared to the DM2 rats (Figure 3).

Principal component analysis

The PCA revealed a separation across the principal component 1 (PC1), which accounted for 23.1% of the observed variation. The C group, which separated to the right, significantly differed from the other groups (C *vs*. DM2 $p < 0.05$ and C *vs*. all the groups receiving phenolic acids $p < 0.01$), which clustered near the plot centre and slightly to the left. This separation was determined mainly by soluble protein as well as by GSH in both forms and its enzymes. The second principal component (PC2) was insignificant (Figure 4).

One of the used models of type 2 diabetes in rodents is the model combining a HFD with intraperitoneal administration of STZ [4, 18]. This model is often used to study the effect of active substances on parameters related to the metabolism of carbohydrates and lipids [5, 19, 24, 41, 44]. As we have presented in our previous study, the animals used in this experiment fed with HFD and injected with STZ developed type 2 diabetes – their mean glucose level exceeded the threshold of 200 mg/dL. The other glycaemia-related parameters, such as insulin and fructosamine concentration in the serum or calculated indices like homeostatic model

assessment - insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI), also confirmed diabetes in the HFD+STZ groups of rats. What is more, the serum lipid profile was also distorted in these animals [80].

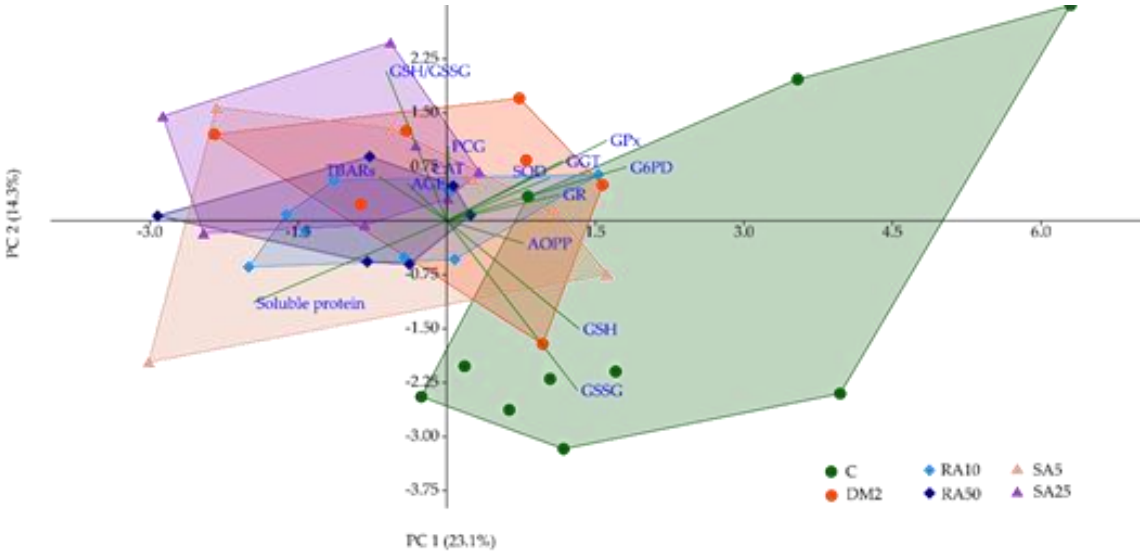

Figure 4.
PCA biplot of biochemical parameters measured in the lenses of the studied rats

The model shows similar organ changes and changes in biochemical parameters that occur in humans with type 2 diabetes [4, 37]. It is also used for model eye diseases, such as diabetic retinopathy, corneal diseases [11, 13, 36] and cataract [62]. Although a more frequently used model of diabetic cataract is the rodent model obtained by a single injection of a high dose of STZ, this model is more similar to type 1 diabetes [42, 50, 77]. It is known that cataract in humans in the course of type 1 diabetes develops rapidly, while in type 2 diabetes, the opacity of the lens usually progresses much slower [47]. This was also confirmed in studies using rat models of diabetes. In rats with type 1 diabetes induced by a single intraperitoneal administration of 60 mg/kg bw STZ, opacity of the lens began to appear after three weeks of diabetes duration, while in rats with type 2 diabetes induced by a HFD administered four weeks prior to a single intraperitoneal administration of 50 mg/kg bw STZ, the onset of cataract was observed after five weeks [62]. Morphological changes in the lens in type 2 diabetes are similar to senile cataract [47].

As the most important non-enzymatic antioxidant of the lens, glutathione regulates the redox state and maintains its transparency [17, 33]. Age-related reduction in the levels of glutathione in the lens leads to damage to its structural proteins, which results in opacity formation [33]. In this study, a significant reduction in the GSH level in the lenses of rats with type 2 diabetes was noted. This is consistent with the reports of other authors on the reduction of GSH content in the lenses as a result of an increased level of oxidative stress in the course of diabetes [57, 64, 74], including type 2 diabetes [62]. GSH can protect proteins from permanent oxidation by attaching to their –SH groups, or as a cofactor of enzymes such as: thioltransferase, glutathione S transferases or glutathione peroxidase [14, 16, 34, 53]. In the present study, a reduction in GSH was observed without changes in the activity of GPx and other glutathione-related enzymes, but at the same time, it was found that the content of lens soluble protein did not decrease. This may prove a direct protective effect of GSH on the lens soluble proteins. It should also be noted that in this study, the GSSG content was reduced in the lenses of diabetic rats compared to control rats, while in the experiment of Su *et al*. [62], an increase in GSSG content was observed in rats. Umapathy *et al*. and Li *et al*. [31, 68] showed that GSSG could be actively exported from the lens to ensure optimal redox balance. The reduction in GSSG level observed in this study, and the lack of changes in the GSH/GSSG ratio may indicate the compensatory defence of the lens against oxidative stress and disturbances in redox homeostasis. It is also worth noting that in this study female rats were used, while the experiment by Su *et al*. [62] was conducted on male animals. Some authors clearly indicate that differences in glutathione metabolism and responses to oxidative stress depend on gender [15, 69]. Of all the enzymes the activity of which was analysed in this study, only CAT activity increased significantly in the eye lenses of the diabetic rats compared to the control rats. The main function of CAT is to decompose H_2O_2 into water and oxygen. The lack of an increase in SOD activity does not indicate the lack of H_2O_2 in the environment, since other enzymes, *e.g.* xanthine oxidase, can also generate H_2O_2 [30].

During the prolonged exposure of the lens to oxidative stress, resulting, for example, from aging or diabetes, the risk of oxidative damage products accumulation within this structure increases [46]. In this experiment, an upward trend in the parameters depicting the oxidative damage: PCG, AOPP, TBARS and AGEs were noted in the lenses of diabetic animals compared to the control rats. Many publications indicate an increase in the TBARS and PCG levels in the lenses of diabetic rats [64, 65, 75]. Even though only an upward trend was observed for the content of oxidative damage products in diabetic rats, the multivariate PCA showed significant differences between the parameters in the lenses of non-diabetic and diabetic rats. On this basis, it can be concluded that the parameters of antioxidant capacity were disturbed, and the parameters of oxidative damage increased in the lenses, which may lead to the formation of the lens opacity after a longer duration of diabetes. Polyphenols, including phenolic acids, inhibit the formation of reactive oxygen species, strengthen the antioxidant defence system, and have anti-inflammatory properties; therefore, they are being studied as potential candidates in the prevention and treatment of chronic eye diseases, such as cataract [76]. This study investigated the effect of RA and SA administered by gavage on oxidative stress parameters in the lenses of rats with experimental type 2 diabetes, which were characterized by hyperglycaemia, hypercholesterolemia and hypertriglycerideaemia [80]. As previously described, the dosages of RA and SA were chosen based on literature data considering the possible dietary intake of these two phytochemicals with a regular diet by an average human. The literature provides information about the range or the mean concentration of both these phenolic compounds in the food and beverages [1, 29, 45, 78-80]. Nevertheless, in the studies conducted on rats, the dose needs to be normalized based on the body surface area using the 6.17 conversion factor [58]. Therefore, the doses used in our study were adjusted so that smaller amounts (10 mg/kg bw RA and 5 mg/kg bw SA) corresponded to the amount possible to be consumed in the diet. Higher dosages can be consumed in the form of dietary supplements. RA at a dose of 50 mg/kg bw and SA at a dose of 25 mg/kg bw were used to test whether they exerted a stronger therapeutic effect than dietary-achievable doses [78-80].

In our previous study [80], we showed that RA and SA, both in lower and higher doses, neither affected the parameters of oxidative stress in blood serum nor had a beneficial effect on the parameters related to glycaemia. Only RA at a dose of 50 mg/kg bw and SA at a dose of 25 mg/kg bw improved the lipid profile in rats with type 2 diabetes. Examination of the parameters of oxidative stress in the cardiac tissue showed that both doses of RA and 25 mg/kg bw of SA decreased the activity of SOD, which was increased due to diabetes, whereas the activity of CAT decreased as a result of the administration of both doses of SA. Moreover, having analysed all the parameters, it was observed that higher doses of the tested phenolic acids showed a normalizing effect on the parameters of oxidative stress in the cardiac tissue of animals with type 2 diabetes [80]. Unfortunately, in the presented study, RA and SA at both lower and higher doses did not increase the GSH content in the lenses or affect TBARS, PCG and AGEs. Yet, RA at a 50 mg/kg bw dose and SA at both doses showed a tendency to reduce AOPP level in the lenses.

We expected that the phenolic acids administered by gavage to the type 2 diabetic rats would have a beneficial effect on redox status in the lenses, similar as in the case of rats in the early period of oestrogen deficiency [81]. A positive effect on the lenses of rats with selenite-induced cataracts has been reported after intraperitoneal administration of RA. It enhanced the content of GSH and the activity of antioxidant enzymes and decreased the level of lipid peroxidation products and parameters of the inflammatory process in the lenses [67]. Subcutaneously administered RA to rats with selenite-induced cataract delayed the appearance of lens opacities and reduced the severity of cataract [8]. RA also reduced the opacity of lenses in an *ex vivo* study in which the test subject were the human lenses obtained during phacoemulsification surgery [8]. On the contrary, in the present study, the level of GSH in the lenses of diabetic rats was not affected by RA or SA when they were administered by gavage. It is worth noting that in the type 2 diabetic rats, neither RA nor SA administered by gavage affected serum non-protein thiol groups (NPSH) levels, which are mainly represented by GSH [80]. On the other hand, our another study showed that the tested phenolic acids increased the concentration of GSH in the serum of the oestrogen-deficient rats [78, 79], which may result in an increase in GSH content in the lenses [81]. The relationship between the GSH content in the lens and the GSH in the serum was confirmed by the study by Whitson *et al*. [72] using a mouse model of the lens glutathione synthesis knockout (LEGSKO), which showed that circulating GSH easily enters the vitreous body and then the lens, and despite the lack of GSH synthesis in the lens itself, GSH levels in the lens remained high. Therefore, the lack of an effect of the tested phenolic acids on the GSH content in the lenses of type 2 diabetic rats may result from no effect of these substances on the GSH (NPSH) level in the serum [80].

Although phenolic acids are commonly considered powerful antioxidants [26], the results of our study indicate that their influence on the parameters of oxidative stress in the lenses of rats with type 2 diabetes induced by HFD and STZ is negligible. The previously observed effect of SA and RA on increasing GSH content in the lenses of rats in the early stage of oestrogen deficiency [81] was not noted in rats with

type 2 diabetes. It seems that the effect of these phenolic acids in the state of chronic hyperglycaemia, hypercholesterolemia and triglyceridaemia is too weak [80] to normalize the disturbed redox balance in the lenses, which may result in the inability to prevent cataract. These results appear to be consistent with studies in humans using oral antioxidant substances, where antioxidants neither prevented cataract nor delayed its formation [38, 39].

Conclusions

As described above, GSH is one of the crucial molecules preventing the lens cataract. In the presented study, two phenolic acids, RA and SA, which are food bioactive compounds, were administered at dietary achievable doses and doses five times higher than dietary. Upon the obtained results it can be concluded that both these phytochemicals, regardless of the dose, do not increase the GSH level which was depleted in the female rats' lenses in the course of type 2 diabetes, when adverse metabolic changes in the body are advanced.

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Conflict of interest

The authors declare no conflict of interest.

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