The Effect of Nano-Epigallocatechin-Gallate on Oxidative Stress and Matrix Metalloproteinases in Experimental Diabetes Mellitus

Adriana Elena Bulboaca 1, Paul-Mihai Boarescu 1,*, Alina Silvia Porfire 2,*, Gabriela Dogaru 3,*, Cristina Barbalata 2, Madalina Valeanu 4, Constantin Munteanu 5, Ruxandra Mioara Râjnovan 6, Cristina Ariadna Nicula 7 and Ioana Cristina Stanescu 8

1 Department of Pathophysiology, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Victor Babeș Street, no. 2-4, 400012 Cluj-Napoca, Romania; adriana.bulboaca@umfcluj.ro
2 Department of Pharmaceutical Technology and Biopharmaceutics, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Victor Babeș Street, no. 41, 400012 Cluj-Napoca, Romania; barbalata.cristina@umfcluj.ro
3 Department of Physical Medicine and Rehabilitation, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Viilor Street, no. 46-50, 400347 Cluj-Napoca, Romania
4 Department of Medical Informatics and Biostatistics, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Louis Pasteur Street, no. 6, 400349 Cluj-Napoca, Romania; mvaleanu@umfcluj.ro
5 Department of Medical Rehabilitation, “BagdasarArseni” Emergency Clinical Hospital Bucharest, Berceni Street, no. 12, 041915 Cluj-Napoca, Romania; office@bioclima.ro
6 Department of Pneumology, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, B.P. Hasdeu Street, no. 6, 400371 Cluj-Napoca, Romania; andra_redro@yahoo.com
7 Department of Ophthalmology, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Clinicilor Street, no. 3-5, 400006 Cluj-Napoca, Romania; niculacr4la6@yahoo.com
8 Department of Neurology, Iuliu Hațieganu University of Medicine and Pharmacy Cluj-Napoca, Victor Babeș Street, no. 43, 400012 Cluj-Napoca, Romania; ioana.stanescu.umfcluj@gmail.com

* Correspondence: boarescu.paul@umfcluj.ro (P.-M.B.); porfire@umfcluj.ro (A.S.P.); dogarugabrielaumf@gmail.com (G.D.); Tel.: +40-752-921-725 (P.-M.B.); +40-264-595-770 (A.S.P.); +40-724-231-022 (G.D.)

Received: 11 January 2020; Accepted: 18 February 2020; Published: 20 February 2020

Abstract: Background: The antioxidant properties of epigallocatechin-gallate (EGCG), a green tea compound, have been already studied in various diseases. Improving the bioavailability of EGCG by nanoformulation may contribute to a more effective treatment of diabetes mellitus (DM) metabolic consequences and vascular complications. The aim of this study was to test the comparative effect of liposomal EGCG with EGCG solution in experimental DM induced by streptozotocin (STZ) in rats. Method: 28 Wistar-Bratislava rats were randomly divided into four groups (7 animals/group): group 1—control group, with intraperitoneal (i.p.) administration of 1 mL saline solution (C); group 2—STZ administration by i.p. route (60 mg/100 g body weight, bw) (STZ); group 3—STZ administration as before + i.p. administration of EGCG solution (EGCG), 2.5 mg/100 g b.w. as pretreatment; group 4—STZ administration as before + i.p. administration of liposomal EGCG, 2.5 mg/100 g b.w. (L-EGCG). The comparative effects of EGCG and L-EGCG were studied on: (i) oxidative stress parameters such as malondialdehyde (MDA), indirect nitric oxide (NOx) synthesis, and total oxidative status (TOS); (ii) antioxidant status assessed by total antioxidant capacity of plasma (TAC), thiols, and catalase; (iii) matrix-metalloproteinase-2 (MMP-2) and -9 (MMP-9). Results: L-EGCG has a better efficiency regarding the improvement of oxidative stress parameters (highly statistically significant with p-values < 0.001 for MDA, NOx, and TOS) and for antioxidant capacity of plasma (highly significant p < 0.001 for thiols and significant for catalase and TAC with p < 0.05). MMP-2 and -9 were also significantly reduced in the L-EGCG-treated group compared with the EGCG group (p < 0.001).

Antioxidants 2020, 9, 172; doi:10.3390/antiox9020172 www.mdpi.com/journal/antioxidants
Conclusions: the liposomal nanoformulation of EGCG may serve as an adjuvant therapy in DM due to its unique modulatory effect on oxidative stress/antioxidant biomarkers and MMP-2 and -9.

**Keywords:** epigallocatechin-gallate; liposomes; diabetes mellitus; oxidative stress

1. Introduction

Consuming green tea has been linked to human health and longevity for centuries. In particular, green tea catechins are involved in many biological processes such as antioxidant activity and modulation of various cellular lipid and protein metabolisms [1]. Green tea contains a great amount of polyphenols (flavonols, flavones, and flavanols) with similar structure, possessing lots of therapeutic active components including catechin, epicatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG) [2]. EGCG is the most active and abundant compound (65% of total catechin content) [3,4].

Green tea therapeutic effects have been studied intensively, proving beneficial in various diseases such as cancer [5], hyperlipidemia [6,7], cardiovascular diseases [8,9], neurodegenerative diseases [10,11], and infectious diseases [12,13]. Some reports also suggest that daily consumption of tea catechins may help in controlling type 1 [14] and type 2 diabetes mellitus [1]. It has been demonstrated that green tea consumption reduces fasting glucose levels, an effect mediated by EGCG [15]. Lipophilic EGCG has been shown to reduce glycemia and serum lipids in experimental diabetes mellitus induced by streptozotocin (STZ) in rats [16].

Type 1 diabetes mellitus (DM) is associated with an autoimmune-mediated destruction of pancreatic beta cells, leading to absolute insulin deficiency [17]. One of the most used experimental models for testing various therapies addressing type 1 DM is based on STZ administration. STZ induces type 1 DM, with destruction of pancreatic beta cells and associated insulin deficiency, as a result of its cytotoxic effect, mediated by increased synthesis of reactive oxygen species (ROS) and subsequent inflammation [18–20]. A protective effect of EGCG on pancreatic beta cells has been already demonstrated in experimental studies [21]; meanwhile, oral chronic administration of EGCG proved to have hypoglycemic and hypolipidemic effects and to reduce oxidative stress in streptozotocin-diabetic rats [22]. EGCG can exert antioxidant, anti-inflammatory, antiangiogenetic, and antifibrotic effects [2]. The catechol or galloyl groups from catechins act as scavengers for metal ions, reducing further production of free radicals [23]. Another essential effect is represented by the scavenging activity for free radicals, through phenoxyl compounds [24]. EGCG treatment can also reduce oxidative stress by increasing the level of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GP), and catalase (CAT), emerging in an antiapoptotic consequence [25].

Matrix metalloproteinases (MMPs) are a family of enzymes (peptidases) involved in degradation and remodeling of extracellular matrix (ECM) [26]. Recent studies reveal that MMPs can regulate chemokines and cytokines synthesis, thus participating in innate immunity processes, inflammation, and angiogenesis [27]. MMPs can be generated by various cell types, such as endothelial cells and mononuclear cells of the immune system [28]. Pathological induction of MMP synthesis is associated with an imbalance between synthesis and degradation of ECM proteins leading to ECM degradation [29]. High glucose ambience influences the MMPs’ increased synthesis and low tissue inhibitors of MMPs (TIMP) activity [30]. Increased levels of MMP-2 and MMP-9 are observed in type 1 diabetic patients and animal models, such as STZ-induced diabetes mellitus in rats [31,32], and are associated with microvascular complications of DM [28].

Analyzing the EGCG therapeutic properties and pharmacokinetic parameters, considerable individual differences and variations between results were noted [33]. EGCG is highly lipophilic, which explains its low bioavailability (0.2% to 2% of the total load ingested by healthy people), mainly because a large amount of the ingested EGCG is degraded by local microbiota and does not enter into the blood circulation [34]. Improvement of bioavailability and stability of EGCG can be obtained by encapsulation
in nanoparticles [35]. Catechin nanoemulsions proved to be stable for long periods of time (120 days at 4 °C) [36]. Liposomes, assembled from phospholipid bilayers similar to cell membranes, are one of the nanoparticles frequently used for drug delivery [23]. Their biphasic character makes them suitable for being carriers for both hydrophilic (in the central aqueous compartment) and hydrophobic (in lipid bilayers) compounds [37,38]. Nanoformulation by encapsulation in liposomes could also facilitate the solubility for hydrophobic particles [4]. Through all of these properties, liposomes can offer an enhanced bioavailability, stability, and shelf life for sensitive ingredients [39].

The aim of this study was to investigate the effect of two forms of EGCG (EGCG solution and liposomal EGCG) on oxidative stress parameters, antioxidant capacity, serum MMP-2 and -9, and pancreatic and liver function in STZ-induced diabetes mellitus in rats.

2. Materials and Methods

2.1. Materials

The substances used for liposomal preparation were: Epigallocatechin-gallate (EGCG) derived from green tea (Sigma-Aldrich, Steinheim, Germany); 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC): N-(carbonyl-methoxypolyethylenglycol-2000)-1,2-distearoylsn-glycero-3-phosphoethanolamine Na-salt (MPEG-2000-DSPE) (Lipoid GmbH, Ludwigshafen am Rhein, Germany); and cholesterol (CHO) obtained from sheep wool (Sigma-Aldrich, Steinheim, Germany). All other solvents and reactive substances were obtained from Sigma-Aldrich, Steinheim, Germany, and had an analytical degree of purity.

2.2. Experimental Model

The study was approved by the Ethic Committee of the University and by the National Sanitary Veterinary Authority number 137/13.11.2018. Twenty-eight male Wistar-Bratislava rats were procured from the Centre of Experimental Medicine, University of Medicine and Pharmacy, Cluj-Napoca, Romania. The rats weighed 200–250 g, were kept in polypropylene cages, with day–night regimen, at constant temperature (24 ± 2 °C) and humidity (60 ± 5%). Free access to food (standardized pellets from Cantacuzino Institute, Bucharest, Romania) and water was provided to all animals. The animals were randomly divided into 4 groups (7 rats/group). The groups were organized as follows:

- group 1—control group (C)—with intraperitoneal (i.p.) administration of 1 mL saline solution,
- group 2—STZ administration by i.p. route (STZ),
- group 3—STZ administration as before + i.p. administration of EGCG solution (EGCG),
- group 4—STZ administration as before + i.p. administration of liposomal EGCG (L-EGCG).

Each medication was dissolved in saline solution (0.9% sodium chloride) and the volume administrated i.p. was 1 mL [19]. The following doses were used: STZ—60 mg/100 g body weight (b.w.) [40]; EGCG in saline solution or in liposomal form were freshly prepared and were administrated i.p. in a dose of 2.5 mg/100 g b.w/day as pretreatment, two consecutive days before STZ administration [41]. Intraperitoneal administration was preferred as a method that improves EGCG bioavailability, compared to low bioavailability with oral administration [42].

Blood samples were taken at 48 h after STZ administration, under ketamine anesthesia (5 mg/100 g bw, i.p. route) from retro-orbital sinus, followed by rat euthanasia by cervical dislocation [43]. Rats with glucose higher or equal to 200 mg/dL were considered to have diabetes mellitus [20].

2.3. Preparation and Physicochemical Characterization of EGCG-Loaded Liposomes

For the preparation of liposomes, we used a modified film hydration method [44,45]. The lipid double-layer components, having a 70 mM concentration (DPPC:MPEG-2000-DSPE:CHO = 4.75:0.25:1 molar ratio), were dissolved in ethanol in a round-bottomed glass flask. Ethanol was evaporated at 45 °C under low pressure; the lipid film product was hydrated with a solution of EGCG diluted in highly purified water, pH = 5.00, at the same temperature. The resulted liposomal
dispersion was then extruded through polycarbonate membranes with 200 nm final pore dimension, with LiposoFastLF-50 equipment (Avestin Europe GmbH, Mannheim, Germany). Unencapsulated EGCG particles were removed by dialysis method, using Slide-A-Lyzer filters (cassettes) with 10 kDa molecular weight cut-off.

To assess the amount of liposomal-loaded EGCG, we used a spectrophotometric method—the reaction with Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) [46]. During this procedure, a dilution of liposomal dispersion with methanol 1:10 (v/v) was made, and a UV-VIS spectrophotometer (Specord 200 Plus, Analytik Jena, Überlingen, Germany) measured the absorbance value.

The size and polydispersity index of liposomes were assessed by dynamic light scattering method (with a 90° scattering angle), and the zeta potential was measured by laser Doppler electrophoresis; a Zetasizer Nano ZS analyzer was used for both assessments (Malvern Instruments Co., Malvern, UK).

The mean liposomal concentration of the L-EGCG solution was about 900 µg/mL, and encapsulation efficiency was over 80%. Liposomal vesicles’ mean size was 170 nm, and polydispersity index was less than 0.2, meaning that the vesicles’ size and uniformity were appropriate to ensure a prolonged circulation in the blood. Aggregative stability was ensured by values of 51.83 mV of the zeta potential.

2.4. Oxidative Stress and Antioxidant Parameters Assessment

Parameters associated with oxidative stress and antioxidant status were determined from collected blood samples. The parameters used to assess oxidative stress were: malondialdehyde (MDA) [47], indirect nitric oxide (NOx) synthesis assessment [48], and total oxidative status (TOS) [49]. Antioxidant status parameters were represented by total antioxidant capacity of plasma (TAC) [50], thiols [51], and catalase [52]. All measurements were performed using a Jasco V-350 UV-VIS spectrophotometer (Jasco International Co, Ltd., Tokyo, Japan). Matrix metalloproteinases (MMPs) were appraised from serum using a rat ELISA kit (Boster Biological technology, Pleasanton, CA, USA) and a Stat Fax 303 ELISA reader (Quantikine, McKinley Place NE, MN, USA).

2.5. Assessment of Beta Pancreatic Cells and Hepatic Cells Function

Glycemia was measured at 48 h after DM induction, as it was previously observed that STZ induces significant beta cell death at 48 after administration [53]. Glycemia was also used as a parameter for pancreatic function changes induced by experimental diabetes mellitus. Hepatic cytolysis was assessed by serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measured by a standardized technique (Vita Lab Flexor E, Spankeren, The Netherlands) [40].

2.6. Data Analysis

The SPSS software package version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and graphic representations. The acceptable error threshold was $p = 0.05$. In order to describe the continuous quantitative data, we used the arithmetic mean and the standard deviation (SD). The distribution of investigated markers in groups was plotted as individual values (circles) and median (line), as recommended by Weissgerber and coauthors [54]. The Kruskal–Wallis ANOVA was used to test the differences in the investigated markers. The Mann–Whitney test was used in post hoc analysis when significant differences were identified by the Kruskal–Wallis ANOVA test.

3. Results

No rat died during the experiment, so the analysis was conducted on all seven rats in each group. All P values for comparison between groups are presented in Supplementary Table S1.

In our experimental model, diabetes mellitus was successfully induced by STZ: all rats that received STZ were definitely diabetic, proven by glycemia >200 mg/dL and values significantly higher in diabetic rats compared to control group: 401.81 (11.31) mg/dL versus 84.27 (2.87) mg/dL, respectively (expressed as mean and standard deviation), with a $p$-value < 0.001. Also, hepatic damage was detected in the STZ group, quantified by significant elevation of transaminases AST and ALT (Table 1).
In our experimental model, diabetes mellitus was successfully induced by STZ: all rats that received STZ were definitely diabetic, proven by glycemia >200 mg/dL and values significantly higher in the STZ-induced DM group compared with control group: 401.81(11.31) mg/dL versus 84.27 (2.87) mg/dL, respectively (expressed as mean and standard deviation), with a p-value of 0.001 in all items, Figure 1a–c, Table 1.

Table 1. Values of oxidative stress parameters, antioxidants levels, glycemia, hepatic enzymes, and matrix metalloproteinases in the four groups, expressed as mean and standard deviation.

| Parameter       | Control (n = 7) | STZ (n = 7) | STZ + EGCG (n = 7) | STZ + L-EGCG (n = 7) |
|-----------------|----------------|------------|-------------------|---------------------|
| MDA [nmol/mL]   | 2.52(0.24)     | 20.94(1.67)| 19.83(1.1)        | 14.1(1.67)          |
| NOx [µmol/L]    | 24.35(2.24)    | 64.34(2.26)| 60.63(2.65)       | 40.36(2.89)         |
| TOS [µmol/L]    | 17.19(1.05)    | 74.22(2.63)| 66.68(3.45)       | 44.84(3.06)         |
| Thiols [mmol /L]| 213.4(6.64)    | 112.33(6.02)| 131.1(3.17)     | 145.64(5.14)        |
| Catalase [U/mL] | 20.12(1.87)    | 10.87(0.87)| 12.81(1.69)       | 15.8(2.42)          |
| TAC [mEq/L]     | 1.41(0.09)     | 0.64(0.06) | 0.83(0.14)        | 1.07(0.13)          |
| Glycemia [mg/dL]| 84.27(2.87)    | 401.81(11.31)| 391.1(10.55)     | 365.3(6.56)         |
| AST [U/L]       | 26.03(2.16)    | 150.37(9.16)| 141.5(9.45)      | 80.67(8.88)         |
| ALT [U/L]       | 24.63(2.23)    | 204.58(9.8) | 193.17(6.57)     | 64.18(3.42)         |
| MMP-2 [ng/mL]   | 86.14(5.96)    | 221(7.19)  | 217.71(7.23)      | 156(6.73)           |
| MMP-9 [ng/mL]   | 19.57(1.27)    | 37(2.24)   | 36.29(2.56)       | 28.14(2.19)         |

MDA = malondialdehyde; NOx = indirect nitric oxide; TOS = total oxidative status; AST = aspartate aminotransferase; ALT = alanine aminotransferase; MMP-2 = matrix metalloproteinase 2; MMP-9 = matrix metalloproteinase 9; STZ = streptozotocin control; STZ + EGCG = STZ and EGCG solution i.p. as pretreatment; STZ + L-EGCG = STZ and liposomal EGCG i.p. as pretreatment.

Oxidative stress parameters (MDA, NOx, and TOS) significantly increased after induction of DM (p-values <0.001 in all items, Figure 1a–c, Table 1). MMP-2 and MMP-9 levels were significantly higher in the STZ-induced DM group compared with control group (p-values <0.001, Figure 4a,b, Table 1). Serum antioxidant capacity, measured by thiol, catalase, and TAC levels, was significantly reduced in diabetic rats compared to control animals (p-values < 0.001 in all items, Figure 2a–c, Table 1).

In the diabetic group pretreated with EGCG, oxidative stress parameters NOx and TOS were significantly reduced compared to the untreated STZ group (with p-values of 0.017 and <0.001, respectively, Figure 1b,c).

![Figure 1. Cont.](image-url)
Antioxidants 2020, 9, x FOR PEER REVIEW 6 of 15

(c)
Figure 1. Distribution of oxidative stress intensity by groups: (a) MDA (malondialdehyde), (b) NOx (indirect nitric oxide), (c) TOS (total oxidative status) on all study groups (7 rats/group). STZ = streptozotocin control; STZ + EGCG = STZ and EGCG solution i.p. as pretreatment; STZ + L-EGCG = STZ and liposomal EGCG i.p. as pretreatment. The symbol–number codes correspond to the p-values < 0.05 as follows: α—STZ compared to control; β—STZ + EGCG compared to control; γ—STZ + EGCG compared to STZ; δ—STZ + L-EGCG compared to control; ε—STZ + L-EGCG compared to STZ; µ—STZ + L-EGCG compared to STZ + EGCG.

(a) (b) (c)

Figure 2. Distribution of plasmatic antioxidant capacity by groups: (a) Thiols, (b) Catalase, (c) TAC (total antioxidant capacity) on all study groups (7 rats/group). STZ = streptozotocin control; STZ + EGCG = STZ and EGCG solution i.p. as pretreatment; STZ + L-EGCG = STZ and liposomal EGCG i.p. as pretreatment. The symbol–number codes correspond to the p-values < 0.05 as follows: α—STZ compared to control; β—STZ + EGCG compared to control; γ—STZ + EGCG compared to STZ; δ—STZ + L-EGCG compared to control; ε—STZ + L-EGCG compared to STZ; µ—STZ + L-EGCG compared to STZ + EGCG.
All antioxidant parameters (thiols, catalase, and TAC) were significantly higher in the STZ-treated group (p-values of < 0.001, 0.026, and 0.017 respectively, Figure 2a–c).

No significant differences were noted in MDA and MMP values between the pretreated group with EGCG compared to the untreated STZ group (Figure 1a, Figure 4a,b). Also, glycemia and liver parameters were not significantly different in the EGCG pretreated group, with the exception of a decrease in ALT (p-value = 0.038, Figure 3c).

In the STZ group pretreated with L-EGCG, all oxidative stress parameters were significantly decreased and serum antioxidant capacity parameters were all increased, with better results compared to the STZ group pretreated with EGCG (p < 0.017, Figures 1 and 2). Also, the L-EGCG solution improved glycemic values and decreased transaminases levels better than EGCG (p < 0.001, Figure 3). The MMP levels were significantly lower in the L-EGCG-treated group compared to the diabetic untreated group or compared to the STZ group pretreated with EGCG (<0.001, Figure 4).
ALT levels, preventing hepatic damage induced by STZ. Furthermore, liposomal EGCG administration another suggested mechanism of EGCG’s protective effect is the increased glucose uptake due to promoting the glucose transporter-4 (GLUT4) translocation in skeletal muscle, through activation of tyrosine phosphorylation of insulin receptors, having an insulin-like effect on H4IIE hepatoma cell lines. Another suggested mechanism of EGCG’s protective effect is the increased glucose uptake due to promoting the glucose transporter-4 (GLUT4) translocation in skeletal muscle, through activation of both phosphoinositol 3-kinase and AMP-activated protein kinase pathways. EGCG also increases tyrosine phosphorylation of insulin receptors, having an insulin-like effect on H4IIE hepatoma cell lines.

The liver is extremely adversely affected in type 1 diabetes mellitus. In our study, we found elevated AST and ALT levels, showing liver damage, in STZ diabetic rats (Table 1, Figure 3). In STZ-induced diabetes, transaminases elevation is the consequence of the toxic effect of STZ on hepatocytes, which induces lipid peroxidation, oxidative stress enhancement, peroxisome proliferation, and mitochondrial dysfunction. Rodriguez et al. identified increased NO levels and hepatic oxidative stress in STZ-induced diabetic rats. In our study, pretreatment with EGCG decreased ALT levels, preventing hepatic damage induced by STZ. Furthermore, liposomal EGCG administration significantly reduced AST and ALT values, confirming the enhanced protective effect of L-EGCG on

Figure 4. Distribution of matrix metalloproteinase (MMP): (a) MMP-2 and (b) MMP-9 on all study groups (7 rats/group). STZ = streptozotocin control; STZ + EGCG = STZ and EGCG solution i.p. as pretreatment; STZ + L-EGCG = STZ and liposomal EGCG i.p. as pretreatment. The symbol–number codes correspond to the p-values < 0.05 as follows: α—STZ compared to control; β—STZ + EGCG compared to control; γ—STZ + L-EGCG compared to control; λ—STZ + L-EGCG compared to STZ; μ—STZ + L-EGCG compared to STZ + EGCG.

The Kruskal–Wallis ANOVA test identified significant differences between the groups with diabetes and EGCG pretreatment for all evaluated parameters (p-values < 0.0001). The post hoc analysis identified significant differences in most of the cases with better protection for the EGCG-treated group, and significantly higher protection when liposomal EGCG solution was used (Figures 1–4).

4. Discussion

4.1. Protective Effects of EGCG on Pancreatic and Hepatic Cell Function in Diabetic Rats

In our study, EGCG reduced blood glucose levels in pretreated animals but the reduction was not statically significant (Table 1, Figure 3). Some of the antidiabetic effects of EGCG are suggested to be the suppression of appetite, adjustment of dietary fat emulsification in the gastrointestinal tract, inhibition of gastrointestinal lipolysis, and reduction of nutrient absorption enzymes. The most significant hypoglycemia was obtained in liposomal EGCG-pretreated groups. This indicates a protective effect of EGCG on pancreatic cell function. Meng et al. showed that EGCG can inhibit inflammation by reducing reactive oxygen species and downregulating the production of inducible nitric oxide synthetase (iNOS). Furthermore, EGCG increases glucose tolerance and decrease HbA1c levels in STZ-induced diabetes in rats, contributing to further prevention of diabetic complications.

The liver is extremely adversely affected in type 1 diabetes mellitus. In our study, we found elevated AST and ALT levels, showing liver damage, in STZ diabetic rats (Table 1, Figure 3). In STZ-induced diabetes, transaminases elevation is the consequence of the toxic effect of STZ on hepatocytes, which induces lipid peroxidation, oxidative stress enhancement, peroxisome proliferation, and mitochondrial dysfunction. Rodriguez et al. identified increased NO levels and hepatic oxidative stress in STZ-induced diabetic rats. In our study, pretreatment with EGCG decreased ALT levels, preventing hepatic damage induced by STZ. Furthermore, liposomal EGCG administration significantly reduced AST and ALT values, confirming the enhanced protective effect of L-EGCG on
hepatic cells. Other studies also demonstrated the hepatic-protective effect of green tea extracts in hepatic injury reflected by decreased serum transaminase levels, and improved structural changes in histopathological examination [64]. Moreover, long-time consumption of EGCG (in healthy Wistar rats) decreases age-induced hepatic damage by lowering the ALT and AST serum levels and improving microscopic changes of the liver tissue due to the aging process [65].

4.2. Effect of EGCG on Oxidative Stress Parameters and Plasmatic Antioxidant Capacity

In this study, increased levels of MDA, NO, and TOS were observed in diabetic rats (Table 1 and Figure 1), together with low levels of antioxidant biomarkers such as thiols, catalase, and TOS (Table 1 and Figure 2). Pretreatment with EGCG and L-EGCG induced protection against STZ toxic effects, as demonstrated by reduction of oxidative stress parameters (Table 1, Figure 1) and by enhancement of antioxidant defense (Table 1, Figure 2), with best results for the liposomal form. STZ-induced diabetes in experimental models is followed by an enhanced production of reactive oxygen species (ROS) and consumption of cell antioxidant systems, as a consequence of necrotic and apoptotic degeneration of pancreatic β cells [66,67]. Hyperglycemia itself is another factor generating intracellular ROS [68]. Oxidative stress (by excessive ROS production, auto-oxidation of glycated proteins, and increased lipid peroxidation) and decreased antioxidant capacity (free radical scavengers and enzymatic systems) are also involved in the pathogenesis of diabetic complications [69–72].

Green tea component EGCG is a flavonoid with antioxidant and anti-inflammatory properties conferred by its particular structure, a flavanol core and two gallocatechol rings, which are able to bind metal ions and scavenge free oxygen radicals. As a consequence, EGCG exerts direct antioxidant effects (scavenger of ROS and cheater of metal ions), but also indirect antioxidant effects (inductor of antioxidant enzymes, such as catalase, and inhibitor of oxydases, such as NADPH—nicotinamide adenine dinucleotide phosphate, lipoxygenase, or xantin-oxydase) [73]. Anti-inflammatory effects of EGCG were also related to the increase of circulating levels of interleukin-10 (an anti-inflammatory cytokine) in nonobese diabetic mice [14]. EGCG can decrease lipid peroxidation in the liver, kidney, and brain, and reduce lymphocyte DNA damage in diabetic mice [74].

EGCG has low bioavailability which can be modified by incorporation in special drug delivery systems. Because of its highly lipophilic nature, EGCG is suitable for incorporation in liposome nanoparticles, composed of phospholipid bilayers. Minnelli et al. showed that pretreatment of adult retinal pigmented epithelium (ARPE) cells with EGCG encapsulated in magnesium liposomes increases the survival of cells exposed to hydrogen peroxide (H₂O₂), with better preserved mitochondria structure on electron microscopy examination, showing the superior antioxidant activity of L-EGCG compared with free EGCG [75]. In this regard, natural antioxidant products could be a promising therapeutic option for prevention of diabetes mellitus and its complications, conferring protection against oxidative damage by liposomal nanostructure encapsulation [69].

4.3. EGCG Effect on Matrix Metalloproteinases

In the present study, serum levels of MMP-2 and -9 increased after DM induction and were better modulated by L-EGCG (Table 1 and Figure 4). In experimental models of DM, increased MMP-2 expression and activity were linked to elevated ROS levels and oxidative stress, with consecutive pancreatic beta cell apoptosis, showing MMP-2’s important role in DM pathogenesis [76]. Thus, inhibition of intracellular MMP-2 expression is an essential target for beta cell protection and DM prevention. There is also a postulated connection between MMP production and inflammatory process and proinflammatory cytokine production associated with DM. Chemokines such as MCP-1 and NF-κB can induce MMP overproduction in DM [77]. After their secretion as inactive forms, proinflammatory molecules contribute to further transformation of MMPs in active forms by different proteases that are implicated in their cleavage [38]. MMPs are also involved in regulation and duration of immune response, endothelial cell function, vascular smooth muscle migration and
proliferation, Ca\(^{2+}\) signaling pathways, and vessel contraction, all of these consistently influencing vascular remodeling in DM [78,79].

Activated inflammatory cells such as leucocytes can contribute to endothelial cell dysfunction and vascular damage by direct and indirect pathways. Indirect loops comprise augmentation of MMP production by proinflammatory cytokines synthesized in activated leucocytes [70].

Activation of MMP-2 and MMP-9 is important in pathogenesis of diabetic microangiopathic complications such as diabetic retinopathy, nephropathy, and neuropathy [39]. Diabetic retinopathy, by inducing apoptosis of retinal endothelial cells and by degrading the junction proteins, is followed by increased vascular permeability [80,81]. In experimental models of DM, increased oxidative stress activates MMP-2, and antioxidant therapies inhibit the development of diabetic retinopathy by modulating retinal MMP-2 levels [32,82]. Diabetic nephropathy, one of the most severe microangiopathy in diabetes mellitus, is also characterized by MMP overexpression and accelerated ECM degradation, both being a hallmark of associated histopathologic changes [30]. MMPs’ increased synthesis can also lead to neuronal injury through blood–nerve barrier (BNB) disruption, contributing to the neuropathic pain associated with diabetic neuropathy [83,84].

The multiple and complex roles exhibited by MMPs are explained by their multiple localizations. MMP-2 and MMP-9 are colocalized in vessel walls and atherosclerotic plaque, being involved in endothelial dysfunction and DM macrovascular complication and vascular remodeling [85,86]. Wang et al. reported a protective effect of EGCG after i.p. administration, by reducing the plasma levels of TNF-\(\alpha\), IL-6, and monocyte chemoattractant protein-1 (MCP-1) [38]. There is also evidence that EGCG can inhibit MMP-2 activation [87]. Multiple compounds of green tea can inhibit MMP-2 and -9, but the most efficient ones proved to be EGCG and epigallocatechin (EGC) [88]. Therefore, we chose the EGCG compound for our experimental study. Moreover, liposomal encapsulation brings an increased bioavailability with better results in reducing oxidative stress biomarkers and MMP plasma level. EGCG reduces MMP-2 activity by targeting the fibronectin type II repeated regions 1 and 3 of MMP-2, binds the amino acids that constitute the exosite of this enzyme, and hinders proper positioning of the substrate [89]. Due to its antioxidants effects and inhibitory action on the protein tyrosine kinases, EGCG reduces MMP-9 activity by reducing its release from the activated neutrophils [90].

From our knowledge, this is the first experimental study addressing liposomal EGCG effects in experimental DM induced by STZ in rats. Decreasing the hepatic and pancreatic damage due to STZ administration is a valuable effect of liposomal EGCG.

4.4. Potential Limitations of the Study

No measurements of EGCG and L-EGCG in the blood or pancreatic and hepatic tissue were done in this study since such quantifications were outside of our aim. Future studies could be conducted to measure the concentration of EGCG and L-EGCG in the blood and tissues. Moreover, oxidative stress parameters and MMPs could be measured in liver and pancreas tissue. Another limitation of our study is that the evaluation of endogenous insulin levels and measurement of HOMA-IR for endogenous pancreatic function were not performed.

Future studies should also investigate the effects of long-term administration of EGCG and L-EGCG on DM and its complications, as this study was focused on assessing their effects 48 h after DM induction.

5. Conclusions

L-EGCG pretreatment reduces oxidative stress biomarkers and MMP plasma levels 48 h after DM induction. Further studies are needed to detect other particularities regarding the EGCG protective mechanisms in order to improve their therapeutic efficiency. Due to the beneficial effects of EGCG nanoformulation proven by this study on oxidative stress, antioxidative defense, and MMP-2 and -9, we propose that L-EGCG could be considered as a novel adjuvant therapy in DM management.
Supplementary Materials: The following is available online at http://www.mdpi.com/2076-3921/9/2/172/s1, Table S1: *p*-values for comparisons between the study groups for all studied parameters.

Author Contributions: Conceptualization, G.D., C.M., and C.A.N.; Data curation, A.E.B., C.M., and I.C.S.; Formal analysis, M.V. and R.M.R.; Funding acquisition, G.D. and I.C.S.; Investigation, A.E.B., A.S.P., C.B., and M.V.; Methodology, P.-M.B., C.B., and C.A.N.; Project administration, A.E.B. and P.-M.B.; Resources, A.S.P., C.B., and I.C.S.; Software, P.-M.B. and M.V.; Supervision, R.M.R.; Validation, P.-M.B., A.S.P., C.M., and R.M.R.; Visualization, G.D. and C.M.; Writing—original draft, A.E.B. and I.C.S.; Writing—review & editing, A.S.P., G.D., and C.A.N. All authors have read and agreed to the published version of the manuscript.

Acknowledgments: The authors would like to thank Olivia Verišean-Rosu for professional English language editing of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Park, J.H.; Bae, J.H.; Im, S.S.; Song, D.K. Green tea and type 2 diabetes. *Integr. Med. Res.* 2014, 3, 4–10. [CrossRef]
2. Chu, C.; Deng, J.; Man, Y.; Qu, Y. Green Tea Extracts Epigallocatechin-3-gallate for Different Treatments. *BioMed Res. Int.* 2017, 2017. [CrossRef] [PubMed]
3. Islam, M.A. Cardiovascular effects of green tea catechins: Progress and promise. *Recent Pat. Cardiovasc. Drug Discov.* 2012, 7, 88–99. [CrossRef] [PubMed]
4. Eng, Q.I.; Thanikachalam, P.V.; Ramamurthy, S. Molecular understanding of Epigallocatechin gallate (EGCG) in cardiovascular and metabolic diseases. *J. Ethnopharm.* 2018, 210, 296–310. [CrossRef] [PubMed]
5. Yuan, J.M. Cancer prevention by green tea: Evidence from epidemiologic studies. *Am. J. Clin. Nutr.* 2013, 98, 1676S–1681S. [CrossRef]
6. Suliburska, J.; Bogdanski, P.; Szulinska, M.; Stepien, M.; Pupek-Musialik, D.; Jablecka, A. Effects of green tea supplementation on total antioxidant, lipids, and glucose values in the serum of obese patients. *Biol. Trace Elem. Res.* 2012, 149, 315–322. [CrossRef]
7. Mozaffari-Khosravi, H.; Ahadi, Z.; FallahTafti, M. The Effect of Green Tea versus Sour Tea on Insulin Resistance, Lipids Profiles and Oxidative Stress in Patients with Type 2 Diabetes Mellitus: A Randomized Clinical Trial. *Iran. J. Med. Sci.* 2014, 39, 424–432.
8. Turek, I.A.; Kozińska, J.; Drygas, W. Green tea as a protective factor in prophylaxis and treatment of selected cardiovascular diseases. *Kardiol. Pol.* 2012, 70, 848–852.
9. Larsson, S.C. Coffee, tea, and cocoa and risk of stroke. *Stroke* 2014, 45, 309–314. [CrossRef]
10. Li, F.J.; Ji, H.F.; Shen, L. A meta-analysis of tea drinking and risk of Parkinson’s disease. *Sci. World J.* 2012, 2012, 923464. [CrossRef]
11. Pervin, M.; Unno, K.; Ohishi, T.; Tanabe, H.; Miyoshi, N.; Nakamura, Y. Beneficial Effects of Green Tea Catechins on Neurodegenerative Diseases. *Molecules* 2018, 23, 1297. [CrossRef] [PubMed]
12. Hauber, I.; Hohenberg, H.; Holstermann, B.; Hunstein, W.; Hauber, J. The main green tea polyphenol epigallocatechin-3-gallate counteracts semen-mediated enhancement of HIV infection. *Proc. Natl. Acad. Sci. USA* 2009, 106, 9033–9038. [CrossRef] [PubMed]
13. De Oliveira, A.; Adams, S.D.; Lee, L.H.; Murray, S.R.; Hsu, S.D.; Hammond, J.R.; Dickinson, D.; Chen, P.; Chu, T.C. Inhibition of herpes simplex virus type 1 with the modified green tea polyphenol palmitoyl-epigallocatechin gallate. *Food Chem. Toxicol.* 2013, 52, 207–215. [CrossRef] [PubMed]
14. Fu, Z.; Zhen, W.; Yuskavage, J.; Liu, D. Epigallocatechin gallate delays the onset of type 1 diabetes in spontaneous non-obese diabetic mice. *Br. J. Nutr.* 2011, 105, 1218–1225. [CrossRef]
15. Kondo, Y.; Goto, A.; Noma, H.; Iso, H.; Hayashi, K.; Noda, M. Effects of Coffee and Tea Consumption on Glucose Metabolism: A Systematic Review and Network Meta-Analysis. *Nutrients* 2019, 11, 48. [CrossRef]
16. Li, T.; Liu, J.; Zhang, X.; Ji, G. Antidiabetic activity of lipophilic (−)-epigallocatechin-3-gallate derivative under its role of α-glucosidase inhibition. *Biomed. Pharm.* 2007, 61, 91–96. [CrossRef]
17. Chiang, J.L.; Maahs, D.M.; Garvey, K.C.; Garvey, K.C.; Hood, K.K.; Laffel, L.M.; Weinzimer, S.A.; Wolfsdorf, J.J.; Schatz, D. Type 1 Diabetes in Children and Adolescents: A Position Statement by the American Diabetes Association. *Diabetes Care* 2018, 41, 2026–2044. [CrossRef]
18. Friederich, M.; Hansell, P.; Palm, F. Diabetes, oxidative stress, nitric oxide and mitochondria function. *Curr. Diabetes Rev.* 2009, 5, 120–144. [CrossRef]
19. Bulboacă, A.E.; Boarescu, P.M.; Bolboacă, S.D.; Blidaru, M.; Feștilă, D.; Dogaru, G.; Nicula, C.A. Comparative Effect of Curcumin versus Liposomal Curcumin on Systemic Pro-Inflammatory Cytokines Profile, MCP-1 and RANTES in Experimental Diabetes Mellitus. *Int. J. Nanomed.* **2019**, *14*, 8961–8972. [CrossRef]
20. Boarescu, P.M.; Boarescu, I.; Bocșan, I.C.; Gheban, D.; Bolboacă, A.E.; Nicula, C.; Pop, R.M.; Răjineanu, R.M.; Bolboacă, S.D. Antioxidant and Anti-Inflammatory Effects of Curcumin Nanoparticles on Drug-Induced Acute Myocardial Infarction in Diabetic Rats. *Antioxidants* **2019**, *8*, 504. [CrossRef]
21. Song, E.K.; Hur, H.; Han, M.-K. Epigallocatechin gallate prevents autoimmune diabetes induced by multiple low doses of streptozotocin in mice. *Arch. Pharm. Res.* **2003**, *26*, 559–563. [CrossRef] [PubMed]
22. Roghani, M.; Baluchnejadmojarad, T. Hypoglycemic and hypolipidemic effects of metalloproteinase and antioxidant activity of chronic epigallocatechin-gallate in streptozotocin-diabetic rats. *Pathophysiology* **2010**, *17*, 55–59. [CrossRef] [PubMed]
23. Granja, A.; Frias, I.; Neves, A.R.; Pinheiro, M.; Reis, S. Therapeutic Potential of Epigallocatechin Gallate Nanodelivery Systems. *BioMed Res. Int.* **2017**, *2017*, 5813793. [CrossRef] [PubMed]
24. Watkins, R.; Wu, L.; Zhang, C.; Davis, R.M.; Xu, B. Natural product-based nanomedicine: Recent advances and issues. *Int. J. Nanomed.* **2015**, *10*, 6055–6074.
25. Othman, A.I.; El-Sawi, M.R.; El-Missiry, M.A.; Abukhalil, M.H. Epigallocatechin-3 gallate protects against diabetic cardiomyopathy through modulating the cardiometabolic risk factors, oxidative stress, inflammation, cell death and fibrosis in streptozotocin-nicotinamide induced diabetic rats. *Biomed. Pharm.* **2017**, *94*, 362–373. [CrossRef] [PubMed]
26. Vu, T.H.; Werb, Z. Matrix metalloproteinases: Effectors of development and normal physiology. *Genes Dev.* **2000**, *14*, 2123–2133. [CrossRef]
27. Löffek, S.; Schilling, O.; Franzke, C.W. Series “matrix metalloproteinases in lung health and disease”: Biological role of matrix metalloproteinases: A critical balance. *Eurrespir. J.* **2011**, *38*, 191–208. [CrossRef]
28. Peeters, S.A.; Engelen, L.; Bijs, J.; Chaturvedi, N.; Fuller, J.H.; Schalkwijk, C.G.; Stehouwer, C.D. EURODIAB Prospective Complications Study Group. Plasma levels of matrix metalloproteinase-2, -3, -10, and tissue inhibitor of metalloproteinase-1 are associated with vascular complications in patients with type 1 diabetes: The EURODIAB Prospective Complications Study. *Cardiovasc. Diabetol.* **2015**, *14*, 31.
29. Phillips, P.A.; McCarroll, J.A.; Park, S.; Wu, M.J.; Pirola, R.; Korsten, M.; Wilson, J.S.; Apte, M.V. Rat pancreatic stellate cells secrete matrix metalloproteinases: Implications for extracellular matrix turnover. *Gut* **2003**, *52*, 275–282. [CrossRef]
30. Xu, X.; Xiao, L.; Xiao, P.; Yang, S.; Chen, G.; Liu, F.; Kanwar, Y.S.; Sun, L. A glimpse of matrix metalloproteinases in diabetic nephropathy. *Curr. Med. Chem.* **2014**, *21*, 3244–3260. [CrossRef]
31. Thraikill, K.M.; Bunn, R.C.; Moreau, C.S.; Cockrell, G.E.; Simpson, P.M.; Coleman, H.N.; Frindik, J.P.; Kemp, S.F.; Fowlkes, J.L. Matrix metalloproteinase-2 dysregulation in type 1 diabetes. *Diabetes Care* **2007**, *30*, 2321–2326. [CrossRef] [PubMed]
32. Kowluru, R.A.; Kamwarr, M. Oxidative Stress and the Development of Diabetic Retinopathy: Contributory Role of Matrix Metalloproteinase-2. *Free Radic Biol. Med.* **2009**, *46*, 1677–1685. [CrossRef] [PubMed]
33. Lee, M.J.; Maliakal, P.; Chen, L.; Meng, X.; Bondoc, F.Y.; Prabhu, S.; Lambert, G.; Mohr, S.; Yang, C.S. Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: Formation of different metabolites and individual variability. *Cancer Epidemiol. Biomark. Prev.* **2002**, *11*, 1025–1032.
34. Li, N.; Taylor, L.S.; Mauer, L.J. Degradation kinetics of catechins in green tea powder: Effects of temperature and relative humidity. *J. Agric. Food Chem.* **2011**, *59*, 6082–6090. [CrossRef] [PubMed]
35. Isemura, M. Catechin in Human Health and Disease. *Molecules* **2019**, *24*, 528. [CrossRef] [PubMed]
36. Tsai, Y.J.; Chen, B.H. Preparation of catechin extracts and nanoemulsions from green tea leaf waste and their inhibition effect on prostate cancer cell PC-3. *Int. J. Nanomed.* **2016**, *11*, 1907–1926.
37. Langer, R. New methods of drug delivery. *Science* **1990**, *249*, 1527–1533. [CrossRef]
38. Wang, S.; Su, R.; Nie, S.; Sun, M.; Zhang, J.; Wu, D.; Moustaid-Moussa, N. Application of nanotechnology in improving bioavailability and bioactivity of diet-derived phytochemicals. *J. Nutr. Biochem.* **2014**, *25*, 363–376. [CrossRef]
39. Mozafari, M.R.; Johnson, C.; Hatziiantoniou, S.; Demetzos, C. Nanoliposomes and their applications in food nanotechnology. *J. Liposome Res.* **2008**, *18*, 309–327. [CrossRef]
40. Bulboacă, A.E.; Porfire, A.S.; Tefas, L.R.; Boarescu, P.M.; Bolboacă, S.D.; Stănescu, I.C.; Bulboacă, A.C.; Dogaru, G. Liposomal Curcumin is Better than Curcumin to Alleviate Complications in Experimental Diabetic Mellitus. *Molecules* 2019, 24, 846. [CrossRef]

41. Qi, S.; Wang, C.; Song, D.; Song, Y. Intraperitoneal injection of (-)-Epigallocatechin-3-gallate protects against light-induced photoreceptor degeneration in the mouse retina. *Mol. Vis.* 2017, 23, 171–178. [PubMed]

42. Ramachandran, B.; Jayavelu, S.; Murhekar, K.; Rajkumar, T. Repeated dose studies with pure Epigallocatechin-3-gallate demonstrated dose and route dependant hepatotoxicity with associated dyslipidemia. *Toxicol. Rep.* 2016, 3, 336–345. [CrossRef] [PubMed]

43. Li, C.; Peng, J.; Hu, R.; Yan, J.; Sun, Y.; Zhang, L.; Liu, W.; Jiang, H. Safety and Efficacy of Ketamine Versus Ketamine-Fentanyl-Dexmedetomidine Combination for Anesthesia and Analgesia in Rats. *Dose Response* 2019, 17. [CrossRef] [PubMed]

44. Porfire, A.; Tomuta, I.; Leucuta, S.E.; Achim, M. Superoxide dismutase loaded liposomes. The influence of formulation factors on enzyme encapsulation and release. *Farmacia* 2013, 61, 865–873.

45. Sylvester, B.; Porfire, A.; Muntean, D.M.; Vlase, L.; Luput, L.E.; Sesarman, A.; Alupei, M.C.; Banciu, M.; Achim, M.; Tomuta, I. Optimization of prednisolone loaded long circulating liposome’s via application of quality by design (QbD) approach. *J. Liposome Res.* 2018, 28, 49–61. [CrossRef] [PubMed]

46. Postescu, I.D.; Tatomir, C.; Chereches, G.; Brie, I.; Damian, G.; Petrisor, D.; Hosu, A.M.; Miclaus, V.; Pop, A. Spectroscopic characterization of some grape extracts with potential role in tumor growth inhibition. *J. Optoelectron. Adv. Mater.* 2007, 9, 564–567.

47. Yagi, K. Assay for blood plasma and serum peroxides. *Methods Enzymol.* 1984, 105, 328–331.

48. Goel, P.; Srivastava, K.; Das, N.; Bhatnagar, V. The role of nitric oxide in portal hypertension caused by extrahepatic portal vein obstruction. *J. Indian Assocpediatrsurg.* 2010, 15, 117–121.

49. Bulboacă, A.E.; Porfire, A.; Barbălată, A.; Bolboacă, S.D.; Nicula, C.; Boarescu, P.M.; Stănescu, I.; Dogaru, G. The effect of liposomal epigallocatechin gallate and metoclopramide hydrochloride co-administration on experimental migraine. *Farmaacia* 2019, 67, 905–911. [CrossRef]

50. Erel, O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin. Biochem.* 2004, 37, 112–119. [CrossRef]

51. Hu, M.L. Measurement of protein thiol groups and glutathione in plasma. *Methods Enzymol.* 1994, 233, 380–385. [PubMed]

52. Aebi, H. Catalase in vitro. *Methods Enzymol.* 1984, 105, 121–126. [PubMed]

53. Haider, R.; Annie, J. Streptozotocin-Induced Cytotoxicity, Oxidative Stress and Mitochondrial Dysfunction in Human Hepatoma HepG2 Cells. *Int. J. Mol. Sci.* 2012, 12, 5751–5767.

54. Weissgerber, T.L.; Milic, N.M.; Winham, S.J.; Garovic, V. Beyond bar and line graphs: Time for a new data presentation paradigm. *PLoS Biol.* 2015, 13, e1002128. [CrossRef] [PubMed]

55. Jia, J.J.; Zeng, X.S.; Song, X.Q.; Zhang, P.P.; Chen, L. Diabetes mellitus and Alzheimer’s disease: The protection of epigallocatechin-3-gallate in streptozotocin injection-induced models. *Front. Pharm.* 2017, 8, 834. [CrossRef] [PubMed]

56. Meng, J.-M.; Cao, S.-Y.; Wei, X.-L.; Gan, R.-Y.; Wang, Y.-F.; Cai, S.-X.; Xu, X.-Y.; Zhang, P.-Z.; Li, H.-B. Effects and Mechanisms of Tea for the Prevention and Management of Diabetes Mellitus and Diabetic Complications: An Updated Review. *Antioxidants* 2019, 8, 170. [CrossRef]

57. Ortsäter, H.; Grankvist, N.; Wolfram, S.; Kuehn, N.; Sjöholm, A. Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. *Nutr. Metab.* 2012, 9, 11. [CrossRef]

58. Ueda-Wakagi, M.; Nagayasu, H.; Yamashita, Y.; Ashida, A.H. Green Tea Ameliorates Hyperglycemia by Promoting the Translocation of Glucose Transporter 4 in the Skeletal Muscle of Diabetic Rodents. *Int. J. Mol. Sci.* 2019, 20, 2436. [CrossRef]

59. Waltner-Law, M.E.; Wang, X.L.; Law, B.K.; Hall, R.K.; Nawano, M.; Granner, D.K. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *J. Biol. Chem.* 2002, 277, 34933–34940. [CrossRef]

60. Kume, E.; Fujimura, H.; Matsuki, N.; Ito, M.; Aruga, C.; Toriumi, W.; Kitamura, K.; Doi, K. Hepatic changes in the acute phase of streptozotocin (SZ)-induced diabetes in mice. *Exp. Toxicol. Pathol.* 2004, 55, 467–480. [CrossRef]
61. Kume, E.; Aruga, C.; Takahashi, K.; Miwa, S.; Dekura, E.; Itoh, M.; Ishizuka, Y.; Fujimura, H.; Toriumi, W.; Doi, K. Morphological and gene expression analysis in mouse primary cultured hepatocytes exposed to streptozotocin. Exp. Toxicol. Pathol. 2005, 56, 245–253. [CrossRef] [PubMed]
62. Kobori, M.; Masumoto, S.; Akimoto, Y.; Takahashi, Y. Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. Mol. Nutr. Food Res. 2009, 53, 859–868. [CrossRef] [PubMed]
63. Rodriguez, V.; Plavnik, L.; Tolosa de Talamoni, N. Naringin attenuates liver damage in streptozotocin-induced diabetic rats. Biomed. Pharmac. 2018, 105, 95–102. [CrossRef] [PubMed]
64. Abolfathi, A.A.; Mohajeri, D.; Rezaie, A.; Nazeri, M. Protective effects of Green Tea Extract against Hepatic Tissue Injury in Streptozotocin-Induced Diabetic Rats. Evid.-Based Complement. Altern. Med. 2012, 2012, 740671. [CrossRef]
65. Niu, Y.; Na, L.; Feng, R.; Gong, L.; Zhan, Y.; Li, Q.; Li, Y.; Sun, C. The phytochemical, EGCG, extends lifespan by reducing liver and kidney function damage and improving age-associated inflammation and oxidative stress in healthy rats. Aging Cell 2013, 12, 1041–1049. [CrossRef]
66. West, I.C. Radicals and oxidative stress in diabetes. Diabet. Med. 2000, 17, 171–180. [CrossRef]
67. Fernandes, S.M.; Cordeiro, P.M.; Watanabe, M.; Fonseca, C.D.; Vattimo, M.F. The role of oxidative stress in streptozotocin-induced diabetic nephropathy in rats. Arch. Endocrinoil. Metab. 2016, 60, 443–449. [CrossRef]
68. De Almeida, D.A.T.; Braga, C.P.; Novelli, E.L.B.; Fernandes, A.A.H. Evaluation of lipid profile and oxidative stress in STZ-induced rats treated with antioxidant vitamin. Br. Arch. Biol. Technol. 2012, 55, 527–536. [CrossRef]
69. Talebanzadeh, S.; Ashrafi, M.; Kazemipour, N.; Erjae, H.; Nazifi, S. Evaluation of the effects of saffron aqueous extract on oxidative stress in the lens of streptozotocin-induced diabetic rats. BRAT 2018, 5, 2133–2141. [CrossRef]
70. Aloud, A.A.; Veeramani, C.; Govindasamy, C.; Alsafi, M.A.; Al-Numair, K.S. Galangin, a natural flavonoid reduces mitochondrial oxidative damage in streptozotocin-induced diabetic rats. Redox Rep. 2018, 23, 29–34. [CrossRef]
71. Schmatz, R.; Belmonte, P.L.; Stefanello, N.; Mazzanti, C.; Spanevello, R.; Gutierres, J.; Bagatini, M.; Curry Martins, C.; Husein Abdalla, F.; da Silva Serres, J.D.; et al. Effects of resveratrol on biomarkers of oxidative stress and on the activity of delta aminolevulinic acid dehydratase in liver and kidney of streptozotocin-induced diabetic rats. Biochimie 2012, 94, e374–e383. [CrossRef] [PubMed]
72. Opara, E.C. Oxidative stress, micronutrients, diabetes mellitus and its complications. J. R. Soc. Health 2002, 122, 28–34. [CrossRef] [PubMed]
73. Bernatoniene, J.; Kopustinskien e, D.M. The Role of Catechins in Cellular Responses to Oxidative Stress. Molecules 2018, 23, 965. [CrossRef] [PubMed]
74. Orsolic, N.; Sirovina, D.; Gajski, G.; Garaj-Vrhovac, V.; Jembrek, M.J.; Kosalec, I. Assessment of DNA damage and lipid peroxidation in diabetic mice: Effects of propolis and epigallocatechin gallate (EGCG). Mutat. Res. 2013, 757, 36–44. [CrossRef] [PubMed]
75. Minnelli, C.; Moretti, P.; Fulgenzi, G.; Mariani, P.; Laudadio, E.; Armeni, T.; Galeazzi, R.; Mobbili, G. A Poloxamer-407 modified liposome encapsulating epigallocatechin-3-gallate in the presence of magnesium: Characterization and protective effect against oxidative damage. Int. J. Pharm. 2018, 552, 225–234. [CrossRef] [PubMed]
76. Liu, C.; Wan, X.; Ye, T.; Fang, F.; Chen, X.; Chen, Y.; Dong, Y. Matrix Metalloproteinase 2 Contributes to Pancreatic Beta Cell Injury Induced by Oxidative Stress. PLoS ONE 2014, 9, e110227. [CrossRef]
77. Macarie, R.D.; Vadana, M.; Ciortan, L.; Tucureanu, M.M.; Ciobanu, A.; Vinereanu, D.; Manduteanu, I.; Simionescu, M.; Butoi, I. The expression of MMP-1 and MMP-9 is up-regulated by smooth muscle cells after their cross-talk with macrophages in high glucose conditions. J. Cell Mol. Med. 2018, 22, 4366–4376. [CrossRef]
78. Smigiel, K.S.; Parks, W.C. Matrix Metalloproteinases and Leukocyte Activation. Prog. Mol. Biol. Transl. Sci. 2017, 147, 167–195.
79. Cui, N.; Hu, M.; Khalil, R.A. Biochemical and Biological Attributes of Matrix Metalloproteinases. Prog. Mol. Biol. Transl. Sci. 2017, 147, 1–73.
80. Mohammad, G. Role of matrix metalloproteinase-2 and -9 in the development of diabetic retinopathy. J. Ocul. Biol. Dis. Inform. 2012, 5, 1–8. [CrossRef]
81. Drankowska, J.; Kos, M.; Kościuk, A.; Marżęda, P.; Boguszewska-Czubara, A.; Tylus, M.; Święch-Zubilewicz, A. MMP targeting in the battle for vision: Recent developments and future prospects in the treatment of diabetic retinopathy. *Life Sci.* 2019, 229, 149–156. [CrossRef] [PubMed]

82. Kowluru, R.A.; Kanwar, M.; Chan, P.S.; Zhang, J.P. AREDS-based micronutrients inhibit retinopathy and retinal metabolic abnormalities in diabetic rats. *Arch. Ophthalmol.* 2008, 126, 1266–1272. [CrossRef] [PubMed]

83. Hughes, P.M.; Wells, G.M.; Perry, V.H.; Brown, M.C.; Miller, K.M. Comparison of matrix metalloproteinase expression during Wallerian degeneration in the central and peripheral nervous systems. *Neuroscience* 2002, 113, 273–287. [CrossRef]

84. Kuhad, A.; Singh, P.; Chopra, K. Matrix metalloproteinases: Potential therapeutic target for diabetic neuropathic pain. *Expert Opin. Ther. Targets* 2015, 19, 177–185. [CrossRef]

85. Raffetto, J.D.; Khalil, R.A. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. *Biochem. Pharmacol.* 2008, 75, 346–359. [CrossRef]

86. Kiugel, M.; Hellberg, S.; Käkelä, M.; Liljenbäck, H.; Saanijoki, T.; Li, X.-G.; Tuomela, J.; Knuuti, J.; Saraste, A.; Roivainen, A. Evaluation of [68Ga]Ga-DOTA-TCTP-1 for the Detection of Metalloproteinase 2/9 Expression in Mouse Atherosclerotic Plaques. *Molecules* 2018, 23, 3168. [CrossRef]

87. Djerir, D.; Iddir, M.; Bourgault, S.; Lamy, S.; Annabi, B. Biophysical evidence for differential gallated green tea catechins binding to membrane type-1 matrix metalloproteinase and its interactors. *Biophys. Chem.* 2018, 234, 34–41. [CrossRef]

88. Demeule, M.; Brossard, M.; Pagé, M.; Gingras, D.; Béliveau, R. Matrix metalloproteinase inhibition by green tea catechins. *Biochim. Biophys. Acta* 2000, 1478, 51–60. [CrossRef]

89. Jha, S.; Kanaujia, S.P.; Limaye, A.M. Direct inhibition of matrix metalloproteinase-2 (MMP-2) by (−)-epigallocatechin-3-gallate: A possible role for the fibronectin type II repeats. *Gene* 2016, 593, 126–130. [CrossRef]

90. Kim-Park, W.K.; Allam, E.S.; Palasuk, J.; Kowolik, M.; Park, K.K.; Windsor, L.J. Green tea catechin inhibits the activity and neutrophil release of Matrix Metalloproteinase-9. *J. Tradit. Complement. Med.* 2016, 6, 343–346. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).