Effects of in vitro supplementation with Syzygium cumini (L.) on platelets from subjects affected by diabetes mellitus

Francesca Raffaelli1*, Francesca Borroni1*, Alessandro Alidori1, Giacomo Tirabassi2, Emanuela Faloia2, Rosa Anna Rabini3, Alessia Giulietti1, Laura Mazzanti1, Laura Nanetti1, & Arianna Vignini1

1Faculty of Medicine, Department of Clinical Sciences, Section of Biochemistry, Biology and Physics, Marche Polytechnic University, Ancona, Italy, 2Department of Clinical and Molecular Sciences, Division of Endocrinology, Azienda Ospedaliero Universitaria, Ospedali Riuniti, Marche Polytechnic University, Ancona, Italy, and 3Diabetology Department, INRCA Geriatric Hospital, Via della Montagnola, Ancona, Italy

Abstract

The aim of this study was to assess the in vitro effects of Syzygium cumini (L.) (Sc) incubation on platelets from patients with diabetes, in order to test its efficacy as a potential adjuvant therapy. This study was performed on 77 patients with diabetes (29 in good (DMgc) and 48 in poor glycemic control (DMpc)) and 85 controls. In patients, platelets were analyzed at recruitment and after in vitro SF incubation (final concentration of 200 μg/ml for 3 hours at 37°C), whereas in controls only basal evaluation was performed. Lipoperoxide and nitric oxide (NO) levels, superoxide dismutase (SOD) and Na+/K+ ATPase activities, total antioxidant capacity (TAC), and membrane fluidity tested by anisotropy of fluorescent probes 1-(4-trimethylaminomethylphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) and 1-6-phenyl-1,3,5-hexatriene (DPH) were determined. Collagen-induced platelet aggregation was also evaluated. In vitro Sc activity counteracts oxidative damage, by improving platelet function through augmented membrane fluidity and Na+/K+ ATPase activity; it also enhances antioxidant system functionality by increasing NO levels, SOD activity, and TAC and by decreasing lipoperoxide levels both in whole samples and in DMgc and DMpc. In addition, a slight tendency towards collagen-induced platelet aggregation decrease after Sc was observed. However, all these parameters, even after improvement, did not reach the levels of control subjects. Our results suggest that Sc may have a preventive and protective effect in oxidative damage progression associated with diabetes mellitus and its complications. If our data will be confirmed, Sc supplementation might become a further tool in the management of this disease, especially in view of its easy availability, safety, low cost, and absence of side effects.

Introduction

Oxidative-stress is involved in the pathogenesis of diabetes mellitus (DM) specifically in the onset of insulin-resistance, progressive insulin deficiency and chronic complications [1, 2]. The imbalance between reactive species and cellular antioxidant defenses can cause these effects either directly or indirectly, acting as a second cellular messenger able to activate stress-inducible cellular pathways [3]. Moreover, pancreatic β-cells have a reduced antioxidant pool, so they are more exposed to oxidative-stress damage [1].

Numerous studies have focused on the use of dietary antioxidants and nutraceuticals in the prevention of diabetic complications [4]. Several authors have demonstrated the effectiveness of supplementation with vitamin E and α-lipoic acid in DM [5, 6]. Lately, the efficacy of supplementation with fermented papaya [7] and resveratrol [8] in DM and its complications has been demonstrated. In recent years, Syzygium cumini (L.) (Sc), a dietary supplement, has received considerable attention for its potential benefits in several diseases [7, 9]. Cumin is extracted from the leaves and seeds of Sc, a tropical evergreen tree belonging to the Myrtacee family. Some authors have explained the chemical composition and antioxidant activities of three anatomically distinct parts of the fruit [10]. It was discovered that acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol, and myricetin [11] are found in Sc and possess antioxidant and free radical scavenging activities [12]. Its leaf extracts contain phenolic compounds, such as ferulic acid and catechin, responsible for anti-oxidant activity [13].

A growing body of evidence indicates the anti-oxidant and anti-diabetic properties of Sc [14]. The extract of Sc seeds seems to have an “acarbose like” hypoglycemic action, by inhibiting the alpha-amylase and alpha-glucosidase enzymes that are physiologically involved in the process of carbohydrate cleavage to monosaccharide units of glucose [15]; inhibition of these enzymes prevents the intestinal absorption of glucose, counteracting the physiological post-prandial increase in glycemia.

One of the most important complications of DM is represented by vascular disease associated with impaired vascular reactivity. In this regard, it is worth noting that platelets are involved in maintaining vascular integrity and, in DM, they show greater adhesion and aggregation [16]. Platelet functions, like the ones

*These authors equally contributed to the work.

Correspondence: Laura Mazzanti, Full Professor, Department of Clinical Sciences, Section of Biochemistry, Biology and Physics, Faculty of Medicine, Marche Polytechnic University, Via Tronto 10, 60128, Ancona, Italy. Tel/Fax: +39 071 2206058. E-mail: lauramazzanti48@gmail.com

Keywords

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History

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involved in the hemostasis process, seem to be regulated by reactive oxygen/nitrogen species. Moreover, increased free radical activity plays a fundamental role in cell injury caused by lipid peroxidation and protein oxidation and is implicated in the pathogenesis of DM-associated vascular disease [16].

To the best of our knowledge, only one study has evaluated the in vitro beneficial effects of Sc extract in platelets; however, the authors considered only certain parameters of oxidative stress, such as catalase, superoxide dismutase, and thiobarbituric acid reactive substances in a relatively small population of DM patients (30 subjects) [16]. Therefore, we aimed to re-evaluate the in vitro anti-oxidant effect of Sc supplementation on platelets by assessing many other direct and indirect parameters of oxidative stress and platelet functionality and involving a larger population of DM subjects and controls; also, we separately examined well- and poorly controlled patients, in order to look for possible different beneficial effects of Sc depending on the degree of chronic hyperglycemia.

Patients and methods

The study was performed on 77 patients affected by type 1 (n = 16) and type 2 (n = 61) DM (35 females and 42 males) consecutively admitted to the Division of Endocrinology of Politechnic University of Marche between May and October 2011. 85 control subjects, who were free of major medical problems, were also considered. Type 2 DM patients were taking oral hypoglycemic agents, while all type 1 DM subjects were undergoing insulin therapy. None of the subjects had cancer, chronic liver disease, cardiovascular, and cerebrovascular diseases, or a secondary form of DM. In addition, no subjects reported episodes of hypoglycemia or were taking immunosuppressive agents. All subjects were non-smokers, non-alcoholics, and followed an unrestricted diet.

This study was performed in accordance with the Declaration of Helsinki as revised in 2001 and it was approved by our institutional Review Board (process 124/2006). Written informed consent was subscribed by all subjects.

Platelet isolation

After an overnight fast, a peripheral venous blood sample was drawn from each subject and immediately mixed with Anticoagulant Citrate Dextrose (36 ml citric acid, 5 mM KCl, 90 mM NaCl, 5 mM glucose, 10 mM EDTA, pH 6.8). Platelets were isolated by differential centrifugation in anti-aggregation buffer (Tris–HCl 10 mm; NaCl 150 mm; EDTA 1 mm; glucose 5 mm; pH 7.4) [17]. Each sample of platelets isolated from diabetic subjects was divided into two aliquots: one remained as it was, while the other was incubated with Sc to a final concentration of 200 μg/ml for 3 hours at 37 °C, according to protocols previously described [8, 16]. Platelets were assessed for Na+/K+ ATPase activity, membrane fluidity by anisotropy of fluorescent probes TMA-DPH and DPH, Nitric Oxide (NO) levels, Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD) Activity and Lipoperoxide levels (TBARs) both at recruitment (T0), and after in vitro incubation (T1). Also, platelet aggregation similarly was assessed. In contrast, in control subjects, only T0 evaluation was performed in all the studied parameters.

Na+/K+ ATPase activity

Na+/K+-activated Mg2+-dependent ATPase activity was determined in platelet plasma membranes according to the modified Kitao and Hattori method [8]. The difference in inorganic phosphate (Pi) hydrolyzed in the presence and in the absence of ouabain was measured [8]. Protein concentration was determined by the Bradford assay using serum albumin as a standard [8] and Na+/K+ ATPase activity was expressed as μmol Pi/mg prot/h.

Membrane fluidity

Platelet plasma membrane fluidity was studied by determining the fluorescence anisotropy (reciprocal of fluidity) of probes 1-(4-trimethylaminophenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), which is incorporated in the lipid–water interface of the membrane bilayer, and 1-6-phenyl-1,3,5-hexatriene (DPH), which is a totally hydrophobic fluorescent probe [8]. Membrane incubation with TMA-DPH and DPH was performed as previously described [18]. Steady-state fluorescence anisotropy (r) of TMA-DPH and DPH was calculated using the following equation:

\[ r = (IvG - Ih)/(Iv + 2Ih) \]

where G is the instrumental factor that corrects the r value for an unequal detection of vertically (Iv) and horizontally (Ih) polarized light. Fluorescence anisotropy is a quantitative index of the freedom of rotation of the probe; a decrease in the r value indicates a higher mobility of probes in the site where it is located, i.e. increased membrane fluidity.

NO levels

NO platelet production was evaluated by the Total Nitric Oxide Assay Kit (Enzo Life Sciences, Pennsylvania, PA). The kit involves the enzymatic conversion of nitrate to nitrite, by the enzyme Nitrate Reductase, followed by the colorimetric detection of nitrite by Griess reaction [19] that absorbs visible light at 540 nm. NO levels were determined using a standard curve generated with known concentrations of NO and expressed as nmol NO/mg protein. Protein concentration was determined by the Bradford assay, using serum albumin as a standard [8].

Total antioxidant capacity measurement

TAC was evaluated in platelets by Total Antioxidant Capacity Assay Kit (Biovision, San Francisco, CA). Briefly, the Cu+ ion, reduced by antioxidants present in the sample, is chelated with a colorimetric probe giving a broad absorbance peak at 570 nm, proportional to the TAC. The results were expressed as nmol/μL Trolox equivalents.

Superoxide dismutase activity

SOD activity was evaluated by colorimetric assay kit (Enzo Life Sciences, Pennsylvania, PA). Briefly, superoxide anion, generated from the conversion of xanthine and oxygen to uric acid and hydrogen peroxide by xanthine oxidase, converts WST-1 (Water Soluble Tetrazolium salts) to WST-1 formazan, that absorbs at 570 nm. SOD is able to reduce the superoxide ion concentration and thereby lowers the rate of WST-1-formazan formation. Absorbance values on the Y axis vs. time, in minutes, in the X axis were plotted and the slopes were calculated for each curve. Therefore, the % inhibition of the change in absorbance read at 450 nm was determined and the results were expressed as U/μL.

Thiobarbituric acid reactive substances levels

Lipoperoxide levels were evaluated by TBARs Assay Kit (Cayman Chemical Company, Ann Arbor, MI). Briefly, lipid peroxides derived from polyunsaturated fatty acids are unstable and decompose to form a complex series of compounds, such as malondialdehyde (MDA). MDA can be quantified following its reaction with thiobarbituric acid (TBA); the MDA–TBA adduct...
formed was measured colorimetrically at 530 nm and values were expressed as μM of MDA.

Collagen-induced platelet aggregation

In vitro platelet aggregation study was evaluated in platelet-rich plasma (PRP) by Born’s method. Platelet aggregation is measured as an increase of light transmittance across a platelet suspension (PRP), kept under agitation at 37°C, after challenge with an agonist, specifically collagen.

Platelet aggregation was induced by collagen (4 μg/ml) and responses monitored for 6 minutes in a dual-channel aggregometer (Chrono-Log, Havertown, PA) at 37°C with a continuous stirring speed of 900 rpm [20].

After challenge with the agonist, activated platelets change their morphology, seen as a small decrease in transmittance and then aggregate, increasing light passage across the suspension, and reaching a maximal aggregation after 4–6 minutes. Platelet aggregation was expressed as a percentage of the light transmission at 6 minutes related to the negative control (platelet-poor plasma) [20].

Biochemical evaluation

The following biochemical parameters were considered: glycaemia, glycated hemoglobin (HbA1c), total cholesterol, high density lipoprotein (HDL) cholesterol, and triglycerides. Assays were performed as described elsewhere [21]. Low density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula [22].

Statistical analysis

Results are expressed as means ± standard deviation (SD). Comparison between controls and patients, as well as between well- and poorly controlled patients was performed with unpaired Student’s t-test, while comparison between T₀ and T₁ was carried out using paired Student’s t-test. Differences were considered significant with p < 0.05. Statistical analysis was performed using SAS statistical package (Statistical Analysis System Institute, Cary, NC).

Results

General and metabolic characteristics

Patients and control subjects did not differ significantly in age, whereas, as expected, controls had significantly lower glycaemia and HbA1C values and a better lipid profile than patients (Table I). Subjects in good (HbA1C < 7.0% or 53 mmol/mol; DMgc) and poor (HbA1C > 7.0% or 53 mmol/mol; DMpc) glycometabolic control did not differ in any of the anthropometric and metabolic parameters, except for HbA1c and fasting glycaemia which were, as expected, significantly higher in DMpc (Table I).

Platelet functionality and oxidative stress markers in total patients before and after supplementation

Na⁺/K⁺ ATPase activity was significantly lower in DM subjects than in controls (Table II). In DM, after Sc supplementation, values increased significantly, but without reaching those of controls (Table II).

The anisotropy of TMA-DPH fluorescent probe, which indicates the outer layer fluidity of platelet membrane, was significantly higher in DM patients than in controls (Table II). In DM, Sc provoked a significant decrease of this parameter, which, however, was still significantly different from the one of controls (Table II). However, this decrease indicated an increase in outer membrane fluidity after Sc supplementation.

Table I. General and metabolic characteristics of enrolled subjects

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 85)</th>
<th>Total patients (n = 77)</th>
<th>DMpc (n = 48)</th>
<th>DMgc (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 ± 11</td>
<td>56 ± 15</td>
<td>57 ± 16</td>
<td>54 ± 13</td>
</tr>
<tr>
<td>Fasting glycaemia (mg/dl)</td>
<td>88 ± 9**</td>
<td>165 ± 39</td>
<td>198 ± 53</td>
<td>131 ± 25*</td>
</tr>
<tr>
<td>HbA1c (%) (mmol/mol)</td>
<td>5.07 ± 0.2**</td>
<td>7.6 ± 0.9</td>
<td>8.7 ± 1.3</td>
<td>6.4 ± 0.5*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>170 ± 17**</td>
<td>184 ± 41</td>
<td>179 ± 40</td>
<td>188 ± 42</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>72 ± 11**</td>
<td>47 ± 13</td>
<td>46 ± 12</td>
<td>48 ± 14</td>
</tr>
<tr>
<td>Total/HDL cholesterol ratio</td>
<td>2.4 ± 0.5**</td>
<td>3.9 ± 3.2</td>
<td>3.9 ± 3.3</td>
<td>3.9 ± 3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>117 ± 18**</td>
<td>137 ± 65</td>
<td>142 ± 75</td>
<td>131 ± 55</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>74 ± 23**</td>
<td>110 ± 36</td>
<td>105 ± 35</td>
<td>115 ± 36</td>
</tr>
</tbody>
</table>

Data are expressed as Means ± Standard Deviation.

DMpc, poorly controlled subjects; DMgc, well controlled subjects; HbA1c, glycated hemoglobin; HDL, high density lipoprotein, LDL, low density lipoprotein.

Statistical comparison between DMpc and DMgc: *p<0.05 vs. DMpc; not significant if not specified.

Statistical comparison of total patients and controls: **p<0.05 vs. total patients; not significant if not specified.

Similarly, DPH fluorescent probe anisotropy, which indicates the fluidity of the inner layer of platelet membrane, was significantly higher in DM subjects than in controls (Table II). In this case, in DM, treatment caused a statistically significant decrease of this marker, although the levels remained significantly higher than the ones of controls (Table II).

Concerning NO levels, values of DM subjects were significantly lower than the ones of controls (Table II). In DM, after Sc supplementation, a significant increase of this parameter was observed, but without reaching the values of control subjects (Table II).

Similarly, antioxidant SOD activity was found to be significantly lower in DM subjects than in controls (Table II). Sc provoked, in DM, a significant increase of this parameter, which, however, was still significantly different from the one of controls (Table II).

TAC values were statistically significant lower in DM subjects than in controls (Table II). In DM, Sc supplementation caused a significant increase of this marker, although the levels remained significantly lower than the ones of controls (Table II).

Platelet peroxidation, measured by lipoperoxide levels through TBARs assay, was significantly higher in DM subjects than in controls (Table II). In DM, Sc supplementation provoked a statistically significant decrease of this parameter, whose values, however, were still significantly higher than the ones of controls (Table II).

Collagen-induced platelet aggregation was significantly higher in DM patients than in controls (Table II). Sc provoked, in DM, a not fully significant decrease of this parameter (p = 0.07), rendering the T₁ value of this variable still significantly higher than the one of controls (Table II).

Platelet functionality and oxidative stress markers in poorly and well-controlled patients before and after supplementation

Na⁺/K⁺ ATPase activity was significantly higher in DMgc compared to DMpc both at T₀ and T₁ (Table II); furthermore, after Sc supplementation, there was a significant increase of the enzyme activity both in DMgc and DMpc (Table II).

The anisotropy of TMA-DPH fluorescent probe showed a statistically significant decrease both in DMgc and DMpc at T₁ compared to T₀ (Table II). Furthermore, both at T₀ and
Table II. Platelet functionality and oxidative stress markers in controls, total diabetic subjects and DMpc and DMgc before (T0) and after (T1) Sc supplementation.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Total patients</th>
<th>DMpc</th>
<th>DMgc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T0</td>
<td>T1</td>
<td>T1</td>
</tr>
<tr>
<td>Na⁺/K⁺ ATPase activity</td>
<td>6.796 ± 0.659</td>
<td>4.177 ± 0.52</td>
<td>5.088 ± 0.24</td>
<td>3.380 ± 0.51</td>
</tr>
<tr>
<td>(µmol Pi/mg prot/h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMA-DPH anisotropy</td>
<td>0.196 ± 0.028</td>
<td>0.312 ± 0.02</td>
<td>0.249 ± 0.01</td>
<td>0.365 ± 0.02</td>
</tr>
<tr>
<td>NO (mmol NO/mg protein)</td>
<td>135.48 ± 12.48</td>
<td>73.09 ± 4.7</td>
<td>111.43 ± 4.7</td>
<td>65.08 ± 5.7</td>
</tr>
<tr>
<td>SOD (U/m)</td>
<td>1.523 ± 0.158</td>
<td>0.736 ± 0.06</td>
<td>0.853 ± 0.03</td>
<td>0.658 ± 0.06</td>
</tr>
<tr>
<td>TAC (nmol/µl Trolox equivalents)</td>
<td>5.621 ± 0.496</td>
<td>2.721 ± 0.24</td>
<td>3.990 ± 0.17</td>
<td>2.252 ± 0.26</td>
</tr>
<tr>
<td>TBARS (µM of MDA)</td>
<td>0.246 ± 0.028</td>
<td>0.482 ± 0.01</td>
<td>0.422 ± 0.03</td>
<td>0.510 ± 0.01</td>
</tr>
<tr>
<td>Collagen-induced platelet aggregation (%)</td>
<td>85.20 ± 5.34</td>
<td>95.40 ± 5.7</td>
<td>93.60 ± 6.8</td>
<td>97.60 ± 5.9</td>
</tr>
</tbody>
</table>

Data are expressed as Means ± Standard Deviation.
DMpc, poorly controlled subjects; DMgc, well controlled subjects; TMA-DPH, 1-(4-trimethylamino)-phenyl-1,3,5-hexatriene; 1-6-phenyl-1,3,5-hexatriene, (DPH); NO, nitric oxide; Sc, Syzygium cumini.

Statistical comparison between T0 and T1 of total patients with T0 of controls: #p < 0.05 vs. T0 (total patients); âp < 0.05 vs. T1 (total patients).
Statistical comparison between T0 and T1 in total patients, DMpc and DMgc: +p < 0.05 vs. T0 (total patients); ±p < 0.05 vs. T1 (total patients); âp < 0.05 vs. T0 (DMpc); **p < 0.05 vs. T0 (DMgc); ***p < 0.05 vs. T0 (DMgc).
Statistical comparison between DMpc and DMgc at T0 and T1; ++p < 0.05 vs. T0 (DMpc); âp < 0.05 vs. T1 (DMpc); not significant if not specified.

Discussion

DM-related chronic hyperglycemia leads to a condition of oxidative stress, due to glucose autoxidation, resulting in an excessive reactive oxygen species (ROS) production, decreased antioxidant defenses, and reduced effectiveness of the enzymes responsible for free radical elimination [23]. Specifically, oxidative stress acts directly on pancreatic β-cells, which are poor in antioxidant enzymes (catalase, glutathione peroxidase, and SOD), by causing apoptosis and indirectly activating stress-inducible cellular pathways; this activation may contribute, together with other pathogenetic mechanisms (advanced glycation protein products formation and lipid peroxidation), not only to the onset of several complications but also to insulin resistance and deficient insulin secretion [3, 24]. In addition, chronic hyperglycemia affects platelet function, by activating protein kinase C (activation effector of platelet), and by reducing, through the glycation of proteins, membrane fluidity, which is essential in the haemostatic process [2]. Hyperglycemia, by inducing oxidative stress, impairs endothelial function and the production of NO and prostacyclin and contributes to the β-cell functional deficit, responsible for the progressive reduction of insulin secretion. Insulin works by inhibiting platelet activation so that insulin deficiency, absolute or relative, appears to be an additional pathogenetic factor responsible for increased platelet adhesiveness and aggregation typical of DM, thus promoting the formation of atherosclerotic plaques [25].

Our study showed that in vitro Sc supplementation induces a significant decrease in oxidative stress on total diabetic platelets, DMgc and DMpc, even if the studied parameters, after improvement, did not reach the levels of control subjects; specifically, we observed a decrease in lipoperoxide levels, as well as an increase in TAC, SOD activity and NO levels. SOD catalyzes the conversion of superoxide anion to hydrogen peroxide and oxygen and is the primary cellular defense against toxic superoxide anion, one of the major cellular oxidants. TAC, instead, is a combined measure of all cellular antioxidant systems, enzymes, proteins, and small molecules. Our study showed, after supplementation, a significant increase in SOD activity and TAC in total diabetic platelets, DMgc and DMpc, highlighting an improvement in intracellular antioxidant defenses. In addition, before and after supplementation, higher levels in SOD activity and TAC were
observed in platelets from DMgc. These defense mechanisms are more effective in DMgc than in DMpc, as the latter must also counteract the effects of failed glycometabolic control. In addition, DMgc, showing lower blood glucose levels, exhibits a lower non-enzymatic glycation of SOD NH2 residues. It is well known that this glycation process may potentially deepen the structure and function of proteins and is positively correlated with blood glucose concentration.

A recent study documented that a dietary supplement of antioxidant vitamins, associated with a change in lifestyle, reduces free radical-induced damage in diabetic subjects with and without complications; in particular, a decrease of thiols, TBARS, and an increase in glutathione S-transferase, ceruloplasmin, SOD, and vitamins C and E was reported [26].

In addition, our study showed a significant decrease, after supplementation, in TMA-DPH and DPH anisotropy in total diabetic platelets, DMgc and DMpc; as anisotropy is inversely proportional to membrane fluidity, the observed decrease in anisotropy could indicate that Sc supplementation induces an increase in platelet membrane fluidity, both in the outer and inner layer. Specifically, TMA-DPH and DPH anisotropy, both at T0 and at T1, showed lower values in DMgc compared to DMpc, suggesting increased fluidity, in the external and internal layer; this could be explained by the lower glycosylation extent and plasma lipoprotein profile alteration, due to the normal blood glucose concentration in DMgc [27]. These data are confirmed by TBARS values, which decreased after Sc supplementation in total patients, DMgc and DMpc. Furthermore, we also observed a significant increase in Na+/K+-ATPase activity after supplementation in the whole sample, as well as in DMgc and DMpc. This impaired Na+/K+-ATPase activity could be related to platelet membrane fluidity increase in the same subjects. Moreover, both at T0 and at T1, Na+/K+-ATPase activity showed higher levels in DMgc than in DMpc; this effect, as observed in SOD activity, can be explained by a lower non-enzymatic glycation of Na+/K+-ATPase NH2 residues and by a lower intracellular ROS production, both due to normal glucose values in DMgc. Na+/K+-ATPase enzymatic activity has been widely studied since this enzyme plays a key role in regulating cellular homeostasis through the maintenance of Na+ and K+ transmembrane gradient [28], and by indirectly influencing Ca2+ transmembrane gradient, involved in the activation and cellular response to platelet aggregating stimuli. The transmembrane Na+/K+-ATPase is also a marker of membrane function and its activity depends on the physicochemical properties and composition of the microenvironment [29]. Our study shows, in fact, an increased Na+/K+-ATPase activity in diabetic platelets incubated with Sc, as this enzyme is located in a more fluid microenvironment that confers greater protection from oxidative damage.

Several studies have shown impaired platelet function in DM, regarding decreased membrane fluidity and Na+/K+-ATPase activity [27, 30]; therefore, an increase of membrane fluidity and Na+/K+-ATPase activity, as observed in our study, highlights the improvement in platelet function, which in turn could slow down the progression of diabetic complications.

Also, our study showed increased NO levels after Sc supplementation in platelets of total patients, DMgc and DMpc. In addition, NO levels were significantly higher, both at T0 and at T1, in DMgc than in DMpc. This could be explained by the higher non-enzymatic glycation on NO synthase NH2 residues of DMpc compared to DMgc, on the one hand, and by the higher intracellular ROS production through the polyol pathway stimulated by excess glucose on the other hand. ROS may potentially alter the structure of any molecule (carbohydrates, lipids, proteins, and nucleic acids) and, specifically, intracellular ROS could alter the structure of NO synthase, which is involved in the synthesis of NO.

Our results suggest that Sc supplementation contrasts oxidative damage associated with DM and its complications, by improving platelet function through increased membrane fluidity and Na+/K+-ATPase activity, as well as by enhancing antioxidant system functionality through increased SOD activity, TAC and NO levels, and decreased lipoperoxide levels.

Finally, in this study, we found that Sc was able to slightly reduce collagen-induced platelet aggregation, in total patients, DMgc and DMpc, although statistical significance was not fully reached; however, consistently with the other findings, collagen-induced platelet aggregation was significantly higher in DMpc than in DMgc both before and after Sc supplementation. This tendency towards reduction, which theoretically results in reduced hyperactivity and hyperaggregability, characteristics of DM, may suggest a new approach of secondary prevention for platelet dysfunction associated complications, mainly microangiopathy. Moreover, since oxidative stress plays a role in causing chronic complications right from the very early stages of DM, Sc supplementation could be proposed, after thorough in vivo testing, even in primary prevention. Sc supplementation as an adjunct to insulin therapy in patients with diabetes is related to its easy availability, safety, low cost, and absence of side effects, unlike many antidiabetic agents.

Before concluding, it should be emphasized that the biochemical mechanisms that lead to the reduction of oxidative stress after treatment with Sc are likely to be mediated by polyphenols, which are contained in Sc [31]. In fact, it has been demonstrated that polyphenols inhibit NADPH oxidase-dependent platelet reactive oxygen species formation or enhance NO generation and/or bioactivity, with a significant inverse correlation between the two variables [32–35].

In conclusion, our study has demonstrated an improvement of oxidative stress markers after Sc supplementation along with a slight positive effect on collagen-induced platelet aggregation. Therefore, Sc intake in the DM diet may have, at first, a preventive and then a protective effect in oxidative damage progression which is associated with DM and its complications; however, its exact protective mechanism warrants further investigation.

Declaration of interest

All authors report no conflicts of interest related to this study. No external funding, apart from the support of the authors’ institution, was available for this study.

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