Protective and anti-angiopathy effects of caffeic acid phenethyl ester against induced type 1 diabetes in vivo

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A B S T R A C T

Objective: This study aims at investigating the anti-diabetic effects of caffeic acid phenethyl ester (CAPE) against induced immunoregulated diabetes in vivo.

Methods: Swiss mice were administered cyclosporine (CsA) 20 mg/kg/day, s.c. for 10 days and simultaneously received multiple low doses of streptozotocin (MLDSTZ) 40 mg/kg/day, i.p. for 5 consecutive days.

Results: Our results showed that administering CAPE (5 μM/kg i.p./every 2 days) to diabetic mice led to a time-dependent decrease in blood glucose levels to 137.1 ± 7.2 from 229.1 ± 12.6 mg/dl and induced a significant increase in serum insulin levels by 93.8% compared with untreated ones. An in vivo anti-inflammatory effect of CAPE treated diabetic mice was observed, based on a significant decrease in IL-1β and IFN-γ (P < 0.01) levels and a highly significant reduction in NO (P < 0.001). An anti-angiogenic effect of CAPE was observed, as determined by a significant serum matrix metalloproteinase (MMP-9) reduction, angiopoietin (Ang-2) reduction and activation of endostatin serum level in the CAPE treated diabetic mice. Furthermore, histopathological examination showed that destroyed pancreatic islets were regenerated and became free of cell infiltration after treatment.

Conclusion: CAPE has a significant anti-diabetic effect on mice in vivo. This anti-diabetic effect may be related to its anti-inflammatory and angiostatic effects. It also reduced angiogenic factors which may shift the equilibrium to the angiostatic effect of CAPE. These findings provide the validity of CAPE as anti-diabetic agent in the special model of CsA/STZ and could be relevant in the future for human diabetes.

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1. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia that results from an absolute or relative insulin deficiency and is associated with long-term complications [1]. Insulin-dependent diabetes mellitus type 1 is an autoimmune disease caused by the selective destruction of pancreatic β-cells [2]. This type of diabetes can be further classified as immune mediated or idiopathic, in which β-cell loss is caused by a T-cell mediated autoimmune attack [3].

Proinflammatory cytokines, such as IL-1β and IFN-γ, are considered critical factors that induce and accelerate β-cell destruction through direct cytotoxic effects, using mechanisms that involve the induction of free radicals and apoptosis-activating pathways in β-cells [4]. However, indirect β-cell destruction may be mediated through mechanisms including the activation of autoreactive T cells [5–7].

Angiogenesis is also a prerequisite in the pathogenesis of diabetes complications [8,9]. Angiogenesis is the generation of new blood vessels from pre-existing ones. Normal angiogenesis depends on the intricate balance between angiogenic factors (such as VEGF, FGF(2), TGF-β, angiopoietins) and angiostatic factors (angiostatin, endostatin, thrombospondins). Therefore, understanding the cellular events involved in angiogenesis and the molecular regulation of these events has enormous clinical implications [10]. This understanding is providing novel therapeutic targets for the treatment of a variety of diseases. Recently, it has been reported that defects in the angiogenic balance may cause a shift towards either excessive angiogenesis or anti-angiogenesis [11]. Furthermore, angiogenesis is essential for proper development and organ homeostasis, such as placental and embryonic growth, collateral formation, wound healing, and granulation. However, angiogenesis is not always healthy and is often associated with pathologic conditions, in which case it is referred to as pathologic angiogenesis [12].

Numerous bioactive compounds, often referred to as nutraceuticals are recently tested for the potential clinical applications. Among the most frequently studied is caffeic acid phenethyl ester (CAPE), a phenolic antioxidant, which is an active anti-inflammatory natural resinous product of honeybees-propolis (bee glue) [13–15]. It has been demonstrated that CAPE may exhibit a significant potential to control hyperglycemia and modulate glucose in vivo [16]. In addition to
being a powerful antioxidant, CAPE was also found to be a specific inhibitor of the transcription nuclear factor-κB (NF-κB), which may account for its anti-inflammatory action. Moreover, there is considerable evidence suggesting that angiogenesis and chronic inflammation are co-dependent; blockage of angiogenesis results in an anti-inflammatory effect [17].

The present study is aimed at investigating the effects of the administration of the CAPE compound on mice induced type 1 diabetes and the angiogenetic parameters such as matrix metalloproteinase-9, angiopoietin as well as, the anti-angiogenic factor—endostatin—which is known to be associated with diabetes. At the same time, correlate their levels with diabetes regression outcome. More details and analysis on the level of cytokines and nitric oxide are necessary to figure out CAPE’s mode of action.

2. Materials and methods

2.1. Experimental animals

Laboratory-bred adult male Swiss mice that were 6–8 weeks of age and weighed 25 ± 2 g were purchased from Cairo University. The mice were housed in the Animal Center, Institute of Genetics, Menoufiya University, Egypt, under constant conditions (12 h light/dark regimen, oriental chow pellet food and water ad libitum). All animal procedures were performed in accordance with the standards of the institutional guidelines for the care and use of experimental animals [18].

2.2. Chemicals and drugs

Cyclosporin A (CsA), Sandimmune® injection, and streptozotocin (STZ) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. IL-1β and IFN-γ detection kits were obtained from Life Technologies ™, Biosource, Belgium. Insulin kits were purchased from Abbott Laboratories, USA. All other chemicals used in this study were purchased from Sigma Co., USA.

2.3. Caffeic acid phenethyl ester

Caffeic acid phenethyl ester was dissolved in DMSO to create a 100 mM stock solution and stored at −20 °C. Serial concentrations in PBS solution were prepared when needed during the mouse treatments.

2.4. Lethality study in Swiss albino mice

Male Swiss albino mice were randomly distributed into several groups of 10 mice each. The mice were treated with different doses (50–300 mg/kg) of caffeic acid phenethyl ester. The number of surviving mice was recorded daily. This process was continued for 30 days. The mice were subjected to experiments in accordance with ethical standards [19].

2.5. Induction of autoimmune type 1 DM

Mice were weighed and ear-notched. STZ was dissolved in cold 0.01 M citrate buffer, pH 4.5, which was always freshly prepared for immediate use. STZ injections were administered intraperitoneally (i.p.), and the doses were determined according to the body weights of the animals. Fifty mice were administered CsA (20 mg/kg/day, s.c.) daily for 10 days prior to STZ treatment and simultaneously received multiple low doses of streptozotocin (MLDSTZ) (20, 40 and 60 mg/kg/day, i.p.) for 5 consecutive days (10 mice/group) [20]. Non-fasting blood samples were collected twice per week by tail bleeding into heparinized tubes. The glucose concentrations in the plasma samples were determined using the enzymatic colorimetric method [21]. Mice were defined as diabetic when their non-fasting blood glucose level (BGL) reached more than 200 mg/dl in two consecutive readings [22].

2.6. Study design

Fourteen days after the last dose of CsA/MLDSTZ co-administration, mice that exhibited signs of diabetes were selected. Diabetic mice were divided into two equal groups (n = 15 for each group), either untreated (group 1, diabetic control) or treated with 5 μM/kg CAPE (group 2, diabetic CAPE). Simultaneously, thirty normal mice were divided into two equal groups (n = 15 for each group) and treated with either saline (group 3, normal saline) or 5 μM/kg CAPE (group 4, normal CAPE).

All treatments were administered i.p., and 10 injections were administered over a period of 21 days. At the end of the treatment period, the mice were anesthetized with ether, and blood was withdrawn by heart puncture and centrifuged at 3000 rpm for 5 min. Serum was separated for the determination of serum insulin levels and other parameters. The pancreas from each mouse was removed and bisected longitudinally. One half was dried carefully with filter paper and homogenized in PBS to prepare a 10% homogenate. The supernatant was removed for the determination of IL-1β, IFN-γ and nitric oxide (NO) levels. The remaining half of each excised pancreas was immersion-fixed in 10% neutral formalin for 24 h. Sections were embedded in paraffin wax, serially sectioned (5 μm), and stained with hematoxylin and eosin (H&E) for histopathological examination [23].

2.7. Determination of the nitric oxide content in pancreatic islet cells

The levels of nitric oxide in the pancreatic islet cells were measured as nitrite and nitrate according to the method of Miranda, which is based on the Griess reaction with a prior reduction step using vanadium chloride to convert nitrates to nitrite ions [24]. The absorbance of the samples was measured at 540 nm using a double-beam spectrophotometer (Shimadzu UVPC 1601, Japan).

2.8. Determination of serum insulin levels

The insulin levels in the serum samples were determined using the micro particle enzyme immunoassay (MEIA) with the Abbott AxSYM® system [25].

2.9. Determination of the IL-1β and IFN-γ content in pancreatic islet cells

The pancreatic islet cells were obtained from different mice groups and the levels of IL-1β and IFN-γ were determined using ELISA kits with monoclonal antibodies specific for IL-1β and IFN-γ. Samples were run in duplicate according to the manufacturer's instructions. The concentrations of cytokines were determined from standard curves prepared using the purified recombinant cytokines provided with the kits.

2.10. Determination of the serum levels of matrix metalloproteinase (MMP-9) and endostatin (sE)

For the assessment of the serum levels of MMP-9 and endostatin, blood samples were collected from each treated and untreated mouse by puncturing the heart. The samples were delivered into plastic tubes that did not contain any anticoagulant and were left to clot. The samples were later centrifuged to obtain serum, which was stored at −70 °C. For the quantitative determination of MMP-9 and endostatin, we used competitive enzyme-linked immunosorbent assay (ELISA), which measures the natural and recombinant forms of the cytokine (Cytokimmune Science Inc., MD). For each sample, 100 μl of serum sample was added to the designated wells. This assay employs the quantitative sandwich
enzyme immunoassay technique. A monoclonal antibody specific for cytokine was pre-coated onto a microplate. Standards and samples were pipetted into the wells, and cytokine was bound by the immobilized antibody. After washing away the unbound substances, an enzyme-linked polyclonal antibody specific for cytokine was added to the wells. Following a wash to remove any unbound antibody, an enzyme reagent and a substrate solution were added to the wells, and a color developed in proportion to the amount of total cytokine (pro and/or active) bound in the initial step. The color development was stopped and the intensity of the color was measured [26].

2.11. Determination of the plasma levels of Angiopoietin 1 (Ang-1) and Angiopoietin 2 (Ang-2)

Plasma samples were collected from each group of mice in a sterile tube containing EDTA. The plasma was separated by centrifugation at 2500 rpm for 10 min in a refrigerated centrifuge. The plasma was stored at −70 °C and thawed before the assay. The enzyme-linked immunosorbent assay (ELISA) was performed using commercially available kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, samples were collected using the anticoagulant ethylene di-amine tetra acetic acid (EDTA) and stored at −70 °C. Plasma samples were added to separate microplates, each containing a specific antibody for Ang-1 and Ang-2. Mixtures were incubated at room temperature for 2 h. Plates were washed four times to remove unbound antigen. Enzyme linked polyclonal antibodies specific for each angiogenic factor were then added, and the mixture was incubated for 2 h, followed by another washing step. Subsequently, the substrate solution was added to the wells. Color development was stopped, and the intensity of the color was measured and compared with a standard curve. The optical density of each well was determined at 570 nm [27].

2.12. Histopathology

The pancreases of the control diabetic group and the diabetic group treated with CAPE were periodically removed and fixed in 10% formaldehyde. The tissues were washed with water, dehydrated through a gradient of increasing ethanol concentrations and embedded in paraffin. Five micrometer thick sections were prepared with the help of a microtome and placed onto glass microscope slides. The sections were stained with hematoxylin and eosin and were subjected to histopathological examination. For evaluation, at least 10 pancreatic tissues were examined for each group [28].

2.13. Statistical analysis

Data are expressed as the means ± standard deviation (SD). Comparisons between two different groups were performed by Mann–Whitney's U test and between more than two groups by Kruskal–Wallis one-way analysis of variance (ANOVA) followed by the Tukey–Kramer test. The GraphPad Software Instat (version 9) was used to carry out the statistical analysis.

3. Results

3.1. Autoimmune type 1 DM model

Streptozotocin-induced diabetes is a well-documented model of experimental diabetes. Co-treatment of mice with CsA/MLDSTZ resulted in autoimmune type 1 DM. This model is confirmed and validated by the time-dependent change in the BGL of 75% of the mice (30 out of 40 mice).

In the present study, the experimental groups demonstrated that a single CsA/MLDSTZ i.p. injection (20 mg/kg) revealed no significant changes in BGL (128.7 ± 6.1 mg/dl) compared with normal mice,
from the first day of injection through 5th day. However, CsA/MLDSTZ (40 mg/kg) induced a gradual and progressive increase in BGL, producing mice with type 1 DM or insulin-dependent diabetes (229.1 ± 12.6 mg/dl) with an accompanying marked reduction in serum insulin levels from the second day of administration. Moreover, CsA/MLDSTZ (60 mg/kg) induced hyperglycemia from the first day of injection (368.5 ± 14.4 mg/dl), which is significant in type 2 or non-insulin-dependent diabetes, with complete damage to the islet cells (Fig. 1).

3.2. Effect of caffeic acid phenethyl ester on blood glucose levels

Treatment of diabetic mice with CAPE 5 μM/kg i.p./every 2 days resulted in a time-dependent decrease in the BGL. This reduction became significantly effective by day 12 of treatment compared with the levels in the untreated mice. By day 21, the BGL was reduced to (137.1 ± 7.2 mg/dl) (Fig. 2), which was significantly different from the value in the diabetic control mice (P < 0.005, ANOVA).

On the other hand, the BGL of control diabetic mice, which did not receive treatment increased throughout the treatment period in a time-dependent manner. Normal mice treated with CAPE 5 μM/kg i.p./every 2 days for 21 days showed non-significant changes in BGL compared with the normal saline group (P > 0.05, ANOVA). Sores and increased irritability have been previously observed in mice that are administered CAPE treatment with 10 μM/kg, so we avoided this dosage in our experimental study.

3.3. Effect of CAPE on serum insulin

Diabetic mice untreated showed a significant decrease (88.7%) in serum insulin levels compared with the normal saline group. On the other hand, treatment of diabetic mice with CAPE 5 μM/kg i.p./every 2 days for 21 days resulted in a significant increase by 93.8% in serum insulin levels. Moreover, normal mice treated with CAPE 5 μM/kg i.p./every 2 days showed a non-significant change in serum insulin levels compared with the normal saline group.

3.4. Effect of CAPE on IL-1β, IFN-γ and NO content in pancreatic islet cells

The untreated diabetic group, showed a significant increase by 81.6%, 53.7% and 92.8% in the pancreatic islets levels of IL-1β, IFN-γ and NO, respectively, compared with the normal saline group. On the other hand, treatment of diabetic mice with CAPE 5 μM/kg i.p./every 2 days for 21 days resulted in significant decreases in IL-1β and IFN-γ levels (P < 0.01, ANOVA) and a highly significant decrease in NO levels (P < 0.001, ANOVA) (43.2%, 26.3% and 58.3%, respectively, compared with the diabetic untreated group). However, there was a non-significant change compared with the normal saline group. Normal mice treated with CAPE revealed a non-significant change in pancreatic islets levels of IL-1β, IFN-γ and NO content compared with the control saline group (Fig. 3).

3.5. In vivo evaluation of serum metalloproteinase-9 and endostatin (sE)

Un-treated diabetic mice elicited a highly significant increase in serum MMP-9 levels with 197.3 ng/ml, which were reduced to 139.1 ng/ml in mice treated with CAPE (P < 0.001), close to the serum levels of normal mice. However, in the untreated diabetic mice, endostatin (sE) levels were significantly reduced to 1.2 ng/ml compared with the normal mice (P < 0.01). In addition, the serum of CAPE-treated diabetic mice, endostatin levels were significantly higher (1.8 ng/ml) (P < 0.001) than the serum levels in the diabetic control group (Table 1). On the other hand, there was a significant correlation between sMMP-9 and the BGL (r = 0.254, P < 0.01, ANOVA) and a highly significant correlation between sMMP-9 and sE, total white blood cells (WBCs), hemoglobin (HB) and the platelet counts of the mice (Table 2).

3.6. Plasma levels of Ang-1 and Ang-2 in treated diabetic mice and diabetic controls

The median and range of the plasma levels of Ang-1 and Ang-2 in normal saline-treated, normal CAPE-treated, diabetic control and CAPE-treated diabetic mice are presented in Table 3. The circulating levels of Ang-1, Ang-2 and the calculated Ang-2/Ang-1 ratio are significantly higher in diabetic mice compared with normal saline mice (P < 0.05, P < 0.01 and P < 0.001). On the other hand, the Ang-2 and Ang-2/Ang-1 ratio were significantly reduced in treated diabetic mice compared with the untreated ones (P < 0.01).

Moreover, there is a significant correlation between Ang-2 and BGL and between the calculated Ang-2/Ang-1 and BGL (P < 0.01, P < 0.01). On the contrary, there is no correlation with Ang-1 (P > 0.05) (Table 4). Furthermore, MMP-9 is strongly correlated with Ang-2 (r = 0.334, P = 0.004) and the calculated Ang-2/Ang-1 ratio (r = 0.321, P < 0.004) but not with Ang-1.

Table 1

Levels of MMP-9 and endostatin serum in normal mice group, diabetic mice group and treated group.

<table>
<thead>
<tr>
<th>Group parameters</th>
<th>Normal group n = 15</th>
<th>Diabetic group n = 15</th>
<th>Treated group n = 15</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>133.4</td>
<td>197.3</td>
<td>139.1</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Median range</td>
<td>79.6–166.4</td>
<td>88.2–216.1</td>
<td>84.5–196.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endostatin (ng/ml)</td>
<td>1.9</td>
<td>1.2</td>
<td>1.8</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Median range</td>
<td>1.1–6.3</td>
<td>1.0–5.4</td>
<td>1.3–9.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P1: Diabetic group vs normal group.
P2: Treated group vs diabetic group.
4. Discussion

Diabetes mellitus type 1 results from the autoimmune destruction of insulin-producing beta cells in the pancreas. Genetic and currently undefined environmental factors participate together in the onset of this disease. In the present study, experimental animals were employed as models for type 1 diabetes induced by CsA/MLDSTZ co-treatment. Moreover, the mice involved in this study were normal and did not have any underlying immune abnormalities that would complicate our study [29].

Propolis obtained from honeybee hives has been used in folk medicine for centuries as an anti-carcinogenic, anti-inflammatory, anti-bacterial and immunomodulatory agent. CAPE is an active component of propolis that attracts the attention of investigators and researchers because of its potential effects in the treatment of a number of disorders and illnesses [30–32].

In this study, to gain more insight into the effects and mechanism of action of CAPE, we performed an in vivo study using an induced diabetes mouse model. Our results showed that CAPE, administered at 5 μM/kg i.p./every 2 days for 21 days, proved beneficial for the host in two ways. First, CAPE induced a marked anti-diabetic activity in type 1 DM, as manifested by the significant decrease in the elevated BGL, which reached almost normal value at the end of the treatment period. Second, the reduction in the BGL appeared parallel to a significant increase in the circulating level of insulin after treatment. This finding seems to be consistent with other similar findings reported by Jung et al., who found that caffee acid induced a significant reduction in the blood glucose and glycosylated hemoglobin levels in treated C57BL/KsJ-db/db mice compared with the control group [33]. Moreover, the beneficial effect of CAPE on autoimmune type 1 diabetes is confirmed by histopathological findings because pancreatic sections from diabetic mice treated with CAPE showed a significant reduction in mononuclear cell infiltration in pancreatic islets and involution of the acinar cells compared with the control diabetic mice, which have marked inflammatory cells (Fig. 4AB).

It is well-known that inflammation is a localized reaction of tissue to infection, irritation, or other injury. However, when inflammation persists or control mechanisms are dysregulated, diseases such as type 1 diabetes can develop. Although, inflammatory cytokines and oxidative stress play a central role in the pathogenesis of acute pancreatitis, CAPE administration 5 μM/kg to Swiss mice for 21 days led to the inhibition of IL-1β, IFN-γ and NO concentrations (43.2%, 26.3% and 58.3%, respectively) compared with untreated diabetic mice. This finding demonstrates the anti-inflammatory and immunomodulatory activity of CAPE. These results are in agreement with Durmus et al., who reported that CAPE treatment inhibits lipid peroxidation and NO overproduction and regulates SOD enzyme activities in diabetic rats [34]; the results are also supported by Wang et al. who documented that a CAPE-treated diabetic group exhibits protective effects against oxidative damage compared with an untreated diabetic group in the sciatic nerve tissues of diabetic rats [35].

Because type 1 DM is characterized by infiltration of the pancreatic islets with activated macrophages and activated T lymphocytes [36], our data indicate that CAPE can inhibit the expression of inflammatory mediators IL-1β, IFN-γ and NO, which participate in the pathogenesis of type 1 DM.

Furthermore, the level of MMP-9 in the serum of untreated diabetic mice was significantly higher (197.3 ng/ml, P < 0.001) compared with the serum of normal mice (133.4 ng/ml). Moreover, we found a highly significant reduction of serum MMP-9 (139.1 ng/ml, P < 0.01) in the treated group compared with the untreated group. This finding seems to be consistent with Kowluru, who illustrated the potential role of MMP-9 in diabetic retinopathy [37]. In addition, the data from our study indicate that the endogenous inhibitor of the angiogenic serum (endostatin) has been shown to be overexpressed (1.8 ng/ml) at significantly higher levels in treated mice compared with untreated (1.2 ng/ml) and close to the value in the serum of normal mice (Table 1). Thus, strategies to neutralize MMP-9 should be considered as supplements to the anti-angiogenic therapeutic strategies that are currently employed in clinical settings. This suggestion is consistent with Rishi and Bhende and Smith and Steel, as these authors mention the use of anti-vascular endothelial growth factor for the prevention of postoperative vitreous cavity hemorrhage after vitrectomy for proliferative diabetic retinopathy [38,39].

Over the past few years, attention has been directed towards the prognostic impact of angiogenic growth factors in diabetes. The results of the present study demonstrate that the levels of plasma Ang-2 (but not Ang-1), like the levels of MMP-9, are selectively elevated in diabetic mice and are associated with indexes of endothelial damage/dysfunction, regardless of vascular disease. Our results showed that Ang-2 and the calculated Ang-2/Ang-1 ratio were reduced in the treated diabetic mice and may be considered as independent prognostic parameters that could predict the efficacy of diabetic treatment strategies. In addition, the levels of Ang-2 and the calculated Ang-2/Ang-1 ratio in the untreated diabetic group were significantly elevated compared with the normal controls. This finding is partly consistent with

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Correlation between investigated angiogenic factors and blood glucose level (BGL), hemoglobin (HB), white blood cell count (WBCs) and platelet counts in the treated mice group.</th>
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<tbody>
<tr>
<td></td>
<td>BGL</td>
</tr>
<tr>
<td>MMP-9</td>
<td>r = 0.254</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.03*</td>
</tr>
<tr>
<td>Endostatin</td>
<td>r = 0.19</td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
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</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Levels of Ang-1 and Ang-2 plasma in normal mice group, diabetic mice group and treated mice group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group parameters</td>
<td>Normal group n = 15</td>
</tr>
<tr>
<td>Ang-1 (ng/ml)</td>
<td>1.63</td>
</tr>
<tr>
<td>Median range</td>
<td>0.669–2.1</td>
</tr>
<tr>
<td>Ang-2 (ng/ml)</td>
<td>2.31</td>
</tr>
<tr>
<td>Median range</td>
<td>0.91–3.6</td>
</tr>
<tr>
<td>Ratio Ang-2/Ang-1</td>
<td>1.42</td>
</tr>
</tbody>
</table>

P1: Diabetic group Vs Normal group.
P2: Treated group Vs Diabetic group.
as anti-diabetic agent in the special model of CsA/STZ and could be useful in the control of the BGL in experimental models. The action of CAPE is accompanied by the shift and elevation of angiostatic and angiogenesis-inhibiting factors.

5. Conclusion

In conclusion, our study has shown that CAPE may be effective in the treatment of autoimmune type 1 DM. The anti-diabetic effect of CAPE seems to be a secondary consequence of its anti-inflammatory and immunomodulatory properties, as it resulted in a significant decrease in the elevated levels of IL-1β, IFN-γ and NO. Furthermore, Ang-2 in circulation may represent an attractive target when introducing CAPE as an anti-angiogenic strategy for the treatment of diabetes as an independent predictor of a favorable prognosis and/or treatment for diabetics. Finally, it has been demonstrated that CAPE has many biological and pharmacological properties. These findings provide the validity of CAPE as anti-diabetic agent in the special model of CsA/STZ and could be relevant in the future for human diabetes.

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Conflict of interest

The authors have no relationships with any person that may be considered a conflict of interest.

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