MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 5(10) pp. 519-528, October, 2017
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

Protective Effects of Caffeic Acid Phenethyl Ester on Type 1 Diabetes: Impact of Metalloproteinase and Angiogenic Inhibitor on Hyperglycemia in Vivo

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Abstract

Type 1 diabetes (T1D) is a syndrome that upsurges the autoimmune destruction of insulin-producing pancreatic β cells. This study aims at investigating the potential and protective role of caffeic acid phenethyl ester (CAPE) on T1D experimental model. Swiss mice were assigned into three groups of eight mice/group. Group 1 mice were normal; Groups 2 is induced diabetic mice by administration of cyclosporin/STZ and group 3 is a diabetic mice treated with CAPE6 µM/kg. Mice group 2 showed marked increases in blood glucose levels. As regard to glycogen hepatic content, a 35.3% reduction was observed in the induced diabetic group 2, this reduction improved to 1.5% in treated group 3. Consequently, a significant elevation of SOD, GSH, and CAT enzymes were seen in the treated group compared to untreated. In addition, serum MMP-9 significantly reduced and TMP-1 appeared to have significantly increased in the treated group compared with untreated. Furthermore, histopathological examination showed marked regenerative changes and normal architecture of islet cell in the treated group compared with untreated once. CAPE has antidiabetic properties related to their anti-inflammatory and angiogenic inhibitor activation effects and may be relevant in the future for human diabetic therapy.

Keywords: Diabetes, Antioxidant enzymes, CAPE, Angiogenesis, Angiogenic inhibitor

Abbreviations

DM: Diabetes mellitus; **T1D:** Type 1 diabetes; **CAPE**: Caffeic acid phenethyl ester **STZ:** Streptotozocine; **ROS**: Reactive oxygen species; **SMC**: Smooth muscle cells **CsA:** Cyclosporin A; **MMP**: Metalloproteinase; **CAT:** Catalase; **GSH:** Glutathione **TIMP-1**: Tissue inhibitor metalloproteinase-1; **SOD:** Superoxide dismutase

INTRODUCTION

Diabetes mellitus (DM), commonly known as diabetes, is a cluster of metabolic issues categorized by hyperglycemia that results from an absolute or relative insulin deficiency and is accompanied with long-standing complications. Additionally, DM is global—affecting both developed and developing countries. DM belongs to a cluster of metabolic diseases designated by hyperglycemia, hyperlipidemia, hyperaminoacidemia, and

hypoinsulinemia that cause reductions in both insulin excretion and function. Type 1 insulin-dependent DM is an autoimmune disease that begins with pancreatic β-cell damage. This category of diabetes can be additionally classified as immune-mediated or idiopathic, in which β-cell damage is produced by a T-cell mediated autoimmune attack.

Angiogenesis is thought of as an uncommon vessel network (microvascular) in response to low oxygen flow or extra incentive agents. The development of angiogenesis includes the limited discharge of angiogenic features from both hypoxic endothelium and supporting pericytes that cause endothelial spread and neovessel sprouting (Kota et al., 2012). Patients with long-standing DM type 1 have irregular blood vessels in glomeruli (Nakagawa et al., 2009), and these findings were consequently found in patients with type 2 diabetes (Seth and Sharma, 2004).

Further, pathophysiological circumstances of diabetes include the incidence of oxidative and nitrosative tension. Numerous studies indicate that reactive oxygen species (ROS) stimulates signaling pathways that enhance angiogenesis (Bhattacharyya et al., 2014;Moris et al., 2017). ROS can be produced through many ways in humans. NAD(P)H oxidases (Nox) exist in vascular EC and smooth muscle cells (SMC) and are a significant source of ROS development from molecular oxygen. Moreover, a widespread variety of angiogenic stimulators can be up-regulated by Nox (Stassi et al., 1997). In diabetes, ROS is comprehensively formed because of either a prolonged inflammatory status or metabolic alterations accompanying this disturbance (e.g., glucose and fatty acid elevation in plasma). ROS triggers the stress-sensitive signaling pathways, which might cause insulin resistance and cell dysfunction, which are two obligatory conditions of diabetes (Blanchetot and Boonstra, 2008).

The aim of diabetic therapy is to induce hypoglycemic activity in the body (Vásquez-Vivar et al., 1998). Anti-diabetic agents can act in different ways, such as stimulating the β-cells of pancreatic islet to release insulin, resisting the hormones that increase blood glucose, increasing the number and sensitivity of insulin receptors, surging the glycogen content, enhancing the use of organ glucose in the tissue and free radical scavenging, resisting lipid peroxidation, correcting the metabolic disorders of lipids and proteins, and promoting microcirculation. However, free radicals unfavorably change lipids, proteins, and DNA and cause a number of human diseases. Hence, an external source of antioxidants is needed to manage the oxidative stress (Palacios-Callender et al., 2007).

At present, herbal medicines have been greatly used in the past few years and the use of natural products to treat diabetes is wide-reaching. More than 1,000 plant types are used as traditional medicines for diabetes (Lobo et al., 2010; Guedes-Martins et al., 2013). The biological actions of plant products used as alternate medicines for treating diabetes are associated with their chemical configuration. Phenolic compounds, flavonoids, terpenoids, coumarins, and other constituents are major components in herbal and/or plant products, and these cause reduction in blood glucose levels (Modak et al., 2007; Tiwari et al., 2014). Numerous classes of natural drugs have been described in the scientific and prevalent literature as having antidiabetic activity (Marles and Farnsworth, 1995). It has been stated that various traditional plants have definite benefits in the treatment of diabetes (Dorababu et al., 2004).

CAPE is a polyphenolic extract that has been patented and has a widespread efficacy. Moreover, it has variable actions that include antioxidant assets. It is documented that CAPE blocks ROS secretion in humans. This study will investigate the potential protective effects of CAPE administration on mice induced type 1 diabetes and the angiogenic parameters of matrix metalloproteinase-9. As well as, the role of anti-angiogenic factor (tissue inhibitor of metalloproteinase 1 [TIMP-1])—expression of which is up-regulated and associated with diabetes. Furthermore, the study measured their levels compared to deterioration of diabetes to appraise the mode of action of CAPEs. Also, the study highlights the mechanism of ROS excretion and pathophysiology in relation to type 1 diabetes combining with antioxidants in experimental study.

MATERIALS AND METHODS

Animals

Adult male Swiss mice weighing 22–25 g were obtained from the animal residence for scientific studies, King Abdulaziz University, (Jeddah, Saudi Arabia). The animals were housed in a pathogen-free environment in the animal residence of faculty of Medicine, Al Baha University, Saudi Arabia, in a 12-h light–dark cycle with meals and water obtainable *ad libitum*. Procedures concerning animals and their maintenance followed institutional rules and national and international laws on the care and use of laboratory animals, with the approval from Al-baha University (Dasyukevich and Solyanik, 2007).

Chemicals and Drugs

Caffeic Acid Phenethyl Ester (CAPE)

Caffeic acid phenethyl ester (CAPE) was obtained from Sigma Chemical Company. The compound was liquefied in DMSO (Sigma, St. Louis, MO) at 100 mM

concentration, prepared into a standard solution, and stored at –20°C. Serialized concentrations in a PBS solution were prepared for the compound for use throughout mice experiments.

Cyclosporin A

CyclosporinA (CsA), Sandimmune® injection, and streptozotocin (STZ) were obtained from Sigma Chemicals Co. (St. Louis, MO). MMP9 and TIMP1 detection kits were obtained from Bioassay Technology laboratory (Harborne, England) and Life Technologies™ (Biosource, Belgium). The rest of the chemicals used were obtained from Sigma Co.

Lethality study in Swiss albino mice

Swiss male albino mice were obtained and divided into groups of 8 mice each. They were administered different dosages of CAPE. The number of live mice was observed daily. This procedure was sustained for 30 days. The mice were subjected to experiments according to moral standards (Broad et al., 1979).

Induction of autoimmune type 1 DM

The mice were assessed and ear-notched. Eight mice were kept as normal control. STZ was liquefied in cold 0.01 M citrate buffer, pH 4.5, which was prepared fresh for instant use. STZ was inoculated intraperitoneally (i.p.), and the dosages were determined in consistent with the body weights of the mice used. Mice were administered CsA (20 mg/kg/day, s.c.). Prior to STZ treatment mice received multiple low doses of streptozotocin (MLDSTZ) (5, 10, 20 and 40 mg/kg/day,i.p.) for 5 sequential days (8 mice/group) (Wright et al., 1995). Non-fasting blood samples were tested twice per week by tail bleeding. Mice were confirmed to have diabetes when their random blood glucose level reached 200 mg/dl or more in two repeated readings compared to normal control (Singleton et al., 2001).

Study design

Fourteen days after CsA/MLDSTZ co-administration, mice that showed symptoms of diabetes were selected (in tables considered as weight zero). Twenty four mice were distributed into equal groups (n = 8 mice/ group) as untreated (group 1, normal control, administered saline only) and induced diabetic untreated (group 2, diabetic untreated). The remaining 8 diabetic mice were treated with CAPE 6µM/kg (group 3, diabetic CAPE treated). All treatments were administered by an intraperitoneal injection, and 12 inoculations were administered over a period of 24 days. At the end of the treatment period (25 days), all mice groups were anesthetized with ether, and blood was withdrawn by heart perforation and centrifuged at 3,000 rpm for 5 min. The serum was separated to determine glucose level and other parameters. The pancreas from each mouse was removed and dissected longitudinally. One portion was dried on a filter paper and homogenized in PBS to prepare a 10% homogenate. The supernatant was drawn for the determination of superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) levels. The other half of each removed 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 (Kendrey and Roe, 1969).

Determination of glucose in mice serum and hepatic glycogen content in homogenate

For glucose level determination, morning blood drop were obtained from tail tip veins during the experimentations from the three groups and the same sample size through a heart puncture at the end of the study. The glucose level concentration was detected using one-touch ultra 2 glucometer LifeScan 6300 Zug, Switzerland) and well-matched blood glucose strip (Henry, 1984) at regular time (Morning samples). Glycogen is a branched polysaccharide of glucose components linked by α-1, 4 glycosidic bonds and α-1, 6 glycosidic bonds. It is produced mainly in the liver and muscle and forms an energy reserve that can be rapidly metabolized to meet a sudden demand for glucose. The most common glycogen metabolism issue occurs in diabetes, in which, because of irregular quantities of insulin, liver glycogen can be unusually accumulated or exhausted. The liver glycogen concentration was measured as described previously. Concisely, 30% KOH was used to digest weighted liver and saturated with Na_2SO_4 for 30 min at 95°C. In order to digest tissue, 95% ethanol was added. The glycogen precipitates were dissolved in water and analyzed by the calorimetric method (Xiang et al., 2014).

Determination of SOD, GSH, and CAT activity in pancreas homogenate

The pancreases were excised and homogenized with 10% (w/v) phosphate buffered saline, pH 7.8. They were centrifuged at 3,000 rpm and 4°C for 20 min. by using a cooling centrifuge. The pure supernatant was aspirated and applied for antioxidant enzyme detection by using a

spectrophotometer (Philip Harris). Nishikimi et al. for SOD detection (Nishikimi et al., 1972). In short, freshly prepared phenazinemethosulfate (0.93 µM) was added to a curette containing 0.1 M sodium pyrophosphate buffer (pH 8.3), nitro blue tetrazolium (0.3 mM), NADH (0.47 mM), and the sample was measured at wavelength 560 nm and expressed as U/mg wet tissue. The reduced GSH level was determined using the method of Prins and Loose (Prins and Loose, 1969). Briefly, the previously mentioned homogenate was prepared as a 0.5-mL mixture with 0.5 mL of tungstate solution, centrifuged at 2000 rpm for 5 min. 200 µL of the supernatant turned into a tube containing Tris buffer followed by the addition of 0.2 mL of 5, 5-dithio-bis-(2)-nitrobenzoic acid reagent. After 30–60 s, the color developed and the optical density was measured at wavelength of 412 nm. GSH content was expressed as µmol/g wet tissue. CAT was measured by the spectrophotometric analysis using the BioVision's Catalase Assay Kit. In brief, catalase reacts with H_2O_2 to yield water and oxygen, and the unconverted H_2O_2 reacts with OxiRed™ probe to develop a product, which can be measured at 570 nm (Colorimetric method) (Walton and Pizzitelli, 2012).

Determination of the serum levels of matrix metalloproteinase (MMP-9) and Tissue Inhibitors of Metalloproteinases 1 (TIMP-1)

The blood samples were drawn from all animal groups for MMP-9 and TIMP1 serum level assessment. The samples were transported to plastic tubes free of anticoagulant and were left to clot. Later, the samples were centrifuged to obtain serum, which was stored at –70°C. For the quantitative determination of MMP-9 and TIMP1, we used competitive enzyme-linked immunosorbent assay (ELISA) (Cytoimmune Science Inc., MD). For each sample, 100 µl of serum sample was added to the designated wells. This assay employs the quantitative sandwich (El-Refaei and El-Naa, 2010).

Histopathological examination of the pancreas

Paraformaldehyde (10%) was used to fix pancreatic tissue section. The sections were stained with H&E. The changes in morphology were examined using a microscope (Eclipse 80i, Nikon, Japan), and the images were acquired with a video camera (DS-Fi1 digital microscope camera, Nikon, Japan) (Awara et al., 2005).

Statistical analysis

The data are expressed as mean ± SEM*. The statistical analysis was carried out using Shapiro-Wilk test for the assessment of the normality of results, one way ANOVA* with LSD* Post Hoc test, and the Pearson correlation coefficient. Values were considered significant at $p < 0.05$. The software SPSS, version 18 was used. *SEM (Standard Error of Mean); ANOVA (analysis of variance); LSD (Least Significant Difference).

RESULTS

Effect of CAPE 6 µM/kg on body weight

A significant body weight loss was observed in diabetic mice group 2 (from 24.06 ± 0.17 g to 17.35 ±0.13 g) which was obviously noticed and recorded until completion of the experiment (Table 1). In group 3, the initial body weight was regained none significantly to 18.71±0.07 g at weight zero, after 25 days CAPE treatment regained weight and almost back to its initial values (24.85 ± 0.16 g) significantly.

Effect of CAPE on blood glucose level (mg/dl) and liver glycogen (mg/g tissue)

Group 1 exhibited no effect on the blood glucose level (BGL). Group 2 showed a significant increase in the BGL from 97 ± 1.55 to 249 ± 4.59 mg/dl. These values were much higher than that of group 1. Table 2 shows a significant reduction in BGL in STZ-induced diabetic mice treated with CAPE in group 3 compared with the diabetic group 2. The diabetic group experienced a significant reduction in content of hepatic glycogen homogenate of 35.3% compared with group 1, while it was reduced to 1.5% after the treatment period with CAPE.

Effect of CAPE on the pancreatic tissue SOD unit/min/mg protein, reduced GSH µmol/g/wet and CAT µ moles activity

The SOD mean activity and reduced GSH and CAT in the pancreatic tissue homogenates of the diabetic group 2 were significantly decreases compared to that of the normal control group. Data exhibited that treatment with CAPE significantly increased the SOD, reduced GSH and CAT enzymes activity (Table 3).

In vivo evaluation of serum metalloproteinase-9 and TMP-1

Mice in the diabetic group 2 showed a significant increase in MMP-9 serum levels with 98.79 ±0.78 ng/mL, but these MMP-9 levels were significantly inhibited with CAPE-treated mice, nearby to the serum levels of normal mice. However, in group 2, TMP-1 levels were

Table 1. Effect of CAPE 6µM/kg on mice body weight

Group	Weight before	Weight zero	Weight after 25 days
Gp.1. Normal Control	**25.31±1.2**	**24.04±0.11 [b]**	**24.03±0.06 [bc]**
Gp.2. Diabetic	**24.06±0.17**	**17.35±0.13 [ac]**	**17.85±0.09 [ac]**
Gp.3. CAPE treated	**24.5±0.13**	**18.71±0.07 [b]**	**24.85±0.16 [ac]**

Data expressed as mean ± SEM. [a]*Significant vs *normal control, [b] significant vs diabetic group, [c] significant vs CAPE treated group.*

Table 2. Effect of CAPE 6µM/kg on blood glucose level mg/dl and liver glycogen mg/gm tissue

Group	Glucose	Glycogen
Gp.1. Normal Control	97±1.55 [bc]	8.03±0.06 [b]
Gp.2. Diabetic	249±4.59 [ac]	5.2±0.07 [ac]
Gp.3. CAPE treated	179±2.36 [ab]	7.91±0.06 [b]

Data expressed as mean ± SEM. [a]*Significant vs *normal control, [b] significant vs diabetic group, [c] significant vs CAPE treated group.*

Table 3. Effect of CAPE 6µM/kg on antioxidant activity

Group	SOD U/mg	GSH µM/g/wet tissue	CAT µmol
Gp.1. Normal Control	**287±2.25 [bc]**	**588±3.09 [b]**	**280±2.23 [bc]**
Gp.2. Diabetic	**201±2.59 [ac]**	**410±4.91 [ac]**	**152±1.36 [ac]**
Gp.3. CAPE treated	**332±5.68 [ab]**	**603±4.36 [b]**	**301±2.28 [ab]**

Data expressed as mean ± SEM*. Significant vs normal control, [b] significant vs diabetic group, [c] significant vs CAPE group.

Table 4. Effect of CAPE 6µM/kg on serum level of MMP-9 and TMP-1

Group	MMP-9 ng/ml	TMP-1 ng/ml
Gp.1. Normal Control	48.53±0.6 [b]	3.76±0.18 [b]
Gp.2. Diabetic	98.79±0.78 [ac]	2.05±0.04 [ac]
Gp.3. CAPE treated	48.18±1.07 [b]	3.86±0.05 [b]

Data expressed as mean ± SEM*. Significant vs normal control, [b] significant vs diseased group, [c] significant vs CAPE group.

significantly reduced to 2.05 ± 0.04 ng/ml compared to that in the normal mice (3.76 ± 0.18). In addition, in the serum of CAPE-treated diabetic mice, the TMP-1 levels were significantly elevated to 3.86 ± 0.05 ng/ml, compared to the serum levels in the diabetic group 2 (Table 4).

Correlation between studied parameters

Figure 1 shows the scatter plots of significantly correlated parameters in the serum and in pancreatic tissue homogenates of all mice. The dense shapes signify the linear regression and correlation coefficient (r), and p is the correlation significance level. There were positive correlations between each pair of the studied parameters: glucose/ TIMP-1, glucose/glycogen weight, TIMP-1/MMP-9 and CAT/GSH/SOD. However, a negative correlation between TIMP-1 and glucose and all other parameters were observed.

DISCUSSION

Type 1 diabetes is caused by an absence or inadequate amount of insulin secretion—the hormone promoting glucose's ability to enter cells and generate energy. Despite the prevalence of type 1 diabetes in infanthood or puberty, it may also affect adults. However, there is evidence that autoimmune activation in a majority of

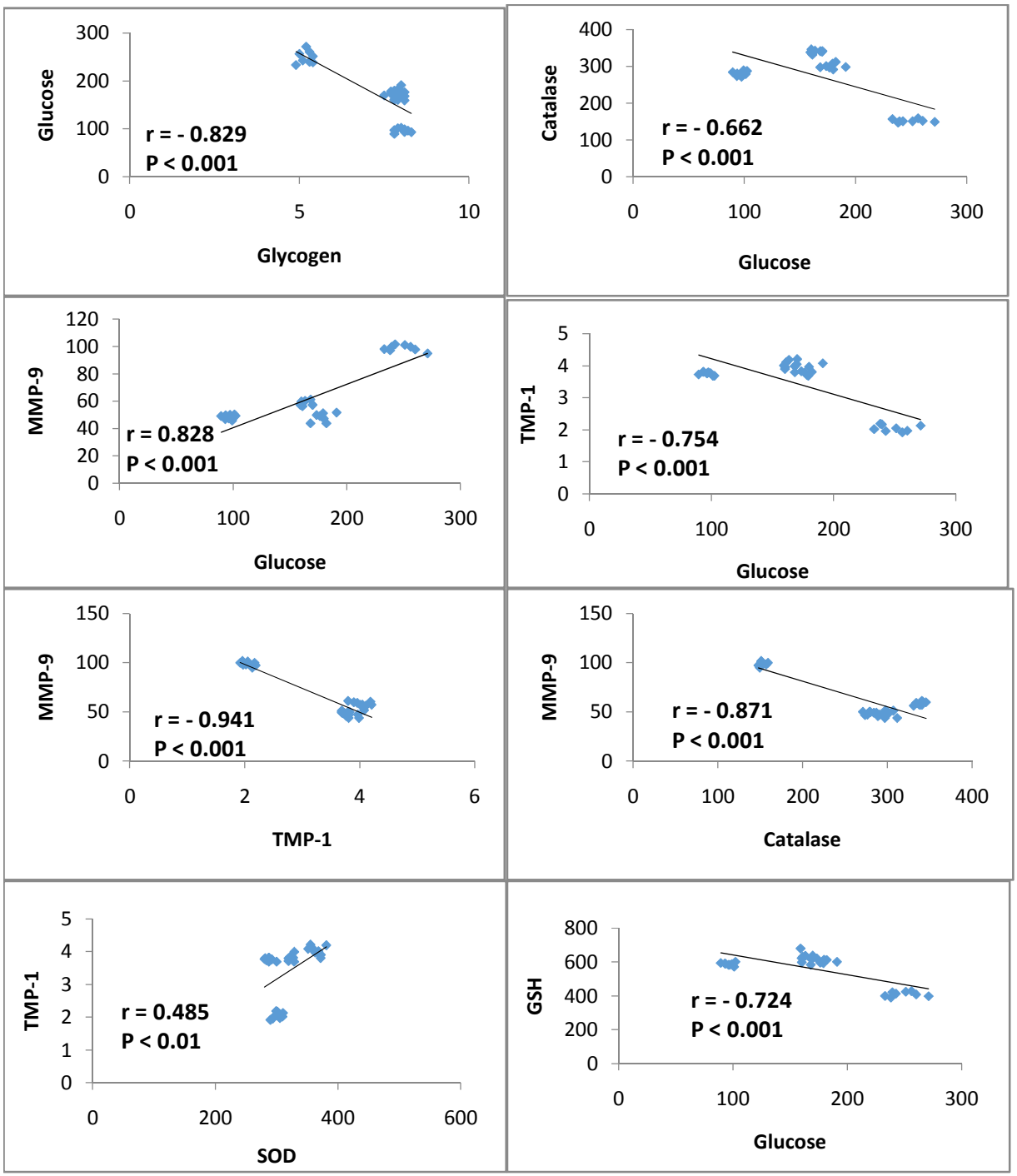

Figure 1. Scatter plots of significantly correlated parameters in the serum and in pancreatic tissue homogenates of all mice groups. The solid lines represent the linear regression and correlation coefficient (*r*), *P* is the correlation significant level and *n* is the total number of samples

people may lead to the development of this type of diabetes.

In the current study, experimental animals were employed as models for T1D induced by CsA/MLDSTZ, the co-treatment presented a number of compensations. Initially, the islet lesions in this model bore a close resemblance to those of human disease. Furthermore, the mice involved in this study were normal and did not have any underlying abnormalities that may alter the results of this study (Serreze et al., 1993).

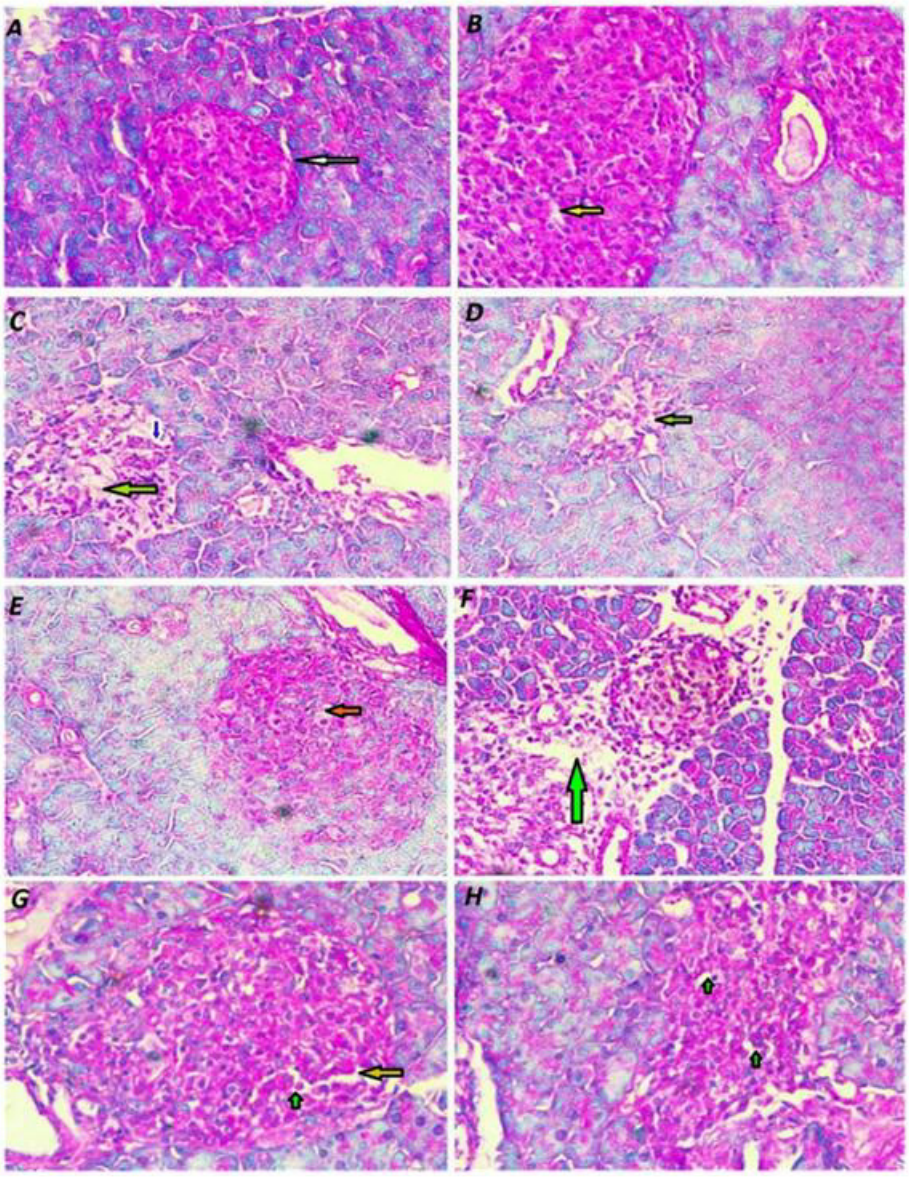

Figure 2. A&B Normal pancreatic tissue; A: rounded contour of islet of Langerhans (arrow) (x400) B: Cells of endocrine area are surrounded by capillary network (H&E, x400). C&D Diabetic group; C: Marked destruction of islets with marked edema and vacuolization (arrow) and inflammatory infiltrates (H&E, x400). D: Marked tissue edema as well as decrease in islet' size and cell number (arrow), (H&E, x200) E, F, G and H: CAPE treated group E: Regenerative changes start to appear in the form of increase in cell number and contour restoration. F: Cellular regeneration, some edema and vacuolization (arrow). G: Rounded to oval islet with restoration of normal contour and its cellular constituents. H: Some glycogenic vacuolization are still present with complete cellular proliferation (H&E, x400).

To gain more insight on the effects and mechanisms of action for CAPE, we performed an *in vivo* study using an induced diabetes mouse model. Our results showed that CAPE, administered at 6 µM/kg intraperitoneal/ twice/week for 24 days, respectively, proved beneficial for the host. CAPE induced a marked gain of body weight as shown by significant increase in the diabetic group from 17.35 ± 0.13 to 24.85 ± 0.16, after the treatment period. Furthermore, antidiabetic activity in type 1 DM, as manifested by the significant decrease in elevated BGL (249 ± 4.59), reached nearby to normal value at the end of the treatment period 179 ±2.36. This finding seems to be consistent with other similar findings reported by Jung et al. who found that caffeic acid induced a significant

reduction in the blood glucose and glycosylated hemoglobin levels in mice treated with C57BL/KsJ-db/db when compared with the control group (Jung et al., 2006).

Thus, the reduction in the BGL seemed to match a significant decrease in the glycogen level of diabetes-induced mice from 5.2 ± 0.07 which elevated to 7.91 ± 0.06, after treatment. Glucose is stored primarily as glycogen in various tissues; with regard to insulin action, glycogen deposition depends on insulin through stimulation of glycogen synthase and/or glycogen phosphorylase inhibition. This in consistent with the findings of Annamala and Augusti wherein they found that alloxan diabetic rabbits treated with glibenclamide showed a significant amelioration in their diabetic condition (Annamala and Augusti, 1980).

The histopathological outcomes confirm the efficacy of CAPE on type 1 diabetes. Changes appear in the form of a renovation of normal islet cell construction, increase in the number of reformative cells, a slight disappearance of glycogenic vacuolizations, and minimization of edematous stroma in the treated diabetic mice. In addition, cellular infiltration in the treated group was less than that in the diabetic group and became more condensed, mainly in the perivascular area (Figure 2). CAPE also showed marked regenerative changes in the form of complete restoration of normal architecture of islet cells and restoration of its number along the cut sections. Scanty lymphocytic infiltrates were seen along the perivascular area. Glycogenic vacuoles and stromal edema are scanty. These results are in agreement with Manikandan (Manikandan et al., 2009), who noted that black tea extract benefits the regeneration of injured pancrease and protects pancreatic beta cells by its antioxidant action against nitrosative stress in streptozotocin-induced diabetes. These results are also consistent with those of El-Kordy and Alshahrani, who proved by using histopathological examination that genistein significantly decreased β-cell loss and improved glucose and insulin levels in diabetic mice (El-Kordy and Alshahrani, 2015).

In the last decade, increase in oxidative stress and reactive oxygen species that attenuate antioxidative effects were reported as major contributors to the pathogenesis of diabetes (Ha andKim , 1999). Pancreatic islet cells are damaged by free radicals. To overcome this, the free radical production site should be bounded by free-radical scavengers, which should be present in high levels or appropriate amounts at the site of free radical production for an extended time (Majithiya et al., 2005). Consequently, increase in the levels of enzymatic and non-enzymatic antioxidants in pancreatic tissue could be beneficial in the treatment of diabetes.

In our study, treatment with CAPE significantly improved the deterioration in the activity of SOD, GSH, and CAT enzymes (Table 3). In accordance with Mouhoubi-Tafinine et al.'s findings, it was shown that oxidative stress was reduced by stimulating the activity and expression of antioxidant enzymes using different honey types and propolis (Mouhoubi-Tafinine et al., 2016). Similar to the improvement of SOD, GSH, and CAT by CAPE, the activity levels in pancreatic tissue homogenates were significantly increased in the CAPE-treated group 3 (Table 3). The improvement in the antioxidant levels after CAPE treatment was previously reported in Russo et al (Russo et al., 2002). In addition to our present findings, it appears that increased antioxidant activity in the pancreatic tissue leads to lowering of the blood glucose level. These data agree with other studies by Ahmad et al. and Abou Khalil et al. in which the correlation between oxidant or antioxidant levels and disease severity was investigated (Ahmad et al., 2013;Abou Khalil et al., 2016). In this regard, mice treated with CAPE showed significant improvement in blood glucose levels, which appeared to be negatively correlated with the improvement of antioxidant (SOD, GSH, and CAT) levels. On the basis of these observations, we hypothesize that the CAPE has a beneficial effect on diabetes type 1 induced in male mice, in part through the improvement of antioxidant activity in the pancreatic tissue.

Moreover, the serum MMP-9 is reduced significantly to 48.18 ng/mL, $p<0.01$ in the CAPE-treated group 3, as shown in Table 4. The MMP-9 serum level in the untreated mice increased significantly up to 98.79 ng/mL ($p <0.001$) compared with the normal mice serum (48.53 ng/mL). This result appears to be in agreement with other findings reported by Norrby et al. that have shown that rats with streptozotocin-induced diabetes mellitus showed a fourfold increase in angiogenic response following mast-cell activation in situ as compared with age-matched normal controls (Norrby et al., 1990). As a result, the reduction in MMP-9 after treatment with CAPE shown in our results appears inconsistent with the result of Marx et al. who proved that MMP-9 levels increase in type 2 diabetic patients with CAD, and treatment of these patients with the antidiabetic PPAR-γ activator rosiglitazone significantly reduces MMP-9 (Marx et al., 2003).

Taken together, these findings can accomplish the following: the tissue inhibitor metalloproteinase-1 (TIMP-1) was revealed to have a significant effect 3.86 ng/ml in CAPE treated group compared with the untreated ones (2.05 ng/mL), and almost closely matched to normal mice levels, Table 4. These results indicate that TIMP-1 may be a beneficial and valuable marker to estimate the longstanding disease activity of diabetes. Furthermore, TIMP-1 itself may be used effectively in the therapy and may be considered an independent factor in diabetic treatment.

Figure 1 shows Pearson's correlation coefficient (r) between serum glucose, MMP-9, and TIMP-1 concent-

rations and the antioxidant parameters (SOD, GSH, and CAT). These correlations suggested that the deficiency in antioxidant production may be related to the glucose level increase in diabetic mice. The MMP-9 concentrations were correlated to the severity of the glucose level elevation and inflammatory conditions. Moreover, TMP-1 elevation is related to inhibition of MMP-9 and significantly correlates to the lowering of blood glucose level.

CONCLUSION

We conclude that administration of CAPE has the potential to act as antidiabetic agents. CAPE improve the type 1 diabetic features, through downregulation of the proinflammatory MMP-9 level in association with elevation of TIMP-1. Moreover, CAPE enhance the antioxidant potential of the pancreatic tissue, attenuate the blood glucose level, and completely restore islet cells. Therefore, it is expected that CAPE resolve, assist, and effective against type 1 diabetes. Clinical trials and advanced research are required to appraise the certain clinical effects of CAPE based on more experimental studies.

ACKNOWLEDGMENT

Our sincere gratitude and thankful to Dr. Ehab S. Atta for his kindly support in histopathology sections, examination and comments.

Conflict of Interest

The author(s) declare(s) that they have no conflicts of interest to disclose.

Funding Support

This study was sponsored by Deanship of Scientific Research in Al Baha University, Kingdom of Saudi Arabia, for their financial and logistical support and for providing necessary guidance concerning project implementation. Project No: 5/1437-2015.

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