Quercetin and Vitamin E ameliorate cardio-apoptotic risks in diabetic rats

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Research Article

Keywords: Mitochondrial permeability transition, mitochondrial lipid peroxidation, diabetes, quercetin, vitamin E

Posted Date: August 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-772041/v1>

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Version of Record: A version of this preprint was published at Molecular and Cellular Biochemistry on January 20th, 2022. See the published version at <https://doi.org/10.1007/s11010-021-04332-w>.

Abstract

Background: Apoptosis is upregulated in all forms of diabetes, with the mitochondria acting as a target in diabetes pathophysiology. Quercetin and vitamin E have shown usefulness in the delay of progression of diabetes-induced complications. However, their effect on the apoptotic process in diabetes mellitus is unknown.

We hypothesize that quercetin treatment in diabetes may decrease the propensity for cardio-myocytic death via regulation of the mitochondria permeability transition (mPT) pore opening.

Methods: Hearts from normal and streptozotocin-induced diabetic rats were used for the study. Low-ionic strength heart mitochondria were used for swelling assay, and mitochondrial lipid peroxidation (mLPO) activity was spectrophotometrically assessed. Levels of cytochrome c, caspase 3 and 9 were determined by immunohistochemistry, while lesions assessed by histology.

Discussion: Diabetic heart mPT pore showed larger amplitude swelling than control, while mLPO levels was increased in diabetic rats relative to control, this resulted in cytochrome c release. This initiated increased caspase 3 and 9 activity in diabetic rats ($p < 0.05$). Histology showed haemorrhagic lesions in diabetic rat hearts. Quercetin and vitamin E treatment reversed these effects, suggestive of their anti-apoptotic effect.

Conclusions: Quercetin and vitamin E protection in diabetes is mediated by mPT pore inhibition and modulation of mitochondrial-mediated apoptosis.

Introduction

Diabetes mellitus (DM) is an epidemic that has affected approximately 382 million people worldwide and expected to increase to about 592 million of the world population in 2035, generating a major world public health burden [1]. 2017). Sadly, the occurrence of diabetes mellitus is affecting a number of organs including the kidney, eyes, and heart [2–4]. Diabetes is a major risk factor in the development of various cardiovascular diseases including heart failure. It has been shown that hyperglycemia associated with this pathological condition causes severe oxidative stress to the cardio-myocytes and thus leads to diabetic cardiomyopathy [5]. The direct association of hyperglycemia with macro- and micro-vascular complications of Type 1 and 2 diabetes has been reported [6]. Cardiovascular disease occurrence in diabetes is significantly high, with studies showing an increased risk of heart failure due to inadequate hyperglycemia control [7]. It is now well established that myocardial cell death by apoptosis is a major determinant in the pathogenesis of cardiomyopathies including ischemia-reperfusion, toxic exposure and various other chronic diseases [8].

It has been demonstrated that apoptosis, a major form of programmed cell death, plays an important role in several pathological disorders involving the heart. The inhibition of this process in myocytes is emerging as a potential therapeutic strategy [9]. Virtually all the apoptotic signaling pathways which take

place in non-cardiac cell types have been shown to also occur during the induction of apoptosis in the heart [10]. It is now clear that apoptosis may take place via at least two pathways including the extrinsic and intrinsic or mitochondrial-mediated pathways [10]. In the mitochondrial mediated pathway, the opening of the mitochondrial permeability transition (mPT) pore or outer mitochondrial membrane permeabilization causes the release of cytochrome c into the cytoplasm thus resulting in the activation of the initiation and executioner caspases, leading to cell [11]. As in other cells, the molecular triggers of cardio-myocytic apoptosis center mainly on the balance between the concentrations of the pro-apoptotic and anti-apoptotic proteins such as bcl 2, bax, cytochrome c and the caspases [11]. It has been revealed that the intrinsic pathway is activated in myocytes by a number of cellular stimuli including hypoxia, ischaemia-reperfusion and oxidative stress, which all enhance the mitochondrial permeability transition, and thus causes an increased permeability of the outer and inner mitochondrial membranes [12].

Observations of Gu *et al.*, demonstrated that the attenuation of early cardiac cell death via suppression of mitochondrial oxidative stress by metallothionein results in a prevention of diabetic cardiomyopathy [13]. Also, Liu *et al.*, reported that the protective effects of N-acetyl-L-cysteine on the heart of streptozotocin (STZ)-induced diabetic rats may be attributable to its protection of the heart against oxidative damage [14]. Furthermore, Kumar *et al.*, showed that multiple antioxidants improve cardiac complications and inhibit cardiac cell death in STZ-induced diabetic rats [15]. Despite the fact that the etiology of ischaemic and reperfusion injury is arguably multifactorial, available evidence indicate clearly that opening of the mPT pore is involved in cardiomyopathy [16]. It has been demonstrated that diabetic heart mitochondria display an enhanced susceptibility to mPT pore opening both in humans and animal models of diabetes [17–18].

Although the exact nature of the component of the mPT pore is still under debate, it is generally accepted that apoptosis, being an orderly regulated process, is a logical therapeutic target [19–20]. In this regard, certain bioactive agents have been shown to have the ability to modulate the opening or inhibition of the pore and as such may be useful in chemoprevention and chemotherapy [21]. In view of the fact that cardiomyocyte apoptosis has been documented as a pivotal form of cell death in ischaemia and reperfusion damage, which are characteristic features of cardiovascular complications in diabetes, it becomes imperative to investigate the influence of certain dietary factors on mitochondrial-mediated apoptosis of the diabetic heart [22]. Vitamin E and quercetin are important dietary antioxidants and we have previously shown that they inhibit mitochondrial mediated apoptosis in the liver of STZ-induced diabetic rats [23]. Low dose quercetin and vitamin E have been shown to be safe for consumption, demonstrating promising pharmacokinetic properties [24–25]. Furthermore, these have been shown to reduce the severity of pancreatic β -cell dysfunction in diabetes through their antioxidant and anti-inflammatory properties [26–27]. The present study was therefore designed to gain insight into the modulatory or protective effects of these dietary components on the heart of STZ-induced diabetic rats via the inhibition of the mPT pore and mitochondrial-mediated apoptosis

Materials And Methods

Chemicals and Reagents

Unless otherwise indicated, all reagents were of the highest purity grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Caspases 9, 3 and cytochrome c antibodies were purchased from Elabscience laboratory (China).

Experimental animals

Animal procedure received approval by the Research Ethics Committee for the Animal Care and Use of the University of Ibadan, Nigeria. Male Wistar rats (100–120 g) used in the study were obtained from the Preclinical Animal House, College of Medicine, University of Ibadan, Nigeria. The rats were kept in temperature controlled (25°C), ventilated cages with 12 hours light/dark cycling and given food and fresh water *ad libitum*.

Body weight determination

The body weight of rats were determined after acclimatization and recorded as the initial weight. The weekly weight obtained for each animal was used to adjust the doses of quercetin for standardization through-out the study period.

Experimental design

Subsequent to 2 weeks acclimatization, diabetes was induced by a single intraperitoneal injection of 45 mg/kg STZ dissolved in 100 mM sodium citrate (pH 4.5) after an overnight fast for rats [28]. Animals which had consistent 72-hour-fasting blood glucose concentrations of ≥ 250 mg/dL were considered diabetic.

Animals were randomly distributed into eight (8) groups with six (6) animals in each group.

Groups I: Normal control rats (NC) that received no special treatment for 28 days

Groups II: NC + oral gavage of 10 mg/kg quercetin (Q10) for 28 days

Groups III: NC + oral gavage of 30 mg/kg quercetin (Q30) for 28 days

Groups IV: NC + oral gavage of 10 mg/kg vitamin E (V) for 28 days

Group V: Diabetic control rats (DC) that were induced with 40 mg/kg STZ and received no special treatment for 28 days.

Group VI: DC + oral gavage of Q10 for 28 days

Group VII: DC + oral gavage of Q30 for 28 days

Group VIII: DC + oral gavage of V for 28 days

These experiments were repeated twice. The number of days used in the study was based on previous studies [29–31]. The doses of quercetin and vitamin E were based on their earlier protective reports in rats [32–34].

Biochemical Assays

After 28 days, rats were sacrificed by cervical dislocation and blood samples obtained through cardiac puncture. However, blood sampling for glucose determination was obtained through ocular puncture after 72 hours by a trained technologist.

The blood was collected into Ethylenediamine tetraacetic acid (EDTA), glucose tubes (sodium fluoride tubes) and used for biochemical analysis. Analysis of Glucose, Cholesterol and Triacylglycerol

Plasma was obtained from blood samples by centrifugation at 3000 rpm for 10 minutes and the supernatant carefully pipetted. Aliquots of the samples were used for glucose determination using glucose oxidase method, while cholesterol and triacylglycerol levels were determined using commercially available kits.

Isolation of rat heart mitochondria

Low ionic strength mitochondria were isolated essentially according to the previous methods with slight modifications using differential centrifugation [35–36]. The heart was aseptically removed and washed with the isolation buffer (220 mM Mannitol, 70 mM Sucrose, 1mM EDTA, 10 mM Tris; pH 7.4). This was weighed and cut into pieces with a pair of scissors. A 10% suspension was prepared and homogenized in a Teflon glass cup. The homogenate was centrifuged twice at 3 000 rpm for 5 min in MSE refrigerated centrifuge. The nuclear debris was discarded and the supernatant was collected. This was centrifuged again at 11 000 rpm for 10 min to obtain the mitochondrial fraction. Mitochondrial pellets were washed twice with washing buffer [220 mM Mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM Tris and 0.2% Bovine Serum Albumin (BSA)] at 10 500 rpm for 10 min. The mitochondria obtained were re-suspended in buffer [220 mM Mannitol, 70 mM Sucrose and 10 mM Tris-HCl], dispensed as aliquots into Eppendorf tubes and kept on ice until use. All procedures were carried out at 4°C.

Measurement of mitochondrial swelling

Mitochondria (0.4 mg/mL) were pre-incubated in the presence of 0.8 µM rotenone and swelling buffer [220 mM mannitol, 70 mM sucrose, and 10 mM Tris (hydroxymethyl) aminomethane (Tris)-HCl (pH 7.4)] for 3½ minutes. This was prior to the addition of 5 mM sodium succinate in the absence of a triggering agent (Ca²⁺) at 25°C. The absorbance change at 540 nm was monitored over a period of 12 min at 30 second interval [37].

Estimation of mitochondrial Lipid Peroxidation (mLPO) determination

The mLPO levels were determined according to previous methods [38]. This was estimated by incubating the mitochondria in a mixture containing 20% (v/v) acetic acid, 0.8% (w/v) thiobarbituric acid and 1.1% (w/v) sodium dodecyl sulphate (SDS) in (1:1). The reaction tubes were placed in a water bath and heated at 95°C for 1 h. The tubes were cooled and butanol added. These were vortex-mixed before centrifugation at 3 000 rpm for 10 min. The organic supernatant was pipetted carefully into clean, dry test tubes and absorbance readings taken at 532 nm against a reagent blank.

Immunohistochemical determination of release of cytochrome c and activation of caspases 3 and 9

Immunostaining was done on heart tissues sliced to 5µM thick. These were embedded in paraffin and rehydrated using methanol and xylene. Citrate (pH 6.0) was used for heat-induced epitope retrieval for 20 minutes, followed by immersion in cold water for 10 minutes. Thereafter, the activities of endogenous peroxidase in the heart sections were blocked with 5% H₂O₂ for 5 minutes. These were incubated overnight at 4°C with 1:200 anticytochrome c, caspase 3 and 9 primary monoclonal antibodies. They were washed and incubated again with horse-radish peroxidase labelled anti-rabbit monoclonal secondary antibodies. The peroxidase binding sites were detected by 0.05% 3'-3'-diaminobenzidine tetrahydrochloride. Counterstaining was performed using Mayer's hematoxylin. Slides were viewed by microscopy and photographed with a digital camera.

Protein Determination

The protein content of the heart mitochondria was determined using BSA as standard [39].

Statistical analysis

Data were analyzed by one way Analysis of Variance using Graph pad prism. Significance was estimated by Tukey post-hoc test and statistical difference of less than $p < 0.05$ was considered significant. The amount of stains in immunohistochemical analysis were quantified using Fiji Software package, version 5.

Results

Quercetin and vitamin E treatment lowered blood glucose, cholesterol and triglyceride levels

Increased blood glucose is a key marker in diabetes pathophysiology, in the study, result (Fig. 1A) shows that the blood glucose in diabetic rats was increased relative to control rats ($P < 0.05$). Oral administration of 10 and 30 mg/kg quercetin decreased the blood glucose by 65 and 61% respectively relative to diabetic rats. Similarly, vitamin E treatment decreased the blood glucose level by 70% in comparison to diabetic rats. It was observed that there was no statistical difference between the treatment regimens. In Fig. 1B, the plasma cholesterol and triglyceride levels were significantly higher in diabetic animals compared with normal animals. Again, treatment with quercetin and vitamin E for 28 days significantly

lowered the levels of both parameters in diabetic rats after 28 days of treatment, with no significant difference in the treatment options.

Figure 1 Effect of quercetin and vitamin E on certain biochemical parameters. (A) Effects of quercetin and vitamin E treatment on blood glucose levels in STZ-induced diabetic rats. (B) Triglycerides and cholesterol levels in STZ-induced diabetic rats previously exposed to quercetin and vitamin E. (C) Effect of vitamin E and quercetin on mitochondrial lipid peroxidation in normal and diabetic rat heart after 28 days of oral administration.

NC (Normal control), DC (Diabetes control), NC + Q10 (Normal control that received 10 mg/kg quercetin), NC + Q30 (Normal control that received 30 mg/kg quercetin), NC + V (Normal control that received 10 mg/kg vitamin E), DC + Q10 (Diabetic control that received 10 mg/kg quercetin), DC + Q30 (Diabetic control that received 30 mg/kg quercetin), DC + V (Diabetic control that received 10 mg/kg vitamin E). Assays were carried out in triplicates, Values are expressed as mean $\pm$ SD. a = $p < 0.05$ compared to DC, b = No statistical difference between DC + Q10 and DC + Q30, c = No statistical difference between DC + Q10, DC + Q30 and DC + V, d = $p < 0.05$ statistical difference between DC + Q10, DC + Q30 and DC + V, e = No statistical difference between NC + Q10, NC + Q30, NC + V. a[#] = No statistical difference between normal control and NC + Q10, NC + Q30 and NC + V. d[#] = No statistical difference between blood glucose at 72 hours and 28 days in NC + Q10, NC + Q30 and NC + V, * = $p < 0.05$ compared to blood glucose at 72 hour. BG (Blood glucose), Trig (Triglyceride level), Chol (Cholesterol level).

Effect of quercetin and vitamin E on mitochondrial Lipid Peroxidation

Mitochondria are prime targets of lipid peroxides in diseases, and the free radicals generated compromise the integrity of the membrane. To assess the impact of diabetes on the mitochondrial membrane, the heart mLPO levels were determined in normal and diabetic control rats. The results in Fig. 1C showed an elevation of mLPO levels in diabetic rats relative to normal rats. Furthermore, an investigation on the effect of quercetin administration on normal rat for 28 days showed no significant effect in mLPO levels (Fig. 1C). Treatment of diabetic rats with 10, 30 mg/kg quercetin and 10 mg/kg vitamin E reduced the mLPO levels by 87, 73, and 58% respectively (Fig. 1C).

Figure 2 Effects of quercetin and vitamin E on the mitochondrial membrane permeability transition pore. (A) Representative profile of the change in absorbance of heart mitochondria respiring on succinate in the presence of rotenone with triggering agent (TA) or without TA (NTA) or Spermine: Inhibitor. (B) Assessment of the Integrity of heart mitochondrial membrane permeability transition pore in STZ-induced diabetic rat respiring on succinate in the presence of rotenone. (C) Effects of quercetin and vitamin E on mitochondrial permeability transition pore of rat heart after 28 days of oral administration. (D) Effects of vitamin E and quercetin on heart mitochondrial permeability transition pore of STZ-induced diabetic rats after 28 days of oral administration.

NC = Normal control, DC = Diabetes control, NC + Q10 = Normal control that received 10 mg/kg quercetin, NC + Q30 = Normal control that received 30 mg/kg quercetin, NC + V = Normal control that received 10 mg/kg vitamin E, DC + Q10 = Diabetic control that received 10 mg/kg quercetin, DC + Q30 = Diabetic control that received 30 mg/kg quercetin, DC + V = Diabetic control that received 10 mg/kg vitamin E.

Quercetin and vitamin E reduced mitochondrial Permeability Transition pore opening in STZ induced diabetic rat heart

Previous studies from our laboratory have shown that quercetin reverses mPT pore opening in the liver of STZ-induced diabetic rats. Therefore, in this study, we determined the intactness of the normal rat heart mitochondria. This was evaluated by incubating 0.4 mg/mL mitochondria in a suspension buffer containing 0.8 μ M, 5 mM succinate at 25°C (pH 7.4) and the rate of decrease in absorbance was monitored at 540 nm over a period of 12 minutes. The results (Fig. 2A) showed that there was no significant change in absorbance of normal rat heart mitochondria but addition of 12 mM Ca^{2+} to the buffer solution caused significant increase in mitochondrial swelling or mPT pore opening which was reduced by 5 mM spermine, a standard inhibitor of the mPT pore opening thus indicating that the mitochondria used in the study were intact. In contrast, the heart mitochondria of STZ-induced diabetic rats showed significant opening of the mPT pore than mitochondria from normal rats (Fig. 2B) which showed no appreciable pore opening. The result of the effects of various doses of quercetin and vitamin E on the status of the mPT pore in heart mitochondria of control animals are shown in Fig. 2C. Here, the results show clearly that quercetin and vitamin E had no significant effect whatsoever on the integrity of mPT pore following oral administration of these substances for 28 days. In order to determine the potency of quercetin and vitamin E in reducing mPT pore opening in diabetic rat heart, 10, 30 mg/kg quercetin and 10 mg/kg vitamin E were administered for 28 days. Results in Fig. 2D showed that mPT pore opening was observed in diabetic rat heart and was reduced by 42, 83 and 50% in animals that received 10, 30 mg/kg quercetin and 10 mg/kg vitamin E, respectively.

Figure 3 Extent of cytochrome c release by the mitochondria from STZ-induced diabetic rat orally exposed to quercetin and vitamin E for 28 days. A) NC (Normal control), B) DC (Diabetic control), C) DC + Q10 (Diabetic control that received 10 mg/kg quercetin), D) DC + V (Diabetic control that received 10 mg/kg vitamin E), E) DC + Q30 (Diabetic control that received 30 mg/kg quercetin). Scale bar = 50 μ M.

a = $p < 0.05$ compared to DC, b = No statistical difference between DC + Q10 and DC + Q30, c = No statistical difference between DC + Q10, DC + Q30 and DC + V.

Quercetin and vitamin E reduced cytochrome c release in diabetic rats

The results of the immunohistochemical analysis of cytochrome c release in hearts of normal and STZ-induced diabetic rats following administration of quercetin and vitamin E are presented in Fig. 3. As shown in the figure, increased cytochrome c release was observed in diabetic rats compared with the normal control rats. Treatment of diabetic rats with 10, 30 mg/kg quercetin and vitamin E showed

reduction in the levels of cytochrome c release by 1.1, 1.3, and 1.5 fold respectively, compared with normal control or diabetic rats.

Quercetin and Vitamin E down regulates caspase 9 and 3 activity in diabetic rats

To investigate the anti-apoptotic effect of quercetin on diabetic rats, we determined the effects of quercetin treatment of diabetic rats on downstream caspase 3 and 9 specific for mitochondrial mediated apoptosis. Here, the results showed that there was a larger extent of caspase 3 and 9 activation in diabetic rats compared with normal control. Treatment with 30 mg/kg quercetin and vitamin E decreased caspase 9 activation by 1.6 and 2.4 fold, respectively, while 10, 30 mg/kg quercetin and vitamin E decreased caspase 3 activation by 1.2, 1.3 and 2.6 folds respectively (Fig. 4).

Figure 4 Caspases 9 and 3 activity in STZ-induced diabetic rat heart following administration of vitamin E and quercetin for 28 days. A) NC (Caspase 9 activity in normal control), B) DC (Caspase 9 activity in diabetic control), C) DC + Q10 (Caspase 9 activity in diabetic control that received 10 mg/kg quercetin), D) DC + V (Caspase 9 activity in diabetic control that received 10 mg/kg vitamin E), E) DC + Q30 (Caspase 9 activity in diabetic control that received 30 mg/kg quercetin), F) NC (Caspase 3 activity in normal control), G) DC (Caspase 3 activity in diabetic control), H) DC + Q10 (Caspase 3 activity in diabetic control that received 10 mg/kg quercetin), I) DC + V (Caspase 3 activity in diabetic control that received 10 mg/kg vitamin E), J) DC + Q30 (Caspase 3 activity in diabetic control that received 30 mg/kg quercetin). Scale bar = 50 μ M.

a = $p < 0.05$ compared to DC, b = No statistical difference between DC + Q10 and DC + Q30, c = No statistical difference between DC + Q10, DC + Q30 and DC + V

Quercetin and vitamin E reduce haemophargic lesions and congestions of coronary vessels in diabetic rats

While normal rat heart showed no visible lesions, diabetes caused haemophargic lesions and congestion of coronary vessels (Fig. 5A and B). Treatment with 10 and 30 mg/kg reduced these complications to mild inflammation (Fig. 5C and E). Furthermore, 10 mg/kg Vitamin E reduced the lesions to multi-focal areas of moderate inflammation (Fig. 5D).

Figure 5 Photomicrographs of sections of the hearts of STZ-induced diabetes rats after oral exposure to quercetin and vitamin E for 28 days. A) NC (Normal control), B) DC (Diabetic control), C) DC + Q10 (Diabetic control that received 10 mg/kg quercetin), D) DC + V (Diabetic control that received 10 mg/kg vitamin E), E) DC + Q30 (Diabetic control that received 30 mg/kg quercetin). Scale bar = 50 μ M.

Blue arrow shows congestion of coronary vessels, green arrow shows hemorrhagic lesion, black arrow shows focal area of inflammation and orange arrow shows multi-focal areas of moderate inflammation involving the myocardium and pericardium

Discussion

The observation that apoptotic cardio-myocyte loss is the most important determinant of mortality and morbidity in various cardiovascular diseases such as heart failure put credence to the postulate that inhibition of cardio-myocyte apoptosis holds promise as an effective strategy for treatment of cardiovascular diseases [40]. Data from work on animal models on the inhibition of mPT pore opening by either cyclosporine A or genetic ablation of cyclosporine D thus providing strong protection from both reperfusion injury and congestive heart failure show clearly that the mPT pore is a promising drug target in human cardiovascular diseases [41]. Diabetic cardiomyopathy is known to be triggered by severe oxidative stress brought about mainly by uncontrolled hyperglycemia in diabetic patients and this effect directly affects the mitochondria [42]. The results obtained in this study show that quercetin and vitamin E sustained glycemic control in diabetic rats during the period of the study. This finding is consistent with the recent reports that dietary supplement ameliorated hyperglycemia in diabetes [43–44]. Several reports have shown that increased triglyceride and cholesterol in diabetes lipidemia would cause cardiovascular disease [45]. The present study confirmed the potency of quercetin treatment in reducing hyperlipidemia, thereby probably may reduce cardiotoxic risk. We have previously shown that the mPT pore is significantly opened in liver mitochondria of STZ-induced diabetic rats and that quercetin and vitamin E inhibited the mPT pore in diabetic rats [23]. The effects of the antioxidants have also been shown to be relevant in testicular protection in diabetes [34]. These antioxidants significantly improved the status of the liver mPT pore and the diabetic complications. This study investigated the effect of quercetin and vitamin E on rat heart mitochondrial-mediated apoptosis via the mPT pore opening in STZ-induced diabetic rats. Our results show that the mPT pore of hearts of STZ-induced diabetic rats is significantly opened as previously reported [46]. The results obtained from experiments on the oral administration of either quercetin or vitamin E showed that the antioxidants significantly inhibited pore opening in diabetic rats. This suggests their cardio-protective role in excessive apoptosis during diabetes, confirming that inhibition of mPT pore restore cardio-protection in diabetic hearts [47]. Our findings from the study also showed that the effect of 30 mg/kg quercetin was the most outstanding in inhibiting heart mPT pore opening in diabetic rats.

Sequel to the fact that quercetin and vitamin E inhibited the mPT pore opening in diabetic condition, effects of quercetin and vitamin E on mLPO was investigated via determination of MDA levels. In this study mLPO level was elevated in diabetic rats while treatment with the antioxidants inhibited the alteration of membrane lipid contents. It has been shown that the mitochondria are prime target and source of lipid peroxides in diseases. Therefore, rescuing this situation as shown by quercetin and vitamin E treatment could help maintain the integrity of cardio-myocytes in diabetic condition.

Studies have shown that induction of oxidative stress is a determinant of apoptosis in diabetes with flavonoids showing antioxidant roles [48–49][10]. These studies give credence to the fact that quercetin and vitamin E show antioxidant role in diabetes. Disturbance of membrane fluidity caused by peroxidation of membrane phospholipid and sensitization of mPT pore enhance cytochrome c release. Which is an irreversible event in the progression to cell death [10]. Elevated cytochrome c release in the

heart sections of diabetic rats confirmed mPT pore opening in the hearts of diabetic rats. Treatment of diabetic rats with quercetin and vitamin E showed reduction in cytochrome c release. The data obtained in this study showed that the presence of cytosolic cytochrome c activated the initiator procaspase 9 and subsequently the executioner caspase 3 and caused cell death. Taken together, the present study showed that quercetin and vitamin E decreased the extent of excessive apoptosis in diabetic rats by reversing the opening of the mPT pore and reducing the release of cytochrome c to the cytosol and thereby decreasing caspase 9 and 3 activation and preventing the death of the cardiomyocytes.

Furthermore, histopathological examinations of the sections of the heart showed reduced lesions as a result of quercetin and vitamin E treatment in STZ-induced diabetic rats.

Researches have shown that over 66% of the active agents in drugs have their natural sources from plant origin, more of these are currently undergoing clinical trials [50–51]. The mechanism of actions of these is by lowering glycemic status. It has been reported that *Trigonella foenum-graecum*, *Ipomea batata*, *Silybum marianum* among others have improved glycemic status in diabetes [51]. Therefore it is unsurprising that vitamin E and quercetin, obtained from dietary sources could modulate glycemic status in diabetes. It is suggested that these dietary molecules should be used as adjuvant therapy for pharmacological management of cardiovascular disease in diabetes.

Conclusion

Study showed the anti-apoptotic role of quercetin and vitamin E in STZ-induced diabetes, therefore these could be probable pharmacologic agent to target downstream intrinsic apoptotic pathway leading to excessive cardio-myocytic death in diabetes.

Declarations

Acknowledgements

The authors acknowledge the contributions of the Director, Biomembrane and Biotechnology Laboratory, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interests

The authors declare no conflict of interest.

Availability of data

All data used in the study are included in the manuscript.

Authors Contribution

Conceptualization, methodology, data curation, investigation, writing, editing, visualization, resources, funding [Oluwatoyin Ojo; Olufunso Olorunsogo]; Data curation, investigation, resources, funding [Ifedayo Obaidu]; Writing, editing [Oluwatosin Obigade]; Conceptualization, supervision, project administration [Olufunso Olorunsogo].

Ethics Approval

All procedures performed using experimental animals were in accordance with the ethical standards of the procedures of the University of Ibadan Ethics Committee, Ibadan, Nigeria.

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Figures

Figure 1

Effect of quercetin and vitamin E on certain biochemical parameters. (A) Effects of quercetin and vitamin E treatment on blood glucose levels in STZ-induced diabetic rats. (B) Triglycerides and cholesterol levels in STZ-induced diabetic rats previously exposed to quercetin and vitamin E. (C) Effect of vitamin E and quercetin on mitochondrial lipid peroxidation in normal and diabetic rat heart after 28 days of oral administration.

Figure 2

Effects of quercetin and vitamin E on the mitochondrial membrane permeability transition pore. (A) Representative profile of the change in absorbance of heart mitochondria respiring on succinate in the presence of rotenone with triggering agent (TA) or without TA (NTA) or Spermine: Inhibitor. (B) Assessment of the Integrity of heart mitochondrial membrane permeability transition pore in STZ-induced diabetic rat respiring on succinate in the presence of rotenone. (C) Effects of quercetin and vitamin E on mitochondrial permeability transition pore of rat heart after 28 days of oral administration. (D) Effects of vitamin E and quercetin on heart mitochondrial permeability transition pore of STZ-induced diabetic rats after 28 days of oral administration.

Figure 3

Extent of cytochrome c release by the mitochondria from STZ-induced diabetic rat orally exposed to quercetin and vitamin E for 28 days. A) NC (Normal control), B) DC (Diabetic control), C) DC+Q10 (Diabetic control that received 10 mg/kg quercetin), D) DC+V (Diabetic control that received 10 mg/kg vitamin E), E) DC+Q30 (Diabetic control that received 30 mg/kg quercetin). Scale bar = 50 μ M. a = $p < 0.05$ compared to DC, b= No statistical difference between DC+Q10 and DC+Q30, c= No statistical difference between DC+Q10, DC+Q30 and DC+V.

Figure 4

Caspases 9 and 3 activity in STZ-induced diabetic rat heart following administration of vitamin E and quercetin for 28 days. A) NC (Caspase 9 activity in normal control), B) DC (Caspase 9 activity in diabetic control), C) DC+Q10 (Caspase 9 activity in diabetic control that received 10 mg/kg quercetin), D) DC+V (Caspase 9 activity in diabetic control that received 10 mg/kg vitamin E), E) DC+Q30 (Caspase 9 activity in diabetic control that received 30 mg/kg quercetin), F) NC (Caspase 3 activity in normal control), G) DC

(Caspase 3 activity in diabetic control), H) DC+Q10 (Caspase 3 activity in diabetic control that received 10 mg/kg quercetin), I) DC+V (Caspase 3 activity in diabetic control that received 10 mg/kg vitamin E), J) DC+Q30 (Caspase 3 activity in diabetic control that received 30 mg/kg quercetin). Scale bar = 50 μ M. a = $p < 0.05$ compared to DC, b= No statistical difference between DC+Q10 and DC+Q30, c= No statistical difference between DC+Q10, DC+Q30 and DC+V

Figure 5

Photomicrographs of sections of the hearts of STZ-induced diabetes rats after oral exposure to quercetin and vitamin E for 28 days. A) NC (Normal control), B) DC (Diabetic control), C) DC+Q10 (Diabetic control that received 10 mg/kg quercetin), D) DC+V (Diabetic control that received 10 mg/kg vitamin E), E) DC+Q30 (Diabetic control that received 30 mg/kg quercetin). Scale bar = 50 μ M.