TRI-E® PREVENTS COGNITIVE IMPAIRMENT AND DEOXYRIBONUCLEIC ACID DAMAGE IN DIABETES MELLITUS

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ABSTRACT

Objective: The aim of the current study is to compare the difference of Tri-E® and ATF potentials in reducing diabetic adverse event due to hyperglycemia that might cause the DNA damage and cognitive impairment indirectly. Methods: Diabetic model sample from Sprague Dawley was developed by combination of twice low-dose streptozotocin injection and high fat diet. The diabetic rat model was treated with Tri-E® and ATF in different groups, control group were provided. The DNA was collected from the whole blood and DNA damage was measured using the comet assay method. While cognitive status was measured using Morris water-maze method. Result: Reduction of the DNA damage and improvement of cognitive function were observed in both ATF and Tri-E® supplemnetations that elevated in diabetic. However, the DNA damage reduction by Tri-E® was one-fold higher compared to ATF supplementation. Conclusion: This study provided data that Tri-E® has superior potential than ATF as a preventative agent to DNA damage and cognitive impairment in diabetes indirectly.

Keywords: Diabetes, Deoxyribonucleic acid damage, Cognitive decline, Comet assay, Tocotrienol.

INTRODUCTION

Globally, diabetes mellitus (DM) is one of most common metabolic disorders characterized by chronic hyperglycemia, due to defects in insulin secretion causing damage, dysfunction, and failure in many vital organs [1-8]. Currently, there are over 150-200 million diabetics worldwide, and this number is expected to rise due to escalation in inactive way of life, consumption of more junk food, and obesity [9,10]. Thereafter, diabetes has to take the place as the fourth killer disease [5]. The prevalence of diabetes in Malaysia has exceeded the projection, according to the data by the International Diabetes Federation [9,10]. According to a study [11], globally, prevalence of diabetes in adults aged 20-79 years, increase to 7.7% by 2030, projected from 6.4% in 2010. These estimates indicate a potential enhancing load of diabetes, mainly in developing countries. In Malaysia, the overall prevalence of DM was estimated to be at 11.6% [11]. Diabetes causes amputees formed among the patients with known diabetes, suffered a stroke event, and eventually renal replacement therapy [12]. Worse still, Type II diabetes mellitus (T2DM) was found diagnosed among children as young as 10 years old [13,14].

Type 1 DM is related to genetic and congenital causes, while T2DM usually is associated with insulin resistance. Prolonged unhealthy diet, a very high sugar diet for instance, causes hyperglycemic condition, which leads to hypercholesterolemia and insulin resistance [15]. Hypercholesterolemia clearly associated with the development of arteriosclerosis with high inflammatory activity [16]. Insulin resistance physiologically occurred when the cells are unable to respond from normal actions of insulin. High plasma glucose causes increase insulin production. This is a normal regulatory mechanism in the human body to increase the glucose uptake by the cells. Factors for elevated plasma glucose include health status, circulating hormones, genetic-inheritance disease, and elevations of gluconeogenesis. Prolonged hyperinsulinemia eventually will lead to hyperglycemia and insulin resistance. Insulin resistance is a known underlying cause that can induce a plethora of interrelated common chronic diseases include diabetes, cardiovascular disease, hypertension, gout, arthritis, and others [17].

Specific decay of brain work such as cognitive decline might be accompanied with DM [15,17,18]. Antioxidants were reported to have preventive properties of the neurodegenerated cells and cognitive decline associated with diabetes. Memory loss in induced diabetic rat could be prevented by vitamin E supplementation [19]. The deficiency of cognitive function includes learning and memory loss, predisposed to damage caused by oxidative activity of synapses in the cerebral cortex and hippocampus, might be improved by vitamin E, an antioxidant, indirectly, by reducing the oxidative effects [20].

Alpha-tocopherol (ATF) gets the highest bioavailability in human body and essential for normal neurologic functions. A study showed that reduced plasma ATF levels were found in participant with cognitive decline [20,21]. This macronutrient has been suggested as a preventive and therapeutic agent in impairment of neurological function [21].

A recent study shows that tocotrienol could be a better agent than ATF or ATF-acetate in the prevention of chronic inflammatory disease [22], as a potent hypcholesterolemic agent [21,23]. Principally, it inhibits the β-hydroxy-β-methylglutaryl coenzyme A reductase activity. Hence, it is a better preventive agent of chronic inflammatory disease-related such as cardiovascular disease. In addition, a study on healthy elderly found that supplementation of tocotrienol-rich fraction (TRF) decreased deoxyribonucleic acid (DNA) damage [24]. The same improvement trend was observed in lipoprotein-lipid profile, with a reduction in markers of protein and lipid damage in the same study [25]. Furthermore, a finding shows that in vivo study using...
lymphocytes showed the significant effect of TRF in reversing oxidative stress induced peroxiredoxin expression [22] and conferred protection against cell death [25].

METHODS

Study design

Animal model was induced to develop diabetic condition with a combination of high-fat diet (HFD) and streptozotocin (STZ) (35 mg/kg) twice intraperitoneal [IP] injections. Control group (Group A) was provided with normal pellet diet (NPD), and citrate buffer (pH 4.4) IP injection was given twice [26,27]. The diabetic rats without treatment were assigned as Group B. Two groups (Group C and D) of diabetic rats were chosen to receive supplementation of Tri-E® and ATF. Supplementation were given orally. The weight and fasting blood glucose (FBG) were evaluated and recorded weekly. Blood collection (2 ml) was done for comet assay.

Glucose measurement

Rat was given anesthetic drug (0.1 ml/100 g b.w) for 5 minutes’ effect. Blood was taken from 1 cm of tip of rat’s tail by pricking the point. Glucose quantification was performed using a glucometer (Accu-Check, Roche, USA). The glucometer’s strip was faced down to prick point to allow the blood flow into the strip until a sound “beep” is heard. After a few second, the reading from the glucometer screen was recorded.

Morris water maze test

The test was performed in a large circular pool (diameter: 180 cm, height: 76 cm) made up of plastic. Pool was filled until the depth of water level was 55 cm. Water was fixed at room temperature (25°C±1°C). A platform was placed, hidden under the water surface (±1.5 cm) so that it becomes invisible to the rats. The color of painted platform was similar to the pool color (black on black). Rats were tested and begun at random point of the pool quadrants. Visual image of the tested rats was recorded for further analysis. Visual cues were hung on the wall intentionally for navigating in the maze, using various objects of highly visible or reflective geometric images such as circles, squares, and triangles. The rats eventually become more capable to identify the location of platform by learning and recalling according to the visual cues relative to the distal of platform location. The duration from the recorded visual image of rats to reach the platform was analyzed and compared between groups [28,29].

Comet assay

A total of 80 ul of 0.6% normal melting point agarose (±60°C) slides was pipetted onto the labeled slides [in a dark environment] and immediately covered with coverslips. The slides then were placed on ice-cold flat tray to allow the agarose to solidify. Then, 0.6% low melting point agarose (LMA) (±37°C) was prepared. 5 ul of fresh whole blood in a microcentrifuge tube was mixed with 70 ul of 0.6% LMA. Coverslip was removed carefully from the slides. The contents in the microcentrifuge tube were rapidly pipetted onto the first agarose layer and spread using a coverslip and allowed to solidify on ice tray. After about half an hour, coverslip was removed and immersed in the freshly prepared and cooled lysing solution for 1 h at 4°C. After one hour, slides were removed from the lysing solution, drained on tissue, and immersed in the electrophoresis buffer (placed horizontally) for 20 minutes to allow the unwinding of the DNA. Electrophoresis was conducted for 20 minutes. Then, slides were removed from the electrophoresis buffer, drained on tissue, and placed on a tray. Neutralization buffer was wisely dropped onto the slides for two times to gently neutralize the excess alkali. The slides were allowed to stand on the tray for 5 minutes. The neutralizing procedure was repeated twice. Then, each slide was drained and added with 30 ul of 20 ug/ml EtBr working solution. Each slide was covered with a new coverslip. The slides were placed in air-tight container placed on wet tissue layer and stored at 4°C to prevent drying of the gel. The slides can be analyzed within one week. Slides were examined at ×200 magnification using fluorescence microscope (AxioCam MRC, Carl Zeiss, Germany). 500 randomly selected non-overlapping cells were analyzed on each slide microscopically by categorizing cells as normal/undamaged cell without tail (score 0), cell with tiny tail (score 1), cells with dim tail (score 2), cell with clear tail (score 3), and only tail (score 4). A total damage score for each slide can be calculated by multiplying the number of cells [N] assigned to each grade of damage by numeric value of the grade and summing over all grades, giving a maximum possible score of 2000, corresponding to 500 cells [30] as simplified by the formula given below.

$$\text{Arbitrary unit \ (total DNA damage) = (score } 0 \times \text{N)) + \ (score } 1 \times \text{N}) + \ (score } 2 \times \text{N}) + \ (score } 3 \times \text{N}) + \ (score } 4 \times \text{N})$$

RESULTS

The weight of rat measured during baseline, Phase I (before injection of the first dose STZ 35 mg/kg), and Phase II (before injection of second dose STZ 35 mg/kg). The values of <t and p>0.05 signified the validity of the test, and there was a significant difference of weight for rat comparing baseline with Phase I and baseline with Phase II. The Fig. 1 shows the rat groups that were fed with HFD have more weight than NPD group. A, the weight increased at 11.7% during Phase I and 22.3% during Phase II compared to the baseline. While in average for group B, C, and D, the weight increased at 17.5% during Phase I and 25.7% during Phase II compared to the baseline.

The baseline glucose reading was normal in all groups; the mean is 5.9 mmol/L. During Phase I, mean FBG in rat fed with HFD and injected of first dose STZ (35 mg/kg b.w) was 7.8 mmol/L compared to the mean of Group A, 4.6 mmol/L. During Phase II, the mean FBG for the group B, C, and D was 15.4 mmol/L compared to Group A. 4.9 mmol/L. During baseline, Phase I and II, Group A has glucose reading within the normal range (4.0-5.5 mmol/L), whereas, in Group B, C, and D, the glucose reading was increased at 32.2% in Phase I and 161.0% in Phase II compared to the baseline (Fig. 2).

The rats could swim for 90 seconds per trial. The mean duration Group A was 54 seconds, Group B was 76 seconds, Group C was 66 seconds, and Group D was 57 seconds to reach the platform during Phase I. While in Phase II, the mean duration to reach the platform in Group A was 72 seconds, Group B was 89 seconds, Group C was 77 seconds, and Group D was 75 seconds. During Phase I, the mean duration of rats to reach the platform for Group B was 40.7%, Group C 22.2%, and Group D 5.6% longer than Group A. While, during Phase II, the mean duration of rats to reach the platform for Group B was 23.6%, Group C was 6.9%, and Group D was 4.2% longer compared to the Group A. During Phase I, Group C was taking 15.8% longer than Group D to reach the platform and also during phase to at 2.7% (Fig. 3).

A total of DNA damage measured using comet assay method showed by Fig. 4. The mean of total damage for a normal rat is 0, diabetic rat without treatment is 339, diabetic rat with ATF is 25, and diabetic rat with Tri-E® is 12. Total DNA damage in Group B was the highest. Group C showed a reduction by 92.6% of total DNA damage, while Group B, C, and D were considered as normal FBG.

DISCUSSION

Diabetes is common complications arising as a chronic disease. In Malaysia, diabetes mellitus is diagnosed to be increased tremendously recently [31]. This matter has affected the quality of life in society as diabetic condition causes a lot of complications include cognitive impairment and DNA damage [17, 32-36].

Injection of STZ (35 mg/kg) was given twice with an interval of 1 week to get the effect of mild damage of pancreas; in line with the characteristic of T2DM. It indicated a combination of failure of pancreas to secrete sufficient insulin and insulin resistant which is commonly caused by obesity, either not a totally damage pancreas or complete zero insulin secretion. However, Group B, C, and D were considered as normal FBG.
According to another previous study, the FBG should be ≥7.8 mmol/L after 4 weeks of STZ injections to be considered as diabetic [26]. These rats already have developed impaired glucose tolerance state. During Phase II, the mean FBG for the Group B, C, and D was 15.4 mmol/L compared to normal group, Group A, 4.9 mmol/L. These data showed that the groups fed with HFD might experience insulin-resistant secondary to obesity. Induction of diabetes was achieved successfully using a combination of HFD and twice injection of STZ, 35 mg/kg b.w with slightly modifications from previous studies [26,27].

In the previous study, the cognitive function was measured by the duration of the rat escaping the water by memorizing the location of the platform relative to the distal visual cues [28,29]. Two locations of platform were placed in two different quadrants (quadrant 2 and 4) so that the rats could learn from every possible angle to memorize the cues randomly [28,29]. Thigmotaxis test was applied to these rats to test the vision and ensure it is not blind [28,29]. According to the current study, Group B had the longest duration to reach platform (77s). It might be due to vision impairment, one of the complications of diabetes and retinopathy. Further investigation on their vision should be done. Rats were allowed to swim until 90s per trial whereas, the mean duration of the diabetic group treated with Tri-E® was significantly less with a comparison to control. Therefore, cognitive impairment showed by diabetic rat was improved with the Tri-E® supplementation. The finding in this study agreed to the previous studies [15,18,29,37,38]. However, supplementation with ATF showed no significance difference with the diabetic group.

The total DNA damage in diabetic group was about 68% higher than in normal rat. It was reduced tremendously (Fig. 4), when comparing the diabetic group with the group with supplements administered. These data show that the DNA damage was reduced in diabetics with the supplementation of ATF and Tri-E®. Supplementation of ATF and Tri-E® showed a reduction to 93% and 96%, respectively, compared to a normal rat. However, supplementation of Tri-E® was lowered by 50% than ATF. Therefore, this study suggested that Tri-E® has more prevention and restoration properties compared to ATF in diabetic rat. Thus, the finding from our study is supported by the finding from the previous studies [15,22,29,38].

CONCLUSION

DM has caused DNA damage. ATF and TRF are known as a source of vitamin E as a powerful antioxidant. Both types of this vitamin E are essential to protect the damage of cell including DNA. The properties of vitamin E include reducing the oxidation process caused by disorder of metabolism in diabetic condition. Diabetic condition is known as a metabolic disorder and causes metabolic problem and cognitive impairment, one of the complications of diabetes and retinopathy. Further investigation on their vision should be done. Rats were allowed to swim until 90s per trial whereas, the mean duration of the diabetic group treated with Tri-E® was significantly less with a comparison to control. Therefore, cognitive impairment showed by diabetic rat was improved with the Tri-E® supplementation. The finding in this study agreed to the previous studies [15,18,29,37,38]. However, supplementation with ATF showed no significance difference with the diabetic group.

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