The Effects of Palm Vitamin E Supplementation on Glycemic Control and Lipid Profile in Streptozotocin-Induced Diabetic Rats

SITI BALKIS BUDIN, ABDUL GAPOR MOHD TOP, WAN NAZAIMOON WAN MOHAMUD, MOKHTAR ABU BAKAR, KHAIRUL OSMAN, MONICA YAU SWEE ENG & JAMALUDIN MOHAMED

ABSTRAK

Dalam kajian ini, kesan pemberian palm vitamin E (PV), terhadap pengawalan status glisemia dan profil lipid pada tikus Sprague Dawley aruhan diabetes telah dikaji. Diabetes diaruh menggunakan streptozotocin secara intravena pada dos 50 mg/kg berat badan. Tikus diabetes dibahagikan kepada dua kumpulan iaitu yang disuplementasi PV (200 mg/kg berat badan/hari) setiap hari dan tanpa suplementasi PV (No PV) Kumpulan bukan diabetes (NDM) pula bertindak sebagai kumpulan kawalan. Selepas suplementasi setiap hari selama lapan minggu, pemberian PV telah berjaya menurunkan aras glukos darah puasa (FBG) dan hemoglobin terglikasi (HbA1c) secara signifikan ($p<0.01$) berbanding kumpulan No PV. Walau bagaimanapun, aras-aras ini masih lebih tinggi ($p<0.001$) berbanding kumpulan kawalan. Kumpulan suplementasi PV juga telah menunjukkan penurunan aras secara signifikan kolesterol total (TC) dan aras lipoprotein berdensiti rendah (LDL-C) ($p<0.001$) serta peningkatan aras lipoprotein berdensiti tinggi (HDL-C) ($p<0.001$) secara signifikan berbanding kumpulan No PV. Sebagai kesimpulannya, kajian ini mendapati bahawa PV berupaya mengawal status glisemia dan memperbaiki keadaan dislipidemia pada tikus diabetes aruhan streptozotocin serta berpotensi mengurangkan komplikasi kardiovaskular akibat diabetes melitus.

Kata kunci: Palm vitamin E, diabetes mellitus, hiperglisemia,dislipidemia, komplikasi kardiovaskular

ABSTRACT

In this study, the effects of palm vitamin E (PV) supplementation on glycemic control and lipid profile in diabetic-induce Sprague-Dawley rats have been evaluated. Diabetes in the rats was induced by a single intravenous streptozotocin (50 mg/kg body weight). The diabetic rats were divided into two groups; supplemented with 200 mg/kg body weight/day of PV and non-supplemented with PV (No PV group). Non-diabetic rats (NDM) formed the control group and only received saline injection. After eight weeks of daily supplementation, PV significantly lowered the fasting blood glucose (FBG) and glycosylated haemoglobin (HbA1c) levels ($p<0.01$) as compared with No PV group. However, these levels were still significantly higher than the control group ($p<0.001$). PV

supplementation group also showed significantly lower levels of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) ($p<0.001$) and higher level of high-density lipoprotein cholesterol (HDL-C) ($p<0.001$) as compared with No PV group. In conclusion, this study suggested that PV may be effective in controlling glycemic status and improving dyslipidemia in streptozotocin-induced diabetic rats and has the potential in reducing cardiovascular complication due to diabetes mellitus.

Key words: Palm vitamin E, diabetes mellitus, hyperglycemia, dyslipidemia, cardiovascular complication

INTRODUCTION

Diabetes mellitus has been recognized as a main independent risk factor for cardiovascular diseases (Klein 1995). There are 100 million people worldwide with diabetes (5% to 8% of overall population), and this number is likely to increase significantly in the near future. Data from clinical studies indicate that most diabetic patients die due to cardiovascular diseases, and atherosclerosis accounts for about 8 to 10% of all diabetic deaths (Gu et al. 1998).

Diabetes mellitus is a complex, progressive disease, which is accompanied by multiple complications. Hyperglycemia has been accepted as being essential for the development of diabetic complications. Recently, there has been interest in the hypothesis that oxidative stress may contribute to the development of complications in diabetes mellitus (Bynes 1991). The oxidative stress is significantly increased in diabetes due to prolonged exposure to hyperglycemia. Chronic hyperglycemia increases the generation of free radicals through non-enzymatic glycosylation, glucose autoxidation, polyol pathways and reduces the capacities of antioxidant defense system (Giuglioano et al. 1996).

The higher incidence of cardiovascular complication is poorly understood. Result of Diabetes Control and Complication Trial (DCCT) (1993) had established that prolonged exposure to hyperglycemia is considered as the primary factor associated with the development of diabetic macrovascular complication in type 1 diabetic patients. The DCCT (1993) and The UK Prospective Diabetes Study (Turner et al. 1998) showed that the improvement of glycemic control, as measured by reduction in HbA1c levels, significantly reduced the risk of development and/or progression of all diabetic complications. DCCT (1993) had also concluded that improving glycemic control would reduce the mortality and morbidity due to cardiovascular diseases in type 1 diabetes mellitus patients.

Dyslipidemia is a primary risk factor for the development of atherosclerosis in the general population and diabetes mellitus (Laakso 1995). Epidemiological surveys have shown that total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels are useful in the assessment of cardiovascular risk in diabetic patients. Dyslipidemia is strongly associated with the glycemic control in which, TC, LDL-C and HDL-C levels are usually normal if glycemic control is adequate (Laakso 1995). Therefore, it is imperative to correct the glycemic

status and dyslipidemia in diabetes mellitus in order to reduce the morbidity and mortality rate due to cardiovascular complication.

Palm vitamin E (PV) is a rich source of vitamin E (having mainly tocotrienol and small amount of tocopherol) and an excellent antioxidant. PV has been used as a nutritional supplementation and also has several therapeutic potential benefits (Theriault et al. 1999). Tocotrienol is a fat-soluble vitamin related to the family of tocopherol. As reflected in their structural similarity, tocopherol and tocotrienol are well recognized for their antioxidant effect (Kamal-Eldin & Appelqvist 1996). In general, antioxidants are suggested to reduced cardiovascular disease by arresting free radical damage and the usage of vitamin E as antioxidant in diabetes mellitus has been extensively studied (Paolisso et al. 1993).

Many studies have been carried out to observe the effect of tocopherol on cholesterol metabolism but the effect of PV on cholesterol metabolism has received much less attention. There are contradictory reports regarding the effects of PV on serum cholesterol levels in man. Qureshi et al. (1991) and Tan et al. (1991) had reported that PV reduced serum TC and LDL-C levels in men whereas Wahlqvist et al. (1992) observed no positive reduction in serum TC and LDL-C levels in men after PV administration. Therefore, it is necessary to study the effect of PV on cholesterol metabolism in order to ascertain whether PV has any effect at all. In this study the effects of PV on glycemic and dyslipidemia were assessed in streptozotocin -induced diabetic rats, in order to obtain more information about the potential of PV in reducing the development of diabetic cardiovascular complications.

MATERIALS AND METHODS

Male Sprague-Dawley rats, (each weighing 260-290 g) were supplied by Animal House of Universiti Kebangsaan Malaysia which located at the Institute of Medical Research. The rats were housed in plastic containers with floors covered with wood shavings. Two rats were placed in each cage. The rats were given a standard laboratory diet and water *ad libitum* for eight weeks. PV was supplied by Malaysian Palm Oil Board of Malaysia (MPOB). Diabetes in the rats was induced by a single intravenous streptozotocin (STZ) injection (Sigma, St Louis, MO, USA), into tail vein at a dose of 50 mg/kg body weight after overnight fasting. Another group of rats, received saline injection only and formed the non-diabetic) group (NDM)

At day three, diabetic rats with fasting blood glucose levels more than 15 mmol/l were selected for the study and divided into two groups: diabetic rats with PV and without PV supplementation (No PV). PV was administered orally at a dose of 200 mg/kg/day, for eight weeks. The remaining groups were left untreated. Food and water intake as well as body weight were recorded weekly.

After eight weeks of supplementation, rats were fasted overnight and blood was withdrawn by cardiac puncture under deep anesthesia with diethyl ether. The blood was collected into EDTA tubes and sodium fluoride tube for fasting blood glucose (FBG) analysis. The blood sample was centrifuged at 3000 rpm for 7 min at 4°C. Fasting blood glucose (FBG) was measured by glucose-oxidize kits (Trace Scientific, Melbourne, Australia. Catalogue no TR 15104) and glycated hemoglobin (HbA1c) was determined by

using a method by Eross et al. (1984) and expressed as a % of total hemoglobin. Determination of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) was carried using kits (Teco Diagnostics, 1286N. Lakeview Ave. Anaheim) and low-density lipoprotein cholesterol (LDL-C) determination was calculated using method by Friedewald et al. (1972).

STATISTICAL ANALYSIS

All results were expressed as mean ± SEM. The data were analyzed by one-way analysis of variance (ANOVA), followed by post hoc LSD multiple comparison test to estimate the significance of different between groups. The difference between groups was considered significant when $p< 0.05$.

RESULTS

The food and water intake of the No PV diabetic group was markedly increased when compared with the control group (NDM) (Fig 1 and 2). Food and water intake, although expressed per rat, were calculated per cage, so that statistical analysis was not legitimate. Figure 1 and 2 show that PV supplementation had considerable effect on water and food consumption throughout the study. Both No PV and PV groups exhibited loss of body weight when compared with NDM group at $p<0.001$. However PV supplementation significantly improved the changes on body weight reduction ($P<0.01$) compared with No PV group (Fig. 3)

Fig. 4 and 5 show FBG and HbA1c levels, respectively. All diabetic rats showed hyperglycemia throughout the study period. PV supplementation induced a significant fall in FBG and HbA1c levels ($p<0.01$) in the diabetic rats as compared to No PV diabetic group. Levels of plasma TC, LDL-C and HDL-C are given in Table 1. Plasma TC and LDL-C concentrations were markedly increased in non-supplemented diabetic rats ($p<0.001$) as compared to control. PV supplementation completely prevented the increase of TC and LDL-C and levels were significantly lower compared with the No PV rats ($P<0.001$). On the other hand the PV supplementation group also has significantly higher levels of HDL-C as compared with No PV group ($p<0.001$).

DISCUSSION

Administration of STZ to rats caused destruction of the β cell of pancreas and lead to reduction of insulin secretion, leading to increase in plasma glucose levels. The diabetic rats also exhibited increment in food intake due to the failure of the cells to obtain glucose as a source of energy. This may further caused the increment in blood glucose level. Consequently, the diabetic rats would get their source of energy from the catabolism of protein and fat, which lead to the reduction in body weight. Hyperglycaemia also leads to polyuria and polydipsia. In this study, all the diabetic rats showed higher levels of glycemic status, food and fluid intake as well as decrease in body weight, which also been reported by Kocak et al. (2000).

After eight weeks of PV supplementation, the glycemic status in streptozotocin-induced diabetic rats was improved by reducing the levels of FBG and HbA1c. Improving the glycemic status could be the reason for the gain in body weight and decrease in food and fluid intake in the PV supplementation rats.

Vitamin E supplementation has been shown to decrease HbA1c and lipid peroxidation in type 1 diabetic patients (Jakus et al. 2000). The study had also suggested that the improvement of glycemic status could be due to the improvement of glucose metabolism. Luostarinen et al. (1995) had reported that supplementation of vitamin E for four weeks was capable to prevent hyperglycemia condition following fish oil intake in healthy volunteers. They had also discovered that vitamin E supplementation could increase the production of insulin and increase insulin: glucose ratio. They had postulated that the antioxidant effects of vitamin E could have the protective effect to β cell destruction, which occurred due to the lipid peroxidation process following the fish oil intake.

Vitamin E supplementation before the induction of diabetes by STZ could prevent the destruction of ß cell and also improved the glycemic status in diabetes rats (Ihara et al. 2000). The supplementation of PV in our study were started at day 3 of STZ induction. Eventhough the destruction of ß cells had occured and caused chronic hyperglycemia, further destruction might be prevented by PV probably through its antioxidant mechanism. In this study the mechanism of action of PV to improve glycemic status could be due to the stimulation of glucose metabolism and also through the antioxidant effects, which protected the pancreas from oxidative damage.

Recent study has also shown that PV has the potential in improving the oxidative stress in diabetic rats (Musalmah et al. 2001). The study also showed that PV could enhance the process of wound healing in diabetic animals, which most probably also through the reduction in oxidative stress levels.

The increase in the glycemic status could probably produce a rise in the values of lipid parameters as shown in the non-supplemented diabetic group. The increase in TC and LDL-C levels and decrease in HDL-C confirm the dyslipidemia pattern in type 1 diabetes mellitus and in agreement with the previous study by Hayashibe et al. (1999). In an other study, HbA1c level was noted as showing positive correlation with TC, LDL-C, triglycerides and VLDL-C in diabetes mellitus patients (Ohta et al. 1998). Thus the data obtained from this study support the relationship of poor glycemic control and higher risk of cardiovascular complications due to rise in plasma concentrations of TC, LDL-C and low concentration of HDL-C. Therefore, it shows that as the metabolic control of the illness worsens, the atherogenic risk increases significantly.

In this study, non-supplemented diabetic rats showed dyslipidemia, however, PV supplementation managed to bring down the levels of TC, LDL-C and increased the level of HDL-C. Therefore by improving the lipid metabolism, PV was able to prevent the pathogenesis of cardiovascular complication in diabetes mellitus.

The data obtained in our study showed PV managed to bring down the atherogenic risk due to the significant low levels of TC, LDL-C and significant rise in HDL-C in plasma concentration compared to the non-supplemented diabetic group. As our results also demonstrate significantly lower levels of FBG and HbA1c in PV supplemented group compared to diabetic group, the improvement of diabetic states could probably cause the improvement in the value of lipid parameters.

In conclusion, our study showed that PV supplementation could improve the glycaemic status and dyslipidemia in streptozotocin induced-diabetic rats and could be used in preventing the pathogenesis of atherosclerosis and cardiovascular complications in diabetes mellitus.

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Siti Balkis binti Budin
Khairul bin Osman
Monica Yau Swee Eng
Jamaludin bin Mohamed
Jabatan Sains Bioperubatan,
Fakulti Sains Kesihatan Bersekutu,
Universiti Kebangsaan Malaysia,
50300
Kuala Lumpur,

Abdul Gapor bin Mohd Top,
Unit Produk Agro,
Lembaga Minyak Sawit Malaysia (MPOB)
No 6 Persiaran Institusi,
Bandar Baru Bangi,
43000,
Kajang.

Wan Nazaimoon binti Wan Mohamud.
Unit Diabetes dan Endokrin,
Pusat Penyelidikan Kardiovaskular, Diabetes dan Pemakanan
Institut Penyelidikan Perubatan
50588, Jalan Pahang,
Kuala Lumpur.

Mokhtar bin Abu Bakar
Jabatan Patologi,
Universiti Kebangsaan Malaysia,
Jalan Yaacob Latif,
Bandar Tun Razak,
56000 Cheras,
Kuala Lumpur

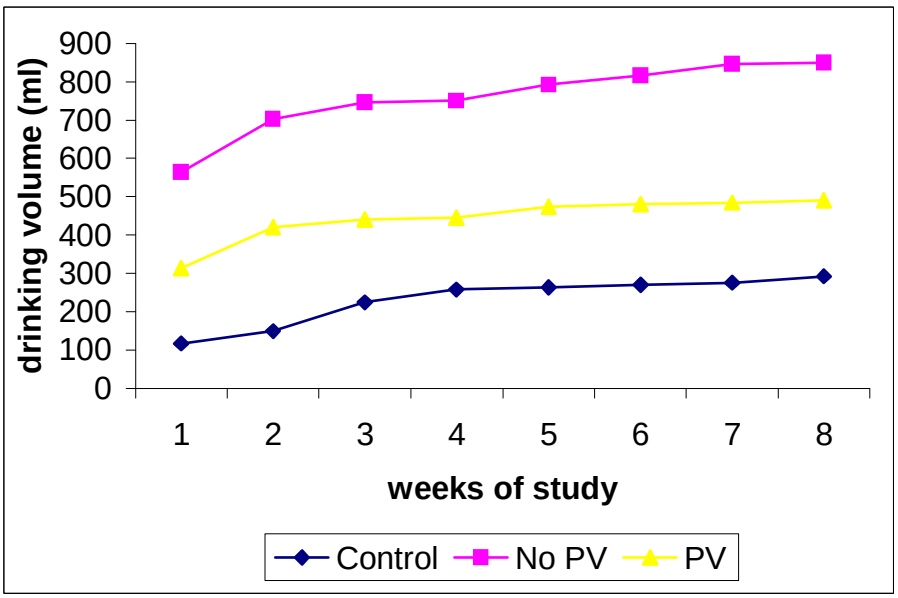

FIGURE 1. Weekly water intake in control (n=7), No PV (n=8) and PV (n=8) groups.

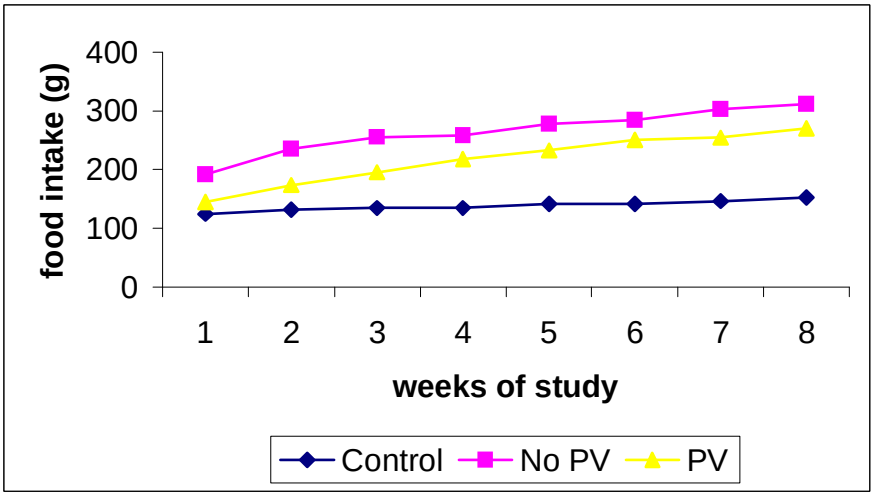

FIGURE 2. Weekly food intake in control (n=7), No PV (n=8) and PV (n=8) groups.

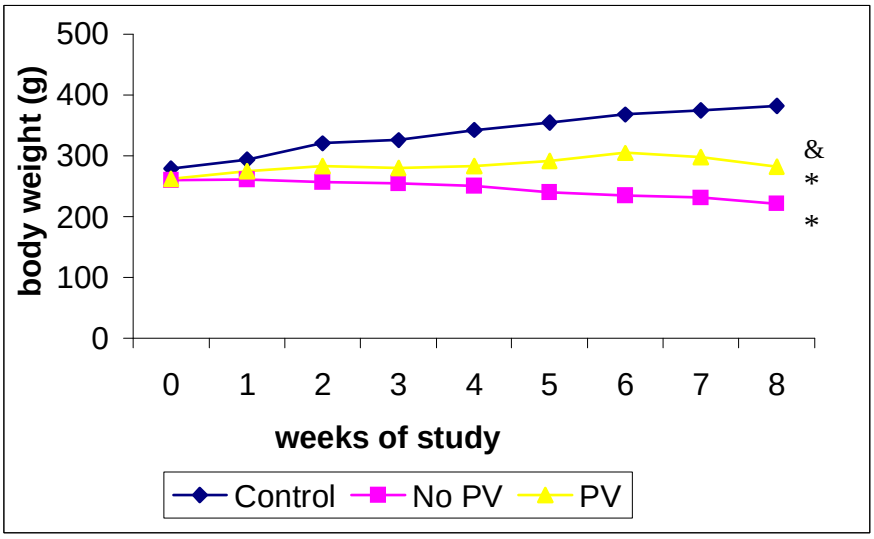

FIGURE 3. Weekly body weight changes in control (n=7), No PV (n=8) and PV (n=8) groups. * P<0.001 vs control; & P<0.01 vs No PV group.

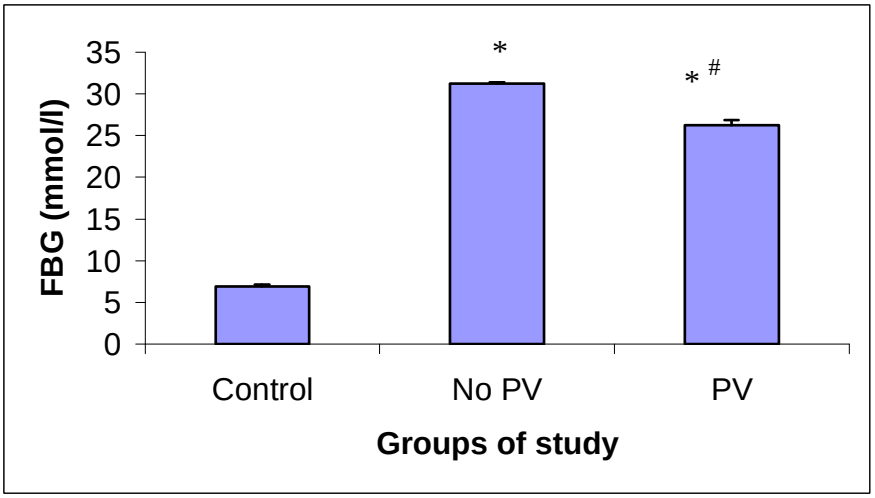

FIGURE 4. FBG levels in the control (n=7), No PV (n=8) and PV (n=8) groups. * P<0.001 vs control group; # P<0.01 vs No PV group.

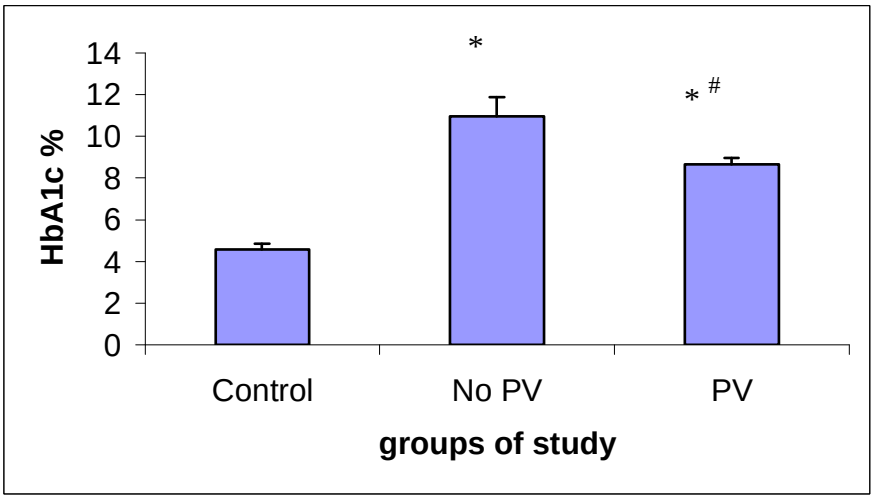

FIGURE 5. Percentage of HbA1c in the control (n=7), No PV (n=8) and PV (n=8) groups. *p<0.001 vs control group; # p<0.01 vs No PV group.

TABLE 1. Levels of TC, LDL-C and HDL-C in control (n =7), No PV (n = 8), and PV (n = 8) after eight weeks of study.

Lipid Profile (mg/dl)	Control	No PV	PV
TC	45.59 ± 2.44	72.11 ± 3.50 ∗	53.38 ± 3.28 &
LDL-C	18.93 ± 1.01	38.94 ± 3.87 ∗	14.53 ± 1.18 $ &
HDL-C	20.84 ± 1.59	4.16 ± 0.58 ∗	17.67 ± 1.27 $ &

Values are means ± SEM. ∗ P<0.001 and $ p<0.05 vs control group; & p<0.001 vs No PV group.