

Polyphenol rich oil palm leaves extract reduce hyperglycaemia and lipid oxidation in STZ-rats

¹Rosalina Tan, R. T., ^{1,3,*}Mohamed, S., ¹Samaneh, G. F., ²Noordin, M. M., ²Goh, Y. M. and ¹Manap, M.Y.A.

¹Faculty of Food Science and Technology, ²Faculty of Veterinary Medicine
Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

³Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor,
Malaysia

Abstract: Diabetes mellitus is a degenerative disease affecting about 25% of people over 40 years and is a risk factor for cardiovascular disease. The oil palm (*Elaeis guineensis*) leaf is a major waste of the palm oil industry, which is among the top vegetable oil industry in the world. This study aims to evaluate the potential anti-diabetic effects of Oil palm leaves (OPL) ethanolic extract on normal and streptozotocin (STZ)-induced hyperglycaemic rats. OPL were administered orally at 50, 100 and 200mg per kg body weight/day) to Sprague Dawley rats and monitored for its glycaemic, lipidemic and antioxidant modulating effects. The Oil palm leaves (OPL) ethanolic extract treatment dose-dependently reduced blood glucose and oxidation in the STZ rats, and restored antioxidants enzymes levels. The optimum dose was 100mg/kg, which effectively reduced liver and kidney damage to the level of normal rats. This is the first study on dietary OPL ethanolic extract ability to modulate physiological responses and show organ protective effects against tissue damage in STZ-induced chronic hyperglycaemic rats. At the doses used OPL showed no adverse or chronic toxicity effects in these rats, indicating its potential use as a new functional food ingredient.

Keywords: *Elaeis guineensis*, streptozotocin, blood glucose, antidiabetic, hypoglycemic

Introduction

Plants represent a good source of potentially useful dietary supplements for improving blood glucose control and preventing long-term complications in diabetics (Gallagher et al., 2003). The main characteristics of diabetes are hyperglycaemia, polyuria, polydipsia and polyphagia, weight loss, muscle weakness and dyslipidemia. Chronic hyperglycaemia is normally accompanied by increased risk to hypertension, oxidative stress, decreased fibrinolytic activity, increased platelet aggregation, and severe atherosclerosis (Reusch, 2003). Many synthetic drugs have been developed for the treatment of hyperglycaemia but there is much interest in discovering cheaper, natural treatments without side effects that can reduce the risk of diabetes.

The World Health Organization estimated that in 2010 diabetes will affect about one quarter billion people worldwide, mostly as non-insulin dependent diabetes mellitus (NIDDM). The number of diabetic patients has increased due to the increasing sedentary life style (Enoki et al., 2007). Treatment of diabetes by insulin and oral hypoglycemic drugs fails to prevent these complications in many patients, warranting

additional research for effective alternative treatments. Recently, there are increasing interest for the development and utilization of antidiabetic plants, owing to their renewable resource and possible beneficial effects besides other economical reasons (Zhao et al., 2007). Knekt et al. (2002), proved in a clinical study that a reduction in risk of type 2 diabetes relate to higher quercetin, and myricetin intakes.

Oil palm (*Elaeis guineensis*) is grown primarily in the tropics and represents the second-largest contributor to human vegetable oil consumption after soybean (Mielke, 1996). The oil palm leaf is a major waste of this oil palm industry. The Oil Palm leaves alcoholic extract (OPL) contains 24.3 mg gallic acid equivalent (GAE)/g dry weight of non toxic, phenolic compounds (mainly common glycosylated flavonoids, catechins and carotenoids) which is higher than green tea (22.5 mg GAE/g dry weight) (Runnie et al., 2003). The OPL effectively inhibited low density lipoprotein (LDL) oxidation better than other edible plant extracts (Salleh et al., 2002). There are currently very little documentations on oil palm leaf (OPL) therapeutic properties. The discovery of the good ex-vivo antioxidant properties of OPL forms the basis of this study, which is to evaluate its potential beneficial effects for chronic

*Corresponding author.

Email: mohamed.suhaila@gmail.com

Tel: +60389468391, Fax: +60389423552

hyperglycaemia (diabetics) where oxidative stress plays a major detrimental role.

Materials and Methods

Extraction

All analytical grades chemicals and drugs were obtained from Sigma-Aldrich (St. Louis, MO). Ketamine and xylazine were from the Veterinary Hospital, Faculty of Veterinary Medicine, UPM. Pyrogallol and dithio-bis(2-nitrobenzoic acid) (DTNB) were from Merck (Malaysia).

Oil palm (*Elaeis guineensis*) leaves obtained from the Universiti Putra Malaysia (UPM) plantation, were chopped and freeze-dried for 24-48hr. The dried leaves was ground to a powder, and extracted with absolute ethanol at a 1:10 (w/v) solvent ratio under continuous agitation for 24hr in the dark. After filtration the residue was re- extracted twice. The pooled extract was vacuum- dried in a rotary evaporator, at 40°C, until the solvent was completely removed to yield a dark green waxy material. The extract was stored in glass vessels, flushed with nitrogen at -20°C.

Animal study

All experimental procedures and animal care had been approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia, and care were taken to minimize pain or discomfort. Male Sprague-Dawley rats weighing 220-300g were housed in clean cages and kept in well ventilated room with a 12 h light/ dark cycle at 22-25°C. The rats were maintained on a standard rat pellets (Gold Coin Sdn. Bhd., Klang, Malaysia) and water provided *ad libitum*. Water was used as the vehicle for dissolving and dispensing OPL (50-200mg OPL /kg/day by oral gavage). The vehicle was given to all rats similar to rats receiving OPL.

Preparation of diabetic rats

Hyperglycaemia was induced in rats by a single intraperitoneal (i.p.) injection of 60mg streptozotocin /kg body weight (bw). Three days after injection, the rats with fasting blood glucose higher than 7.8mmol/L were considered hyperglycaemic/diabetic. The rats were divided into seven groups (50 diabetic and 20 normal rats); (i) NC: Normal control rats (ii) N100-OPL: Normal rats receiving 100mg/kg OPL (iii) DC: Diabetic control rats (iv) D50-OPL: Diabetic rats receiving 50mg OPL (v) D100-OPL: Diabetic rats receiving 100mg OPL (vi) D200-OPL: Diabetic rats receiving 200mg OPL (vii) GB: Diabetic rats receiving glibenclamide (GB) orally (30mg/kg b.w.) in aqueous solution. Feed and water were removed

from cages 12 h before blood were collected from the rats' tails for blood glucose (glucometer Accu-chek sensor, Roche, USA) on the 1st day and every 3 days. Changes in body weights were recorded in the fasting state. The animals were sacrificed after blood collection following euthanasia on the 35th day of the experiment.

Plasma and Red Blood Cells Preparation

Two ml of blood was drawn via cardiac puncture from each rat under ketamine/xylazine (ketamine – 50 mg/kg, xylazine 5 mg/kg) combination anaesthesia. The blood were collected in heparinised tubes and kept on ice before being centrifuged at 3000xg for 10 minutes to separate the red blood cells (RBC) from the plasma. The RBC was washed and centrifuged three times with 0.9% NaCl at 3000xg for 10 minutes, respectively. The RBC and plasma was kept at -80°C for further use.

Biochemical and histopathological analysis

Total protein, triglyceride (TG), aspartate amino transferase (AST), alanine amino transferase (ALT) levels in plasma were determined on Hitachi 902 automated analyzer. The thiobarbituric acid reactive substances (TBARS) concentration, SOD and CAT were measured according to established methods (Zhang et al. 1997; Aebi, 1974; Marklund and Marklund, 1974 respectively).

For histopathological analysis, liver and kidney tissue sections (5 µm) were deparaffinised and processed for routine hematoxylin-eosin (H&E) staining. Ten randomly selected fields of each section were observed with a light microscope under 100 x magnifications. The detailed quantitative histological observations were made on battlement (zigzag) manner on the least 20 fields per slide and the percentage of necrotic and inflamed cells of liver, abnormal hepatocytes or glomerulus and tubules were calculated.

Data analysis

The results were expressed as means ± SEM. The statistical analysis was carried out using one-way analysis (ANOVA) followed by Tukey test. All procedures were performed at 95% confidence level.

Results and Discussions

Blood glucose levels

The STZ increased the fasting blood glucose from 5 mmol/L to more than 12 mmol/L within 3 days, and to about 22 mmol/L after 1 week. Administration of OPL significantly ($p < 0.05$) attenuated this blood sugar increase dose dependently in STZ rats

(Figure 1). The OPL showed no hypoglycaemic or other apparent toxic effects in normoglycaemic rats. The blood sugar in all normoglycaemic rats were unchanged throughout the study, while all untreated STZ rats showed an increasing trend.

Body weight changes

The untreated hyperglycaemic rats (DC) showed significant ($p < 0.05$) weight loss by about 40% when compared to normal rats by the 35th day (Fig 2.). Glibenclamide treatment to STZ rats did not significantly reduce this weight loss. However, OPL significantly ($p < 0.05$) prevented the weight loss in STZ rats, dose dependently, and caused significant weight gains in normal rats. Based on the body weights, the apparent optimum dose is 100mg/kg body weight.

During the course of the experiment, 20 rats died; 6 from untreated STZ group, 4 from the D50-OPL group; 3 from the GB group; and 1 each from the D100-OPL and D200 OPL groups. The OPL treatment not only reduced weight loss but significantly reduced the mortality rate where at dose 100 and 200mg/kg showed very significant lower mortality compared to the untreated STZ group. At 100 and 200mg/kg bw, the mortality was improved by 71% compared untreated STZ group (DC).

Oxidative stress status

The oxidative stress status, measured as TBARS were significantly lower in the plasma of OPL treated rats compared to the untreated DC rats. DC rats had 250% more TBARS compared to NC and both the OPL and GB treatment normalised the TBARS levels (Table 1). TBARS level were highest in DC rats and lowest ($p < 0.05$) in the D100-OPL group, pointing

to the OPL in-vivo antioxidative effects. Chronic hyperglycaemia caused the endogenous antioxidant enzymes activities (erythrocyte SOD and catalase) to decrease. The OPL treatment dose- independence restored the antioxidant enzymes activities to near normal levels in STZ rats ($p < 0.05$) (Table 1).

Total protein and creatinine levels were significantly low in DC rats. STZ rats treated with OPL and GB significantly ($p < 0.05$) restored the serum total protein and creatinine to normal levels (Table 2). AST and ALT were significantly high in DC rats and both OPL and GB treatments significantly re-establish them to normal levels. Revival of serum total protein, AST, ALT and creatinine of diabetic rats towards normal levels indicated that the OPL produced no adverse effect on the liver and kidney functions. A significant decrease in serum triglycerides (TG) was observed in DC rats, while OPL treatments restored them to normal levels. Table 3 shows the effect of OPL on the liver and kidneys of normal and diabetic rats. In the liver, the changes caused after induction of diabetes are granular cytoplasm, dilated sinusoids, shrunken nuclei and inflammation, which was reduced after dietary supplementation with OPL. Table 3 also shows the protective effects of *Elaeis guineensis* extract on diabetic rats liver and kidney through histopathological enumeration of the cells. The DC livers and kidneys showed major injury. All rats in the diabetic groups exhibited similar pathological changes in the kidney. Lesions of the diabetic kidney in DC groups involved the glomeruli including capillary basement membrane thickening with expansion of the mesangium showed in Figure 3. These changes lead to initial hyperfiltration eventually leading to renal insufficiency or complete kidney failure. The percentage of abnormal cells in OPL treated STZ-

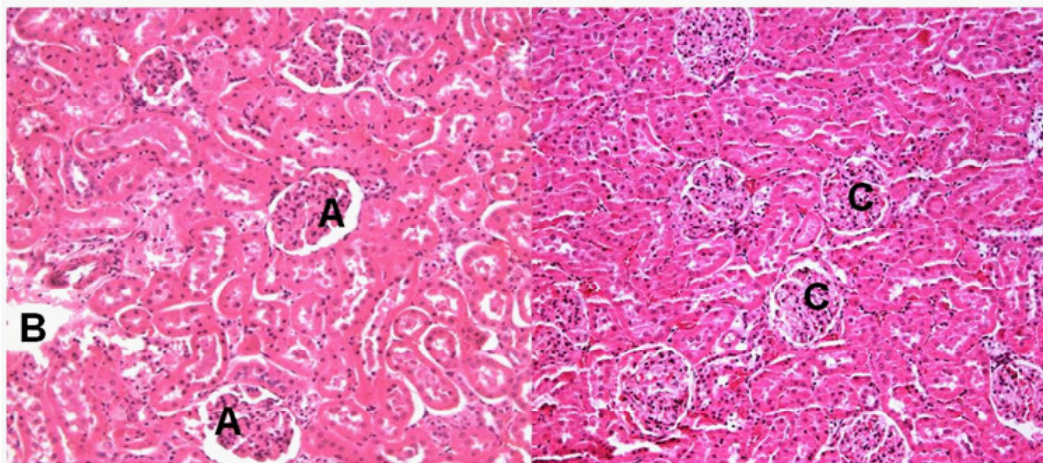


Figure 3. Photomicrograph of rat's kidney from DC group at necropsy. An expansion of mesangium (A), loss of glomeruli (B) were observed as compared to kidney in OPL treated group (C) (H&E, X 200)

Table 1. The antioxidant markers of different groups of rats fed with *Elaies guineensis* leaf ethanolic extract.

TREATMENT	Catalase (k/s/mg protein)	SOD(U/ g protein)	TBARS(μ M/ g protein)
NC	0.006 \pm 0.001 ^b	1.971 \pm 0.173 ^{bc}	0.159 \pm 0.034 ^b
N100OPL	0.006 \pm 0.001 ^b	3.580 \pm 1.300 ^c	0.224 \pm 0.038 ^b
DC	0.002 \pm 0.001^a	0.211 \pm 0.056^a	0.405 \pm 0.168^c
GB	0.006 \pm 0.001 ^b	2.021 \pm 0.537 ^c	0.156 \pm 0.020 ^b
50mg OPL/kg	0.004 \pm 0.000^a	1.085 \pm 0.247 ^b	0.181 \pm 0.060 ^b
100mg OPL/kg	0.006 \pm 0.000 ^b	1.764 \pm 0.268 ^c	0.070 \pm 0.004 ^a
200mg OPL/kg	0.005 \pm 0.002 ^b	2.449 \pm 0.456 ^c	0.185 \pm 0.049 ^b

SOD: Superoxide Dismutase; TBARS: Thiobarbituric acid Reactive substances

(i) NC: Normal control rats (ii) N100-OPL: Normal rats receiving 100mg/kg OPL (iii) DC: Diabetic control rats (iv) D50-OPL: Diabetic rats receiving 50mg OPL (v) D100-OPL: Diabetic rats receiving 100mg OPL (vi) D200-OPL: Diabetic rats receiving 200mg OPL (vii) GB: Diabetic rats were receiving glibenclamide (GB) orally (30mg/kg b.w.) in aqueous solution. Bar represent means \pm SEM of ten rats for each group (n=10).

^{a-c}, Mean with different letters within each row are significantly different (P<0.05).

Table 2. The effect of *Elaies guineensis* leaf ethanolic extract on liver and kidney markers

TREATMENT	TG (mg/dl)	TOTAL PROTEIN (g/dl)	AST (U/L)	ALT (U/L)	Creatinine (μ mol/L)
NC	1.55 \pm 0.09 ^b	75.38 \pm 0.59 ^b	211.1 \pm 8.5 ^b	53.56 \pm 3.75 ^b	67.38 \pm 2.05 ^b
N100OPL	1.71 \pm 0.08 ^b	75.43 \pm 0.69 ^b	220.4 \pm 16.8 ^b	52.01 \pm 2.63 ^b	60.71 \pm 2.26 ^b
DC	0.59 \pm 0.17 ^a	62.50 \pm 5.94^a	439.1 \pm 27.2^a	129.8 \pm 15.1^a	57.33 \pm 5.33^a
GB	0.91 \pm 0.17 ^a	73.00 \pm 0.58 ^b	178.0 \pm 7.71 ^c	89.7 \pm 12.1 ^b	52.60 \pm 2.29^a
50mg/kg	2.02 \pm 0.23 ^b	77.50 \pm 0.76 ^b	228.4 \pm 13.4 ^b	78.34 \pm 9.37 ^b	64.50 \pm 1.70 ^b
100mg/kg	1.65 \pm 0.49 ^b	74.56 \pm 1.49 ^b	225.0 \pm 13.3 ^b	79.60 \pm 7.69 ^b	71.11 \pm 4.48 ^b
200mg/kg	2.34 \pm 0.28 ^b	71.88 \pm 0.64 ^b	261.6 \pm 5.9 ^b	64.85 \pm 1.27 ^b	63.13 \pm 2.02 ^b

TG: TRIGLYCERIDES, AST: aspartate amino transferase, ALT: alanine amino transferase

(i) NC: Normal control rats (ii) N100-OPL: Normal rats receiving 100mg/kg OPL (iii) DC: Diabetic control rats (iv) D50-OPL: Diabetic rats receiving 50mg OPL (v) D100-OPL: Diabetic rats receiving 100mg OPL (vi) D200-OPL: Diabetic rats receiving 200mg OPL (vii) GB: Diabetic rats were receiving glibenclamide (GB) orally (30mg/kg b.w.) in aqueous solution. Data are expressed as means \pm SEM (n= 10). p< 0.05, as compared to control diabetic.

^{a-c}, Mean with different letters within each row are significantly different (P<0.05).

Table 3. The protective effects of *Elaies guineensis* leaf extract on liver and kidney histopathology of diabetic rats

Treatment	Kidney damage (%)	Liver necrotic & inflamed cells (%)
OPL 50mg/kg	31.7 \pm 3.9 ^a	24.40 \pm 2.76 ^a
OPL 100mg/kg	25.4 \pm 3.1 ^a	25.20 \pm 1.94 ^a
OPL 200mg/kg	40.1 \pm 5.6 ^a	34.73 \pm 1.50 ^a
NC	30.0 \pm 4.8 ^a	54.00 \pm 2.08 ^b
DC	80.2 \pm 5.5 ^c	88.00 \pm 1.53 ^c

(i) NC: Normal control rats (ii) N100-OPL: Normal rats receiving 100mg/kg OPL (iii) DC: Diabetic control rats (iv) D50-OPL: Diabetic rats receiving 50mg OPL (v) D100-OPL: Diabetic rats receiving 100mg OPL (vi) D200-OPL: Diabetic rats receiving 200mg OPL (vii) GB: Diabetic rats were receiving glibenclamide (GB) orally (30mg/kg b.w.) in aqueous solution. Data are expressed as means \pm SEM (n= 10). p< 0.05, as compared to control diabetic.

^{a-c}, Mean with different letters within each row are significantly different (P<0.05).

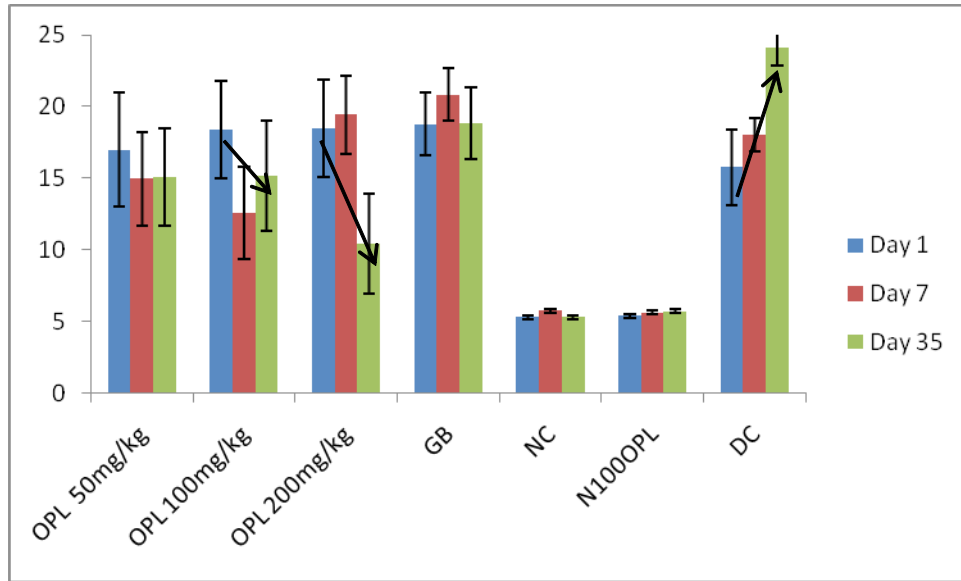


Figure 1. Effects of *Elaias guineensis* leaf ethanolic extract on the Blood glucose levels of different groups of rats at different time intervals.

[(i) NC: Normal control rats (ii) N100OPL: Normal rats receiving 100mg/kg OPL (iii) DC: Diabetic control rats (iv) OPL50mg/kg: Diabetic rats receiving 50mg OPL (v) OPL100mg/kg: Diabetic rats receiving 100mg OPL (vi) OPL 200mg/kg: Diabetic rats receiving 200mg OPL (vii) GB: Diabetic rats were receiving glibenclamide (GB) orally (30mg/kg b.w.) in aqueous solution]

Bar represent means ± SEM of ten rats for each group (n=10). p < 0.05 as compared to diabetic control.

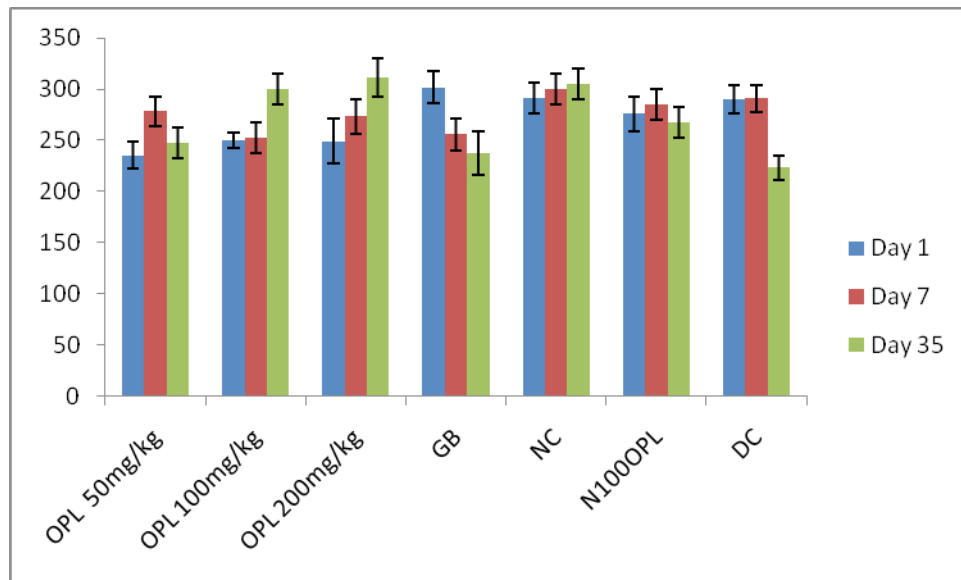


Figure 2. Effect of *Elaias guineensis* left ethanolic extract on body weight (g) changes in rats.

[(i) NC: Normal control rats (ii) N100OPL: Normal rats receiving 100mg/kg OPL (iii) DC: Diabetic control rats (iv) OPL50mg/kg: Diabetic rats receiving 50mg OPL (v) OPL100mg/kg: Diabetic rats receiving 100mg OPL (vi) OPL 200mg/kg: Diabetic rats receiving 200mg OPL (vii) GB: Diabetic rats were receiving glibenclamide (GB) orally (30mg/kg b.w.) in aqueous solution]

Bar represent means ± SEM of ten rats for each group (n=10). p < 0.05 as compared to diabetic control.

rat livers and kidneys were insignificantly different to NC liver and kidney levels. Few damages in the form of swelling of hepatocytes and vacuolation that were observed in OPL treated rats. In normal rats no significant difference was found between NC rat and OPL treated rat for hepatocyte and nephron abnormalities.

Streptozotocin-induced hyperglycaemia is a well-documented model of experimental diabetes. STZ was known to destroy insulin producing pancreatic cells. Low dose of STZ- diabetic induction rat model would appear to represent a good experimental NIDDM diabetic state and provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia (Like and Rossini, 1976). Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level. Diabetes induced by STZ is associated with the characteristic loss of body weight which is due to muscle wasting (Swanston-Flatt et al., 1990). Body weight and mortality improvements by OPL treatments in the study may be a reflection of the improved health of the diabetic groups.

This is the first report of the good anti-hyperglycaemic effects of OPL in STZ- induced diabetic rats. The OPL may possess diverse biological activities and pharmacological function, but currently there is no documentation of OPL's glycaemic and lipidemic modulating properties. Leaves often contain substantial amounts of antioxidants, flavonoids and polyphenols and the present study suggests that antioxidant action may be an important property of OPL associated with the anti-hyperglycaemic effect. The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control (Gray and Flatt, 1997). In this study, the antioxidant and blood glucose lowering effects of OPL could be due to its flavonoids content. However not all flavonoids have anti-hyperglycaemic activities, depending on their phenolic structure. Some leaf extracts are toxic. A reduction in risk of type 2 diabetes was clinically proven to relate with higher quercetin, and myricetin intakes (Knekt et al., 2002). Some flavonoids are known to help heal free radical- mediated diseases such as diabetes (Czinner et al., 2000). Flavone materials have various biological effects which includes immunomodulating, anti-oxidant, lipidaemic, blood vessel and glycaemic modulating properties (Hollman and Katan, 1999).

Insulin-dependent diabetes mellitus cause chronic microvascular, macrovascular and neurologic complications resulting in morbidity and mortality (sudden death, death from hyperglycaemia

or hypoglycaemia, fatal or non-fatal myocardial infarction, angina, heart failure, stroke, renal failure, peripheral vascular disease, amputation, vitreous haemorrhage, retinopathy, blindness, cataract extraction). Intensive treatments for maintaining blood glucose concentrations close to the normal range could decrease the frequency and severity of complications, such as development of retinopathy, microalbuminuria (high urinary albumin and neuropathy (Shamoon et al., 1993; Turner 1998). The chief adverse event associated with intensive therapy is severe hypoglycaemia. The OPL were shown in this study to not cause hyperglycaemia.

The final products of lipid peroxidation can be evaluated by the TBARS levels (Requena et al., 1997). The balance between both the enzymatic and non-enzymatic antioxidant activities in the intracellular levels are important for the health and survival of organism (Grazioli et al., 1998). These enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), while the non enzymatic are ascorbic acid (vitamin C), α - tocopherol (vitamin E), glutathione (GSH), β - carotene, and vitamin A (Stahl et al., 1998).

The roles of oxidative stress and antioxidants in organs and tissue damage have been reportedly widely in diabetics (West, 2000). The diabetogenic action of STZ can be prevented by the SOD, CAT and other hydroxyl radical scavengers; suggesting that diabetes involves superoxide anion and hydroxyl radicals (Ames et al., 1993). In this study, STZ treatment significantly increased lipid peroxides and decreased antioxidant enzymes activities in the plasma of the rats, confirming that STZ- induced diabetes is accompanied by an increased generation of reactive species (Winiarska et al., 2006). Lipid peroxidation in STZ- induced diabetes was elevated from the reduction in the levels of reduced glutathione, a potent endogenous antioxidant.

The generation of reactive oxygen species (oxidative stress) play important roles in the etiology of diabetic complications. Many biochemical pathways strictly associated with hyperglycemia (namely glucose autoxidation, polyol pathway, prostanoid synthesis, protein glycation), subsequently increase free radicals production. Exposure of endothelial cells to prolonged hyperglycaemia leads to amplified production of superoxide anion, which may extinguish nitric oxide, a potent endothelium-derived vasodilator that participates in the general homeostasis of the vasculature, causing reduced endothelial-dependent relaxation and delayed cell replication (Giugliano et al., 1996). These effects are

reversed by dietary antioxidants such as OPL.

Radical-mediated damage to proteins may be initiated by autoxidation of lipids and sugars. The consequent protein oxidation reactive products include protein hydroperoxides. Oxidized proteins are frequently functionally useless and are more susceptible to proteinases. However, some oxidized proteins have damaging actions as observed in aging, diabetes, atherosclerosis and neurodegenerative diseases. Protein oxidation occasionally plays controlling roles in cellular remodelling and cell growth (Dean et al., 1997). Thus dietary supplementation with antioxidants such as OPL help could prevent this oxidative damage to proteins.

Liver and kidney beneficial or toxic effects of the supplement were assessed through serum markers namely total protein, AST, ALT and creatinine. The serum AST and ALT activities in STZ-diabetic rats were significantly above normal, as previously reported (Greive et al., 2003), and OPL treatments to diabetic rats prevented this increases, indicating here, that OPL effectively inhibited diabetic nephropathy and hepatopathy. Diabetic nephropathy is measured by excess urinary albumin excretion and abnormal serum creatinine levels. Measurement of the aminotransferases (ALT and AST) is of toxicological importance as changes in their activities are indicative of tissue damage by toxicants or in disease conditions (Singh et al., 2001). ALT and AST are intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury.

This study provides evidence of some antioxidative protective effect of OPL against extensive damages in the kidneys and liver under oxidative stress or hyperglycaemia. The kidney exhibits a characteristic pattern of changes in the glomerulus during diabetes (Shafirir and Sima, 2000). These important hyperglycaemic target organs play important roles in regulating chronic hyperglycaemia. Diabetes causes carbohydrate metabolism changes that adversely affect their functions. Hyperglycaemia caused increase in fluid intake and increased water excretion, resulting in over burdening to the kidneys. The possible beneficial or toxic effects of OPL can be monitored from the kidney function test. The creatinines were used as indices of changes in glomerular filtration rate. Significant elevations in plasma creatinine levels occur only when there are at least 75% non-functional nephrons. Here, the creatinine levels were significantly lower in the DC and GB groups indicated increased fluid intake and excretion while OPL treated rats were in the normal ranges. This together with the reduced number of abnormal glomerulus compared to the untreated DC group, illustrate the

protective effect of OPL on the glomerulus. The DC rats possessed many abnormal glomeruli signifying glomerulus damage, which includes some glomeruli loss and shrunken glomerulus. The free radicals may alter the structure and function of the kidney, especially the glomerulus and cause diverse types of glomerular lesions pathophysiology, ranging from inflammatory to apoptosis. All the livers and kidneys of OPL treated normal rats were normal without any observable injury indicating the non-toxic nature of OPL. Hepatic fat accumulation which caused hepatocyte necrosis in the liver is a well-recognized complication of diabetes with reported frequency of 40-70% (Levinthal et al., 1999). In STZ rats' liver, vacuolation and a significant number of hepatocyte necrosis was observed whereas STZ+OPL rats liver were slightly better than normal. The high antioxidant content of OPL may be associated to the organs protective effect.

Flavonoids are synthesised from phenylalanine and generally affects various enzymes and gene expression. Flavonoids toxicity to animal cells is low, and are major functional components of many herbal preparations for medical use since ancient times. The daily intake of flavonoids with normal food, is about 1-2 g (Havsteen, 2002) and this can be supplemented or increased by OPL consumption.

Conclusion

The results of this study strongly suggest that OPL is potentially useful for the alleviation of diabetic and secondary complications diabetics. It is well known that certain flavonoids exhibit hypoglycaemic activity and are able to help regenerate the beta cells of the pancreas. The significant antihyperglycaemic effect of OPL is probably due to its flavonoids contents. Further work is needed to investigate the actual active components in the OPL. The present investigation reports the first anti-hyperglycaemic activity of OPL which may be a new potential alternative in the treatment and management of diabetes.

Acknowledgements

We thank the Malaysian government for the provision of the research grant, and all the staffs for their technical supports and help in the animal study.

References

- Aebi H, 1974. Catalase Bergmeyer, H.V (ed). Methods of Enzymatic Analysis. Chemie, Weinheim, FRG, pp.673.

- Ames, B.N., Shigenaga, M.K. and Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. of the National Academy of Sciences of the United States of America* 90: 7915-7922.
- Czinner, E., Hagymási, K., Blázovics, A., Kéry, Á., Szoke, Á. and Lemberkovic, Á., 2000. In vitro antioxidant properties of *Helichrysum arenarium* (L.) Moench. *Journal of Ethnopharmacology*. 73: 437-443.
- Dean, R.T., Fu, S., Stocker, R., Davies, M.J. 1997 Biochemistry and pathology of radical-mediated protein oxidation *Biochemistry Journal*. 324 (1): 1-18
- Enoki, T., Ohnogi, H., Nagamine, K., Kudo, Y., Sugiyama, K., Tanabe, M., Kobayashi, E., Sagawa, H. and Kato, I., 2007. Antidiabetic activities of chalcones isolated from a Japanese herb, *Angelica keiskei*. *Journal of Agriculture and Food Chemistry* 55: 6013-6017.
- Gallagher, A.M., Flatt, P.R., Duffy, G. and Abdel-Wahab, Y.H.A., 2003. The effects of traditional antidiabetic plants on in vitro glucose diffusion. *Nutrition Research* 23: 413-424.
- Gray, A.M. and Flatt, P.R., 1997. Nature's own pharmacy: The diabetes perspective. *Procedure Nutrition Society* 56: 507-517.
- Grazioli, V., Schiavo, R., Casari, E., Marzatico, F., Rodriguez Y Baena, R. and Gaetani, P., 1998. Antioxidant enzymatic activities and lipid peroxidation in cultured human chondrocytes from vertebral plate cartilage. *FEBS Letters* 431: 149-153.
- Greive, K.A., Osicka, T.M., Russo, L.M. and Comper, W.D., 2003. Nephrotic-like proteinuria in experimental diabetes. *American Journal of Nephrology* 23: 38-46.
- Giugliano, D., Ceriello, A., . and Paolisso, G. 1996 Oxidative stress and diabetic vascular complications *Diabetes Care* 19 (3): 257-267.
- Havsteen, B.H. 2002 The biochemistry and medical significance of the flavonoids *Pharmacology Theraphy* 96 (2-3): 67-202.
- Hollman, P.C.H. and Katan, M.B., 1999. Dietary flavonoids: Intake, health effects and bioavailability. *Food Chemistry and Toxicology*. 37: 937-942.
- Knekt, P., Kumpulainen, J., Järvinen, R., Rissanen, H., Heliövaara, M., Reunanen, A., Hakulinen, T., . and Aromaa, A. 2002 Flavonoid intake and risk of chronic diseases *American Journal of Clinical Nutrition* 76 (3): 560-568.
- Levinthal, G. N., and Tavill, A. S. Liver Disease and Diabetes Mellitus. *Clinical Diabetes* 17: 1- 21.
- Like, A.A. and Rossini, A.A., 1976. Streptozotocin induced pancreatic insulinitis: new model of diabetes mellitus. *Science* 193:415-417.
- Marklund, S., . and Marklund, G. (1974). Involvement of the superoxide anion radicals in auto-oxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* 47: 469-474.
- Mielke, T., 1996. Outlook for oils and fats from 1996 onwards with special emphasis on oil palm and kernel oil. PAC Seminars of the Palm Oil Res. Inst. of Malaysia (PORIM).
- Requena, J.R., Fu, M.X., Ahmed, M.U., Jenkins, A.J., Lyons, T.J., Baynes, J.W. and Thorpe, S.R., 1997. Quantification of malondialdehyde and 4-hydroxynonenal adducts to lysine residues in native and oxidized human low-density lipoprotein. *Biochemistry Journal* 322: 317-325.
- Reusch, J.E.B., 2003. Diabetes, microvascular complications, and cardiovascular complications: What is it about glucose? *J.Clin. Invest.* 112:986-988.
- Runnie I, Noordin Mohamed Mustapha, Radzali Musec, Azizah Hamid, Hapizah Nawawid, Mahinda Y. Abeywardena, and Suhaila Mohamed. 2003. Antioxidant and Hypocholesterolemic Effects of *Elaeis guineensis* Frond Extract on Hypercholesterolemic Rabbits – ASEAN Food Journal 12 [3]: 137-148
- Salleh, MN, Runnie, I., Roach, P.D., Mohamed, S., . and Abeywardena, M.Y. 2002. Inhibition of low-density lipoprotein oxidation and up-regulation of low-density lipoprotein receptor in HepG2 cells by tropical plant extracts *Journal of Agriculture and Food Chemistry* 50 (13): 3693-3697.
- Shafir, E., and Sima, A. A. F. Diabetic animals for research into the complications: A general overview in Sima A. A. F., *Animal models and chronic complications*, The Netherlands, pp. 1- 42.
- Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumria, M.M.L., Ranganathan, S. and Sridharan, K., 2001. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. *Journal Ethnopharmacology* 76: 269-277.
- Shamoon, H., Duffy, H., Fleischer, N., Engel, S., Saenger, P., Strelzyn, M., Litwak, M., . and Gonzalez, J. 1993 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus *New England Journal of Medicine* 329 (14):

977-986.

Stahl, W., Junghans, A., De Boer, B., Driomina, E.S., Briviba, K. and Sies, H., 1998. Carotenoid mixtures protect multilamellar liposomes against oxidative damage: Synergistic effects of lycopene and lutein. *FEBS Letters* 427: 305-308.

Swanston-Flatt, S.K., Day, C., Bailey, C.J. and Flatt, P.R., 1990. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia* 33: 462-464.

Turner, R. 1998 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33) *Lancet* 352 (9131): 837-853.

West, I.C., 2000. Radicals and oxidative stress in diabetes. *Diabetes Medicine* 17: 171-180.

Winiarska, K., Fraczyk, T., Malinska, D., Drozak, J. and Bryla, J., 2006. Melatonin attenuates diabetes-induced oxidative stress in rabbits. *Journal of Pineal Research* 40: 168-176.

Zhao, R., Li, Q., Long, L., Li, J., Yang, R. and Gao, D., 2007. Antidiabetic activity of flavone from Ipomoea Batatas leaf in non-insulin dependent diabetic rats. *Int. Journal of Food Science and Technology* 42: 80-85.

Zhang SZ, Wang XY, . and Qi Z.M: 1997 Studies on the mechanism and its effects of selenium and vitamin E in the free radicals metabolism of animals with selenium deficiency. *Husbandary and Veterinary Science* 28: 23-27.