DISCUSSION
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Diabetes mellitus is an endocrine disorder in which glucose metabolism is impaired because of total loss of insulin after destruction of pancreatic beta cell or because of inadequate release of insulin from the pancreatic beta cell or insensitivity of target tissue to insulin. The fundamental mechanism underlying hyperglycemia involved over production (excessive hepatic glyconeogenesis and gluconeogenesis) and decrease utilization of glucose by the tissue (Saeed et al., 2008). The present study is to investigate the effect of *Mukia maderspatana* and *Raphanus sativus* on the treatment of diabetes and diabetes related complications.

Preliminary phytochemical analysis reveals the presence of phenolics and flavonoids in extracts and fractions of *Mukia maderspatana* and *Raphanus sativus*. Phenolic compounds have been reported to have antiallergic, antioxidant, anti-inflammatory, anticancer, antiviral and antibacterial activities (Iwai, 2009; Serkedjieva, 2009). Flavonoids also long been recognized to possess antiallergic, antiproliferative and anticarcinogenic activities. They have been shown to affect a large variety of enzymes, to possess important enzyme inducing activities, free radical antioxidant properties and in particular to affect cellular protein phosphorylation (Middleton, 1994).

Total phenolic and flavonoid contents will serve as a means to evaluate the quality of the extracts or fractions with respect to its reproducibility. In the present study, the total phenolic content (g %) of EEMM and EERS are found to be 4.75±2.53 and 4.26±1.34 of gallic acid equivalents, respectively. The total flavonoid content (g %) of EEMM and EERS are found to be 13.62±0.48 and 14.74±0.68 of rutin equivalents, respectively.

Inhibition of α-glucosidase and α-amylase enzymes, involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients (Ali et al., 2006). Natural enzyme inhibitors would likely offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia, due to lower abdominal side effects arising from excessive inhibition of pancreatic α-
amylose, which results in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Kwon et al., 2006).

The results of the present study are in agreement with those of previous studies, which have demonstrated that the *Mukia maderspatana* and *Raphanus sativus* have α-amylase inhibitory activity and inhibition potential against α-glucosidase.

Altered glucose transport associated with defective Glut-4 translocation and impaired insulin signaling cascade has been evidenced as one of the major defects in insulin resistance in type 2 diabetes. L6 muscle cell line is a suitable *in vitro* model used to study the glucose transport activity since skeletal muscle is the major site for primary glucose disposal and glucose utilization (Koivisto et al., 1991). Earlier reports on L6 myotubes demonstrated the maximum glucose uptake activity by troglitazone and rosiglitazone, respectively (Ciaraldi et al., 1995; Yonemitsu et al., 2001). The present findings also reveal an increase in glucose uptake, thus reinforced the enhanced glucose transport by extracts and fractions of the selected plants.

The single high dose STZ-induced diabetic rat is one of the animal models to study human type 1 diabetes mellitus. In this model, diabetes arises from irreversible destruction of the β-islet cells of the pancreas, causing degranulation or reduction of insulin secretion. Here, the insulin is markedly depleted, but not absent (Omuru and Satu, 1964).

Normally, diabetes is detected by measuring glucose blood levels. Diabetic rats have impaired glucose tolerance. Additional load of glucose is found to impair the tolerance further. Therefore, the present research investigates the short-term FBG and OGTT during the drug treatment. From the results obtained, it was obvious that fasting blood glucose concentrations dramatically elevated together with corresponding deteriorating oral glucose tolerance in diabetic rats. The extract and fractions show a significant (p<0.001) time-dependent decrease in blood glucose level after oral administration at 7, 14 and 21 days when compared to the diabetic control group.
From the data obtained in OGTT, the EEMM and EERS produce a significant attenuation (p<0.001) in blood glucose level at 60 min to 180 min when compared with diabetic control. However, the fractions of the CFMM, BFMM, CFRS and BFRS have no significant effect on the blood glucose at 30 min, but produce a significant attenuation at 120 min and 180 min (p<0.001). The test suggests that the selected plants act by increasing peripheral utilization of glucose.

The daily administration of extract and fractions to STZ-diabetic rats twice a day for 3 weeks caused a statistically significant reduction in fluid and food intake and an increase in the body weight in STZ-diabetic rats. This could be due to the improved glycemic control.

Glucose-6-phosphatase catalyzes the final step in glucose production by the liver and kidney. Streptozotocin increases the expression of glucose-6-phosphatase mRNA, which contributes to the increased glucose-6-phosphatase activity in diabetes mellitus (Liu et al., 1994). Over production of glucose by the liver is the major cause of fasting hyperglycemia in both insulin-dependent and non-insulin-dependent diabetes mellitus. Partially pancreatectomized diabetic rats (90%) have been reported to have a 5-fold increase in the messenger RNA and a 3 to 4-fold increase in the protein level of the catalytic subunit of hepatic glucose-6-phosphatase. Prolonged hyperglycemia may thus result in overproduction of glucose via increased expression of this protein (Massillon et al., 1996).

Similarly, in the present study also both the extracts and fractions control the increase in the blood glucose in STZ-diabetic rats by decreasing the activity of glucose-6-phosphatase in the liver. This could be one of the mechanisms for the suppression of blood glucose concentration in the diabetic rats. Other workers have also reported that extracts of some plants such as Zizyphus spina-christi significantly reduced serum glucose level, liver phosphorylase and glucose-6-phosphatase activities, and significantly increased serum pyruvate level and liver glycogen content after 4 weeks of treatment (Glombitza et al., 1994). Hence the suppression of glucose- 6-phosphate hydrolysis could also be one of the reasons for the antihyperglycemic effect of the plants in STZ-diabetic rats. Similar effects were reported for other hypoglycemic agents such as Vanadate (Mosseri et al., 2000).
Insulin deficiencies in STZ induced diabetic rats are associated with hypercholesterolemia and hypertriglyceridemia. Insulin deficiency may be responsible for dyslipidemia because insulin has an inhibitory action on HMG-Co A reductase, a key enzyme that is rate limiting in the metabolism of cholesterol rich LDL particles (Gold et al., 1970). Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver. In diabetic rats, there is a decrease in lipoprotein lipase activity resulting in impaired clearance of VLDL and chylomicron from plasma (Bagdade et al., 1968).

In the present study, both the extracts and fractions significantly (p<0.001, p<0.01) decreases both serum cholesterol and triglyceride levels in diabetic rats. There is also a significant (p<0.001, p<0.01) decrease in serum LDL and VLDL levels by treatment with extract and fractions compared to diabetic control animals. It also produces significant (p<0.001, p<0.01) change in serum HDL level by treatment compared to diabetic control. Since lipid abnormalities accompanying with premature atherosclerosis is the major cause of cardiovascular disease in diabetic patients, an ideal treatment for diabetes, should have a favourable effect on lipid profile in addition to glycemic control (Saeed et al., 2008).

Oxidative stress plays a major role in pathogenesis of both types of diabetes mellitus. Free radical production caused by hyperglycemia may occur via at least three different routes: non enzymatic glycation, autooxidation of glucose and impairs cellular activation of polyol pathway. High levels of free radicals and simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and lipid peroxidation (Wolf and Dean, 1987). The increase in oxygen free radicals in diabetes could be due to rise in blood glucose levels. The antioxidant enzymes include SOD and CAT and play a role as protective enzyme in the defense system. In the present study, diabetic control rats show a significant decrease in SOD and CAT, compared to normal rats indicating dysfunction in antioxidant defense system in diabetes mellitus.

Hypoinsulinemia in diabetes increases the activity of fatty acyl coenzyme A oxidase, which initiates β-oxidation of fatty acids, resulting in lipid peroxidation (Bruch and Thayer, 1983). The enormous increase in lipid peroxidation leads to the alteration of
the trans bilayer fluidity gradient which could hamper the activities of membrane-bound enzymes and receptors (Oberley, 1988).

The products of lipid peroxidation such as lipid radicals and lipid peroxides are extremely harmful to most of the cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damage (Elangovan et al., 2000). It has been suggested that oxidative stress plays an important role in the development of chronic complications of diabetes (Tatsuki et al., 1997). In the present study extracts/fractions increased the CAT and SOD enzyme level, whereas, the level of TBARS was decreased significantly.

In the present study, the antihyperglycemic effect of both the extracts and fractions of *Mukia maderspatana* and *Raphanus sativus* are investigated using the obese-diabetic rat model by high-fat feeding and low streptozotocin injection. The rats fed with HFD can result in insulin-resistant mainly through Randle or glucose–fatty acid cycle (Zhang et al., 2003).

Furthermore, high-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes (Reed et al., 2000). There is no significant variation in plasma insulin concentrations between diabetic and normal rats. However, because fasting blood glucose is significantly higher in diabetic rats, it has been suggested that insulin resistance develops in these animals. The rat model, therefore exhibits hyperglycemia, hyperlipemia and insulin resistance that would closely reflect the natural history and metabolic characteristics of humans and it is further sensitive to pharmacological testing.

Hyperglycemia, the most important feature of diabetes mellitus, is in itself very dangerous for diabetic patients. It impairs the prooxidant/antioxidant balance, reducing antioxidant levels and increasing free radicals, which can damage the pancreatic β-cells and induce insulin resistance (The Diabetes Control and Complications Trial Research Group, 1993). In the present study, the antihyperglycemic effect of extract and fractions could control hyperglycemia, the key feature of the diabetes and thus indicating that both the plants can control the development of diabetes.
Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile. In uncontrolled type 2 diabetes mellitus, there will be an increase in TC, LDL-c and VLDL-C and TC with decrease in HDL-C which contributes to the coronary artery disease (Arvind et al., 2002). Raised plasma FFA level plays a major role in the pathogenesis of insulin resistance and type 2 diabetes (Kovacs and Stumvoll, 2005). In addition, hypertriglyceridemia is also an important maker of insulin resistance (Schwartz, 2006). In the present study, a rise in blood glucose was accompanied with the lipid metabolism disorder. A marked increase in serum lipids and lipoprotein in diabetic rats was observed in diabetic rats. A regular administration of the extracts/fractions for 3 weeks lowered the TC, TG, LDL-C and VLDL-C values while elevate the HDL-C value. The alterations in lipid and lipoprotein profiles during experimental diabetes were restored to near normal level.

Earlier studies have also shown that there is a close relationship between the increase of free radicals, blood glucose and lipid peroxidation (LPO) in the progress of diabetes (Reddy et al., 2005). Diabetics usually exhibit high oxidative stress due to persistent and chronic hyperglycemia, results in depleting the activity of antioxidative defense system and thus promote free radicals generation (Kamalakannan and Prince, 2006).

Oxygen free radicals could react with polyunsaturated fatty acids which lead to LPO. Increased LPO impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (Arulselvan and Subramanian, 2007). In the present findings, the concentrations of MDA decreases significantly (p<0.001) after treating with extracts and fractions, indicating the inactivation of LPO reactions and the decreased free radical generation.

Furthermore, in the present study, the activities of CAT and SOD decreases in diabetes group as reported earlier which could be due to the inactivation caused by STZ-generated ROS. Treatment of diabetes with the extracts and fractions reverses the activities of these enzymatic antioxidants, which might be due to decreased oxidative stress as evidenced by decreased LPO.

The present findings suggests that extracts and fractions of Mukia maderpsatana and Raphanus sativus elicit antioxidant properties by attenuating the lipid peroxidation caused by various forms of free radicals and this possibly affects lipid profile.
Additionally, the present results might be correlated with previous evidence of its constituents. In earlier studies, workers have isolated some flavonoids from *Mukia maderspatana* and *Raphanus sativus*, including kaempferol and quercetin (Retnam and De Britto, 1998; Kamil and Kalina, 1997). The synergistic properties of the flavonoids might be contributing and thus helpful in the prevention of diabetic complications through improving dyslipidemia.