Hypolipidemic activity of an aqueous extract of Morinda citrifolia fruit in normal and streptozotocin-induced diabetic rats
(Aktiviti hipolipidemik ekstrak akuas buah Morinda citrifolia pada tikus normal dan tikus diabetik diaruh dengan streptozotocin)

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Key words: Morinda citrifolia, hypolipidemic, normal rats, streptozotocin-induced diabetic rats

Abstract
This study was undertaken to investigate the hypolipidemic activity of Morinda citrifolia fruit extract. Aqueous extract of M. citrifolia in concentrations ranging from 0.25 g/kg (low dose), 0.50 g/kg (medium dose) and 1.00 g/kg (high dose) were orally administered on streptozotocin-induced diabetic rats for 6 weeks. The hypolipidemic effect of M. citrifolia extract in rats was determined by measuring the total lipid, total cholesterol and triglyceride concentrations in blood (plasma) and liver tissue. The administration of medium dose of M. citrifolia extract had significantly ($p < 0.05$) reduced the cholesterol content in blood and liver of normal rats as compared to the normal control rats. For the diabetic rats, the administration of medium and high doses of M. citrifolia extracts successfully reduced the plasma triglyceride level significantly ($p < 0.05$) as compared to diabetic control rats. The concentration of triglyceride and total lipid in the liver were also decreased significantly ($p < 0.05$) in the diabetic rats which had received high dose of M. citrifolia extract as compared to control rats. Results showed that M. citrifolia exhibits the potential in lowering the concentration of certain lipid components in blood and tissue of experimental rats.

Introduction
Diabetes mellitus is a major disease affecting nearly 10% of the world population (Joy and Kuttan 1999) and is long considered to be a serious endocrine syndrome (Alarcon-Aguilara et al. 1998). Prevalence of diabetes worldwide was expected to increase to 5.4% (300 million people) by the year 2025 (King et al. 1998). This disease is a complex metabolic disorder which affects the use of all major nutrients such as carbohydrate, fat and protein (Indirani and Uliyar 1987; Bilbis et al. 2002).

One of the important nutrients that is closely related to diabetes mellitus is lipid. Diabetes can cause a state of hyperlipidemia coupled with impaired essential fatty acid metabolism, which has adverse consequences, thus contributing to the development of diabetic complications (Sima 2000; Dhandapani et al. 2002). The alteration in the lipid metabolism is associated with the secondary complications
of diabetes such as atherosclerosis and cardiovascular system disease (Sener et al. 2002). Lowering of plasma lipid levels through dietary or drug therapy seems to decrease the risk of vascular diseases (Scott 1999).

In Malaysia, one of the popular herbs that has been traditionally used to treat diabetic patients without much scientific evidence for its efficacy is *Morinda citrifolia* or locally known as 'mengkudu' (Muhammad and Mustafa 1994). It is believed to reduce high blood pressure, alleviate pain, stimulate the immune system and increase body energy (Heinicke 1985; Muhammad and Mustafa 1994; Solomon 1998; Indu and Ng 2000). Our previous studies showed that an aqueous extract of *M. citrifolia* reduces the blood glucose level in diabetic rats (Hadijah et al. 2004). Besides hyperglycaemia, the levels of plasma lipids are usually raised in diabetes mellitus (Eddouks et al. 2005). Thus, this present study was undertaken to investigate the effect of an aqueous extract of *M. citrifolia* fruit on lipid constituents in plasma and liver of normal and streptozotocin-induced diabetic rats.

**Materials and methods**

**Preparation of extract**

The fully mature fruits of *M. citrifolia* were collected from MARDI Station, Serdang. According to Rohani and Rosalizan (2005), the maturity index of *M. citrifolia* fruit was at the yellow-white stage which is 15–16 weeks after the fruit set. The fresh fruits were blended, using Waring blender, with distilled water at a ratio of 1:1 (v/w). For example, 1 kg of fruits was mixed with 1 litre of water. The mixture was centrifuged at 2,000 rpm for 10 min to get clear juice which was then stored at 4 °C. The juice was administered daily to the rats for 6 weeks.

**Experimental animals**

Male Sprague-Dawley rats with an average body weight of 200–230 g were obtained from Animal House, Universiti Kebangsaan Malaysia (UKM). They were fed with a standard rat chow diet (Barastoc, Australia) and water *ad libitum*. All rats were acclimatized to the animal facility for one week before the commencement of the experiment. Diabetes was induced by intramuscular injection of streptozotocin (Sigma Co., USA.) dissolved in 0.9% saline at 65 g/kg of body weight according to procedures by Peungvicha et al. (1997) and Teixera et al. (1997).

After four days of the injection, rats were fasted overnight and blood glucose level was measured with glucometer (Precision Q.I.D., Abbott, UK) using tail blood samples. This method permits the measurement of blood glucose levels with minimal injury to the rat (Teixera et al. 1997). Rats with blood glucose levels of more than 8.3 mmol/litre were classified as diabetic and included in the experiment (Sener et al. 2002). Streptozotocin produces a diabetic condition by destroying the pancreatic β cells which secrete insulin, an important hormone for glucose metabolism (El-Fiky et al. 1996).

In this present study, the rats were divided into two major groups i.e. normal and diabetic groups. The normal rats were then further subdivided into four groups (G1, G2, G3 and G4) consisting of six rats per group. Meanwhile, the diabetic rats were subdivided into five groups (GA, GB, GC, GD and GE) with five rats per group. The G1 of normal and GA of diabetic rats were given distilled water and served as a control for the purpose of comparison. The G2, G3 and G4 of normal and GB, GC, GD and GE of diabetic rats were given low dose of extract (0.25 g/kg of body weight), medium dose (0.50 g/kg of body weight) and high dose of *M. citrifolia* extract (1.0 g/kg of body weight) respectively.

Glibenclamide (5.0 g/kg of body weight) was orally administered to the GE rats. Glibenclamide is a medicinal drug used to control diabetes in human. It was reported that the administration of glibenclamide reduced the blood glucose by stimulating the
secretion of insulin from pancreatic β cells and the inhibition of glucagon secretion from pancreatic α cells (Gilman et al. 1990; Peungvicha et al. 1998). Distilled water, *M. citrifolia* extracts and glibenclamide were administered daily for 6 weeks by using needle gavage (force-feeding) to all rats.

**Plasma and tissue preparations for biochemical assays**

**Plasma**  Blood samples were collected from the posterior *vena cava* of rats after fasting for 15 h and under ether anesthesia at the final stage of the experiment. The blood samples were then transferred into EDTA tube and centrifuged (1,500 g for 10 min at 4 °C) to obtain the plasma fraction. Plasma samples were kept at –20 °C until analysis. The plasma samples were analysed for total cholesterol and triglyceride concentrations using Chemistry Analyser (Cobas Mira, Roche, UK) at Faculty of Medicine and Health Science, Universiti Putra Malaysia.

**Liver**  The liver of each rat was immediately collected after the rats were euthanized. They were carefully washed with NaCl solution (0.9%) and stored in liquid nitrogen. Livers were then kept at –80 °C until further assays. For analysis, livers were cut into small pieces and were homogenized in cold Tris-HCl with 1.15% KCl (pH 7.4) in a glass Teflon homogenizer for six strokes according to the procedure by Campbell et al. (1978). The homogenate was then centrifuged at 1,500 g for 10 min at 4 °C and the supernatant was collected and used for the determination of total lipids (Elleston and Caraway 1976), total cholesterol (Tietz 1976) and triglycerides (Giegel et al. 1975).

**Statistical analysis**

The significant differences between the control and extract-treated groups were determined using SAS (ANOVA followed by Duncan New Multiple Range Test). All values are expressed as group mean ± standard error of mean (SEM). The minimal level of significance accepted was *p* <0.05.

**Results**

**Effect of *M. citrifolia* extract on the plasma cholesterol and triglyceride**

The administration of medium dose of the extract (0.50 g/kg) significantly reduced (*p* <0.05) the cholesterol concentration (2.01 ± 0.10 mmol/litre) by 26.10% in normal rats as compared to the control rats (2.72 ± 0.22 mmol/litre) (*Table 1*). However, the high dose of extract (1.00 g/kg) did not change the cholesterol concentration significantly, indicating that the hypocholesterolemic activity appeared not to be dose-dependent.

Meanwhile, the plasma cholesterol levels were not affected by the administration of extracts in all extract-treated diabetic rats (*Table 2*). There was no significant difference in cholesterol levels among the four groups of diabetic rats, except in the glibenclamide-treated diabetic rats. The supplementation of glibenclamide had reduced the cholesterol levels.

**Table 1.** The effect of *Morinda citrifolia* extracts on the cholesterol and triglyceride levels in the plasma of normal rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (mmol/litre)</th>
<th>Triglyceride (mmol/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 - Control</td>
<td>2.72 ± 0.22a</td>
<td>0.56 ± 0.06a</td>
</tr>
<tr>
<td>G2 - 0.25 g/kg extract</td>
<td>2.18 ± 0.09ab</td>
<td>0.67 ± 0.08a</td>
</tr>
<tr>
<td>G3 - 0.50 g/kg extract</td>
<td>2.01 ± 0.10b*</td>
<td>0.68 ± 0.04a</td>
</tr>
<tr>
<td>G4 - 1.00 g/kg extract</td>
<td>2.16 ± 0.30ab</td>
<td>0.59 ± 0.04a</td>
</tr>
</tbody>
</table>

Mean values in the same column with the same letter is not significantly different (*p* <0.05) according to DMRT (*Significantly different as compared to G1*)

Values are mean ± SEM, *n* = 6
Hypolipidemic activity of Morinda citrifolia

content significantly (p <0.05) as compared to control diabetic rats (38.91% reduction). These results showed that all dosages of M. citrifolia were not effective in reducing the cholesterol levels in the plasma of diabetic rats.

The concentrations of triglyceride were also not affected by the administration of M. citrifolia extracts in all extract-treated normal rats. There was no significant difference between the extract-treated normal rats when compared to control rats (Table 1). On the other hand, the plasma triglyceride levels of untreated diabetic rats (GA) were significantly higher as compared to the other group of diabetic rats as shown in Table 2. The administration of M. citrifolia to all diabetic rats (GB, GC and GD) was effective to reduce the triglyceride concentrations by 76.04%, 62.67% and 58.99% respectively, when compared to the untreated diabetic rats (GA). Similar result also found in the GE rats which has been supplemented by the glibenclamide. The percentage of triglyceride reduction was 76.04%, which was similar to the low dose of M. citrifolia extract.

Effect of M. citrifolia extract on total lipids, total cholesterol and triglyceride in the liver

The administration of M. citrifolia extract did not affect the total lipids and triglyceride concentrations in the liver of normal rats (Table 3). The results indicated no significant difference in extract-treated rats when compared to the control. However, the liver cholesterol concentration was reduced significantly (p <0.05) by 24.27% when a high dose of extract (1.00 g/kg) was administered to the normal rats (2.59 ± 0.33 mmol/litre) as compared to the control rats (3.42 ± 0.25 mmol/litre).

Meanwhile, only the supplementation of high dose of M. citrifolia extract had reduced the total lipids and triglyceride contents significantly (p <0.05) in the liver of the diabetic rats (Table 4). The total lipids were reduced by 8.68%, whereas the triglyceride was reduced by 45.6%. The low and medium doses of extract were not

Table 2. The effect of Morinda citrifolia extracts on the cholesterol and triglyceride levels in the plasma of diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (mmol/litre)</th>
<th>Triglyceride (mmol/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA - Control</td>
<td>2.39 ± 0.48a</td>
<td>2.17 ± 0.61a</td>
</tr>
<tr>
<td>GB - 0.25 g/kg extract</td>
<td>2.16 ± 0.09ab</td>
<td>0.52 ± 0.03b*</td>
</tr>
<tr>
<td>GC - 0.50 g/kg extract</td>
<td>2.07 ± 0.19ab</td>
<td>0.81 ± 0.09b*</td>
</tr>
<tr>
<td>GD - 1.00 g/kg extract</td>
<td>2.24 ± 0.17ab</td>
<td>0.89 ± 0.06b*</td>
</tr>
<tr>
<td>GE - 5 g/kg glibenclamide</td>
<td>1.46 ± 0.12b*</td>
<td>0.52 ± 0.09b*</td>
</tr>
</tbody>
</table>

Mean values in the same column with the same letter is not significantly different (p <0.05) according to DMRT (*Significantly different as compared to GA)
Values are mean ± SEM, n = 5

Table 3. The effect of Morinda citrifolia extracts on the total lipids, cholesterol and triglyceride levels in the liver of normal rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total lipids (g/g)</th>
<th>Cholesterol (g/g)</th>
<th>Triglyceride (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 - Control</td>
<td>18.99 ± 2.66a</td>
<td>3.42 ± 0.25a</td>
<td>0.75 ± 0.10a</td>
</tr>
<tr>
<td>G2 - 0.25 g/kg extract</td>
<td>18.55 ± 1.49a</td>
<td>3.15 ± 0.23ab</td>
<td>0.93 ± 0.15a</td>
</tr>
<tr>
<td>G3 - 0.50 g/kg extract</td>
<td>18.51 ± 0.89a</td>
<td>3.12 ± 0.20ab</td>
<td>0.96 ± 0.12a</td>
</tr>
<tr>
<td>G4 - 1.00 g/kg extract</td>
<td>17.58 ± 1.76a</td>
<td>2.59 ± 0.33b*</td>
<td>0.96 ± 0.17a</td>
</tr>
</tbody>
</table>

Mean values in the same column with the same letter is not significantly different (p <0.05) according to DMRT (*Significantly different as compared to G1)
Values are mean ± SEM, n = 6
effective. However, the liver cholesterol content in all diabetic rats treated with *M. citrifolia* extracts (GB, GC and GD) were not significantly different from the GA diabetic rats. This result was similar to the plasma cholesterol levels in diabetic rats as the cholesterol levels were also not affected by the extracts. The supplementation of glibenclamide (a reference drug) to diabetic rats also did not show any significant changes in the total lipid, cholesterol and triglyceride concentrations.

**Discussion**

Most recent studies on the treatment of diabetes have focused on the potential use of plant with hypoglycemic and hypolipidemic effects (Jung et al. 2006). Abnormalities in lipid profile are one of complication in diabetes mellitus, which is found in about 40% of diabetic patients (Ravi et al. 2005). Reports also have shown that the diabetic animals including man have an elevated level of plasma triglyceride (hypertriglyceridemia) and it is the most common lipid abnormality in diabetes (Peungvicha et al. 1997; Shuichi and Hiroyuki 2000). Hypertriglyceridemia is also related to insulin resistance and glucose intolerance (Gingsberg 1994).

In this present study, it can be seen that the plasma triglyceride level was increased significantly (*p* <0.05) in diabetic control rats (2.17 ± 0.61 mmol/litre) as compared to normal control rats (0.56 ± 0.06 mmol/litre) (**Tables 1 and 2**). The administration of *M. citrifolia* extract at all dosages reduced the plasma triglyceride levels significantly in diabetic rats. Whereas only the highest dose of *M. citrifolia* extract (1.00 g/kg) managed to reduce the liver triglyceride content.

Previous reports showed the marked hyperlipidemia that characterizes the streptozotocin-diabetic rats (Riyad et al. 1988; Choi et al. 1991). Yasni et al. (1991) reported that the increment of plasma triglycerides was due to increased production of chylomicron in the intestine or insufficient action of peripheral lipoprotein lipase in the diabetic condition. Chylomicron is an essential lipoprotein to carry the triglyceride molecules in the blood that has been absorbed from intestine. Other lipid metabolism disturbance occurred in diabetes is the increased mobilization of fatty acids from adipose tissue (Singh et al. 1987). The excessive fatty acids will enter the liver and are esterified to form triglycerides (Hem 1977), hence, increasing its level in the liver. Therefore, one of our aims in this study was to determine whether the *M. citrifolia* extract could reduce the triglyceride levels in the plasma and liver of diabetic rats.

The present findings showed that the administration of *M. citrifolia* extracts to diabetic rats at all dosages (0.25, 0.50 and 1.00 g/kg) decreased the triglyceride levels in plasma while at dosage of 1.00 g/kg decreased triglyceride level in liver, indicating a reduction in the hypertriglyceridemia. The concentration of liver triglyceride was also increased in diabetic rats as reported by Rai et al. (1997). The increment in triglyceride was due to the

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total lipids (g/g)</th>
<th>Cholesterol (g/g)</th>
<th>Triglyceride (g/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA - Control</td>
<td>16.25 ± 0.71a</td>
<td>2.65 ± 0.35a</td>
<td>1.25 ± 0.32a</td>
</tr>
<tr>
<td>GB - 0.25 g/kg extract</td>
<td>16.17 ± 0.27a</td>
<td>2.42 ± 0.15a</td>
<td>0.88 ± 0.08ab</td>
</tr>
<tr>
<td>GC - 0.50 g/kg extract</td>
<td>15.73 ± 0.32ab</td>
<td>2.45 ± 0.36a</td>
<td>0.85 ± 0.03ab</td>
</tr>
<tr>
<td>GD - 1.00 g/kg extract</td>
<td>14.84 ± 0.19b*</td>
<td>2.28 ± 0.21a</td>
<td>0.68 ± 0.07b*</td>
</tr>
<tr>
<td>GE - 5 g/kg glibenclamide</td>
<td>15.11 ± 0.31ab</td>
<td>1.76 ± 0.24a</td>
<td>0.85 ± 0.07ab</td>
</tr>
</tbody>
</table>

Mean values in the same column with the same letter is not significantly different (*p* <0.05) according to DMRT (*Significantly different as compared to GA*)

Values are mean ± SEM, n = 5
impairment of insulin secretion that occurs in diabetes.

It was observed that the high dose of *M. citrifolia* extract (1.0 g/kg) managed to reduce the total lipids concentration in the liver of diabetic rats. An increase in the total lipids of liver in STZ-induced diabetic rats may indicate increase of synthesis of lipids and storage capacity, which may have caused an increase in plasma triglycerides (Ravi et al. 2005). The low dose (0.25 g/kg) and medium dose (0.50 g/kg) which have been used in this study may not be effective to reduce the total lipids in the liver of rats.

Diabetes is often associated with an increasing level of cholesterol (Peungvicha et al. 1997) but from the present findings, cholesterol concentration was not influenced by the *M. citrifolia* extracts in the diabetic rats. This result was supported by Marshall (2000) who reported that the cholesterol concentration in the diabetic patients are normally maintained in the normal range or slightly increased. Thus, the administration of *M. citrifolia* extract has no beneficial effect in lowering the cholesterol levels both in plasma and liver of diabetic rats.

On the other hand, the cholesterol levels in plasma and liver of normal rats have been significantly decreased. It was shown that the medium dose of *M. citrifolia* extract (0.50 g/kg) managed to reduce the plasma cholesterol level in normal rat. The low dose of extract (0.25 g/kg) was not effective, whereas, the high dose (1.00 g/kg) was also not effective (not to be dose-dependent). However, the high dose of *M. citrifolia* managed to reduce the cholesterol level in the liver. Both low dose (0.25 g/kg) and medium dose (0.50 g/kg) of extracts were not effective in reducing the liver cholesterol of normal rats. These results suggested that certain dosages of *M. citrifolia* had reduced the plasma and liver cholesterol in normal rats. Goh et al. (1995) and Solomon (1998) had also claimed that *M. citrifolia* has the ability to reduce blood cholesterol concentration in human. However, further study is needed to verify the hypocholesterolemic effect of *M. citrifolia* extract by using hypercholesterolemic or high fat diet rats.

The mechanism of hypolipidemic effect of *M. citrifolia* as shown in the study, is yet to be established. Phytochemistry studies found that *M. citrifolia* has some chemical constituents such as alkaloids, flavonoids, flavone glycoside (Goh et al. 1995; Indu and Ng 2000) and citrifolinin, a form of glycoside (Sang et al. 2001). Similar results were also noted in the aqueous extract of *Capparis spinosa* fruit (Eddouks et al. 2005). Chemical studies on *Capparis spinosa* fruit reported the presence of alkaloids, flavonoids and glucosonates (Calis et al. 2002) and likely contributed to the observed hypolipidemic activity of its extract.

Flavonoids are plant polyphenols frequently found in fruits, vegetables and grains (Merken and Beecher 2000). The hypolipidemic activity of flavonoids from various sources has been reported by several studies (Koshy et al. 2001; Anila and Vijayalakshmi 2002; Jung et al. 2006). This compound also has been identified as the anti-diabetic components in a number of traditional ethnic remedies (Jung et al. 2006). Meanwhile, the alkaloids and glycoside compounds also have anti-diabetic and anti-hyperlipidemic activities (Ur-Rahman and Zaman 1989; Ravi et al. 2004; Ravi et al. 2005). Thus, it is possible that these active compounds are responsible for the hypolipidemic activities observed in *M. citrifolia*.

Findings from this study also showed that not necessarily all the dosages used (0.25, 0.5 and 1.0 g/kg) were effective towards lowering lipid levels in plasma and liver. This shows that the dosage level is closely related to the active components present in the extract. However, it is possible that there are other active components in *M. citrifolia* that may also has the ability to reduce the lipid levels, but yet to be determined in future phytochemistry study.
Conclusion

*Morinda citrifolia* fruit extract exhibited the hypolipidemic effect by reducing certain lipids component in the plasma and liver of normal and streptozotocin-induced diabetic rats at certain dosages. Results showed that *M. citrifolia* has a potential to reduce certain lipid components. However, more scientific studies need to be conducted to determine the active ingredients involved in the mechanism of reducing lipid components in blood.

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References


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Abstrak
Aktiviti hipolipidemik ekstrak buah mengkudu, Morinda citrifolia telah dikaji. Ekstrak berakuas M. citrifolia pada kepekatan 0.25 g/kg (dos rendah), 0.50 g/kg (dos sederhana) dan 1.0 g/kg (dos tinggi) telah diberi secara oral kepada tikus normal dan tikus diabetik selama 6 minggu. Kesah hipolipidemik M. citrifolia pada tikus telah ditentukan dengan mengukur aras jumlah lipid, jumlah kolesterol dan kepekatan trigliserida di dalam plasma darah dan tisu hati. Pemberian ekstrak M. citrifolia pada dos sederhana menurunkan kandungan kolesterol darah dan hati secara signifikan (p <0.05) pada tikus normal berbanding dengan tikus normal kawalan. Manakala pada tikus diabetik pula, pemberian ekstrak M. citrifolia pada dos sederhana dan tinggi telah menurunkan plasma trigliserida secara signifikan (p <0.05) berbanding dengan tikus diabetik kawalan. Kandungan trigliserida dan jumlah lipid di dalam hati juga telah dapat diturunkan secara signifikan (p <0.05) pada kumpulan tikus diabetik yang diberi ekstrak M. citrifolia pada dos tinggi. Kajian ini mendapati ekstrak M. citrifolia berpotensi menurunkan kepekatan beberapa komponen lipid tertentu di dalam darah atau tisu tikus kajian.

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