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Hypoglycemic activity of *Morinda citrifolia* extract in normal and streptozotocin-induced diabetic rats

(Aktiviti hipoglisemik ekstrak *Morinda citrifolia* pada tikus normal dan tikus diabetes diaruh dengan streptozotosin)

H. Hadijah*, M.Y. Ayub**, H. Zarida*** and A. Normah*

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

Abstract

The hypoglycemic activity of *Morinda citrifolia* extract was tested through an acute and subchronic study. In the acute study, a single dose of *M. citrifolia* extract (0.25 g/kg) was orally administered to normal and diabetic rats for an oral glucose tolerance test. In the subchronic study, an aqueous extract of *M. citrifolia* in concentrations of 0.25 g/kg (low dose), 0.50 g/kg (medium dose) and 1.0 g/kg (high dose) of body weight were orally administered to normal and diabetic rats for 6 weeks. Glibenclamide, a reference drug was also orally administered to the diabetic rats at a dosage of 5 mg/kg for both studies.

In the acute hypoglycemic study, the blood glucose level in normal and diabetic rats did not change significantly as compared to their relative controls. A similar result was also observed in the glibenclamide-treated rats. In the subchronic study, blood glucose levels were reduced significantly (p <0.05) in normal and diabetic rats at medium (0.5 g/kg) and high (1.0 g/kg) dose of *M. citrifolia* extracts as compared to the controls. Supplementation of glibenclamide also reduced the glucose level significantly (p <0.05) in diabetic rats.

Introduction

Morinda citrifolia or locally known as 'mengkudu' is among the well-known medicinal plants in Malaysia (Muhammad and Mustafa 1994). It has been reported to be traditionally used for the alleviation of various diseases including diabetes mellitus (Bailey and Day 1989; Muhammad and Mustafa 1994). Before the introduction of insulin in 1922, the treatment of diabetes mellitus relied on the use of traditional plants based on folk medicine (Akhtar and Ali 1984; Gray and Flatt 1997). Although insulin has become one of the most important medicine, efforts are continuing to find insulin substitutes from plant sources for the treatment of diabetes mellitus (Erenmemisoglu et al. 1995). However a few medicinal plants have scientific explanations, thus the World Health Organization (WHO 1980) recommended the herbal treatments for diabetes need further evaluation and attention.

A study done by Heineckie (1986) found an alkaloid substance in morinda extract called xeronine. A glycoside, citrifolinin also has

*Food Technology Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

**Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43650 Bandar Baru Bangi, Selangor, Malaysia

***Faculty of Medicinal & Health Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Authors' full names: Hadijah Hassan, Ayub Mohd. Yatim, Zarida Hambali and Normah Ahamad

E-mail: hadijah@mardi.my

been identified and isolated from *M. citrifolia* fruit (Sang et al. 2001). Active compounds such as alkaloid and glycoside have been reported to show beneficial effect in reducing blood sugar (Ur-Rahman and Zaman 1989). Previous study on the leaves extract of *Morinda lucida* Benth proved the hypoglycemic activity in streptozotocin-induced diabetic rats (Olajide et al. 1999). Based on this information, the present study was undertaken to determine the hypoglycemic activity of another species of morinda namely *M. citrifolia* by measuring the blood glucose level in normal and streptozotocin-induced diabetic rats in acute and subchronic study.

Materials and methods

Preparation of extract

The fruits of *M. citrifolia* were collected randomly from MARDI Station in Serdang, Selangor. The fresh fruits (seedless) were blended with distilled water at a ratio of 1:1 (w/v). The cloudy juice was centrifuged at 2 000 rpm for 10 min to get a clear supernatant. The supernatant was then stored at 4 °C.

Experimental animals

Male *Sprague-Dawley* rats (200–250 g) obtained from Animal House (Universiti Kebangsaan Malaysia) were used for the study. They were fed with a standard rat chow diet and water ad libitum. All rats were acclimatized to the animal facility for one week before starting the experiment. Diabetes was induced by intramuscular injection of streptozotocin (Sigma Co., USA) dissolved in 0.9% saline at 65 mg/kg of body weight (Peungvicha et al. 1997; Teixera et al. 1997) after overnight fasting. Rats with blood glucose levels above 8.3 mmol/litre measured by blood glucose sensor (Precision QID, UK) were classified as diabetic and were included in the experiment (Lamela et al. 1986; Sener et al. 2002).

The rats were divided into two major groups; normal and diabetic. In the acute study, normal rats were further subdivided into two groups (G1, G2) of six rats per group. The G1 normal rats were given distilled water by an oral feeding and served as control. The G2 rats were given an aqueous extract of *M. citrifolia* orally at 0.25 g/kg of body weight. Meanwhile, the diabetic rats were subdivided into three groups (G1, G2, G3). The G1 and G2 diabetic rats were given the same treatment as the normal group. Glibenclamide, a reference drug was orally administered to the G3 diabetic rats at 5 mg/kg of body weight (Peungvicha et al. 1998; Ahmad et al. 2000).

In the subchronic study, *M. citrifolia* extracts in concentrations ranging from 0.25 g/kg (low dose), 0.50 g/kg (medium dose) and 1.0 g/kg (high dose) were repeatedly administered orally to normal (3 groups) and diabetic rats (3 groups) for 6 weeks. The control group of rats (normal and diabetic) was only given distilled water. Glibenclamide was orally administered to diabetic rats at the same dosage as in the acute study.

Blood glucose determination

All rats were fasted for about 15 h before the blood glucose from samples of the tail blood was measured. In the acute hypoglycemic study, oral glucose tolerance test (OGTT) was done to evaluate the blood glucose changes in rats. The first blood samples were taken 30 min before the rats received any treatments (fasting blood glucose). Immediately afterwards, distilled water (as control), *M. citrifolia* extract at 0.25 g/kg or glibenclamide was orally administered to the respective groups of rats as described earlier. Thirty minutes after administrating the extract, glucose solution in concentration of 50% (w/v) was orally administered to each rat at single dose (1.25 g/kg of body weight).

Blood samples were taken from the tip of the tail before any treatment (–30 min), 0 min (just before glucose administration), 30, 60, 90, 120 and 150 min after glucose

administration for glucose measurement by the blood glucose sensor (Precision QID., UK). Whereas in the subchronic hypoglycemic study, tail blood samples were taken at initial stage (0 day, before any treatment) and final stages of an experiment (after 6 weeks).

Statistical analysis

The significant differences between the control and extract-treated groups were analysed using Student's t-test. All mean values are expressed as group means ± standard error of mean. The minimal level of significance accepted was $p < 0.05$.

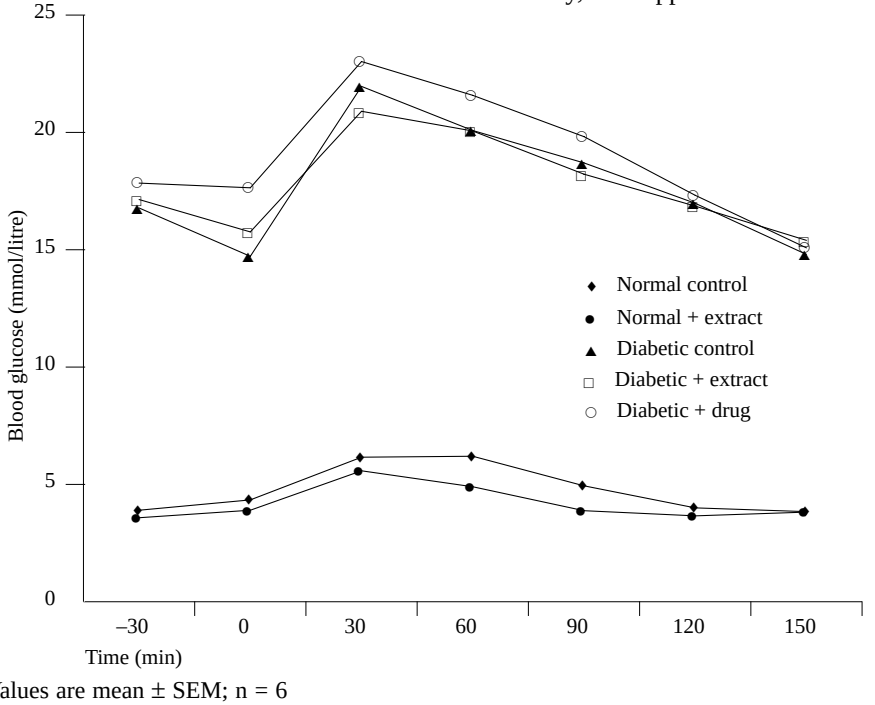
Results and discussion

OGTT in normal and diabetic rats

Results of the effect of *M. citrifolia* extract in OGTT are shown in *Figure 1*. The blood glucose levels of normal and diabetic rats reached a peak at 30 min after the administration of glucose solution and gradually decreased to the pre-glucose load level. The administration of *M. citrifolia* extract at a single dose (0.25 g/kg) did not significantly ($p > 0.05$) reduce the blood glucose level in normal rats as compared to the controls.

In the diabetic rats, the blood glucose levels were about three times higher than those of the normal rats *(Figure 1)*. Streptozotocin produces diabetic states by destroying the pancreatic β cells that secrete insulin, an important hormone for glucose metabolism (El-Fiky et al. 1996). As shown in *Figure 1*, the administration of *M. citrifolia* extract (0.25 g/kg) also did not significantly ($p > 0.05$) reduce the blood glucose levels in diabetic rats.

A similar result was observed in the glibenclamide-treated diabetic rats. In this study, the supplementation of an oral



Values are mean ± SEM; n = 6

ns = No significant differences for all treatments as compared to control groups ($p > 0.05$) according to Student's t-test

Figure 1. Effect of ***Morinda citrifolia*** *extract (0.25 g/kg) on blood glucose level in normal and diabetic rats (oral glucose tolerance test)*

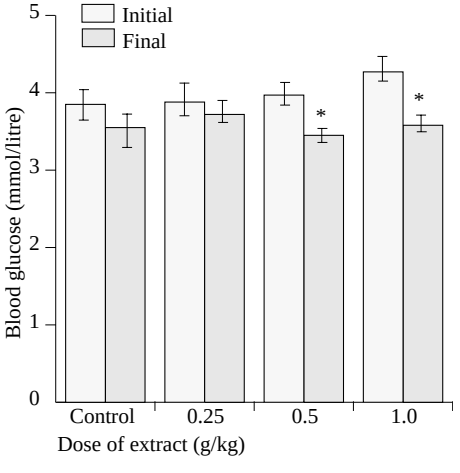
hypoglycemic drug, glibenclamide did not produce a significant decrease in the blood glucose level of diabetic rats probably because there may not have been enough active β pancreatic cells to secrete insulin as they were already destroyed by the streptozotocin (Peungvicha et al. 1998). The administration of a single dose of *M. citrifolia* extract also was not effective enough to lower the blood glucose levels of rats.

Subchronic administration of** Morinda citrifolia **extracts in normal and diabetic rats

Supplementation of medium (0.50 g/kg) and high dose (1.0 g/kg) of *M. citrifolia* extracts reduced final blood glucose levels significantly ($p < 0.05$) in normal rats as compared to initial blood glucose levels *(Figure 2)*. Similar results were also observed in extract-treated diabetic rats as shown in *Figure 3*. In both groups of rats, administration of the lowest dose of *M. citrifolia* extract (0.25 g/kg) failed to reduce the blood glucose levels ($p > 0.05$). This may be due to inadequate dosage of extract to stimulate the pancreatic β cells for insulin secretion.

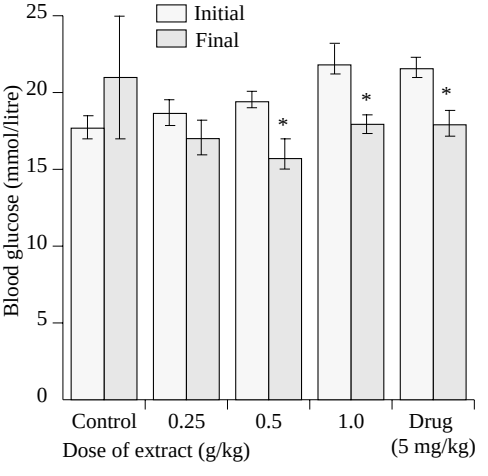
The administration of reference drug, glibenclamide also reduced blood glucose level significantly ($p < 0.05$) in diabetic rats *(Figure 3)*. It was reported that the administration of glibenclamide reduces the blood glucose by stimulating the secretion of insulin from pancreatic β cells and inhibition of glucagon secretion from pancreatic α cells (Gilman et al. 1990; Peungvicha et al. 1998).

The results of the present study indicated that an aqueous extract of *M. citrifolia* produced hypoglycemic activity (blood sugar lowering effect) in normal and diabetic rats in subchronic experiment. The mechanisms involved in reducing blood sugar levels by the extracts were not fully elucidated. However, based on the previous study, an anti-diabetic plant has certain active components that could exert a beneficial effect in the diabetic state by improving or mimicking insulin action or by enhancing insulin secretion (Gray and Flatt 1997).



Values are mean ± SEM; n = 6
*Final values are significantly different ($p < 0.05$) as compared to initial values according to Student's t-test

*Figure 2. Effect of **Morinda citrifolia** extracts administration for 6 weeks on blood glucose level in normal rats*



Values are mean ± SEM; n = 5
*Final values are significantly different ($p < 0.05$) as compared to initial values according to Student's t-test

*Figure 3. Effect of **Morinda citrifolia** extracts administration for 6 weeks on blood glucose level in diabetic rats*

Noor and Ashcroft (1998) also reported that some anti-diabetic plants act as an insulinotropic agent that can stimulate insulin release and potentiate glucose-induced insulin secretion. One of the active components that have hypoglycemic property is glycoside (Ur-Rahman and Zaman 1989). Two glycosides had been isolated successfully from the fruit of *M. citrifolia* (Mingfu et al. 1999), showing that this plant has potential to be used as anti-diabetic agent.

Conclusion

An aqueous extract of *Morinda citrifolia* exhibited blood's lowering activity in normal and diabetic rats in the subchronic study. The repeated doses of extracts throughout 6 weeks of experiment were more effective as compared to a single dose of extract, indicating the accumulative effect. Further study should be carried out to determine and understand the mechanisms of *M. citrifolia* extracts in lowering blood glucose. In addition, an extension of these findings to non-insulin dependent diabetic models (diabetes type II), which is a more common type of diabetes, deserves attention.

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Abstrak

Aktiviti hipoglisemik ekstrak *Morinda citrifolia* telah dikaji melalui ujian akut dan subkronik. Dalam ujian akut, dos tunggal ekstrak *Morinda citrifolia* (0.25 g/kg) telah diberikan secara oral kepada tikus normal dan diabetes melalui ujian toleransi glukosa. Dalam ujian subkronik, ekstrak *M. citrifolia* dengan kepekatan dos dari 0.25 g/kg (dos rendah), 0.50 g/kg (dos medium) dan 1.0 g/kg (dos tinggi) telah diberikan secara oral kepada tikus normal dan diabetes selama 6 minggu. Glibenclamide, sejenis dadah rujukan diberikan secara oral kepada kumpulan tikus diabetes pada dos 5 mg/kg dalam kedua-dua kajian.

Dalam ujian hipoglisemik akut, aras glukosa darah pada tikus normal dan diabetes tidak berubah secara signifikan apabila dibandingkan dengan tikus kawalan. Hasil yang sama juga telah diperhatikan pada kumpulan tikus yang dirawat dengan glibenclamide. Dalam ujian hipoglisemik subkronik, aras glukosa darah telah dapat diturunkan dengan signifikan (p <0.05) pada tikus normal dan diabetes yang telah diberi ekstrak *M. citrifolia* pada dos sederhana (0.5 g/kg) dan tinggi (1.0 g/kg) berbanding dengan tikus kawalan. Pemberian glibenclamide juga telah berjaya menurunkan aras glukosa darah dengan signifikan (p <0.05) pada tikus diabetes.

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