Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(5): 275-279

Received: 08-07-2016
Accepted: 09-08-2016

Halimatul Saadiah Mohammad Noor
A) Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
B) Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

Nor Hadiani Ismail
A) Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
B) Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

Noraini Kasim
A) Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
B) Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

Rozaini Mohd Zohdi
B) Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.
C)Department of Life Science, Faculty of Pharmacy, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

Abd. Manaf Ali
Department of Biotechnology, Faculty of Agriculture and Biotechnology, Universiti Sultan Zainal Abidin, 20300 Kuala Terengganu, Terengganu, Malaysia

Correspondence
Halimatul Saadiah Mohammad Noor
A) Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
B) Atta-ur-Rahman Institute for Natural Product Discovery, UiTM Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

Hypoglycemic and glucose tolerance activity of standardized extracts *Ficus deltoidea* varieties in normal rats

Halimatul Saadiah Mohammad Noor, Nor Hadiani Ismail, Noraini Kasim, Rozaini Mohd Zohdi and Abd. Manaf Ali

Abstract
Ficus deltoidea or its local Malaysian name "Mas Cotek" is a popular herbal plant with a long history of the use among the Malays to control blood sugar levels. Assessment of the seven different varieties of *F. deltoidea* available in the Peninsular Malaysia for *in vitro* α-glucosidase inhibitory has revealed different levels of activity warranting further *in vivo* studies. The acute toxicity test showed no signs of morbidity or mortality and the median lethal dose (LD_{50}) of the extracts for all varieties was higher than 2000 mg/kg body weight. Histopathological assessments of the kidney and liver did not show any abnormalities. Administration of the extracts at two different doses, 250 and 500 mg/kg in normal rats indicate *F. deltoidea* did not produce severe hypoglycemia. However, in oral glucose tolerance test (OGTT), the leaves extracts showed significant reduction in plasma glucose level after 30 minutes, albeit to different levels with the most effective being *F. deltoidea* var. *intermedia*.

Keywords: Diabetes, *Ficus deltoidea*, glucose tolerance

Introduction
The prevalence of diabetes has been escalating worldwide, in both developed and developing nations. Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose concentration, caused by insulin deficiency and often combined with resistance. The number of diabetic cases is projected to increase to 300 million by 2025 [1]. The World Health Organization (WHO) has estimated that in the year 2030, Malaysia would have a total of 2.48 million people with diabetes. Although there are plenty of antidiabetic agents available, some produce serious side effects such as hypoglycemic coma and hepatorenal disturbance [2]. Some insulin secretagogues or sensitizers are associated with tremendous weight gain [3]. In addition, such antidiabetic drugs are not safe for use during pregnancy [4]. Thus the search of safer products for management of diabetes, especially from natural origin is very important. *Ficus deltoidea* (Moraceae) is an evergreen shrub reaching to 2 meters of height, with whitish grey bark, broadly spoon-shaped to obovate leaves and spherical or round figs [5]. *F. deltoidea* has been traditionally claimed to have antidiabetic property and has been used as traditional remedy for diabetes management based on the ethnobotanical approaches [6]. There are at least seven varieties of *F. deltoidea* available in Malaysia. In the current work, we analyzed the chemical profiles using high performance liquid chromatography (HPLC) and evaluated the *in vitro* α-glucosidase inhibitory activities of the seven varieties as well as *in vivo* toxicity, hypoglycemic effect and oral glucose tolerance properties in normal rats.
Previous studies have illustrated the glucose lowering effect of aqueous *F. deltoidea* extract in normal rats [7] and mild diabetic rats [8]. However, these reports did not specify the variety of *F. deltoidea* used. In this work, we established an optimized extraction protocol using aqueous ethanol as extraction solvent and compared HPLC profiles which were found to differ significantly among the seven varieties (Figure 1). *In vitro* α-glucosidase inhibitory activity of the extracts showed varying degree of activity (Table 1), which is not surprising since these extract have relatively different chemical profiles. Good inhibition were found for varieties *trengganuensis, kunstleri, intermedia* and *deltoidea* with percentage inhibitory of 67.43, 66.48, 63.61 and 57.55%, respectively followed by weak inhibition for varieties *motleyana* and *bilobata*. Variety *angustifolia* did not show any inhibition at all. The above observation led us to a comparative *in vivo* study of these varieties looking into hypoglycemic and glucose

tolerance activity in normal rat model. However, due to the scarcity of varieties *motleyana* and *bilobata*, only five varieties were compared.

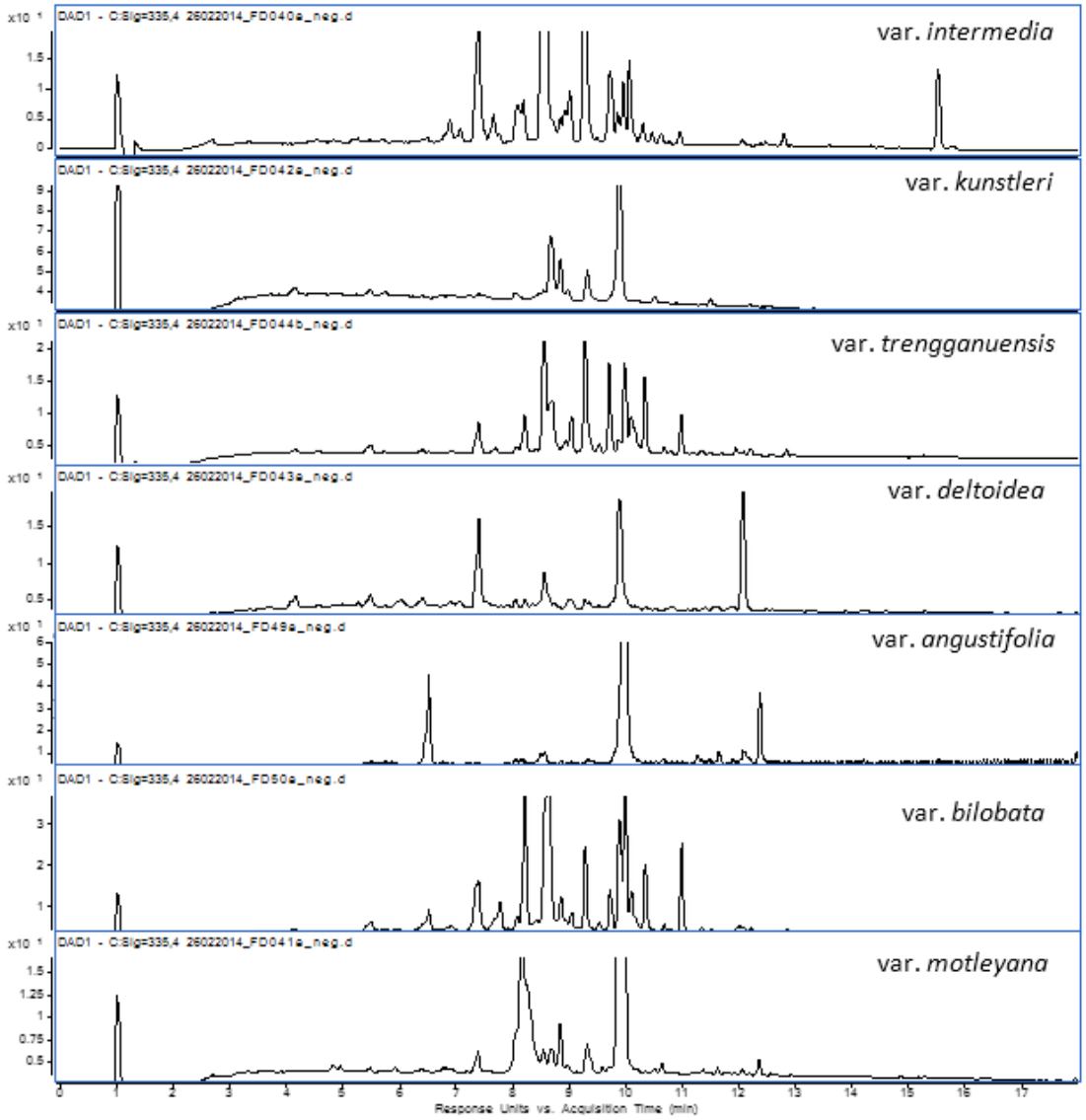

Fig 1: UV 335 nm chromatogram of seven *F. deltoidea* varieties using High performance Liquid Chromatography (HPLC).

Table 1: α- Glucosidase Inhibitory Activity of *F. deltoidea* ethanolic aqueous extracts (at 100 ppm extract).

***F. deltoidea* variety**	**% of inhibition**
intermedia	63.61 ± 8.59
kunstleri	66.48 ± 14.04
trengganuensis	67.43 ± 6.24
deltoidea	57.55 ± 6.27
angustifolia	No inhibition
bilobata	14.37 ± 6.91
motleyana	21.48 ± 4.17
Acarbose	No inhibition

Materials and Methods

Plant material

Five varieties of *F. deltoidea* leaves used in the study were *trengganuensis*, *intermedia*, *kunstleri*, *deltoidea* and *angustifolia*. Varieties *trengganuensis*, *deltoidea* and *kunstleri* were collected in Kuala Terengganu, Terengganu while variety *intermedia* were collected from Cameron Highland, Pahang. Otherwise, variety *angustifolia* was collected from Batu Pahat, Johor.

Extract preparation

Ethanolic aqueous extracts of the different varieties of *F. deltoidea* was prepared by soaking the dried leaves powder into 50% ethanol (100g/L) and sonicated at 40 ºC for 30 minutes. The combined suspension was filtered and evaporated using rotary evaporator under vacuum to dryness. The extract was then freeze dried to remove residual water.

Animal

Animal protocol was approved by UiTM Committee on Animal Research & Ethics (UiTM CARE). Healthy Sprague Dawley male rats used in were kept in Laboratory Animal Facility and Management (LAFAM), Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus. Rats between 8-12 weeks of age and weighing about 300-400 g were housed in individual ventilated cage (IVC) maintained under standard condition (12h light and 12h dark cycle; 25 ± 30 ºC; 35-60 % humidity).

Acute toxicity studies

Starved overnight rats (12h) were divided into four groups (n=3) and were orally fed with *F. deltoidea* extract in increasing dose levels of 5, 50, 300 and 2000 mg/kg body weight using G18 animal feeding needle. The rats were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days for any lethality or death. The rats were weight the day of dosing, weekly intervals thereafter and at the time of death or sacrificed ((OECD/ OCDE) guideline for the testing of chemical 423, adopted on 17th December 2001)

Hypoglycemic effect

Rats were fasted 12 hours prior to test. After fasting period, rats were administered with *F. deltoidea* extracts and metformin at two different doses, 250 and 500 mg/kg orally using G18 animal feeding needle. Blood samples were taken from rat tail tip before (0 hour) and after 30, 60 120 minutes and continued to 4 and 6 hours after extracts or metformin administration.

Oral glucose tolerance test (OGTT)

OGTT was performed on overnight fasted (18h) normal rats [10]. Rats divided into three groups (n=6) were administered drinking water, ethanolic extracts of *F. deltoidea* 250 and 500 mg/kg, respectively for each varieties of *F. deltoidea*. Fasting blood glucose was checked in fasted normal rats (-30 mins) followed by oral administration of *F. deltoidea* ethanolic aqueous extracts suspended in 1% carboxymethylcellulose (CMC). Thirty minutes later (at 0 hour), rats of all groups were given glucose (2g/kg) orally. Blood samples were collected from the rat tail tip prior to glucose administration (0 min) and 15, 30, 60, 90 and 120 minutes after glucose administration.

Statistical analyses

Standard deviation (x ± s) was used to represent numerical variables while one-way ANOVA was used to compare the mean values of multiple samples. SPSS17.0 statistical software was employed to analyze the experimental data.

Results and Discussion

Acute toxicity study revealed the non-toxic nature of the leaves extracts of the *F. deltoidea* varieties tested. No mortality or behavioral changes were observed in rats treated with 2000 mg/kg of the extracts indicating that the LD_{50} is higher than this dose. Histopathological examination revealed normal architecture and no significant change were observed on the liver and kidney (Figure 2 and 3)

A: Control; B: Treated group (2000 mg/kg)

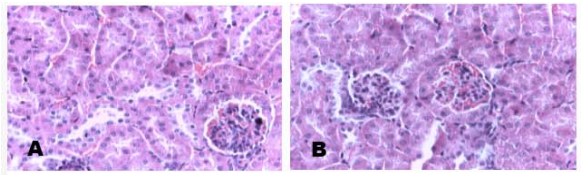

Fig 2: Histophatology of kidney

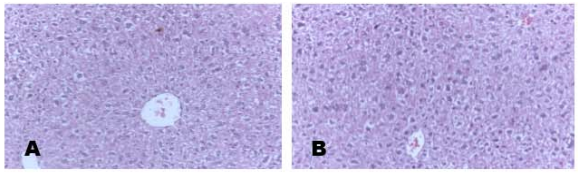

Fig 3: Histophatology of liver

Among all varieties tested, the hypoglycemic effect was maximally observed with variety *trengganuensis* at dose 250 mg/kg and *intermedia* at dose 500 mg/kg compared to metformin at those doses (Table 2). Percentage of reduction in glucose levels after 2 hours administration at dose 250 mg/kg of varieties *intermedia*, *kunstleri*, *trengganuensis* and *deltoidea* were 10.90, 4.80, 23.88, 2.56% respectively, while at dose 500 mg/kg were 14.81, 2.51, 3.85, 7.12%, respectively. Metformin cause a significant (P<0.001) reduction of 16.78% at dose 250 mg/kg and 12.40% at dose 500 mg/kg in glucose levels 2 hours after its administration. Control rats and groups of rats treated with variety *angustifolia* did not exhibit any significant alteration in their glucose levels through the duration of the experiment. For the period of 6 hours on blood glucose assessment, normoglycemic level of blood glucose in the normal rats was maintained after oral administration of extract *F. deltoidea* varieties (Table 3).

Table 2: Hypoglycemic effect of 250 and 500 mg/kg ethanolic aqueous extracts *F. deltoidea* varieties and metformin after 30, 60 and 120 minutes administration in normal rats.

Treatment Group	Dose of extract (mg/kg)	Basal	30m	60 m	120 m
C	-	4.83 ± 0.21	4.97 ± 0.13	5.12 ± 0.32	5.40 ± 0.78
FDI	250	3.53 ± 0.64	3.42 ± 0.56	3.35 ± 0.55	3.17±0.55(10.90)**
	500	5.40 ± 0.44	5.19 ± 0.33	5.00 ± 0.31	4.60±0.50(14.81)**
FDK	250	3.33 ± 0.93	3.22 ± 0.41	3.30 ± 0.11	3.17±0.15(4.80)**
	500	3.97 ± 0.25	3.97 ± 0.04	4.07 ± 0.36	3.87±0.67(2.51)**
FDT	250	4.77 ± 0.85	4.48 ± 0.60	4.19 ± 0.43	3.63 ± 0.57(23.88)**
	500	5.20 ± 0.10	5.15 ± 0.04	5.08 ± 0.03	5.00 ± 0.17(3.85)**
FDD	250	3.90 ± 0.26	3.94 ± 0.15	3.78 ± 0.66	3.80 ± 1.30 (2.56)**
	500	4.63 ± 0.45	4.55 ± 0.44	4.41 ± 0.46	4.30 ± 0.44 (7.12)**
FDA	250	3.63 ± 0.49	3.81 ± 0.49	3.98 ± 0.71	4.33 ± 0.92 (0)
	500	3.67 ± 0.32	3.76 ± 0.40	3.85 ± 0.49	4.03 ± 0.67 (0)
MET	250	4.53 ± 0.95	4.32 ± 0.76	4.08 ± 0.59	3.77±0.15 (16.78)**
	500	4.03 ± 0.32	3.80 ± 0.64	3.77 ± 0.75	3.53±0.67 (12.40)**

Table 3: Hypoglycemic effect of 250 and 500 mg/kg ethanolic aqueous extracts *F. deltoidea* varieties and metformin after 2, 4 and 6 hours administration in normal rats.

Treatment Group	Dose of extract (mg/kg)	Basal	2h	4h	6h
NC	-	4.83 ± 0.21	5.40 ± 0.78	5.90 ± 0.30	5.97 ± 0.86
FDI	250	3.53 ± 0.64	3.17 ± 0.55	3.97 ± 1.24	4.73 ± 0.32
	500	5.40 ± 0.44	4.60 ± 0.50	4.63 ± 0.06	4.50 ± 0.53
FDK	250	3.33 ± 0.93	3.17 ± 0.15	3.03 ± 0.57	3.47 ± 0.47
	500	3.97 ± 0.25	3.87 ± 0.67	3.77 ± 0.45	4.00 ± 0.75
FDT	250	4.77 ± 0.85	3.63 ± 0.57	3.33 ± 0.23	3.47 ± 0.49
	500	5.20 ± 0.10	5.00 ± 0.17	4.13 ± 0.15	4.27 ± 0.60
FDD	250	3.90 ± 0.26	3.80 ± 1.30	3.97 ± 0.31	4.07 ± 0.15
	500	4.63 ± 0.45	4.30 ± 0.44	4.30 ± 0.20	3.97 ± 0.25
FDA	250	3.63 ± 0.49	4.33 ± 0.92	3.90 ± 0.26	4.13 ± 0.25
	500	3.67 ± 0.32	4.03 ± 0.67	3.73 ± 0.15	3.97 ± 0.42
MET	250	4.53 ± 0.95	3.77 ± 0.15	3.80 ± 0.20	4.07 ± 0.31
	500	4.03 ± 0.32	3.53 ± 0.67	3.97 ± 0.21	3.90 ± 0.36

C: Control; FDI: *F. deltoidea* var. *intermedia;* FDK: *F. deltoidea* var. *kunstleri*; FDT: *F. deltoidea* var. *trengganuensis*; FDD: *F. deltoidea* var. *deltoidea;* FDA: *F. deltoidea* var. *angustifolia;* MET: Metformin.

Note: Values in bracket indicate percentage lowering of blood glucose relative to basal reading
***p*<0.001 compared with normal control group.

In oral glucose tolerance test (OGTT), the highest increase in blood glucose levels was observed 30 min after glucose administration and starts to reduce significantly after 30 minutes onward. Areas under the glucose curve ($AUC_{Glucose}$) for each individual rat were calculated to determine the increment of blood glucose concentration from 0 to 120 minutes. The results showed that extract of variety *intermedia* at dose of 500 mg/kg significantly attenuated $AUC_{Glucose}$ value by 15.74% (*p*<0.01) compared with the control group. (Table 4).

Table 4: $AUC_{Glucose}$ value (mmol/L); Calculation of area under the curve in OGTT (Trapezoid rule).

Treatment Group	Dose of extract (mg/kg)	AUC $_{Glucose}$ value (mmol/L) (mean ± SD)
Normal Control	-	945.00 ± 32.41
intermedia	250	874.00 ± 31.61 (7.51)*
	500	796.25 ± 40.05 (15.74)*
kunstleri	250	858.00 ± 54.08 (9.21)*
	500	855.38 ± 44.88 (9.48)*
trengganuensis	250	919.88 ± 53.70 (2.65)*
	500	852.00 ± 32.22 (9.84)*
deltoidea	250	895.00 ± 40.57 (5.29)*
	500	878.63 ± 40.21 (7.02)*
angustifolia	250	932.38 ± 51.35 (1.34)*
	500	900.63 ± 32.23 (4.70)*
Metformin	250	816.25 ± 27.40 (13.62)***
	500	808.88 ± 34.71 (14.40)***

Note: Values in bracket indicate percentage of $AUC_{Glucose}$ attenuation relative to control group.
**p*<0.05 and ****p*<0.001 compared with control group.

In this study, all the varieties tested except *angustifolia* exhibit a significant hypoglycemic effect in normal rats and this was proven during 2 hours after administration of the plant extracts. However for a period of 6 hours after the extracts administration, normoglycemic level of blood glucose was maintained. This indicates that *F. deltoidea* did not produce severe hypoglycemia which should be avoided in diabetic patient. Glucose tolerance results give an evaluation as tendency of the extract to improve glucose tolerance activity. Significant reduction of the blood glucose level in OGTT was found after administration of *F. deltoidea* extract indicates that there are antihyperglycemic compounds in the extracts. Metformin act as positive control to identify the differentiation with the plant extracts as the potential in reducing blood glucose concentration as metformin is a conventional antihyperglycemic agent. On the two doses tested, 500 mg/kg produced expected higher glucose tolerance activities due to higher dose which correlate to the glucose tolerance activity. The lower of glucose tolerance activity of *F. deltoidea* extracts compared to the metformin could be due to combination of bioactive and non-bioactive constituents. In contrast, metformin consists of single constituents and its antihyperglycemic activity has been scientifically proven [9]. Further evaluations need to be carried out to assess hypoglycemic/anti-hyperglycemic effect of the plant extracts for a longer duration to avoid dismissing the effectiveness of the extracts if the plants have a late onset of activity. Target compound present in the extract should also be isolated and tested *in vivo* to evaluate the potential of the compound in reducing glucose concentration in blood.

Effect of 250 and 500 mg/kg ethanolic aqueous extract *F. deltoidea* varieties in blood glucose of normal rats and area under the curve (AUC Glucose value (mmol/L)) indicate that the most relevant extracts comparable to metformin were varieties *intermedia* followed by *trengganuensis*, *kunstleri* and *deltoidea*.

Acknowledgements
The author wish to thank Ministry of Agriculture for given the grant entitled "*In vivo* Antidiabetic Studies of Standardized Extracts *Ficus deltoidea* Varieties" under the file number of 100-RMI/MOA 16/6/2 (6/2015).

References

1. Hodavance MS, Ralston SL, Pelczer I. Beyond blood sugar the potential of NMR-based metabolomics for human diabetes type 2: the horse as a possible model. Analytical Bioanalytical Chemistry 2007; 387:533-537.
2. Suba V, Murugesan T, Arunachalam G, Mandal SC, Saha BP. Antidiabetic potential of *Barleria lupulina* extract in rats. Phytomedicine 2004; 11:202-205.
3. Hays NP, Galassetti PR, Coker RH. Prevention and treatment of type 2 diabetes: Current role of life style, natural products and pharmacological interventions. Pharmacology Therapeutics 2008; 118:181-191.
4. Rahman AU, Zaman K. Medicinal plants with hypoglycemic activity. Ethnopharmacology 1989; 26:1-55
5. Corner EJH. The complex of *Ficus deltoidea*; a recent invasion of the sunda shelf. Philosophical Transactions of the Royal Society 1969; 256:281-317.
6. Adam Z, Hamid M, Ismail A, Khamis S. Effect of *Ficus deltoidea* extracts on hepatic basal and insulin-stimulated glucose uptake, Journal of Biological Sciences. 2009; 9(8):796-803.
7. Adam Z, Hamid M, Ismail A, Khamis S. Effect of *Ficus deltoidea* aqueous extract on blood glucose level in normal and mild diabetic rats, Malaysian Journal of Health Sciences. 2007; 5(2): 9-16.
8. Aminuddin N, Sin CY, Chee ES, Nee KL, Renxin L. Blood glucose lowering effect of *Ficus deltoidea* aqueous extract, Malaysian Journal of Health Science. 2007; 26(1): 73-78.
9. Chehade JM, Mooradian AD. A rational approach to drug therapy of type 2 diabetes mellitus. Drugs 2000; 60(1): 95-113.
10. Bonner-Weir S. Morphological evidence of pancreatic polarity of beta cells within islets of Langerhans. Diabetes 1988; 37: 616-621.