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## Inhibitory Properties of *Ficus deltoidea* on $\alpha$ -Glucosidase Activity

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**Abstract:** The present study was undertaken to evaluate the potential of *Ficus deltoidea* to inhibit the enzyme,  $\alpha$ -glucosidase in the small intestine, as this is known to be an antidiabetic mechanism. The results of the *in vitro* study had shown that hot aqueous, ethanolic and methanolic extracts of *Ficus deltoidea* inhibit rat intestine  $\alpha$ -glucosidase activity significantly, in a concentration dependent manner. The IC<sub>50</sub> values of these extracts are 4.15, 2.06 and 1.72 mg mL<sup>-1</sup>, respectively. From a study of the enzyme kinetics, all extracts exhibited a mixed-type inhibition mechanism against the activity of  $\alpha$ -glucosidase. In an animal study, all extracts, when used at a dose of 1000 mg kg<sup>-1</sup> b.wt., reduce postprandial hyperglycemia following sucrose administration. This was true in normal and in diabetic rats, as shown by the significant attenuation of the value of AUC<sub>Glucose</sub> compared to the control group. The methanolic extract is the most potent inhibitor of  $\alpha$ -glucosidase, followed by the ethanolic and hot aqueous extracts. We suggest that the inhibition of  $\alpha$ -glucosidase in the small intestine in part mediates the antidiabetic property of *Ficus deltoidea*.

**Key words:** *Ficus deltoidea*, antidiabetic,  $\alpha$ -glucosidase, mixed-type inhibition mechanism, postprandial hyperglycemia

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### INTRODUCTION

Postprandial hyperglycemia is a condition in which the blood glucose level rises in an exaggerated manner following a meal. It can induce the elevation of the level of glycosylated hemoglobin (HbA<sub>1c</sub>), thereby contributing to the development of macro and microvascular complications that have been associated with diabetes (Baron, 1998). Postprandial hyperglycemia is also a direct and independent risk factor for cardiovascular disease (Ceriello, 2005). Moreover, it is implicated in the development of type II diabetes mellitus and may lead to dysfunction of the pancreatic  $\beta$ -cells (Baron, 1998). Therefore, correcting postprandial hyperglycemia is critical in the early treatment of diabetes mellitus, as it can suppress the levels of HbA<sub>1c</sub> and prevent diabetic complications such as cardiovascular disease (Baron, 1998; Ceriello, 2005).

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Apart from insulin therapy, postprandial hyperglycemia can be treated therapeutically through delaying the digestion of carbohydrate, thereby reducing the rate of absorption of glucose from the small intestine (Uebanso *et al.*, 2007). This is done by inhibiting enzymes that are responsible for carbohydrate digestion such as  $\alpha$ -amylase and  $\alpha$ -glucosidase in the digestive organs (Coniff and Krol, 1997). The  $\alpha$ -amylase catalyzes the first step in the breakdown of complex carbohydrates (polysaccharides) and  $\alpha$ -glucosidase catalyzes the breakdown of oligosaccharides and disaccharides to simple absorbable sugars (Fujisawa *et al.*, 2005). The  $\alpha$ -glucosidase inhibitors are oral antidiabetic agents that are used widely in the treatment of postprandial hyperglycemia (Kim *et al.*, 2005). Acarbose, the first of the  $\alpha$ -glucosidase inhibitors to be discovered, has been reported to reduce postprandial hyperglycemia and the level of HbA1c by approximately 50 mg dL<sup>-1</sup> and 0.86%, respectively (Li *et al.*, 2005). However, the use of this drug is associated with non-desirable effects such as gastrointestinal problems (Coniff and Krol, 1997), lethal ileus (Odawara *et al.*, 1997) hepatotoxicity (Andrade *et al.*, 1998) and renal tumors (Charpentier *et al.*, 2000). Due to these side effects, there is a need for searching of new effective  $\alpha$ -glucosidase inhibitors that have fewer side effects. Plants are considered safe for use and to have fewer side effects. Therefore, they have been widely used in the search for new antidiabetic agents (Li *et al.*, 2005). The  $\alpha$ -glucosidase inhibitors derived from plants have been studied and isolated widely (Seo *et al.*, 2005; Du *et al.*, 2006; Lysek *et al.*, 2006).

*Ficus deltoidea*, from the Moraceae family, is commonly used as a medicinal plant in Malaysia (Mat-Salleh and Latif, 2002). The plant is native to Southeast Asia and the Philippines (Forest *et al.*, 2003). Based on ethnobotanical approaches, it has been claimed that *F. deltoidea* has antidiabetic properties and it has been used traditionally as a treatment for diabetes. Nevertheless, scientific evidence to confirm its efficacy and its possible mode of action is still lacking. Sucrose has been reported to induce stronger hyperglycemia than other dietary carbohydrates, including starch (Uebanso *et al.*, 2007). Therefore, we have used sucrose as the substrate to measure the effect of the fractions and extract of *F. deltoidea* on the activity of  $\alpha$ -glucosidase. The effect of these extracts on the postprandial blood glucose level was also evaluated in normal and diabetic rats following sucrose administration.

## MATERIALS AND METHODS

This study was conducted from January to July, 2009 at Medical Technology Division, Malaysian Nuclear Agency.

### Chemicals

Rat intestine acetone powder was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan) and 0.9% saline was purchased from B. Braun Medical Inc., (Pennsylvania, USA). Maleic acid, TRIS-hydrochloride, sodium hydroxide, acarbose and sucrose were purchased from the Sigma Chemical Co. (St. Louise, USA). Ethanol, methanol and n-butanol were purchased from JT Baker Reagent Chemicals (New Jersey, USA). Sulphuric acid, diethyl ether and chloroform were purchased from Merck (Darmstadt, Germany).

### Plant Material and the Extraction Procedure

Plants of *F. deltoidea* were collected at Sungai Tengi Selatan, Selangor, Malaysia. The plants were identified by a taxonomist from the Biodiversity Unit of the Institute of Bioscience, Universiti Putra Malaysia. A specimen (SK1467/07) was deposited at the herbarium of the above institute. The leaves of *F. deltoidea* were oven dried at 45°C and

ground to a fine powder. Hot aqueous extracts were prepared by boiling the powdered sample in distilled water for 3 h ( $100 \text{ g L}^{-1}$ ) by changing the water every hour. The combined suspension was filtered using Whatman filter paper No. 54 and freeze dried to give the powdered form. Ethanolic and methanolic extracts were prepared by soaking the sample powder ( $100 \text{ g L}^{-1}$ ) in 70% ethanol and 90% methanol, respectively, for 3 days at room temperature. The solvent was changed daily. The combined suspension was filtered using Whatman filter paper No. 54 and evaporated to dryness under reduced pressure at  $30^\circ\text{C}$ . To prepare the chloroform fraction, a third quarter of the total yield of the methanolic extract was acidified with 5% sulphuric acid and extracted with chloroform (3×3:1). The acidified chloroform extract was separated from the aqueous portion using a separation funnel and evaporated to dryness under reduced pressure at  $30^\circ\text{C}$ . The aqueous portion was then extracted with n-butanol (3×3:1). The n-butanol fraction was separated from the aqueous portion using a separation funnel and evaporated to dryness in the fume hood.

#### ***In vitro* $\alpha$ -Glucosidase Inhibition Assay**

The *in vitro*  $\alpha$ -glucosidase inhibition assay was performed according to the method of Ohta *et al.* (2002) with slight modification. To prepare the crude  $\alpha$ -glucosidase solution, we suspended 100 mg of rat-intestinal acetone powder in 1 mL of 0.9% saline and sonicated the suspension three times, for 1 min each time. The suspension was centrifuged at 3000 rpm for 30 min. The supernatant containing the crude  $\alpha$ -glucosidase solution was separated and used as our source of the enzyme. The protein content was measured using commercial Bradford protein assay kits from Invitrogen (California, USA).

#### **The Assay of $\alpha$ -Glucosidase Activity in the Presence of $5000 \mu\text{g mL}^{-1}$ *F. deltoidea***

The reaction mixture consisted of 350  $\mu\text{L}$  of 100 mM maleate buffer pH 6.0, 50  $\mu\text{L}$  of 50 mM sucrose and 50  $\mu\text{L}$  of either  $5000 \mu\text{g mL}^{-1}$  *F. deltoidea* or  $65 \mu\text{g mL}^{-1}$  acarbose, placed in 1.5 mL tubes. Negative control tubes contained all of the above, except that the test substances were replaced with distilled water. The mixtures were pre-incubated for 5 min at  $37^\circ\text{C}$ . To initiate the enzyme reaction, 50  $\mu\text{L}$  of crude  $\alpha$ -glucosidase was added to each tube and the reaction was incubated for a further 60 min at  $37^\circ\text{C}$ . The enzyme reaction was terminated by adding 500  $\mu\text{L}$  of 2 M Tris-Maleate-NaOH buffer at pH 7.4. The glucose that was produced during the reaction was measured using the Megazyme Glucose Test Kit, obtained from Megazyme International (Wicklow, Ireland).

#### **Dose Response Evaluation and Determination of the $\text{IC}_{50}$ Value**

We determined the  $\text{IC}_{50}$  values of the *F. deltoidea* extract or the fractions that showed greater than 50% inhibition against  $\alpha$ -glucosidase activity. The crude  $\alpha$ -glucosidase solution was incubated with 50 mM sucrose and increasing concentrations of *F. deltoidea* ( $156$ - $5000 \mu\text{g mL}^{-1}$ ) or acarbose ( $4.06$ - $130 \mu\text{g mL}^{-1}$ ). The concentration of *F. deltoidea* or acarbose that caused 50% inhibition ( $\text{IC}_{50}$  value) of the  $\alpha$ -glucosidase activity was determined through a nonlinear regression analysis of the dose response curve.

#### **Kinetics Analysis of the Inhibition of $\alpha$ -Glucosidase**

To determine the mechanism of inhibition of  $\alpha$ -glucosidase by *F. deltoidea*, we measured the enzyme activity in the absence or presence of two different concentrations (2 and 3  $\text{mg mL}^{-1}$ ) of the *F. deltoidea* extracts with increasing concentrations of sucrose (0, 1.25, 2.5, 5.0 and 10.0 mM). The type of inhibition of the *F. deltoidea* extracts against the activity of  $\alpha$ -glucosidase was analyzed using Lineweaver-Burk plots.

### **The Effect of the Extracts of *F. deltoidea* on Postprandial Blood Glucose Levels Following Sucrose Loading**

We used normal male adult and streptozotocin-induced diabetic Sprague Dawley rats weighing 200-250 g in this study. The animals were bred in house at the Animal House of the Malaysian Nuclear Agency. The animals were fed using a standard laboratory pellet diet and water was supplied *ad libitum*. All animal procedures were approved by the Animal Care and Use Committee (ACUC), of the Faculty of Medicine and Health Science, Universiti Putra Malaysia (ACUC no: UPM/FPSK/PADS/BR/UUH/F01-00208). Diabetes was induced using a single intravenous (tail vein) injection of sterile streptozotocin (60 mg kg<sup>-1</sup> b.wt., 100 mg mL<sup>-1</sup> in distilled water) given under ether anesthesia. The streptozotocin solution was prepared immediately before use, as the drug is very labile in solution. The fasting blood glucose was checked 7 days after the injection and the rats that had blood glucose levels at 13.0 mmol L<sup>-1</sup> or above were considered diabetic and used as the experimental animals (Kesari *et al.*, 2006).

The rats were fasted 12 h before the test. After the fasting period, the animals were divided randomly into five equal groups (seven rats in each group). Group I (the control rats) were given sucrose (2 g kg<sup>-1</sup> b.wt.) orally using intragastric gavage. Group II was given sucrose (2 g kg<sup>-1</sup> b.wt.) combined with acarbose (50 mg kg<sup>-1</sup> b.wt.) orally using intragastric gavage. Groups III, IV and V were given sucrose (2 g kg<sup>-1</sup> b.wt.) combined with (respectively) the ethanolic and methanolic and hot aqueous extracts of *F. deltoidea* (1000 mg kg<sup>-1</sup> b. wt.) orally using intragastric gavage. Blood samples were collected for the measurement of glucose concentration immediately before (0 min) and 30, 60, 90 and 120 min after, the tested materials had been administered. The areas under the glucose curve (AUC<sub>Glucose</sub>) during the 120 min observation period were calculated using computer calculator software provided by Thomas Wolever, from the Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada (Jalil *et al.*, 2008).

### **The Collection of Blood and the Biochemical Analysis**

Blood samples were collected from the tip of the rats tails under mild ether anesthesia. The glucose level was determined using an electronic glucometer, Accu Check Advantage from Roche Diagnostic (Indianapolis, USA).

### **Statistical Analysis**

All results are expressed as the Mean±SD for a given number of observations. Statistical analysis were done according to Musa (1993). The data were analyzed using one way Analysis of Variance (ANOVA), followed by Tukey's post hoc test. The group means were considered significantly different at the level of p<0.05.

## **RESULTS**

### **α-Glucosidase Inhibition in the Presence of 5000 µg mL<sup>-1</sup> *F. deltoidea***

Table 1 shows the results of a preliminary evaluation of the inhibitory activity of 5000 µg mL<sup>-1</sup> *F. deltoidea* and 65 µg mL<sup>-1</sup> acarbose against the activity of rat intestine α-glucosidase. All *F. deltoidea* extracts and fractions show inhibitory activity against α-glucosidase. Among the *F. deltoidea* extracts and fractions, the methanolic extract had the highest inhibitory activity, followed by the ethanolic extract, the hot aqueous extract, the n-butanolic fraction and the chloroform fraction. The ethanolic, methanolic and hot aqueous extracts inhibited the activity of the enzyme more than 50% relative to the untreated control at these concentrations. Therefore, these extracts were evaluated further by determining the

Table 1: Inhibitory effect of *F. deltoidea* against rat intestine  $\alpha$ -glucosidase activity

Tested materials	$\alpha$ -glucosidase inhibition (%)	IC <sub>50</sub> value (mg mL <sup>-1</sup> ) / (95% C.I.)
Hot aqueous extract <sup>a</sup>	53.04±3.37	4.15±0.25 (3.53-4.77)
Ethanollic extract <sup>a</sup>	75.83±2.79	2.06±0.22 (1.52-2.62)
Methanolic extract <sup>a</sup>	80.12±2.19	1.72±0.58 (0.27-3.19)
Chloroform fraction <sup>a</sup>	6.49±2.70	ND
N-butanolic fraction <sup>a</sup>	26.06±4.34	ND
Acarbose <sup>b</sup>	86.12±2.33	0.033±0.003 (0.028-0.039)

<sup>a</sup>*F. deltoidea* extracts and fractions were preliminary tested at a concentration of 5000  $\mu$ g mL<sup>-1</sup>. <sup>b</sup>Reference standard, acarbose was preliminary tested at a concentration of 65  $\mu$ g mL<sup>-1</sup>. The *F. deltoidea* or acarbose were pre-incubated with maleate buffer and sucrose for 5 min at 37°C. Subsequently, the crude  $\alpha$ -glucosidase solution was added into each tube and further incubated for 60 min at 37°C. The enzyme reaction was terminated by adding Tris-Maleate-NaOH buffer. The glucose that was produced during the reaction was measured using the Megazyme Glucose Test Kit. The percentages of  $\alpha$ -glucosidase inhibition at the mentioned concentration were expressed the Mean±Standard Deviation of four replicates. Test substances that showed greater than 50% inhibition against  $\alpha$ -glucosidase activity were further evaluated for determination of IC<sub>50</sub> value. The IC<sub>50</sub> value was expressed as the mean ± standard deviation of three independent experiments. ND: Not determined

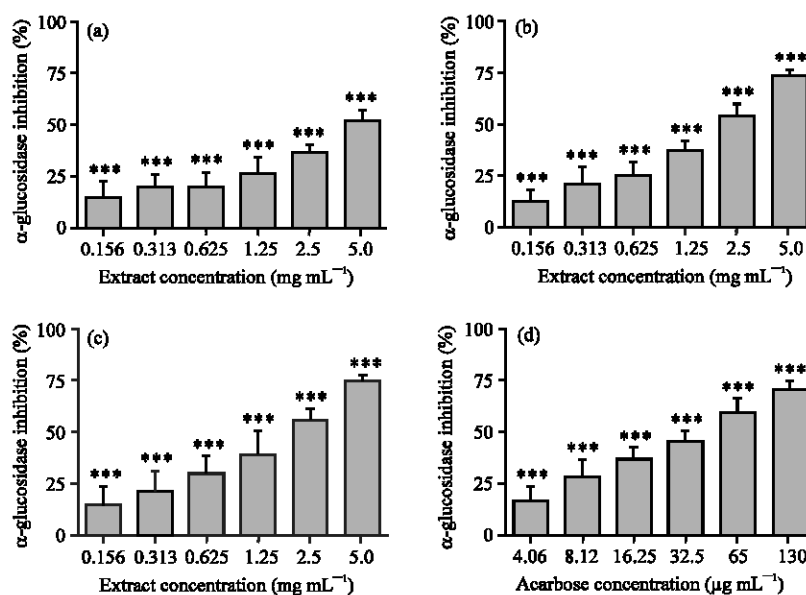


Fig. 1: Dose response inhibition of the (a) *F. deltoidea* hot aqueous extract, (b) the ethanollic extract, (c) the methanolic extract and (d) acarbose against rat intestine  $\alpha$ -glucosidase activity. The data are expressed as the Means±SD of four replicates from three independent experiments. \*\*\*p<0.001 compared to the control incubation (0% inhibition)

IC<sub>50</sub> values through a dose response evaluation. The inhibitory activity of all the *F. deltoidea* extracts was less than that of acarbose, which inhibited the activity of  $\alpha$ -glucosidase the most.

### The Dose Response Evaluation and the Determination of the IC<sub>50</sub> Value

The results from the dose response evaluation of the inhibition of rat intestine  $\alpha$ -glucosidase activity by the ethanollic extract, methanolic and hot aqueous extracts of *F. deltoidea* and acarbose are shown in Fig. 1a-d. The *F. deltoidea* extracts (0.156-5 mg mL<sup>-1</sup>) and acarbose (4.06-130  $\mu$ g mL<sup>-1</sup>) exhibited a concentration-dependent

inhibition effect against the activity of rat intestine  $\alpha$ -glucosidase and evoked a significant inhibition ( $p < 0.001$ ) at all concentrations evaluated. The  $IC_{50}$  values were determined using a nonlinear regression analysis of the dose response curve. The methanolic extract had the lowest  $IC_{50}$  value, followed by the ethanolic extract and the hot aqueous extract (Table 1). Nevertheless, the  $IC_{50}$  value of these extracts was much higher than that of acarbose.

### Kinetic Analysis of the Inhibition of $\alpha$ -Glucosidase

The enzyme kinetics were analyzed to determine the inhibitory mechanism of the *F. deltoidea* extracts against  $\alpha$ -glucosidase. Analysis of the Lineweaver-Burk plots revealed that all the extracts of *F. deltoidea* possess a mixed-type inhibition mechanism against rat intestine  $\alpha$ -glucosidase (Fig. 2). The values of  $V_{max}$  and  $K_m$  were also determined from the

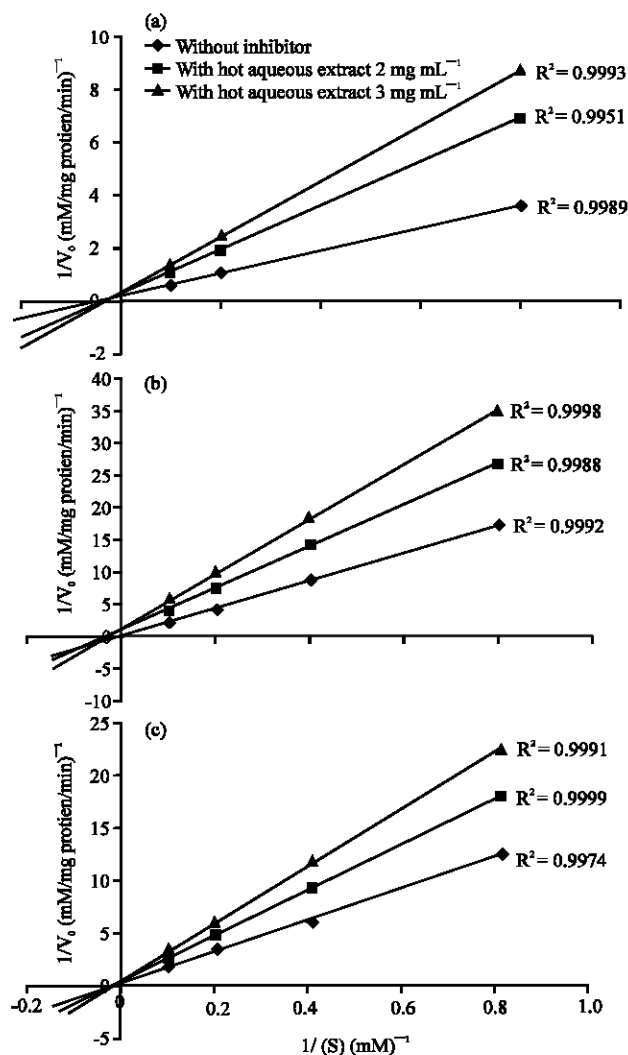


Fig. 2: Lineweaver-Burk plots of the activity of  $\alpha$ -glucosidase in the presence of different concentrations (0, 2 and 3  $mg\ mL^{-1}$ ) of the *F. deltoidea*, (a) hot aqueous extract, (b) the ethanolic extract and (c) the methanolic extract

Table 2: Kinetics analysis of  $\alpha$ -glucosidase inhibition by *F. deltoidea* extracts

<i>Ficus deltoidea</i> extracts	Concentration (mg mL <sup>-1</sup> )	V <sub>max</sub> (mM mg <sup>-1</sup> protein min <sup>-1</sup> )	K <sub>m</sub> (mM)
Hot aqueous	0	9.05±0.44 (7.98-10.17)	47.57±5.49 (33.95-61.20)
	2	6.77±0.66 (5.13-8.40)	52.03±11.81 (22.68-81.37)
	3	5.65±0.05 (5.54-5.76)	49.80±12.15 (19.61-79.99)
Ethanollic	0	4.10±0.21 (3.58-4.63)	81.38±5.23 (34.37-128.80)
	2	1.66±0.14 (0.44-2.87)	59.29±8.44 (38.32-80.26)
	3	0.80±0.16 (0.40-1.20)	34.59±6.02 (19.65-49.54)
Methanolic	0	2.61±0.71 (0.84-4.37)	24.58±2.23 (4.50-44.66)
	2	2.10±0.53 (0.77-3.40)	34.12±3.27 (26.00-42.23)
	3	1.68±0.30 (0.95-2.42)	37.63±2.27 (17.23-58.02)

The *F. deltoidea* extracts at different concentrations (0, 2 and 3 mg mL<sup>-1</sup>) were pre-incubated with increasing concentration of sucrose (0, 1.25 and 2.5, 5.0 and 10.0 mM) for 5 min at 37°C. Subsequently, the crude  $\alpha$ -glucosidase solution was added into each tube and further incubated for 60 min at 37°C. The enzyme reaction was terminated by adding Tris-Maleate-NaOH buffer. The glucose that was produced during the reaction was measured using the Megazyme Glucose Test Kit. The results were expressed as the Mean±Standard Deviation from three independent experiments. The values of V<sub>max</sub> and K<sub>m</sub> were determined through double reciprocal Lineweaver-Burk plot analysis. The values in bracket indicate 95% confidence intervals

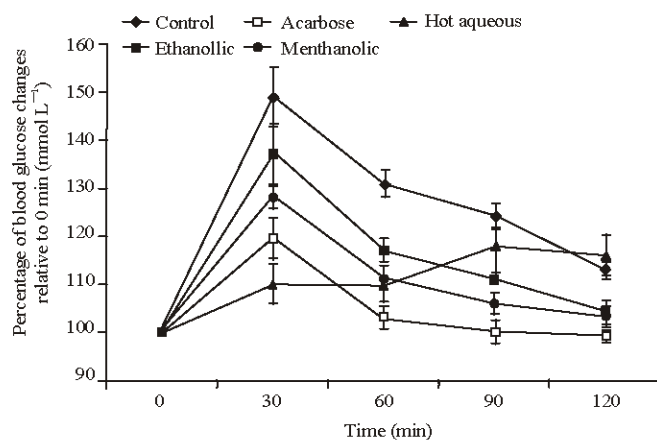


Fig. 3: The effect of the *F. deltoidea* extracts at a dose of 1000 mg kg<sup>-1</sup> b. wt. on the blood glucose levels following the administration of sucrose in normal rats. The data are expressed as the Means±SD, n = 7. The level of significance was set at p<0.05, relative to 0 min of the respective treatment group. The range of baseline (0 min) blood glucose levels was 4.30- 5.05 mmol L<sup>-1</sup>

Lineweaver-Burk plots and are shown in Table 2. We found that for all extracts, the V<sub>max</sub> values decreased with increasing concentrations of the *F. deltoidea* extracts. There is no consistent trend in the K<sub>m</sub> values for the extracts. For the hot aqueous extract, the K<sub>m</sub> value is increased at 2 mg mL<sup>-1</sup> but decreases again at 3 mg mL<sup>-1</sup>. For the ethanollic extract, the K<sub>m</sub> values decreased with increasing concentrations of the extract. In contrast, the K<sub>m</sub> values for the methanolic extract increased in proportion to the concentration of the extract.

### The Effects of *F. deltoidea* Extracts on the Postprandial Blood Glucose Level

The effects of the *F. deltoidea* extracts and acarbose on the postprandial blood glucose level following the oral administration of sucrose in normal rats are shown in Fig. 3. The baseline values (0 min) for the blood glucose levels of all treatment groups lay in the range 4.30-5.05 mmol L<sup>-1</sup>. The highest increase in the blood glucose level was observed 30 min after



the administration of sucrose for all the groups. In the control group, the blood glucose levels increased significantly, by 49% ( $p < 0.001$ ), 31% ( $p < 0.001$ ) and 24% ( $p < 0.001$ ) after 30, 60, 90 and 120 min of sucrose administration, respectively, compared to the baseline levels (0 min). In the acarbose-treated group, the blood glucose level increased significantly, by 19% ( $p < 0.001$ ), 30 min after the administration of sucrose. It decreased to a level similar to the baseline after 120 min. The blood glucose level of the group that was treated with the hot aqueous extract increased in a time-dependent manner following the administration of sucrose. A significant increase in the blood glucose level (17%,  $p < 0.05$ ) was observed 90 min after the administration of sucrose.

Following treatment with the ethanolic extract, the blood glucose level increased significantly, by 37% ( $p < 0.001$ ) after 30 min and by 17% ( $p < 0.05$ ) after 60 min of sucrose administration. After this, the blood glucose level decreased in a time-dependent manner after 90 and 120 min. In the group that was treated with the methanolic extract, the blood glucose level increased significantly, by 28% ( $p < 0.001$ ) and 11% ( $p < 0.01$ ) after 30 and 60 min of sucrose administration, respectively. Thereafter, the blood glucose level decreased in a time-dependent manner to a level that was similar to the baseline. Acarbose and the hot aqueous extract were able to prevent the blood glucose level from increasing to as high a level as was found in the control group (Fig. 3). Acarbose prevented the blood glucose levels from increasing by 21% ( $p < 0.01$ ), 22% ( $p < 0.001$ ), 20% ( $p < 0.001$ ) and 12% ( $p < 0.001$ ) after 30, 60, 90 min and 120 min of sucrose administration, respectively, compared to the levels observed at the corresponding times for the control group. The hot aqueous extract prevented the blood glucose levels from increasing by 23% ( $p < 0.001$ ) and 12% ( $p < 0.001$ ) after 30 and 60 min of sucrose administration, respectively, compared to the levels observed at the corresponding times for the control group.

Figure 4 shows the effect of the *F. deltoidea* extracts and acarbose on the postprandial blood glucose level changes following the oral administration of sucrose in the streptozotocin-induced diabetic rats. The baseline level (0 min) of the blood glucose levels for all the treatment groups lay in the range 17.27-17.90 mmol L<sup>-1</sup>. The highest increase in

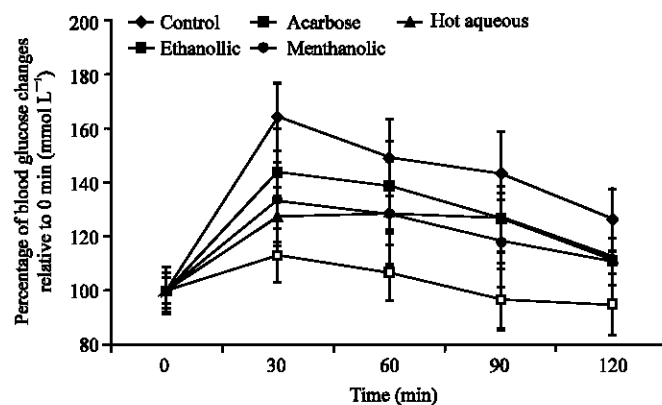


Fig. 4: The effect of the *F. deltoidea* extracts at a dose of 1000 mg kg<sup>-1</sup> b. wt. on the blood glucose levels following the administration of sucrose in the streptozotocin-induced diabetic rats. The data expressed as the Means±SD, n = 7. The level of significance was set at  $p < 0.05$ , relative to 0 min of the respective treatment group. The range of baseline (0 min) blood glucose levels was 17.39-17.90 mmol L<sup>-1</sup>

blood glucose level was observed 30 min after the administration of sucrose in all the groups, except for the group that was treated with the hot aqueous extract, where the highest increase in blood glucose was observed after 60 min of sucrose administration. In the control group, the blood glucose level increased significantly, by 62% ( $p<0.001$ ), 47% ( $p<0.001$ ), 42% ( $p<0.001$ ) and 25% ( $p<0.01$ ) after 30, 60, 90 and 120 min of sucrose administration, respectively, compared to the baseline level (0 min). In the group that had been treated with acarbose, we observed no significant increase in the blood glucose level. Treatment with the hot aqueous extract resulted in a time-dependent increase in blood glucose following the administration of sucrose until 60 min of sucrose administration. Significant increases, by 27% ( $p<0.001$ ), 29% ( $p<0.001$ ) and 27% ( $p<0.001$ ) were observed 30, 60 and 90 min after the administration of sucrose, respectively.

Following treatment with the ethanolic extract, the blood glucose level was significantly increased by 44% ( $p<0.001$ ), 39% ( $p<0.001$ ) and 27% ( $p<0.01$ ) after 30, 60 and 90 min of sucrose administration, respectively. For the group that was treated with the methanolic extract, the blood glucose level was increased significantly by 33% ( $p<0.001$ ) and 28% ( $p<0.01$ ) after 30 and 60 min of sucrose administration, respectively. Acarbose and the methanolic extract showed a preventive effect against the increase in blood glucose compared to the control group at all time intervals. However, in the group that was treated with the hot aqueous extract, an increase in blood glucose was prevented only after 30 min of sucrose administration. Acarbose prevented the blood glucose levels from increasing by 33% ( $p<0.001$ ), 30% ( $p<0.001$ ), 34% ( $p<0.001$ ) and 25% ( $p<0.001$ ) after 30, 60, 90 and 120 min of sucrose administration, respectively, compared to the levels observed at the corresponding times in the control group. Use of the methanolic extract prevented the blood glucose levels from increasing by 20% ( $p<0.001$ ), 15% ( $p<0.05$ ), 18% ( $p<0.01$ ) and 13% ( $p<0.05$ ) after 30, 60, 90 and 120 min of sucrose administration, respectively, compared to the levels observed at the corresponding times in the control group. Meanwhile, the hot aqueous extract prevented the blood glucose levels from increasing by 23% ( $p<0.001$ ) after 30 min of sucrose administration, compared to the levels observed at the corresponding time in the control group.

The areas under the glucose curve ( $AUC_{Glucose}$ ) for each individual rat of the normal and diabetic groups were calculated to determine the increases in the blood glucose concentrations from 0 to 120 min of the observation period (Table 3). For the normal rats, the ethanolic, methanolic and hot aqueous extracts attenuated the  $AUC_{Glucose}$  values significantly by 45.0% ( $p<0.05$ ), 41.7% ( $p<0.05$ ) and 54.9% ( $p<0.001$ ), respectively, compared to the control group. For the diabetic rats, the ethanolic, methanolic and hot aqueous extracts attenuated the  $AUC_{Glucose}$  values significantly, by 49.6% ( $p<0.001$ ), 33.8% ( $p<0.01$ ) and 60.8% ( $p<0.001$ ),

Table 3: The effect of *F. deltoidea* extracts on the area under glucose curve following oral sucrose administration

Treatment group	Dose (mg kg <sup>-1</sup> b.wt.)	Normal rats	Diabetic rats
Control	-	136.3±37.8 (101.3-171.3)	936.0±133.4 (812.6-1059.0)
Acarbose	50	44.2±15.1*** (25.5-62.9)	117.5±74.2*** (55.4-179.5)
Hot aqueous	1000	75.0±36.3* (29.5-120.5)	471.7±212.8*** (274.9-668.5)
Ethanolic	1000	79.4±18.8* (56.0-102.7)	620.6±223.9** (433.4-807.7)
Methanolic	1000	61.5±14.2** (38.9-84.1)	366.5±116.2*** (224.5-488.5)

Test substances were given concurrently with 2 g kg<sup>-1</sup> b.w. of sucrose to the 12 h fasted rats orally using intragastric gavage. Blood samples were collected just before (0 min), 30, 60, 90 and 120 min after test substances and sucrose administration for the measurement of glucose levels. The areas under glucose curve ( $AUC_{Glucose}$ ) of each individual rat were calculated using computer calculator software provided by Thomas Wolever, from the Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada. The results of  $AUC_{Glucose}$  values were expressed as the Mean±Standard Deviation, n = 7. The values in bracket indicate 95% Confidence Intervals. \*\* $p<0.01$  and \*\*\* $p<0.001$  compared to the control group

respectively, compared to the control group. However, the attenuation of the  $AUC_{\text{Glucose}}$  values by these extracts was less than that caused by acarbose, which attenuated the  $AUC_{\text{Glucose}}$  values by 67.6% ( $p < 0.001$ ) and 87.4% ( $p < 0.001$ ), relative to the control group, in normal rats and diabetic rats, respectively. Of the three extracts that were evaluated, the methanolic extract was found to be most potent in correcting hyperglycemia after the administration of sucrose, in both the normal and the diabetic condition.

## DISCUSSION

*Ficus deltoidea* has been used for a long time as traditional medicine to counter high blood glucose levels. However, studies on its efficacy and possible mode of action are still needed. Earlier studies have shown that an aqueous extract of *F. deltoidea* reduced the blood glucose levels in normal rats (Aminudin *et al.*, 2007) and in mildly diabetic rats (Adam *et al.*, 2007). An ethanolic extract of *F. deltoidea* has been shown to reduce postprandial hyperglycemia in normal and streptozotocin-induced diabetic rats whereas the hot aqueous extract reduced postprandial hyperglycemia only in the normal rats (data not shown). Moreover, *F. deltoidea* did not produce severe hypoglycemia and has no effect on the fasting blood glucose levels in normal rats (Adam *et al.*, 2007). This is desirable for an antihyperglycemic agent, as it has been noted that severe hypoglycemia can cause seizures, coma, or death and may even induce permanent brain damage (Ryan and Becker, 1999). Studies on the mechanism of the antidiabetic action of this plant have shown that *F. deltoidea* enhances the basal and insulin-stimulated uptake of glucose into liver cells. Insulin-mimetic and insulin-sensitizing activities have also been demonstrated during the facilitation of glucose uptake into liver cells (Adam *et al.*, 2009).

The present study is an extension of previous research and was carried out to evaluate whether *F. deltoidea* possesses other of antidiabetic mechanisms, such as the inhibition of  $\alpha$ -glucosidase in the small intestine. Although, less potent than standard reference, acarbose, *F. deltoidea* extracts inhibit the activity of rat intestine  $\alpha$ -glucosidase significantly *in vitro* and reduce postprandial blood glucose levels in normal and diabetic rats following the administration of sucrose. This result suggests that antidiabetic compounds may be present in *F. deltoidea*, which exerts its antidiabetic effect through the inhibition of  $\alpha$ -glucosidase.

Carbohydrate is the largest component of the human diet, but only monosaccharides (fructose and glucose) can be absorbed from the small intestine. Therefore, complex carbohydrates (polysaccharides) need to be degraded into monosaccharides to be absorbed. This degradation is mediated by  $\alpha$ -glucosidase, a key enzyme in carbohydrate digestion (Coniff and Krol, 1997; Li *et al.*, 2005). This enzyme, located on the brush-border surface membrane of the upper small intestine, is a typical exo-acting carbohydrase which hydrolysis the terminal glucose moiety from the non-reducing end of their substrates, i.e., maltose or sucrose (Ernst *et al.*, 2005).

Acarbose has a chemical structure that is similar to that of the oligosaccharides that are derived from starch digestion. This structure enables acarbose to bind competitively and reversibly to the oligosaccharide-binding site of  $\alpha$ -glucosidase, thereby preventing enzymatic hydrolysis and delaying the absorption of glucose from food into the circulatory system. This reduces postprandial hyperglycemia, improves the glycemic condition and leads to a reduction in the HbA<sub>1c</sub> level (Coniff and Krol, 1997; Evans and Rushakoff, 2002). In this study, acarbose was used as a positive control to compare the effectiveness of *F. deltoidea* extracts against  $\alpha$ -glucosidase. The results showed that the inhibitory action of *F. deltoidea* on  $\alpha$ -glucosidase was less potent than that of acarbose, for all of the

parameters that were evaluated. These observations can easily be explained if the *F. deltoidea* extracts contain a mixture of compounds: some compounds that are not bioactive may be mixed in with the active compounds in the extracts. This would reduce the concentration of active compounds in the extract and decrease the ability of the extracts to inhibit  $\alpha$ -glucosidase. Unlike the *F. deltoidea* extracts, acarbose is a single, nitrogen-containing pseudotetrasaccharide and its inhibitory effect on  $\alpha$ -glucosidase has been well documented by Coniff and Krol (1997) and Evans and Rushakoff (2002).

A preliminary evaluation was performed to obtain a general overview of the inhibitory effect of *F. deltoidea* extracts against  $\alpha$ -glucosidase. Of the five extracts and fractions of *F. deltoidea* that were evaluated, the ethanolic, methanolic and hot aqueous extracts were able to inhibit 50% or more of the activity of  $\alpha$ -glucosidase activity, relative to the control. These extracts were therefore evaluated further by measuring the  $IC_{50}$  values using a dose response curve. Among these three extracts, the methanolic extract appears to be the most potent inhibitor of rat intestine  $\alpha$ -glucosidase, as shown by the highest percentage of inhibition of  $\alpha$ -glucosidase at  $5000 \mu\text{g mL}^{-1}$  and the lowest  $IC_{50}$  value (Table 1). The next most potent inhibitors were the ethanolic and hot aqueous extracts.

We studied the enzyme kinetics to elucidate the type of inhibition exhibited by the *F. deltoidea* extracts against  $\alpha$ -glucosidase and demonstrated that all extracts exhibited a mixed-type inhibition mechanism. For mixed-type inhibition, the lines for different concentrations of inhibitors in the Lineweaver Burk plots intersect to the left of the  $1/v_0$  axis. Moreover, with increasing concentrations of the inhibitors, the maximum velocity of the reaction ( $V_{max}$ ) will decrease whereas the binding affinity between the enzyme and the substrate, which is inversely measured by the  $K_m$  value, may decrease or increase (Voet *et al.*, 1998). In the present study, all *F. deltoidea* extracts exhibited the characteristics listed above as shown by Lineweaver Burk plots of the extracts (Fig. 2) and the kinetic analysis of these plots (Table 2). Mixed-type inhibition occurs when inhibitors bind to both enzymes and to the enzyme-substrate complexes. The enzyme-substrate-inhibitor complexes are unproductive complexes and cannot form products (Jakubowski, 2008). In addition, mixed-type inhibitors bind to enzyme sites that participate in both substrate binding and catalysis and this reduces the catalytic activity of the enzymes and suppresses the formation of products (Voet *et al.*, 1998). This could be the reason why, when increasing the concentrations of the inhibitors or extracts in the dose response evaluation, the inhibition of  $\alpha$ -glucosidase activity was increased-indirectly indicating that the formation of glucose was suppressed.

From the *in vivo* study, it has been shown that the *F. deltoidea* extracts have the ability to reduce postprandial hyperglycemia following the oral administration of sucrose in normal and streptozotocin-induced diabetic rats. However, this reduction was less than that observed when using acarbose. In both the normal and diabetic rats, treatment with the ethanolic and methanolic extracts gave similar glucose curves to those of the control group and the group that was treated with acarbose. In all the groups mentioned, for both conditions, the level of blood glucose increased maximally after 30 min of sucrose administration and decreased in a time-dependent manner thereafter.

Treatment with the hot aqueous extract gave a different type of glucose curve in the normal and diabetic rats. In the normal rats, the blood glucose level increased time dependently until 90 min after the administration of sucrose, but it decreased slightly after 120 min in the normal rats (Fig. 3). However, in the diabetic rats, the blood glucose level increased maximally after 60 min of sucrose administration and decreased in a time-dependent manner thereafter (Fig. 4). There is possibility that the blood glucose level of the group of

normal rats that was treated with the hot aqueous extract could have decreased further if the observations had continued for 180 or 240 min. The inability of the treatment with the hot aqueous extract to reduce the blood glucose level after the administration of sucrose may be due to the presence of oligosaccharides or disaccharides in the hot aqueous extract that are not present in the ethanolic and methanolic extracts. These carbohydrates may contribute to the amount of glucose absorbed in addition to that derived from the degradation of sucrose. However, this suggestion needs further confirmation. Even though treatment with the hot aqueous extract did not bring the blood glucose level back to the baseline levels after 120 min, it nevertheless, prevented the blood glucose levels from increasing as high as the levels seen for the control group. In normal rats, this preventive effect was observed after 30 and 60 min of sucrose administration, whereas in the diabetic rats, it occurred only after 30 min of sucrose administration. The degree of control of the blood glucose levels observed in the group that was treated with the hot aqueous extract after 30 min of sucrose administration was similar in both the normal and the diabetic rats. Moreover, the preventive effect in normal rats at this time interval was the highest of all the extracts and the acarbose (Fig. 3). Use of the methanolic extract also prevented an increase in the blood glucose levels. This effect occurred at all time intervals in the diabetic rats, but no such effect was observed in the normal rats. The degree of prevention of a rise in the blood glucose levels by the methanolic extract was less than that observed when acarbose was used, at all time intervals.

The areas under the glucose curve ( $AUC_{\text{Glucose}}$ ) for each individual rat were calculated to determine the increase in the blood glucose concentration during the 120 min observation period. These calculations had shown that all extracts of *F. deltoidea* had the ability to attenuate the  $AUC_{\text{Glucose}}$  value significantly, both for the normal and diabetic rats. The highest attenuation of the  $AUC_{\text{Glucose}}$  values (%) was shown by the methanolic extracts. Therefore, we suggest that methanolic extracts possess the highest corrective effect on postprandial hyperglycemia after the administration of sucrose, both in the normal and diabetic rats (Table 3).

The  $\alpha$ -glucosidase inhibitory activity of the plant *Sophora flavescens* was linked to the presence of flavonoids (Kim *et al.*, 2006) and the inhibitory activity of *Rosa damascena* was linked to the presence of flavonoids and tannins (Gholamhuseinian *et al.*, 2009). According to Zunoliza *et al.* (2009), *F. deltoidea* var. *angustifolia* contain flavonoids and tannins. In this study, the same variety of *F. deltoidea* was used and there is possibility that these secondary metabolites are responsible for the inhibitory property of this plant on  $\alpha$ -glucosidase enzyme. Among all extracts and fractions that were evaluated, the methanolic extract appears to be the most potent inhibitor of  $\alpha$ -glucosidase. The methanolic extract of *F. deltoidea* var. *angustifolia* contains 87 mg g<sup>-1</sup> of total flavonoids and 179 mg g<sup>-1</sup> of total tannins, which are higher concentrations than are found in the aqueous extract which contains only 27 mg g<sup>-1</sup> of total flavonoids and 96 mg g<sup>-1</sup> of total tannins (Zunoliza *et al.*, 2009). This could be the reason for the higher  $\alpha$ -glucosidase inhibition of the methanolic extracts than for the other extracts in this study. Further experiments are required to correlate the  $\alpha$ -glucosidase inhibitory activity of the *F. deltoidea* extracts with the presence of flavonoids and tannins in this plant. However, no report on the flavonoid and tannin contents of the ethanolic extract of this plant is available. Considering that the potential of ethanolic extract to inhibit  $\alpha$ -glucosidase was between that of the methanolic and hot aqueous extracts, we suggest that the flavonoid and tannin contents of the ethanolic extract of  $\alpha$ -glucosidase activity lies between that of these two extracts.

There are other plants that also exhibit high levels of inhibitory effects against  $\alpha$ -glucosidase in their methanolic extracts. These include *Tournefortia hartwegiana*

(Ortiz-Andrade *et al.*, 2007) *Pelvetia babingtonii* de Toni (Ohta *et al.*, 2002) and *Punica granatum* (Li *et al.*, 2005). The  $\alpha$ -glucosidase inhibitory activity of *Tournefortia hartwegiana* was related to the presence of triterpenoids in this plant (Ortiz-Andrade *et al.*, 2007). However, the  $\alpha$ -glucosidase inhibitory activities of *Pelvetia babingtonii* de Toni and *Punica granatum* were not correlated with any secondary metabolites of the plants. Other plants that have been screened and reported to possess  $\alpha$ -glucosidase inhibitory effects include *Scutellaria baicalensis* (Gao *et al.*, 2004), *Tussilago farfara* L. (Gao *et al.*, 2008) *Mimordica charantia* (Uebanso *et al.*, 2007) and Pine bark (Kim *et al.*, 2005). Based on the results of the present study, *F. deltoidea* can be included in this group. The potential usefulness of *F. deltoidea* in the management of postprandial hyperglycemia and diabetes mellitus needs to be evaluated further.

This study has shown that extracts from *F. deltoidea* possess  $\alpha$ -glucosidase inhibitory activity *in vitro* and that these extracts ameliorate postprandial hyperglycemia following the administration of sucrose in normal and diabetic rats. Therefore, we suggest that the blood glucose lowering effect of *F. deltoidea* is mediated partly by the inhibition of the  $\alpha$ -glucosidase activity in the small intestine.

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