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Effect of *Ficus deltoidea* Aqueous Extract on Blood Glucose Level in Normal and Mild Diabetic Rats

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ABSTRAK

Ficus deltoidea atau nama tempatannya 'Mas Cotek' telah dipercayai secara tradisional mempunyai aktiviti hipoglisemia. Dalam kajian ini, aktiviti hipoglisemia ekstrak akuas Ficus deltoidea pada tikus normal dan diabetik ringan (aruhan streptozotocin) telah dikaji. Ekstrak pada dos berbeza (100, 500 dan 1000 mg/kg) telah diberi secara oral kepada kedua-dua kumpulan dalam keadaan puasa dan pasca prandial. Keputusan menunjukkan bahawa ekstrak akuas Ficus deltoidea tidak mempunyai kesan hipoglisemia pada tikus normal dan tikus diabetik ringan puasa. Pada tikus diabetik ringan pasca prandial, ekstrak akuas Ficus deltoidea pada dos 1000 mg/kg menunjukkan menunjukkan aktiviti hipoglisemia selepas 2 ($p < 0.01$), 4 ($p < 0.05$) and 6 ($p < 0.01$) jam pengambilan ekstrak. Metformin, 500 mg/kg juga menunjukkan aktiviti hipoglisemia selepas 2 ($p < 0.05$), 4 ($p < 0.01$) and 6 ($p < 0.01$) jam pengambilan. Oleh sebab itu, kami mencadangkan bahawa mekanisme tindakan ekstrak akuas Ficus deltoidea mungkin melalui peningkatan pengambilan glukos oleh tisu otot serta pengurangan glukoneogenesis pada hepar.

Kata kunci: Diabetis mellitus, Ficus deltoidea, dos akut, kesan hipoglisemia, tikus diabetis aruhan streptozotocin

ABSTRACT

Ficus deltoidea, which is locally known as 'mas cotek,' has been traditionally believed to have hypoglycaemic activity. In this study, hypoglycaemic activity of aqueous extract of Ficus deltoidea in normal and mild diabetic rats (streptozotocin induced) was evaluated. The extract was administered orally at different doses (100, 500 and 1000 mg/kg) to both groups in fasting and post prandial state. The result shows that the aqueous extract of Ficus deltoidea did not have any hypoglycaemic activity in normal rats as well as in fasting mild diabetic group. In post prandial mild diabetic rats, aqueous extract of Ficus deltoidea at the dose of 1000 mg/kg show hypoglycaemic activity after 2 ($p < 0.01$), 4 ($p < 0.05$) and 6 ($p < 0.01$) hours extract administration. Metformin, 500 mg/kg also shows hypoglycaemic activity after 2 ($p < 0.05$), 4 ($p < 0.01$)

and 6 ($p < 0.01$) hours administration. Therefore, we suggested that mechanism of action of the Ficus deltoidea aqueous extract maybe through enhancement of glucose uptake to muscle tissue and reduce hepatic gluconeogenesis.

Key words: Diabetes mellitus, Ficus deltoidea, acute dose, hypoglycaemic effect, streptozotocin-induced diabetic rats

INTRODUCTION

Diabetes mellitus remains the major global health problem even though there is abundant hypoglycaemic agents today (Kecskemeti et al. 2002). The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 and the total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild et al. 2004). In Malaysia, the prevalence of diabetes is found to be 8.2% (Rugayah 1997).

In developing countries, plants have been used extensively to alleviate the symptoms of diabetes for many centuries. Recent scientific investigations have confirmed the efficacy of many of these plants, some of which are remarkably effective. In Malaysia, few plants have been scientifically investigated and reported to have antidiabetic properties. They are *Morinda citrifolia*, *Averrhoa bilimbi*, *Tinospora crispa*, *Parkia speciosa*, *Andrographis paniculata*, *Gynura procumbens* and *Orthosiphon stamineus* (Mafauzy 2004). However, there are still plants with antidiabetic properties, which have not yet been studied. One of which is *Ficus deltoidea*, which is locally known as "Mas cotek." This plant belongs to the family of Moraceae. It is an evergreen shrub or small tree (Forest et al. 2003). The whole plant including the roots, bark, leaves and fruits, is believed to have medicinal properties.

The aim of this study was to investigate the hypoglycaemic effect of the aqueous extracts of *Ficus deltoidea* in normal and mild-diabetic rats.

MATERIALS AND METHODS

CHEMICALS

Streptozotocin (STZ), glibenclamide, metformin and carboxymethylcelulose (CMC) were purchased from Sigma Chemicals (St. Louis, USA). One touch gluco-meter (Accu-chek advantage) of Roche Diagnostics, Germany was purchased from local pharmacy.

PLANT MATERIAL AND PREPARATION OF THE AQUEOUS EXTRACT OF FICUS DELTOIDEA

Ficus deltoidea were collected from Sungai Tengi Selatan, Selangor. The specimen was identified by a taxonomist at Biodiversity Unit, Institute of Biological Sciences, University Putra Malaysia. A voucher specimen was deposited at the IBS herbarium Biodiversity Unit, Institute of Biological Sciences, University Putra Malaysia with voucher number SK1467/07. The leaves of *Ficus deltoidea* were air-dried and grounded to a fine mesh. A total of 100 g of the mesh were boiled with distilled water for 3 hours. The resulting supernatant was cooled and filtered using Whatman filter paper. The filtrate was then spray dried to give a powder form.

EXPERIMENTAL ANIMALS

Adult male Sprague Dawley rats weighing 150-200 g were purchased from Institute of Medical Reseach, Kuala Lumpur. The animals were kept in Malaysian Nuclear Agency Animal House and fed on a standard laboratory pellet diet with water supplied *ad libitum*. Mild diabetic rats were induced by a single intravenous (tail vein) injection of a freshly prepared STZ solution (60 mg/kg body weight) in Water for Injection (WFI) given under ether anesthesia. Fasting blood glucose (FBG) was checked 7 days after injection and only rats with FBG from 6.0 – 9.0 mmol/L were considered mild diabetic and used as the experimental animals. Other rats with FBG less than 6.0 mmol/L and more than 9.0 mmol/L were excluded from the study (Kesari et al. 2006).

BIOLOGICAL ASSAY

Different doses of the of *Ficus deltoidea* aqueous extract (100, 500 and 1000 mg/kg) and standard drug (glibenclamide, 3 mg/kg and metformin, 500 mg/kg) were tested for hypoglycemic activity in fasting and post-prandial state of normal and mild diabetic rats. The test was carried out in different batches of rats. In every batch, animals were divided into six equal groups (five rats in each group). Control rats (group I) were given vehicle (1% CMC) orally. Group II–IV were treated orally with aqueous extract of *Ficus deltoidea* suspended in 1% CMC at doses of 100, 500 and 1000 mg/kg, respectively. Group V-VI were treated orally with 3 mg/kg of glibenclamide and 500 mg/kg of metformin, respectively.

In fasting state, rats were fasted for 16 hours prior to test. In post-prandial state, rats had free access to water and laboratory pellet diet but were fasted 1 hour prior to test. Blood glucose levels were estimated before and after 2, 4 and 6 hours of *Ficus deltoidea* aqueous extract administration. Blood samples were collected from rat tail tip under mild ether anesthesia. The glucose level was determined by using electronic glucometer (Accu-chek advantage, Roche Diagnostics, Germany).

STATISTICAL ANALYSIS

Results were presented as mean ± standard deviation of blood glucose. Statistical analysis was performed using GraphPad Instat 3 software. Data were evaluated using one way ANOVA and groups were considered significantly different if $p < 0.05$. Dunnet post hoc test was done if a significant value was obtained for ANOVA.

RESULTS

The effect of *Ficus deltoidea* aqueous extract on blood glucose level (BGL) of fasting normal rats is presented in Table 1. There are no significant differences between post treatments hours as compared to pretreatment hours in all groups tested. In postprandial normal rats, there was a significant increase (21.28%; $p < 0.05$) in BGL after 6-hour of administration of *Ficus deltoidea* (500 mg/kg). Instead, a significant decrease (11.57%; $p < 0.05$) was observed after 4-hour administration of metformin (Table 2).

In fasting mild diabetic rats, all doses of *Ficus deltoidea* did not produce any significant hypoglycemic effects whereas in glibenclamide treated group, a significant fall of 26.61% ($p < 0.05$) and 45.56% ($p < 0.01$) in BGL was observed after 4-hour and 6-hour administration, respectively (Table 3). Metformin also produces significant hypoglycemic effects after 2-hour (40.44%; $p < 0.05$) and 6-hour (46.57%; $p < 0.01$) administration of *Ficus deltoidea* aqueous extract as compared to pretreatment hours.

TABLE 1. Hypoglycemic effect of *Ficus deltoidea* aqueous extract in fasting normal rats (mean ± S.D.)

Treatment	Dose (mg/kg b.w)	Blood glucose level (mmol/L)			
		Pre-treatment	Post-treatment (hr)		
		0 hr	2	4	6
1% CMC	-	4.93 ± 0.26	4.83 ± 0.48	4.78 ± 0.71	4.83 ± 0.50
	100	4.75 ± 0.81	4.93 ± 0.49	4.80 ± 0.83	4.63 ± 0.77
Extract	500	5.18 ± 0.39	4.83 ± 0.74	4.68 ± 0.54	4.40± 0.56
	1000	4.13 ± 0.30	4.48 ± 0.46	4.43 ± 0.39	4.75 ± 0.65
Glibenclamide	3	4.15 ± 0.59	4.38 ± 0.46	3.83 ± 0.30	3.58 ± 0.49
Metformin	500	4.50 ± 0.50	4.45 ± 0.17	4.43 ± 0.17	4.30 ± 0.73

TABLE 2. Hypoglycemic effect of *Ficus deltoidea* aqueous extract in postprandial normal rats (mean ± S.D.)

Treatment	Dose (mg/kg b.w)	Blood glucose level (mmol/L)			
		Pre-treatment	Post-treatment (hr)		
		0 hr	2	4	6
1% CMC	-	8.98 ± 0.46	9.85 ± 3.08	8.35 ± 0.99	8.08 ± 1.30
	100	9.53 ± 1.13	9.35 ± 1.07	8.93 ± 1.95	8.7 ± 1.17
Extract	500	8.27 ± 0.95	9.35 ± 0.21	9.30 ± 0.89	10.03 ± 0.06*
	1000	7.64 ± 1.15	8.16 ± 0.61	8.38 ± 0.08	7.60 ± 0.44
Glibenclamide	3	8.83 ± 1.04	7.45 ± 0.64	8.10 ± 1.22	7.48 ± 0.63
Metformin	500	8.30 ± 0.49	7.52 ± 0.33	7.34 ± 0.44*	7.7 ± 0.60

* $P < 0.05$ as compared to pretreatment hour.

TABLE 3. Hypoglycemic effect of *Ficus deltoidea* aqueous extract in fasting mild diabetic rats (mean ± S.D.)

Treatment	Dose (mg/kg b.w)	Blood glucose level (mmol/L)			
		Pre-treatment	Post-treatment (hr)		
		0 hr	2	4	6
Contrrol (1% CMC)	-	6.60 ± 1.44	6.18 ± 2.13	4.76 ± 1.47	5.12 ± 1.34
	100	6.54 ± 1.59	7.56 ± 2.3	6.38 ± 1.92	5.86 ± 2.15
Extract	500	7.18 ± 3.32	6.38 ± 1.59	4.42 ± 1.17	4.78 ± 1.4
	1000	7.1 ± 1.51	6.73 ± 1.47	5.75 ± 1.57	5.88 ± 1.99
Glibenclamide	3	6.54 ± 0.66	5.28 ± 0.93	4.80 ± 1.13*	3.56 ± 0.42**
Metformin	500	8.16 ± 2.85	4.86 ± 0.54*	6.38 ± 1.02	4.36 ± 0.73**

* $P < 0.05$, ** $P < 0.01$ as compared to pretreatment hour.

In postprandial mild diabetic rats, *Ficus deltoidea* (100 mg/kg) produces a significant fall of 32.91% ($p < 0.05$) in BGL after 6-hour treatment (Table 4). A significant hypoglycemic effect was also observed in rats treated with *Ficus deltoidea* (1000 mg/kg) after 2-hour (19.90%; $p < 0.01$), 4-hour (12.62%; $p < 0.05$) and 6-hour (25.87%; $p < 0.01$) administration. Metformin also produces significant fall of 39.98% ($p < 0.05$), 60.02% ($p < 0.01$) and 64.49% ($p < 0.01$) in BGL after 2-hour, 4-hour and 6-hour treatment, respectively.

TABLE 4. Hypoglycemic effect of *Ficus deltoidea* aqueous extract postprandial mild diabetic rats (mean ± S.D.)

Treatment	Dose (mg/kg b.w)	Blood glucose level (mmol/L)			
		Pre-treatment	Post-treatment (hr)		
		0 hr	2	4	6
Control (1% CMC)		23.35 ± 246	18.57 ± 2.80	19.50 ± 2.25	18.23 ± 2.35
Extract	100	18.23 ± 1.98	15.60 ± 2.14	14.00 ± 3.02	12.23 ± 4.29*
	500	17.40 ± 4.47	15.68 ± 4.63	13.40 ± 3.73	10.76 ± 5.21
	1000	20.6 ± 0.44	16.50 ± 0.36**	18.00 ± 0.53*	15.27 ± 1.97**
Glibenclamide	3	21.98 ± 4.97	18.73 ± 2.38	17.38 ± 5.61	13.45 ± 6.16
Metformin	500	18.36 ± 3.87	11.02 ± 4.65*	7.34 ± 2.56**	6.52 ± 2.91**

* $P < 0.05$, ** $P < 0.01$ as compared to pretreatment hour.

DISCUSSION

Ficus deltoidea has been traditionally believed to have hypoglycaemic activity. In this study, the hypoglycaemic activity of *Ficus deltoidea* aqueous extract in different glycemic state; normoglycaemic and mild hyperglycaemic with different prandial states; fasting and 1-hour post prandial was investigated.

In order to determine the mechanism of action of aqueous extract of *Ficus deltoidea*, two conventional hypoglycaemic agents, glibenclamide and metformin were used as positive controls. Glibenclamide is one of the hypoglycaemic agents belonging to the second generation of sulphonylureas group. The mechanism of action of glibenclamide is through depolarization of the pancreatic beta cells by blocking the ATP-sensitive potassium channels causing influx of calcium and stimulation of insulin secretion (Luzi and Pozza 1997). Metformin has been reported to be an effective antihyperglycemic agent with the main mechanism of action through enhancement of glucose uptake in muscle and reduce hepatic

gluconeogenesis, thereby reducing glucose level in blood stream (Chehade and Mooradian 2000).

In normal rats, the result shows that aqueous extract of *Ficus deltoidea* as well as glibenclamide (3 mg/kg) did not show significant reduction in BGL, both in fasting and post prandial condition; whereas meformin has a significant hypoglycaemic activity after 4 hour ($p < 0.05$) administration in post prandial state. The results indicate all doses of aqueous extract of *Ficus deltoidea* did not have hypoglycaemic activity in normal rats both in fasting and post prandial condition.

In fasting mild diabetic rats, all doses of *Ficus deltoidea* aqueous extract did not produce any significant hypoglycaemic effect. Instead, glibenclamide show significant hypoglycaemic effects after 4 ($p < 0.05$) and 6 ($p < 0.01$) hours administration. The same results were also observed with metformin after 2 ($p < 0.05$) and 6 ($p < 0.01$) hour administration. This result suggest that, in fasting condition of mild diabetic group, all doses of *Ficus deltoidea* aqueous extract did not had any hypoglycaemic activity as shown by glibenclamide and metformin.

In post prandial mild diabetic rats, aqueous extract of *Ficus deltoidea* (100 mg/kg) significantly reduce BGL after 6 ($p < 0.05$) hour administration whereas at 1000 mg/kg, BGL was significantly reduced after 2 ($p < 0.01$), 4 ($p < 0.05$) and 6 ($p < 0.01$) hour administration. Metfomin (500 mg/kg) also gave the similar result. However, unlike metformin, glibenclamide did not produce any significant hypoglycaemic effects. The results show that the aqueous extract of *Ficus deltoidea* at concentrations 500 and 1000 mg/kg exhibits the same activity as that of metformin but not glibenclamide. From this observation, we can suggest that the BGL lowering effect of aqueous extract of *Ficus deltoidea* at concentration 100 mg/kg after 6 hour administration and at 1000 mg/kg after 2, 4 and 6 hour administration in post prandial mild diabetic rats may be caused by enhancement of glucose uptake in muscle and reduction of hepatic gluconeogenesis, but not through stimulation of insulin secretion. Further evaluations at cellular level by using cell line are being carried out to confirm this suggestion.

CONCLUSION

In conclusion, aqueous extract of *Ficus deltoidea* did not have any hypoglycaemic activity in normal rats as well as in fasting mild diabetic group. Aqueous extract of *Ficus deltoidea* at concentration 1000 mg/kg have the hypoglycaemic activity in post prandial mild diabetic rats and the mechanism of action of the extract is suggested may be through enhancement of glucose uptake in muscle tissue and reduction of hepatic gluconeogenesis.

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