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Blood Glucose Lowering Effect of *Ficus deltoidea* Aqueous Extract

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ABSTRACT *Ficus deltoidea* has traditionally been used to cure several pathological conditions including diabetes. However, none of these claims have been scientifically researched thus far. In this preliminary study, the blood glucose lowering activity of *F. deltoidea* was monitored through the oral glucose tolerance test. Post-treatment of the aqueous extracts of both the leaves and fruits of *F. deltoidea* at 50mg/kg dosage significantly reduced the external glucose load. Both extracts were found to be non-toxic as shown by the brine shrimp toxicity test.

ABSTRAK *Ficus deltoidea* telah digunakan secara tradisional untuk merawat pelbagai keadaan patologi termasuk diabetes. Walaubagaimanapun, setakat ini semua kegunaan dan tanggapan ini masih belum dikaji secara saintifik. Dalam kajian awal ini, aktiviti penurunan paras glukosa darah akan dipantau melalui kaedah ujian toleransi glukosa oral. Rawatan dengan ekstrak akues daun dan buah *F. deltoidea* pada dos 50mg/kg secara signifikan akan menurunkan paras glukosa. Kedua-dua ekstrak didapati tidak toksik berdasarkan kepada keputusan ujian ketoksikan 'brine shrimp'.

(*Ficus deltoidea*, aqueous extract, hypoglycemia)

INTRODUCTION

Ficus deltoidea Jack is a plant belonging to the family Moraceae, found in various parts of the world. Its synonym is *Ficus diversifolia* Blume and common names are Mistletoe fig and Mistletoe rubber plant [1, 2]. This plant is native to Southeast Asia that includes Thailand, Sumatra, Java, Borneo, Philippines and Malaysia particularly Sabah and Sarawak [3, 4]. In Peninsular Malaysia, the distribution includes Johor (Batu Pahat), Pahang (Tahan River), Malacca (Ayer Keroh and Mt. Ophir), Selangor (Klang Gates) and Perak (Relau Tujor (Wray) and Lumut) [5]. *F. deltoidea* is widely known in Malaysia as Mas Cotek or Emas Cotek referring to the golden spots on the surface of its leaves. Other names are Telinga Beruk, Telinga Gajah and Serapat Angin. In Sabah and Sarawak it is also called 'Sempit-sempit' or 'Agoluran', 'Tabat Barito' in Indonesia and 'Kangkalibang' in Africa [4, 6, 7].

According to Neal [8], plants in this genus are all woody, ranging from trees and shrubs to climbers. In Malaysia, it is usually found as a dioecious shrub or a small tree that grows up to 3 - 5m. In the wild, it usually grows as an epiphyte or sometimes on the ground [4, 9]. The leaves are dark green, found in deltoid, elliptic, obovate, spathulate or rhomboid shape ranging from 2 x 1.5 cm to 16.5 x 10 cm in size [4]. Propagation can occur directly from the minute seed and many can propagate from cuttings. Trees usually begin life as epiphytes on other trees [2].

There are more than 60 species of Ficus identified [2, 10] and *F. deltoidea* is one of many species that has been cultivated in various parts of the world as a houseplant or as an ornamental shrub [2]. It makes nice attractive foliage and is said to be the only Ficus species able to produce fruits when it is cultivated indoors [2, 11]. Morphologically, *F. deltoidea* can be differentiated as a male plant or female plant based on its leaf shape. In general, the male plants usually possess small leaves in contrast to the female plants leaves, as depicted in figure 1 [12]. Male plants produces very small (0.3 cm across) yellowish fruits that turn orange as they ripen, whilst the female plant produces round-shaped (up to 1.5 cm in diameter) green fruits which turn red when ripe. Taxonomic classification divide this species into six varieties which are *Ficus deltoidea var bilobata, Ficus deltoidea var angustifolia, Ficus deltoidea var intermedia, Ficus deltoidea var kunstleri, Ficus deltoidea var motleyan* and *Ficus deltoidea var trengganuuensis* [7, 13]. Phytochemical analyses have shown the presence of flavonoids, tannins, triterpenoids, phenols [6, 7] and proanthocyanins [7]. Alkaloids and steroids one the other hand, were not commonly found in this plant [7, 14].

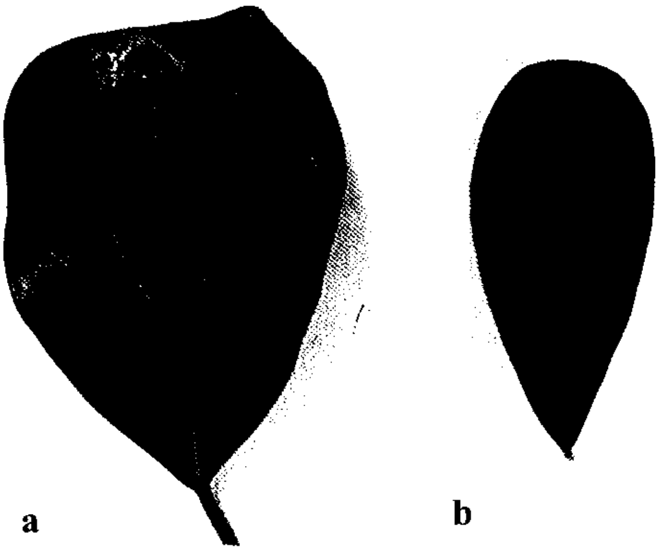

Figure 1. Morphological differences between a) the female leaf and b) the male leaf. The female leaf is usually bigger, thicker and the surface is shinier. The golden spots on the surface of the female leaf are usually more pronounced compared to the male leaf.

F. deltoidea is a reputed traditional medicine in Peninsular Malaysia with claims to having the ability to promote good health. A decoction of the leaves is taken after childbirth as a tonic [4], for birth control [15] and to improve blood circulation and promote constriction of the uterus [6, 16]. It has also been claimed to reduce blood cholesterol, prevent migraine and as an aphrodisiac [16]. Beside the leaves, part of the bark and stem have also been used to cure many diseases such as pneumonia [6, 16], diabetes mellitus, high blood pressure, skin diseases and gout [6, 17]. However, these medicinal uses have only been described in folk medicine and even though it is apparently effective, there is no experimental or clinical data available to support these claims. It is, therefore deemed interesting to start examining the various usage of this plant. In this paper we will report our preliminary research findings, regarding the potential of this plant in lowering blood glucose level as observed by the oral glucose tolerance test.

MATERIALS AND METHODS

Plant Material Preparation

Plant parts used in this study were the leaves and fruits of *F. deltoidea*, supplied by Mr Nasnan Jajuli (Delto Medicama Plantation). Both leaves and fruits were air-dried separately, cut into small pieces and then pulverized.

Aqueous Extraction

Each pulverized plant part was weighed (100 grams) and boiled twice with 1L of distilled water for four hours. The aqueous extracts were then brought to concentration by boiling using slow heat (approximately at 60°C) and later subjected to freeze-drying (yield 7.36% and 11.61%, w/w, dry weight basis for leaves and fruits respectively) and stored in a capped bottle until further use. Doses of 50, 100 and 200mg/kg of the leaves aqueous extract (LAE) and fruits aqueous extract (FAE) were prepared in 1 ml of distilled water.

Oral Glucose Tolerance Test

Healthy ICR strain mice aged between 8 - 10 weeks (weighing 35 - 40 g) were used throughout this study. All mice were housed under environmentally controlled conditions with 12 hours light and dark cycle with free access to pellets and tap water. Prior to the oral glucose tolerance test (OGTT), mice were grouped (five animals each), fasted overnight, but allowed free access to water. Blood was collected from their tail veins and blood glucose levels were directly measured using Accu-Chek® Advantage glucose meter and strips (Roche Diagnostics (M) Sdn Bhd). Immediately afterwards, all mice were given oral glucose load of 3g/kg each. After 30 minutes, blood glucose levels were measured again and immediately followed by oral administration of distilled water, tolbutamide (T) and *F. deltoidea* aqueous extracts according to this design: Group 1 - control (1 ml of distilled water), Group 2 (50 mg/kg LAE), 3 (100 mg/kg LAE), 4 (200 mg/kg LAE), 5 (50 mg/kg FAE), 6 (100 mg/kg FAE), 7 (200 mg/kg FAE) and 8 (20 mg/kg T). Blood glucose levels were monitored again at 30 minutes, 1½ and 2½ hrs after the treatment (denoted as 60, 120 and 180 min).

Brine Shrimp Toxicity Bioassay

Brine shrimp (*Artemia salina*) toxicity bioassay was conducted according to McLaughlin *et al.* [18] as described by Orech *et al.* [19]. A small container was divided into two compartments; one was covered and darkened whilst the other was supplied with a light source. Artificial seawater (38.0g NaCl in 1L distilled water) was poured into the container and a teaspoon of brine shrimp eggs were added into the dark compartment. The eggs were left at room temperature for 48 hours. Hatched larvae will swim through a small opening on the divider towards the illuminated compartment. Different concentrations of LAE and FAE were prepared using distilled water (5 ml) in triplicates (5, 10, 25, 50, 75 and 100mg/ml). Newly hatched brine shrimp nauplii were transferred into test tubes containing the prepared extracts (10 each). All test tubes were allowed to stand at room temperature and observation was done in 8 hours at every 2 hours interval. The number of dead and surviving brine shrimp was recorded and lethal concentration that killed 50% of the brine shrimp (LC_{50}) for each extract was determined.

RESULTS

Effect of *F. deltoidea* extracts towards oral glucose tolerance test

Tables 1 and 2, demonstrates the effects of both *F. deltoidea* aqueous extracts towards blood glucose level. Reduction of the blood glucose levels however, is not dose dependant with some of the lower dosage showing a better hypoglycaemic potential. Based on these data, maximum hypoglycaemic activity was achieved at 180 minutes showing approximately 17.6, 4.0, 12.5% (LAE) and 14.8, 6.0, 15.5% (FAE) reduction for each dosage compared to the control value. The percentages were calculated based on the starting endogenous level. As reference, a prescribed drug (T) was used and it showed comparable hypoglycaemic effect.

Brine shrimp toxicity bioassay

From this bioassay, the toxicity level of *F. deltoidea* aqueous extracts could be determined. Table 3 summarizes the LC_{50} values for LAE and FAE, each showing 30.0 and 50.5 mg/ml respectively. These values are very high indicating extremely low toxicity values for both plant parts.

DISCUSSION

Effect on oral glucose tolerance test

Prior to oral glucose tolerance test, the endogenous levels of blood sugar following 12 hours fasting were monitored (time 0). The normal fasting sugar level ranges from 3.9 – 5.5 mmol/L. Oral administration of glucose load (3g/kg) to fasted rats produced a rapid elevation in the post-prandial blood glucose level, followed by a progressive decline until they nearly reached the endogenous level at the end of the 180 minutes observation. Post-treatment of both extracts (LAE and FAE), following glucose load markedly reduced the elevated blood glucose level after 2½ hrs of administration compared to the control group. This observation was comparable to that of Tolbutamide (T); a drug used in treating diabetic patients. The reduction effect however, did not occur in dose-dependent manner. Maximum hypoglycaemic activity was demonstrated by LAE at 50mg/kg dosage (17.6% reduction) followed by 200 and 50mg/kg dosages of FAE (15.5 and 14.8% reduction respectively).

For both extracts however, there was a noticeable drop in the reduction percentage observed in groups treated with 100mg/kg dosage ($p < 0.01$). The reduction percentages were only 4 and 6% for each LAE and FAE even though they portrayed a very similar blood glucose lowering pattern at the beginning. Regardless of this unexplained observation, both extracts showed remarkable hypoglycaemic potential with LAE showing a more pronounced effect compared to FAE ($p < 0.05$) even when low dosage (50mg/kg) was used.

Table 1. Effect of 30 minutes post-treatment with 50, 100 and 200mg/kg of *F. deltoidea* leaves aqueous extract towards oral glucose tolerance test

TIME (min)	BLOOD GLUCOSE (mmol/L)				
	CONTROL	**50mg/kg**	**100mg/kg**	**200mg/kg**	**PD**
0	4.93 ± 1.13	4.98 ± 1.21	4.34 ± 1.41	4.52 ± 1.73	4.74 ± 0.56
30	16.04 ± 1.34	14.01 ± 2.15	15.76 ± 2.93	19.02 ± 1.59	15.04 ± 1.52
60	10.79 ± 1.84	10.40 ± 2.02	10.38 ± 1.86	13.14 ± 2.71	10.04 ± 1.52
120	6.26 ± 1.19	5.47 ± 1.08	7.49 ± 1.08	7.56 ± 1.37	4.18 ± 0.62
180	5.69 ± 1.09	4.69 ± 1.09	5.46 ± 1.29	4.98 ± 1.19	nd

Values described are mean ± S.D of three experiments, n = 5. nd – not determined; value is less than 3.6mmol/L , therefore it was not readable by the glucose meter. Statistical analysis was done using ANOVA ($p<0.05$).

Table 2. Effect of 30 minutes post-treatment with 50, 100 and 200mg/kg of *F. deltoidea* fruits aqueous extract towards oral glucose tolerance test

TIME (min)	BLOOD GLUCOSE (mmol/L)				
	CONTROL	**50mg/kg**	**100mg/kg**	**200mg/kg**	**PD**
0	4.93 ± 1.13	4.51 ± 1.26	4.51 ± 0.93	4.91 ± 1.91	4.74 ± 0.56
30	16.04 ± 1.34	16.00 ± 1.99	14.90 ± 0.95	18.46 ± 1.63	15.04 ± 1.52
60	10.79 ± 1.84	11.01 ± 1.80	12.96 ± 1.68	14.72 ± 1.99	10.04 ± 1.52
120	6.26 ± 1.19	5.63 ± 0.53	6.15 ± 0.61	6.73 ± 1.79	4.18 ± 0.62
180	5.69 ± 1.09	4.85 ± 0.40	5.35 ± 0.91	4.81 ± 1.17	nd

Values described are mean ± S.D of three experiments, n = 5. nd – not determined; value is less than 3.6mmol/L , therefore it was not readable by the glucose meter. Statistical analysis was done using ANOVA ($p<0.05$).

Table 3. Brine shrimp toxicity evaluation of the leaves and fruits of *F.deltoidea* aqueous extracts after 8 hours observation

F. deltoidea extract	LC_{50} (mg/ml)
LAE	30.0 mg/ml
FAE	50.5 mg/ml

Values obtained following triplicate brine shrimp toxicity tests

Thus far, no known bioactive chemical constituent has been isolated from this plant that facilitates the blood glucose lowering effect as demonstrated. It might be possible that these plant extracts (both LAE and FAE) contain bioactive(s) that act as an insulin secretagogue and/or sensitize insulin receptors as proposed for several plants extracts [20] and some sulphonylureas [21]. Arambewala and his research team also suggested this hypothesis when explaining their work on antidiabetic activities of *Piper betle* [22]. As this is a preliminary investigation, it is still too early to predict the mode of action. It could be possible to suggest that the anti-diabetic activity shown by these extracts is not dependent to endocrine pancreas and insulin; the extracts themselves might have insulinomimetic activity, a similar mode of action reported with hot water extract of *Piper sarmentosum* [23] and 50% ethanolic extract of *Piper longum* [24]. However; this could only be explained by further investigation using streptozotocin-induced diabetic rat as the subject.

Brine shrimp toxicity bioassay

The initial brine shrimp bioassay was studied using a lower range of concentration (50 - 1000μg/ml, 1 – 10mg/ml; data not shown). Due to the low level of toxicity, the bioassay was repeated using a higher concentration range (5 - 100mg/ml). From the results, both *F. deltoidea* extracts showed considerably high LC values (30.0 and 50.5 mg/ml respectively). This indicates the suitability and safety of these extracts for consumption. The fruit extract (FAE) however, showed a higher LC value compared to the leaf extract (LAE) suggesting that the former is less toxic.

In conclusion,. the results of this study demonstrated the hypoglycaemic potential of the aqueous extracts of leaves and fruits from *F. deltoidea* towards blood glucose level in non-diabetic mice. The brine shrimp toxicological test showed high LC_{50} values indicating the non-toxic nature of both the aqueous extracts (LAE and FAE). Further anti-diabetic and toxicological studies are now underway to gather more conclusive data on the efficacy and safety of these extracts.

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