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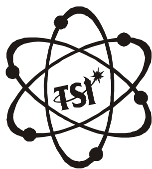

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Effect of *Ficus deltoidea* leaves on glycolytic enzymes in liver of normal and streptozotocin-induced diabetic rats

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ABSTRACT

Ficus deltoidea from the Moraceae family is commonly called "mas cotek" by the Malays. The Malay folklore medicines believed that consuming a decoction of the vascular bundle of *Ficus deltoidea* led to a lowering of the blood glucose level of diabetic patients. To establish its antidiabetic activity, the present study was undertaken to evaluate the effect of *Ficus deltoidea* leaves on key glycolytic enzymes in the liver of streptozotocin-induced diabetic rats. Diabetes was induced in 1 month old rats by single intravenous injection of STZ at a dose of 65mg/kg body weight. After a week, they were checked for fasting blood glucose concentrations to confirm the status of diabetes.The non-diabetic and streptozotocin (STZ)-induced diabetic rats were then treated with the aqueous and ethanolic extracts (200mg/kg) for 2 weeks. The blood glucose concentrations were measured for 2 weeks upon these administrations. A significant increase was observed in fasting blood glucose level in untreated diabetic rats. Diabetic rats also showed a significant decrease in the activities of hepatic hexokinase (HK), glucokinase (GK) and phosphofructokinase (PFK). However, after 14 days treatment with the plant extracts, the activities of hepatic HK, GK and PFK were found to significantly increase. From these results, one may tentatively suggest that *Ficus deltoidea* may have beneficial effects in diabetes mellitus by increasing glycolysis and may be source for new generations of antidiabetic drugs. © 2009 Trade Science Inc. - INDIA

KEYWORDS

Diabetes;
Ficus deltoidea;
Glycolytic enzymes.

INTRODUCTION

Diabetes mellitus is a syndrome, which affects more and more people in all countries over the world. It is the fourth or fifth leading cause of death in the world[1]. In Malaysia, diabetes is a growing concern. According to Muhajir et al.[2], the prevalence of diabetes was 0.65% in 1960, 6.3% in 1986, 10.5% in 1996 and 12% in 2005. Clearly, the incidence of diabetes among Malaysians is on the rise. Special attention on the scientific research is needed to control this syndrome.

Ficus deltoidea belongs to the Moraceae family, which includes other plants with proven antidiabetic potency such as *Ficus bengalensis* and *Ficus hispida*[3,4]. Various parts of *Ficus deltoidea* have been used for various medicinal purposes including the treatment of diabetes mellitus. However, there is little report of their antidiabetic activity either in experimental ani-

mal or in humans. For this reason, the study was designed primarily to evaluate the effects of aqueous and ethanolic extracts of *Ficus deltoidea* leaves on the activities of key glucose metabolic enzymes in the livers of non-diabetic and diabetic rats.

METHODS AND MATERIALS

Plant material

The dried plant sample obtained from BIO MAS trading. A voucher number (SK1510/2007) is housed in the Phytomedicinal Herbarium, Institute of Biosciences, Universiti Putra Malaysia, Serdang, Selangor. The leaves were chopped into small pieces.

Preparation of plant extract

Aqueous extract of *Ficus deltoidea* leaves

Ficus deltoidea leaves (200g) were chopped into small pieces, extracted with 1000ml distilled water by continuous hot extraction at 60°C for 6 hours. The mixture was then filtered to obtain the extract. The extract was completely lyophilized by continuous freeze drying operation for 54 hours, yielding 20g of crude extract. A dark semi-solid material was stored at 4°C until usage.

Ethanolic extract of *Ficus deltoidea* leaves

Ficus deltoidea leaves (500g) were extracted with 80% ethanol (2.5L) for 3 days at room temperature (27°C). The mixture was then filtered to obtain the extract. The extract was concentrated at 40°C to yield 50g of crude extract. A dark semi-solid material was stored at 4°C. The crude extracts was first dissolved in water and used in this study[5].

Animals

The study was conducted on thirty-six 2-month-old male *Sprague Dawley* rats weighing 200-250g. The animals were housed in colony cages (six rats per cage), at ambient temperature of 25±2°C with 12-h-light/12-h-dark cycle. Before and during the experiment, rats were fed with normal laboratory pellet diet and water *ad libitium*. After randomization into various groups, the rats were acclimatized for a week in the new environment before initiation of experiment.

Induction of experimental diabetes

After one week of acclimatization, the rats were subjected to a 16-hour fast. Diabetes was induced in rats by a single intravenous injection of streptozotocin (STZ) (Sigma, St. Loius, MO) into tail vein at a dose of 65mg/kg. The STZ was freshly dissolved in 0.1M citrate buffer (pH 4.5)[6]. The induction of STZ-diabetes was confirmed by determination of blood glucose level on the seventh day STZ administration.

EXPERIMENTAL

A total of 36 rats were divided into six groups of six animals each: groups 1-3 include normal rats while groups 4-6 include diabetic rats. Group 1 serves as normal control group and received only saline, group 2 received the aqueous leaf extract twice daily at a dose of 200mg/kg while group 3 received ethanolic leaf extract twice daily at a dose 200mg/kg. The diabetic groups (4-6) paralleled the normal rats. Starting from treatment day zero, saline, aqueous and ethanolic extract of *Ficus deltoidea* leaves were administered orally by gavages twice daily for 14 consecutive days. On day 15, the animals were euthanized using chloroform. The livers were removed immediately, washed with ice cold saline and stored at -80°C. These tissues were used for the assay of hepatic hexokinase, glucokinase and phosphofructokinase.

Biochemical measurement

A portion of the liver tissues was dissected out washed with ice-cold saline immediately and were homogenized in 0.1M Tris-HCl buffer (pH 7.4) for the assay of key enzymes of carbohydrate metabolism. The homogenate was centrifuged at 10000rpm to remove the debris and the supernatant was used as enzyme source for the assays of hexokinase[6], glucokinase[7] and phosphofructokinase[8].

Statistical analysis

Data for activity of hepatic enzymes and blood glucose were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. Data are expressed as means±standard error of mean (SEM). A p-value of less than 0.05 is considered as statistically significant.

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RESULTS AND DISCUSSION

Fasting blood glucose

Before the treatment with saline, aqueous and ethanolic extracts from *Ficus deltoidea* leaves, the fasting blood glucose concentrations of the streptozotocin-induced diabetic rats were more than 4 times higher than those of the non-diabetic rats (Figure 1). However, after 14 days of repeated treatment, the blood glucose levels of the aqueous and ethanolic extract-treated diabetic rats were significantly decreased. In contrast, the fasting blood glucose levels of untreated diabetic rats remained high throughout this investigation. Clearly, injection of streptozotocin at a dose of 65mg/kg body weight had successfully increased the fasting blood glucose level. Streptozotocin injection produced diabetes mellitus via destruction of the β-cells of islets of Langerhans as proposed by[9].

Glycolytic enzymes

Activities of HK, GK and PFK are known to be very sensitive signs of the glycolytic pathway[10]. In the present study, levels of glycolytic enzymes in liver were shown to decrease in the diabetic controls. As clearly shown in Figure 2, Figure 3 and Figure 4, the injection of STZ at a dose of 65mg/kg body weight successfully suppressed the activities of glycolytic enzymes. This concurs with the previous findings by[11] and[12] who found that relative deficiency of insulin in chemical model of type II diabetes caused the suppression of HK, GK and PFK activities.

Figure 2 indicates that the activities of hexokinase were significantly depressed in untreated diabetic rats. Hexokinase activity was decreased by approximately 53.16%. The decrease in hexokinase activities observed in diabetic rats could be due to the loss of insulin receptors and production of glycated proteins[13,14]. The present project found that the activities of HK in diabetic rats treated with AE and EE increased significantly compared to the untreated rats (Figure 2). After 14 days, the hexokinase activity of the DA (diabetic treated with aqueous extract) and DE (diabetic treated with ethanolic extract) groups significantly increased by 40.21% and 62.37% respectively. The significant

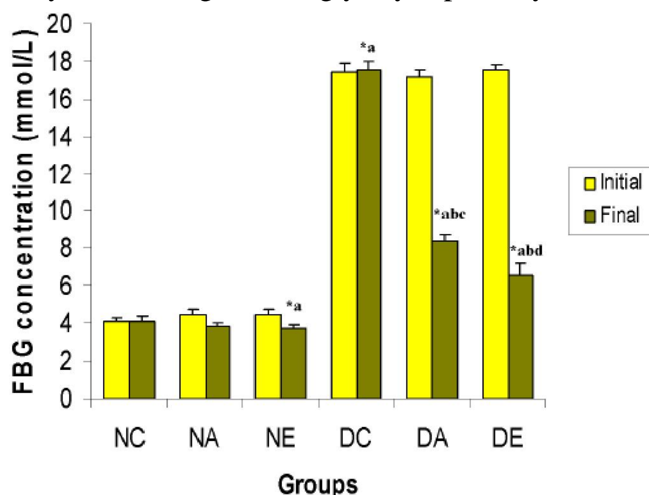

Figure 1 : Effect of aqueous and ethanolic extracts of *Ficus deltoidea* on fasting blood glucose concentration in normal and streptozotocin-induced diabetic male albino rats. Data are expressed as means±S.E.M., *n*=6; *a, statistical significance versus normal control (p<0.05);*b, statistical significance versus diabetic control (p<0.05); *c, statistical significance versus normal treated with aqueous extract (p<0.05); *d, statistical significance versus normal treated with ethanolic extract (p<0.05). NC (normal treated with saline), NA (normal treated with aqueous extract), NE (normal treated with ethanolic extract), DC (diabetic treated with saline), DA (diabetic treated with aqueous extract), DE (diabetic treated with ethanolic extract)

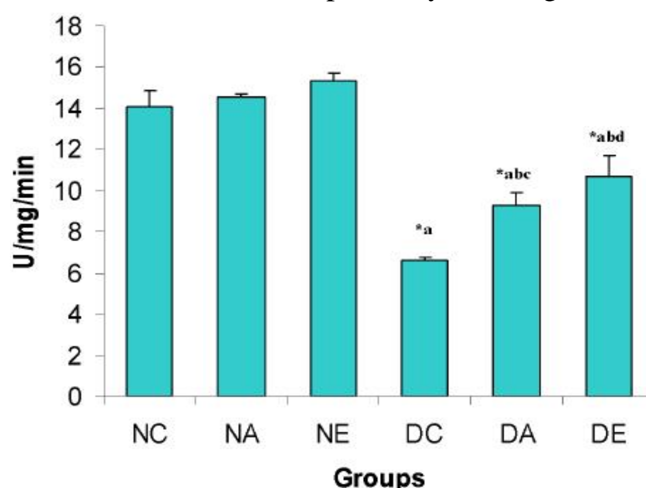

Figure 2 : Effect of aqueous and ethanolic extracts of *Ficus deltoidea* on hexokinase activity in normal and streptozotocin-induced diabetic male albino rats. Data are expressed as means±S.E.M., *n*=6, *a, statistical significance versus normal control (p<0.05); *b, statistical significance versus diabetic control (p<0.05); *c, statistical significance versus normal treated with aqueous extract (p<0.05); *d, statistical significance versus normal treated with ethanolic extract (p<0.05); U, µmol reduction of NAD^+ per minute. NC (normal treated with saline), NA (normal treated with aqueous extract), NE (normal treated with ethanolic extract), DC (diabetic treated with saline), DA (diabetic treated with aqueous extract), DE (diabetic treated with ethanolic extract)

increase in the activity of hexokinase could reflect a flux into the pentose phosphate pathway that in turn may increase the utilization of glucose for NADPH and possibly energy production.

Hepatic glucokinase activity is the most sensitive indicator of the glycolytic pathway in a diabetic state[15]. The present work shows that the activity of glucokinse decreased significantly by 56.79% in diabetic rats. Grover et al.[12], also reported a decrease in the enzymatic activity of glucokinase among diabetic animals which may result in the depletion of liver glycogen. Insulin could control either the activity of GK or its synthesis. Therefore, treatment with exogenous insulin would return GK to a normal physiological level[16]. The present study shows that AE and EE of *Ficus deltoidea* leaves could also play a similar role in bringing GK activity to normal physiological level (Figure 3).

Phosphofructokinase is a key rate-limiting enzyme in glycolysis and represents a major control point in the metabolism of glucose[17]. In the present work, intravenous injection of STZ suppressed the activity of PFK in diabetic rats by approximately 58.70% (Figure 4). Vats et al.[15], had also reported a decrease in PFK activity during diabetes. One reason could be due to the loss of regulation by the enzyme in an attempt to provide adequate sustainable energy. Repeated treatments with AE and EE of *Ficus deltoidea* leaves on non-diabetic rats decreased the activity of PFK (Figure 4). The reduction could probably be due to the decrease in fructoses-2,6-bisphosphate, and important allosteric modulator of the enzyme. The fructoses-2,6-bisphosphate modulates the gluconeogenic counterpart fructose-1,6-bisphosphatase, causing an increase in the enzyme activity and thus increase in glucose-6-phosphatase activity too. Moreover, the results could also be due to the different types of water-soluble active principles with a diversed range of biochemical activities.

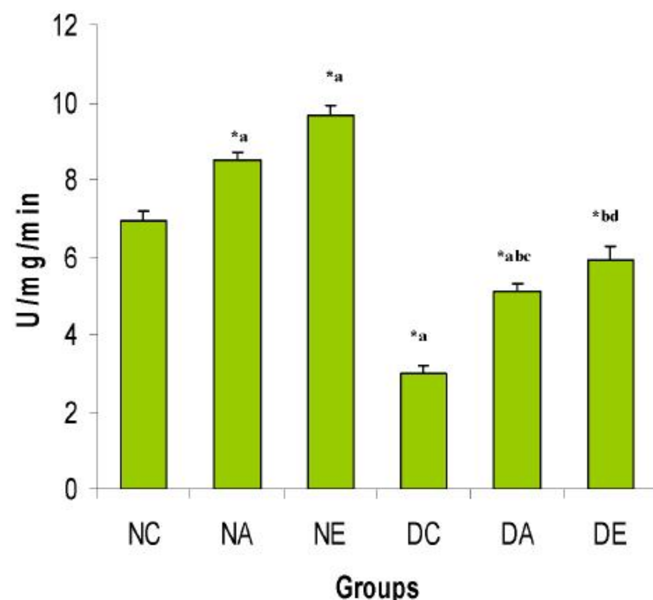

Figure 3 : Effect of aqueous and ethanolic extracts of *Ficus deltoidea* on glucokinase level in normal and streptozotocin-induced diabetic male albino rats. Data are expressed as means±S.E.M., *n*=6; *a, statistical significance versus normal control ($p<0.05$); *b, statistical significance versus diabetic control ($p<0.05$); *c, statistical significance versus normal treated with aqueous extract ($p<0.05$); *d, statistical significance versus normal treated with ethanolic extract ($p<0.05$); U, µmol reduction of $NADP^+$ per minute. NC (normal treated with saline), NA (normal treated with aqueous extract), NE (normal treated with ethanolic extract), DC (diabetic treated with saline), DA (diabetic treated with aqueous extract), DE (diabetic treated with ethanolic extract)

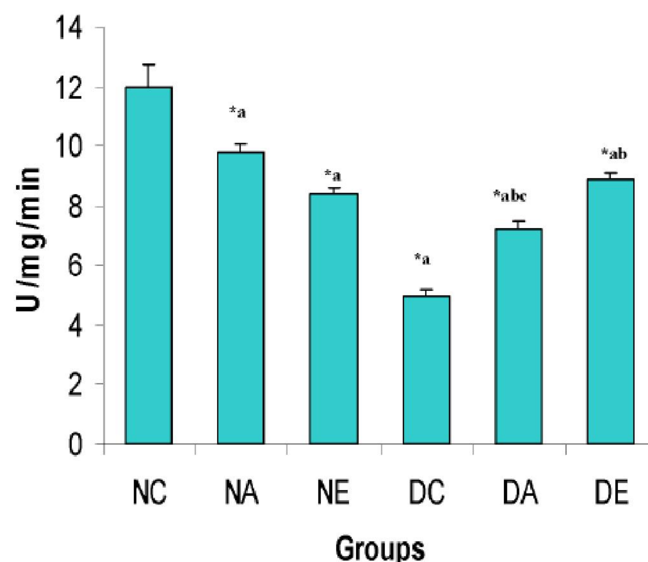

Figure 4 : Effect of aqueous and ethanolic extracts of *Ficus deltoidea* on phosphofructokinase level in normal and streptozotocin-induced diabetic male albino rats. Data are expressed as means±S.E.M., *n*=6; *a, statistical significance versus normal control ($p<0.05$); *b, statistical significance versus diabetic control ($p<0.05$); *c, statistical significance versus normal treated with aqueous extract ($p<0.05$); *d, statistical significance versus normal treated with ethanolic extract ($p<0.05$); U, µmol conversion of NADH to NAD^+ per minute. NC (normal treated with saline), NA (normal treated with aqueous extract), NE (normal treated with ethanolic extract), DC (diabetic treated with saline), DA (diabetic treated with aqueous extract), DE (diabetic treated with ethanolic extract)

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