Antidiabetic activity of the leaves of *Tetracera indica* Merr. (Dilleniaceae) *in vivo* and *in vitro*

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In folk remedies, the leaves of *Tetracera indica* are used to treat diabetes in Malaysia. This study was aimed at investigating the antidiabetic potential of *T. indica* leaves *in vivo* and *in vitro* to prove its usefulness in diabetes. Aqueous (AQ) and methanol (MEOH) extracts of *T. indica* leaves were administrated to normal and alloxan induced diabetic male albino rats (Sprague Dawley strain). Two doses of each extract (250 and 500 mg/kg B.W) were evaluated for antidiabetic activity *in vivo*. The blood glucose levels were measured at 0, 2, 4, 6 and 8 h after oral administration of extracts. Comparison was made with glibenclamide (GLBC). For the *in vitro* method, the effect of both extracts on the lipid accumulation of 3T3-L1 adipocytes was analyzed by using Oil Red O staining. In addition, 2-deoxy-D-[3H] was used to measure the effect of the extracts on glucose uptake activities. Both extracts exhibited antihyperglycemic activity in alloxan induced diabetic rats, however in normal rats no hypoglycemic activity was observed, when compared with both +ve and -ve controlled groups. The LD50 of both extracts was found to be more than 5000 mg/kg body weight and no lethal toxicity was observed within this range. For *in vitro* analysis, AQ extract was found to reduce triglyceride accumulation on 3T3-L1 cells in a dose-dependent manner whereas cells treated with MEOH extract significantly induced lipid accumulation. Besides, 2-deoxy-D-[3H] glucose uptake activities were significantly different at dose 200 µg/mL (*P* < 0.05 compared to DMSO control). Moreover, the data were significant (*P* > 0.05) with MDI (inducer) and metformin which were used as the positive control for the *in vitro* assay.

**Key words:** *Tetracera indica* Merr., dilleniaceae, antidiabetic activity, *in vivo*, *in vitro*, rats, adipocytes.

**INTRODUCTION**

Diabetes mellitus is no longer an epidemic that can be ignored. It is a complex disorder characterized by a relative or absolute insufficient secretion dependent diabetes mellitus (IDDM) or concomitance resistance of the metabolic action of insulin on target tissue, non insulin diabetes mellitus (NIDDM) (Alberto and Swapnil, 2001). The prevalence of diabetes for all age-groups worldwide has been estimated to be 2.8% in 2000 and expected to reach up to 4.4% in 2030 (Sarah et al., 2004). The International Diabetes Federation (IDF) has estimated that 246 million people worldwide suffer from diabetes and this figure is projected to increase to 380 million by the year 2025 (International Diabetes Federation, Diabetes Atlas, Third Edition, 2006). The
prevalence of diabetes in Asian populations has increased rapidly in recent decades. In 2007, more than 110 million individuals in Asia were living with diabetes, with a disproportionate burden among the young and middle aged.

Similarly, rates of overweight and obesity are increasing sharply, driven by economic development, nutrition transition, and increasingly sedentary lifestyles (Lee et al., 2007).

The number of diabetics in Malaysia has increased by almost 80% in the last 10 years from 1996-2006 to 1.4 million adults above the age of 30. Ninety five percent of diabetic patients suffer from type 2 diabetes, of which 50 percent will develop cardiovascular disease within 10 years (http://thestar.com.my/news/story.asp?file=/2010/1/11/nation/20100111140301&sec=nation).

In the measurement of diabetes mellitus, several approaches are often employed which include dietary intervention, use of different classes of oral but safe hypoglycemic agents, insulin injection, aerobic exercise and food supplements (Davis and Granner, 2001). However, in developing countries and in some other developed countries, these therapeutic options are expensive, and not readily accessible, especially to the poor. Beside these, the therapeutic regimen are considered rigid, multi-pharmaceutical and often associated with intolerable side effects. These factors militate against effective management/treatment of the patients. In view of these shortcomings, herbal pharmacotherapy is often explored by these patients. Plants are frequently considered to be less toxic and free from side effects than synthetic ones (Jung et al., 2006). Recently, the search for appropriate anti-diabetic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs responsible for unrevealing serious side effects among diabetics (Rates, 2001).

_Tetracera indica_ (Houtt. Ex Christm. and Panz.) Merr. (DILLENIACEAE) (T. indica), (synonyms: Assa indica Christm and Panz) (Malay name: Mempelas minyak or Mempelas paya), is a large, woody, rain forest climber of Malaysia and Indonesia. It has a medium sized and simple leaves that arrange alternately with each other. Its flowers are white in color with small pink calyces. The flowers also have a nice fragrance. It has fruits that taste kind of sour and look like berries (Christophe, 2002).

In folk remedies, different parts of _T. indica_ have been found to act effectively in the treatment of fever, flu, sinus symptoms, skin rashes, itching, piles, mouth ulcer, diarrhea, insect bites and diabetes. In Machang, Kelantan, Malaysia, decoction of the climbing stems and roots is drunk to reduce high blood pressure and the leaves, crushed and mixed with water, are applied on the whole body to treat fever (Ong and Nordiana, 1999). Temuan tribe in Selangor, Malaysia uses its roots concoction to treat high blood pressure and high fever, while leaves and root pounded together is used to treat skin itching (Faridah and Nurulhuda, 1999). Moreover, its shoot ground, wrapped in banana leaves are heated then applied to treat headache (Latiff and Zakri, 1996). Some undocumented claims have been recorded in the field study of _T. indica_ leaves in the management of diabetes in different parts of Pahang DM, Malaysia. Furthermore, _T. indica_ is also one of the active ingredients in a local herbal medicine viz, Plantisol, which is widely prescribed to treat diabetes in Malaysia by the local herbalists. Other active ingredients of Plantisol are _Barringtona racemosa_, _Pithecellobium jirina_, _Tinospora crispa_ and _Andrographis paniculata_. (http://www.klik4sihat.com/kencingmanis). Phytochemical examination of _T. indica_ leaves has already revealed the presence of four terpenoids viz., β-sitosterol, lupeol, betulin, betulinic acid (Dan and Dan, 1980) and a lone flavonoid viz., 5, 7-dihydroxy-8-methoxyflavone (Wogonin) (Harrison et al., 1994).

The wide usage of _T. indica_ leaves by the local traditional practitioners to cure diabetes and its undocumented claims in the treatment of diabetes in Malaysia have prompted us to evaluate the efficacy of _T. indica_ leaves in the management of diabetes mellitus _in vivo_ and _in vitro_ to confirm its effectiveness as well as to prove its undocumented traditional antidiabetic claims scientifically.

**MATERIALS AND METHODS**

**Collection of plant material**

Fresh _T. indica_ leaves (15 kg) were procured from the local garden Taman Pertanian, Indera Mahkota, 25200-Kuantan, Pahang Darul Makmur, Malaysia. The plant was identified and authenticated by the taxonomists at Taman Pertanian and its sample was deposited in the herbarium of Faculty of Pharmacy, IIUM, Kuantan, Pahang DM, Malaysia (voucher specimen number QPC-017) for future references.

**Preparation of polar extracts of the leaves of _T. indica_**

15 kg fresh leaves were dried in a laboratory dryer within a temperature range (30 to 40°C) and were pulverized to a crude powdered form by applying Fritsch Universal Cutting Mill-PULVERISETTE 19-Germany and the grinded material was made ready for the extraction process 4.7 kg (31.33%).

**Aqueous extract (AQ) of the leaves of _T. indica_**

1.5 kg powdered (pulverized) material was soaked in 4 L of sterile double distilled water in a tightly closed round bottle flask at room temperature for a period of 24 h and filtered through Buchner funnel. The whole process was repeated 3 times to ensure the maximum yield of water soluble components and further heated at 95°C on water bath for 1 h to ascertains maximum yield of water soluble compounds. The extract was freeze dried to give a final yield of 130 g aqueous (AQ) extract (8.86%).
Methanol (MEOH) extract of the leaves of *T. indica*

3 kg of crude powdered material was soaked in 9 L analytical grade distilled methanol for 24 h, filtered through Buchner funnel, concentrated in a reduced pressure using Buchi rotary evaporator. Recovered methanol was again poured into the already extracted powdered material, filtered and concentrated. The entire process was repeated about 4 times till the plant material stopped giving colouration and eventually freeze-dried giving a final yield of 367.5 g MEOH extract (12.25%).

**Experimental animals**

Adult albino rats of Sprague Dawley (SD) strain, each weighing 150 to 260 g, bought from Mikro Makmur Enterprises, Kuantan Pahang, Malaysia, were used for this study. The animals were grouped and housed in an air conditioned, maintained under standard condition of temperature 25 ± 2°C with 12 h/12 h dark and light cycle. They were allowed to acclimatize for a period of 2 weeks, given free access to standard dry pellet diet and water ad libitum, prior to the experiment.

**Acute toxicity evaluation of AQ and MEOH extracts of *T. indica***

Twelve male albino rats (SD) (n = 6), weighing 200 to 280 g were administered a grade dose of 5000 mg/kg b.w. of both AQ and MEOH extracts, after 16 h fasting, following up and down procedure according to (OECD guideline 2008). The animals were observed at 1 h after administration of extracts, and also at 6 h and up to 24 h for any sign of toxicity viz., weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing and mortality (Hamid et al., 2008; Umar et al., 2010).

**Induction of diabetes**

Diabetes mellitus was induced in the rats by single intraperitoneal injection of 160 mg/kg B.W of freshly prepared alloxan monohydrate in normal saline. In order to prevent fatal hypoglycemia due to massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h followed by 5% glucose solution bottles in their cages for a period of 24 h. After 4 days, the animals showing blood glucose level ≤ 14.50 mmol/L were considered diabetic and used for the study. All experiments were conducted under strict observance of animal ethics guidelines after getting the permission (IIUM/305/20/4/10) from the Institutional Animal Ethics Committee of International Islamic University Malaysia, Faculty of Medicine, Kuantan Campus, IIUM, and Malaysia.

**Experimental design**

Twelve different groups of animals were designated to check the antidiabetic efficacy of AQ and MEOH extracts of the leaves of *T. indica*, respectively. Experiment consisted of 72 male albino rats of the Sprague Dawley (SD) strain, divided equally (n = 6) among the AQ and MEOH extracts and fasted overnight. Respective groups were divided as follows:

- **Group 1**: Diabetic Control Rats
- **Group 2**: Normal Control Rats
- **Group 3**: Normal Rats/250 mg/kg B.W AQ Ext
- **Group 4**: Normal Rats/500 mg/kg B.W AQ Ext
- **Group 5**: Diabetic Rats/250 mg/kg B.W AQ Ext
- **Group 6**: Diabetic Rats/500 mg/kg B.W AQ Ext
- **Group 7**: Normal Control/Emulsion
- **Group 8**: Normal Control/250 mg/kg B.W MEOH Ext
- **Group 9**: Normal Control/500 mg/kg B.W MEOH Ext
- **Group 10**: Diabetic Rats/250 mg/kg B.W MEOH Ext
- **Group 11**: Diabetic Rats/500 mg/kg B.W MEOH Ext
- **Group 12**: Diabetic Rats/GLBC (0.25 mg/kg B.W).

The MEOH extract prepared in the form of emulsion (oil: water, 6:4) and AQ extract in double-distilled sterilized water, were administered to the experimental animals by gastric intubation using forced feeding needle. Blood samples from the tail vein were collected for the measurement of blood glucose at 0 (base line), 2, 4, 6 and 8 h after administering the extracts. Blood glucose was measured using one touch Ultra glucometer (LifeScan, USA) and the results were compared with those of 12th group treated with glibenclamide (0.25 mg/kg B.W) a standard oral hypoglycemic agent (Ji et al., 2006). The rats were randomly distributed for the aqueous (AQ) and methanol (MEOH) extracts viz., untreated diabetic control rats (Group 1), diabetic rats receiving the AQ or MEOH extracts at 250 mg/kg B.W (Groups 5, 10), 500 mg/kg B.W (Groups 6, 11), diabetic rats treated with glibenclamide (GLBC) 0.25 mg/kg B.W (Group 12), normal control rats (Group 2), normal rats receiving the AQ or MEOH extracts at 250 mg/kg B.W (Groups 3, 8), normal rats receiving AQ or MEOH extracts at 500 mg/kg B.W (Groups 4, 9). In the case of MEOH extract, Group 7 was administered the emulsion (oil: water 6:4) only and in case of AQ extract, Group 2 was administered double-distilled sterilized water to confirm any untoward, anti-diabetic or cumulative effect by both emulsion as well as double-distilled sterilized water in the rats, respectively (Umar et al., 2010; Yankuzo et al., 2011).

**Cell culture**

3T3-L1 preadipocyte cells were obtained from the American Type Culture Collection, USA and were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% foetal bovine serum (FBS). Cells were subcultured every 3 to 4 days at approximately 80% confluence. Cells were then seeded onto 12-well plates at a density of 2 x 10⁵ cells/well. Two days post confluence (defined as day-0), cells were stimulated to differentiate with differentiation medium containing DMEM with 10% FBS, MDI [0.5 mM 3-isobutyl-1-methyl-xanthine (IBMX), 0.25 µM dexamethasone, 1 µg/mL insulin] for 2 days. In the course of screening adipocyte differentiation-inhibitory activity, 3T3-L1 preadipocytes were treated with differentiation medium in the presence of various concentrations of test extracts at day-0. Day 2, differentiating media was replaced with 10% FBS/DMEM medium containing 1 µg/mL insulin and incubated for another two days (day 4). Thereafter, the cells were maintained in 10% FBS/DMEM medium for an additional 4 days (day 8) with medium changes every 2 days. All media contained 1% penicillin-streptomycin (10,000 U/mL). Cells were maintained at 37°C incubator in a humidified atmosphere of 5% CO₂ (Nidhina et al., 2011).

**In vitro concentration dose design (cell viability)**

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Mosmann, 1983). mature adipocytes were seeded in 96-well plates and grown until confluence. Test materials were dissolved in dimethyl sulfoxide (DMSO). Cells were then incubated with either 0.01% DMSO or extracts (10, 20, 50 and 100 µg/mL). Preliminary study showed that DMSO at a final concentration of < 0.1% in media did not affect cell viability or differentiation. Experiment was allowed to proceed for 48 h at 37°C in humidified 5% CO₂ atmosphere. Cells were then washed twice with phosphate buffer saline (PBS). After that, 20 µL of MTT stock solution (5 mg/mL) was added to each well and the
plates were further incubated for 4 h at 37°C. 100 µL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the water-insoluble purple formazan crystals. After 1 h, the absorbency was measured at 570 nm and reference wavelength of 630 nm with a microplate reader.

**Adipocyte differentiation**

Eight days after the differentiation induction, cells were washed three times with PBS and fixed with 10% formalin for 1 h at room temperature. After fixation, cells were washed with PBS once and stained with freshly diluted Oil Red O solution (3 parts of 0.6% oil red o in isopropanol and 2 parts of water) for 1 h. Cells were then washed twice with distilled water and visualized under a microscope. Images were captured on an Olympus microscope. For quantitative analysis, Oil Red O staining was dissolved with isopropanol and optical density was measured at 520 nm by multilwell plate reader (TECAN) (Shin et al., 2010).

**Glucose uptake activity assay**

Glucose uptake activity was analyzed by measuring the uptake of radiolabeled glucose according to procedure performed by Bai et al. (2010). Briefly, > 90% fully differentiated adipocytes grown in 12-well plates were washed twice with serum-free DMEM and incubated for 3 h at 37°C with 1 mL of serum-free DMEM. The cells were then washed three times with Krebs - Ringer HEPES (KRPH) buffer (118 mM NaCl, 5 mM KCl, 1.3 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4 and 30 mM HEPES, pH 7.4) and incubated with 0.9 mL of KRPH buffer for 30 min at 37°C. Insulin and test extracts including the control was added, and the cells were incubated at 37°C for a further 60 min. Glucose uptake was initiated by the addition of 0.1 mL of KRPH buffer containing 2-deoxy-D-[6H]glucose (0.037 MBq; Perkin Elmer) and glucose (0.001 mM). After 60 min, glucose uptake was terminated by washing the cells three times with ice-cold PBS. The cells were lysed through incubation for 20 min at 37°C with 0.7 mL of 1% Triton X-100. Levels of radioactivity in the cell lysates were determined using a Tri-Carb 2000TR liquid scintillation counter.

**Phytochemical characterization of AQ and MEOH extracts of the leaves of T. indica**

Total phenolic content in AQ and MEOH extracts of T. indica leaves was determined by the Folin-Ciocalteu assay (McDonald et al., 2001; Kim et al., 2007) with some modifications. Briefly, 0.25 mL of plant extracts (0.1 mg/mL) were mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and allowed to stand for 5 min at room temperature. Then, 2 mL of 1 M Na2CO3 was added and the mixture was incubated at room temperature for 2 h. Finally, total phenolic content was estimated at 765 nm using a spectrophotometer (Jenway 6405 UV/vis Dunmow, Essex, UK). A standard curve was prepared using gallic acid (0-250 mg/L) in methanol: water (50:50, v/v). Total phenolic values are expressed as gallic acid equivalents (GAE) mg/g of plant extracts. All determinations were performed in triplicate.

Total flavonoid content in AQ and MEOH extracts of T. indica leaves was estimated using the Dowd method as adapted by Arvouet-Grand et al. (1994). Briefly, 2.5 mL of 2% AlCl3 in methanol was mixed with 2.5 mL of plant extract (0.1 mg/mL). The mixture was allowed to stand for 10 min at room temperature and the total flavonoid content was determined by UV spectrophotometer at 415 nm using a quercetin (0 to 250 mg/L) standard curve. Total flavonoids values are expressed in terms of quercetin equivalents (QE) mg/g of plant extract. All determinations were performed in triplicate.

Thin layer chromatography (TLC) evaluation of AQ and MEOH extracts of T. indica leaves was carried out on silica gel 60 F254, 0.2 mm thickness aluminium plates, in benzene:acetone (B:A) (9:1, 5:1 and 3:1), toluene:ethylformate:formic acid (T:E:F) (5:4:1), benzene:pyridine:formic acid (B:P:F) (39:6:5), chloroform:methanol:formic acid (C:M:F) (90:05:06) and benzene:acetone:formic acid (B:A:F) (3:1:0.1), respectively to confirm the presence of different class of phytochemicals by using different class of selected reagents (Harborne, 1998).

**Determination of free radical scavenging activity (antioxidant activity) of T. indica**

Free radical scavenging activity of AQ and MEOH extracts of T. indica leaves was measured by the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical according to the method adopted by Shimada et al. (1992) and Mensor et al. (2001) with slight modification. Briefly, 100 µL from each polar extracts (MEOH and AQ) methanolic solution at different concentrations (12.5-100 µg/mL) was added to 200 µL solution of DPPH (25 mg/mL) in methanol and the reaction mixture was shaken vigorously. Blank solution was prepared by adding 100 µL of methanol into 200 µL solution of DPPH (25 mg/mL) and used as a blank sample solution in 96 well plates. The samples were kept in the dark for 30 min at ambient temperature and decrease in absorption was measured in multi-detector micro plate reader (infinite M 200 Nanoquant) at 517 nm. The experiment was carried out in triplicate and radical scavenging activity was calculated by the following formula:

% Inhibition = \[ \frac{A_b - A_a}{A_b} \times 100 \]

Where, Aa = Blank sample and Ab = Extract sample

L-Ascorbic at different concentrations (12.5-100 µg/mL) was used as positive control. IC50 was obtained by linear regression analysis of the dose response curve b/w % inhibition and concentrations.

**Statistical analysis**

Results are presented as means ± standard error of experiments. Data were analyzed by repeated measurements and one way ANOVA using SPSS version 19. Values of p < 0.05 were considered statistically significant.

**RESULTS**

**Plant extraction and phytochemical characterization and antioxidant activity of AQ and MEOH extracts of the leaves of T. indica**

Upon extraction at room temperature, 1.5 kg pulverized plant material yielded 130 g freeze dried AQ extract (8.66%) and 3 kg pulverized plant material yielded 367.5 g freeze dried MeOH extract (12.25%). TLC examination of these extracts was characterized by analyzing their contents of phenols and flavonoids, the main compounds reputed as to be responsible for the antidiabetic properties of many plants (Jung et al., 2006). Phytochemical TLC examination of MEOH and AQ extracts of T. indica leaves in BA, TEF, BPF, CMF and BAF solvent systems showed the presence of more than
nine spots of phenolic compounds and five terpenoidal compounds.

The chemical characterization of AQ extract resulted in 5.25 ± 0.07 GAE mg/g of phenolic compounds, 6.23 ± 0.02 QE mg/g of flavonoids, whereas the chemical characterization of MEOH extract was 6.45 ± 0.07 GAE mg/g of phenolic compounds, 7.15 ± 0.05 QE mg/g of flavonoids.

The AQ and MEOH extracts of *T. indica* leaves at various concentrations (12.5 to 100 µg/mL) were tested for antioxidant activity using the DPPH assay. The results exhibited free radical scavenging activities for both MEOH and AQ extracts in different concentrations during analysis. The mean values for inhibition ranged from 35.886 to 72.965% and 12.416 to 89.857% for MEOH and AQ extracts, respectively. The IC_{50} inhibition of both extracts was calculated based on the results obtained using L-Ascorbic acid, as reference standard for MEOH and AQ extracts, respectively. The results showed that MEOH and AQ extract contained IC_{50} inhibition of 38.087 and 49.363, respectively.

### Determination of non-toxic concentrations of AQ and MEOH extracts of the leaves of *T. indica* in vivo

Acute toxicity study revealed about the safe nature for both AQ and MEOH extracts of *T. indica* leaves. Experiment was conducted on normal healthy rats. No mortality in rats was observed by both extracts and their behaviour also appeared normal in all the animals. Neither lethality nor any toxic reaction among animals was found at any dose selected until the conclusion of study.

### Determination of antidiabetic activity of AQ and MEOH extracts of the leaves of *T. indica* in vivo

Freshly prepared alloxan monohydrate was administered by single intraperitoneal injection of 160 mg/kg B.W and diabetes was manifested in rats after 4 days. The effect of different doses of AQ and MEOH extracts on fasting blood glucose levels in normal rats and alloxan induced diabetic male albino rats (SD) have been summarized in Table 1. The fasting blood glucose levels in all the animals were observed in the range of 14.3 to 31.8 mmol/L.

The fasting glucose level of diabetic untreated rats (Group 4) was significantly higher than that of normal untreated rats (Group 1). AQ extract of the leaves of *T. indica* at the dose of 250 mg and 500 mg/kg B.W, revealed the highest antihyperglycemic activity at 8 h post treatment, respectively. While the antihyperglycemic activity of AQ extract at all the selected doses in diabetic rats was found to be less than the oral hypoglycemic agent, glibenclamide (GLBC). The MEOH extract at 500 mg/kg B.W exhibited the highest antihyperglycemic activity in diabetic rats after 6 and 8 h post treatment, respectively than that of the GLBC. However, 250 mg/kg B.W of MEOH extract exhibited the highest antihyperglycemic activity at 8 h post treatment. MEOH extract revealed a significant reduction in blood glucose level (that is, antihyperglycemic activity) in the diabetic rats at 4, 6 and 8 h for 250 mg/kg B.W and 6 and 8 h for 500 mg/kg B.W post treatment, respectively.

MEOH extract exerted highest antihyperglycemic effect (58.2%) and (55.3%) (p < 0.05) at 500 mg and 250 mg/kg B.W at 8 h post treatment, respectively, however, AQ extract displayed highest antihyperglycemic effect (77.4%) and (74.1%) at 250 mg and 500 mg/kg B.W at 8 h post treatment, respectively. The significant antihyperglycemic activity of MEOH and AQ extracts was found to be comparable with a known oral synthetic hypoglycemic drug, glibenclamide (GLBC) (Table 1).

Different doses of MEOH and AQ extracts of the leaves of *T. indica*, produced a significant fall in the blood glucose levels of diabetic rats (anti-hyperglycemic effect) (Figure 1) and the same doses did not exhibit any

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**Table 1.** Effect of different doses of AQ and MEOH extracts of the leaves of *T. indica* on blood glucose levels (mmol/L) in normal and diabetic rats at different time intervals (h). p* = < 0.05, p** = < 0.005, GLBC= Glibenclamide.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Groups/doses</th>
<th>0 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diabetic control rats</td>
<td>29.3 ± 3.8</td>
<td>27.4 ± 2.6</td>
<td>27.1 ± 3.1</td>
<td>28.2 ± 3.3</td>
<td>26.1 ± 4.1</td>
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<td>2</td>
<td>Normal control /distilled H₂O</td>
<td>4.4 ± 0.4</td>
<td>3.9 ± 0.4</td>
<td>4.4 ± 1.2</td>
<td>3.6 ± 0.7</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>3</td>
<td>Normal rats/250 mg/kg B.W AQ Ext</td>
<td>5.2 ± 0.6</td>
<td>4.5 ± 0.6</td>
<td>5.7 ± 0.4</td>
<td>5.7 ± 0.8</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>Normal rats/500 mg/kg B.W AQ Ext</td>
<td>4.5 ± 0.5</td>
<td>3.9 ± 0.4</td>
<td>4.8 ± 0.9</td>
<td>4.0 ± 0.4</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>Diabetic rats/250 mg/kg B.W AQ Ext</td>
<td>21.2 ± 2.6</td>
<td>18.1 ± 2.4**</td>
<td>13.4 ± 1.0**</td>
<td>9.9 ± 1.6**</td>
<td>8.8 ± 1.8**</td>
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<tr>
<td>6</td>
<td>Diabetic rats/500 mg/kg B.W AQ Ext</td>
<td>22.6 ± 2.2</td>
<td>16.6 ± 1.8**</td>
<td>12.7 ± 1.7**</td>
<td>9.2 ± 2.1**</td>
<td>6.9 ± 1.2**</td>
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<td>7</td>
<td>Normal control/emulsion</td>
<td>5.3 ± 1.0</td>
<td>4.4 ± 0.5</td>
<td>4.8 ± 1.3</td>
<td>3.9 ± 0.9</td>
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<tr>
<td>8</td>
<td>Normal control /250 mg/kg B.W MEOH Ext</td>
<td>3.9 ± 0.5</td>
<td>6.1 ± 1.0</td>
<td>3.3 ± 0.9</td>
<td>3.7 ± 0.9</td>
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</tr>
<tr>
<td>9</td>
<td>Normal control/500 mg/kg B.W MEOH Ext</td>
<td>6.0 ± 1.0</td>
<td>4.5 ± 0.6</td>
<td>4.3 ± 1.5</td>
<td>5.3 ± 2.4</td>
<td>4.4 ± 1.5</td>
</tr>
<tr>
<td>10</td>
<td>Diabetic rats/250 mg/kg B.W MEOH Ext</td>
<td>18.9 ± 2.9</td>
<td>12.8 ± 4.7**</td>
<td>11.4 ± 2.4**</td>
<td>9.1 ± 2.2**</td>
<td>6.6 ± 2.8**</td>
</tr>
<tr>
<td>11</td>
<td>Diabetic rats/250 mg/kg B.W MEOH Ext</td>
<td>22.0 ± 4.2</td>
<td>14.7 ± 2.1**</td>
<td>11.4 ± 1.5**</td>
<td>7.4 ± 2.6**</td>
<td>4.8 ± 0.8**</td>
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<tr>
<td>12</td>
<td>Diabetic rats/GLBC (0.25 mg/kg B.W)</td>
<td>27.1 ± 7.8</td>
<td>14.0 ± 7.6**</td>
<td>11.3 ± 5.6**</td>
<td>8.2 ± 4.0**</td>
<td>5.8 ± 2.7**</td>
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Figure 1. Percentage fall (%) on glucose level of different doses of AQ and MEOH extracts of *T. indica* leaves on diabetic rats. (Note: Grp 1_diabetic rats/250 mg AQ ext; Grp 2_diabetic rats/500 mg AQ ext; Grp 3_diabetic rats/500 mg MEOH ext; Grp 4_diabetic rats/0.25 mg GLBC; Grp 5_diabetic rats/250 mg MEOH ext; Grp 6_diabetic control).

Figure 2. Effect of different concentrations of MEOH and AQ extracts of *T. indica* on the proliferation of 3T3-L1 adipocytes. Cell viability after treatment was determined by MTT assay. Assays were performed at least three times with 3 replicates for each treatment. *p < 0.05, **p < 0.01 compared with differentiated control.

Determination of antidiabetic activity of AQ and MEOH extracts of *T. indica* leaves in vitro

Effect of AQ and MEOH extracts of *T. indica* leaves on the viability of 3T3-L1 cells

The viability assay did not show any cytotoxic effects up to 500 µg/mL for AQ and MEOH extracts on 3T3-L1 cells (Figure 2). In addition, both extracts decreased the cell population growth in a dose dependent manner with significant reduction at dose 1 mg/mL. The cytotoxic effect of MEOH extracts on 3T3-L1 cells was observed at 1 mg/mL.

Effect of AQ and MEOH extracts of the leaves of *T. indica* on adipocyte differentiation of 3T3-L1 preadipocyte

Eight days after the differentiation, morphological changes of increased and decreased lipid accumulation were observed in these cells (Figure 3). MEOH extract of the leaves of *T. indica* was found to enhance lipid accumulation in the cytoplasm of treated cells while AQ extract of the leaves of *T. indica* was found to inhibit the hypoglycemic effect in normal non-diabetic rats in comparison to control.
adipogenesis process with increased concentration (Figure 4). As shown by the oil red o elution, *T. indica* MEOH extract induced intracellular fat accumulation in a concentration dependent manner with a 1.50 fold, 1.56 fold and 1.61 fold increased at concentration 100, 200 and 500 µg/mL compared to DMSO control. Besides, treatment with *T. indica* AQ extract significantly inhibited the adipogenesis process with up to 56.4% of inhibition relative to MDI-treated control cells at dose 500 µg/mL.

**Effect of AQ and MEOH extracts of the leaves of *T. indica* on glucose uptake stimulation in differentiated 3T3-L1 adipocytes**

The results showed that both extracts (MeOH and AQ) were found to stimulate glucose uptake in mature 3T3-L1 adipocytes (Figure 5) with *T. indica* MEOH extract showed a more promising uptake. In addition, both extract’s effects at dose 200 µg/mL were significant with MDI and metformin action.

**DISCUSSION**

Since antiquity, diabetes has been treated with plant medicines. A number of experimental and clinical studies have shown the efficacy of various herbs in lowering blood glucose in diabetes. These herbal preparations exhibit their beneficial effects by different mechanisms which may or may not affect insulin release (Hui et al., 2009). In view of traditional use of *T. indica* in treating diabetes, the present study was carried out to prove the traditional claims scientifically.

Currently, different kinds of synthetic drugs viz., biguanides, diphenylalanine derivatives, glucosidase inhibitors, meglitines, sulphonylureas, and thiazolidinediones in addition to insulin, are widely used in the management of diabetes all over the world. However, due to untoward side effects, the efficacies of these drugs are quite controversial and there is a strong demand for new but safe drugs for the treatment of diabetes efficaciously (Thirunavukkarasu et al., 2003). Plants have been suggested as a rich, as yet unexplored...
source of potentially useful antidiabetic drugs. However, only a few have been subjected to detailed scientific exploration due to a lack of mechanism based available in vitro assays (Koehn and Carter, 2005; Bnouham et al., 2006; Frode and Medeiros, 2008).

A number of experiments have shown the beneficial effects of medicinal plants in the management of diabetes mellitus. Numerous mechanisms of actions have been proposed for these plant extracts. Some reports have linked their effects to the activity of pancreatic cells (synthesis, release, cell regeneration/revitalization) (Yi et al., 2009) or the increase in the inhibitory effect against
insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts (Meng et al., 2009; Angel et al., 2010). Others have suggested that the mechanisms may involve improved glucose homeostasis (Ahmad et al., 2000), increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen (Yuan et al., 1998) and/or decrease of glycogenolysis acting on enzymes (El-Missiry and El Gindy, 2000) inhibition of intestinal glucose absorption (Nicola et al., 1996), reduction of glycemic index of carbohydrates (Frati et al., 1998) and reduction of the effect of glutathione (Raza et al., 1996).

In our study, different doses of AQ and MEOH extracts of the leaves of T. indica produced a significant fall in the blood glucose levels of diabetic rats without showing any hypoglycemic effect in animals. Although, both AQ and MEOH extracts of the leaves of T. indica exhibited significant antihyperglycemic activity in the diabetic rats, the MEOH extract statistically proved more potent in comparison to standard oral antidiabetic drug (Glibencamamide), however, there was no hypoglycemic activity observed with the both extracts in normal rats.

Adipocyte differentiation is a crucial process to maintain the normal function of adipocyte (Shin et al., 2010). Many lipid droplets were observed in mature 3T3-L1 cells stimulated by the inducers (Hata et al., 2008). The formations of lipid droplets serve as the marker for adipocyte differentiation process. In this study, we found that MEOH extract of the T. indica leaves dose-dependently enhanced adipocyte differentiation in 3T3-L1 cells. In the absence of inducer cocktail (MDI), the MEOH extract was still able to differentiate adipocyte formation in 3T3-L1. Hence, it is suggested there is an insulin mimicking activity of the MEOH extract on adipocyte. Accordingly, stimulation of glucose uptake is another insulin mimicking activity of the extract. In contrast, the AQ extracts of the leaves reduced the adipocyte differentiation process. Since inhibition of adipocyte differentiation has been suggested as an important approach for treatment and prevention of obesity (Shin et al., 2010), we propose that T. indica AQ extracts can also be used for the management of diabetes-obesity related diseases. Besides, glucose uptake in insulin-responsive tissues such as skeletal muscle and adipose tissue is essential for the maintenance of whole body glucose homeostasis (Choi et al., 2009). Enhanced glucose transport and utilization can lower the blood glucose both in animal and human. Metformin and sodium orthovanadate which are two antidiabetic drugs known to increase cellular glucose utilization, on leptin secretion, glucose uptake, and lactate production in isolated cultured rat adipocytes (Mueller et al., 2000) was used as the positive control. Our results show that both extracts can significantly stimulate glucose uptake (P < 0.05) compared to DMSO control cells. Furthermore, the treated adipocytes also showed significant result with metformin and MDI treated cells.

The phytochemical screening revealed the presence of flavonoids and sterols/triterpenoids which play a major role in controlling free radicals and diabetes (Jung et al., 2006; Umar et al., 2010; Yankuzo et al., 2011). Oxidative stress is one of the pathophysiological mechanisms that develops diabetes and leads to its complications. Chronic excess of glucose causes toxic effects on structure and impairment of organs function due to free radical generation (Kaneto et al., 2006). Both extracts were found to exhibit strong antioxidant activity which could be another key factor for their ameliorating effect on antidiabetic activity. Moreover, flavonoids are known to regenerate the damaged β-cells in the alloxan induced diabetic rats and are considered effective antihyperglycemic agents (Chakravarthy et al., 1982; Hif and Howell, 1984). Flavonoids, especially quercetin, have been reported to possess antidiabetic activity. It is reported that quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in streptozotocin-induced diabetic rats (Vessal et al., 2003). Also in another study, it has been reported that quercetin stimulates insulin release and enhances Ca2+ uptake from isolated islets cell which suggest a place for flavonoids in noninsulin-dependent diabetes (Hif and Howell, 1985). The antidiabetic effect of T. indica extracts may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. In this study, the antihyperglycemic activity caused by GLBC in alloxan-induced diabetic rats is an indication of the presence of some beta cells, as GLBC is known to stimulate insulin secretion from beta cells. The T. indica may have stimulating effect on the remnant β-cells.

Many new bioactive principles isolated from plants having antihyperglycemic effects have shown antidiabetic activity equal and even more potent than the known oral hypoglycemic agents such as daonil, tolbutamide and chloropropamide. However, many other active agents obtained from plants have not been well characterized and documented. More investigations and sincere efforts are still sought to evaluate the precise mechanism of action of medicinal plants with antidiabetic effect at the molecular level (Bnouham et al., 2006).

Conclusion

Based on this study we can conclusively construe that the polar extracts (AQ and MEOH extracts) of the leaves of T. indica do possess significant antihyperglycemic agents which might provide the lead as a novel class of therapeutics in the management of diabetes and results of our study also justify the utility of T. indica by the local traditional practitioners in the treatment of diabetes infirmities in Malaysia; however, further pharmacological and toxicological studies at the molecular and clinical levels are still warranted to confirm the true antidiabetic
potential of the leaves of *T. indica*.

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