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Original Article

Improvement of spatial learning and memory, cortical gyrification patterns and brain oxidative stress markers in diabetic rats treated with *Ficus deltoidea* leaf extract and vitexin

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ABSTRACT

Despite the fact that *Ficus deltoidea* and vitexin played important roles in controlling hyperglycemia, an effective mitigation strategy dealing with cognitive deficit observed in diabetes, little is known about its neuroprotective effects. The study is aimed to determine changes in behavioral, gyrification patterns and brain oxidative stress markers in streptozotocin (STZ)-induced diabetic rats following *F. deltoidea* and vitexin treatments. Diabetic rats were treated orally with metformin, methanolic extract of *F. deltoidea* leaves and vitexin for eight weeks. Morris water maze (MWM) test was performed to evaluate learning and memory functions. The patterns of cortical gyrification were subsequently visualized using micro-computed tomography (micro-CT). Quantification of brain oxidative stress biomarkers, insulin, amylin as well as serum testosterone were measured using a spectrophotometer. The brain fatty acid composition was determined using gas chromatography (GC). Biochemical variation in brain was estimated using Fourier transform infrared (FT-IR) spectroscopy. Results showed that oral administration of *F. deltoidea* extract and vitexin to diabetic rats attenuated learning and memory impairment, along with several clusters of improved gyrification. Both treatments also caused a significant increase in the superoxide dismutase (SOD) and glutathione peroxidase (GPx) values, as well as a significant reduction of TBARS. Strikingly, improvement of cortical gyrification, spatial learning and memory are supported by serum testosterone levels, fatty acid composition of brain and FT-IR spectra.

1. Introduction

There is a considerable amount of scientific data that consistently links diabetes mellitus (DM) to neurological consequences, in particular, to cognitive function.[1–3] It is now recognized that oxidative stress is a key factor linking DM with cognitive impairment in experimental animals and humans.[4–6] Excessive levels of reactive oxygen species (ROS) by hyperglycemia cause damage to cellular components such as DNA, neuronal proteins and lipid peroxidation.[7,8] Despite intensive glycemic control, evidence of a series of neurobehavioral shifts pertaining to DM[9,10] highlighted the need for interventions to reduce the deleterious effect of diabetes on cognition.

Considering the pathophysiologic mechanism of the cognitive impairments consequences of DM, it is important to determine the pattern and magnitude of this complication. Clinical studies have demonstrated that diabetes not only impairs the integration of neural systems, but also alters the brain anatomy.[11] As cortical gyrification patterns are thought to orchestrate cognitive functions,[12] the combined analysis of data from behavioral and biochemical test as well as micro-CT imaging may provide insight into central aspects of pathology that could be present exclusively in the brain. It is becoming increasingly evident that micro-CT has tremendous potential for general mapping of the mouse and rat brain.[13,14] Micro-CT has emerged as an attractive diagnostic

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imaging technique that enables noninvasive, high-resolution, *in vivo* and *ex vivo* imaging.[15] Thus, exploring cortical gyrification using micro-CT imaging technique may provide additional clues with respect to the underlying anatomical feature which correlates cognitive ability and decline.

Approach to analyze biochemical variation in rat brain by FT-IR may establish conclusively the link between the molecular fingerprint and diabetes-related cognitive change.[16] FT-IR has proved to be a powerful tool for studying molecules changes that occur in diseases. It possesess many advantages such as very small sample volume requirement, good precision over the entire physiological range, being reagent-free, can rapidly and noninvasively detect changes in the biochemical composition of cells and tissues (at the molecular level).[17] These techniques have been used to probe chemical changes in serum,[18,19] plasma,[20] brain,[21,22] pancreas,[23] urine[24] and bone[25–27] of human and animal.

Many efforts have been made to elucidate the mechanisms and therapeutic properties of plant-derived neuroprotective agents. In recent, special interest has been paid to medicinal plants with antioxidant and antidiabetic properties.[28] This is due to the fact that hyperglycemia-induced oxidative stress plays a key role in accentuating the progression of cognitive impairment in diabetes. In Malaysia, the decoction of *Ficus deltoidea* leaves has been extensively used as an herbal medicine to normalize blood sugar, afterbirth tonic to contract the uterus and vaginal muscles as well as a dietary supplement used for treating leucorrhoea in humans.[29] In recent, a great deal of attention has been focused on leaves due to the high antioxidant.[30,31] showed the presence of more than 20 varieties of flavonoids in the leaves of *F. deltoidea*, in which vitexin has recently been isolated, identified, and evaluated.[32] Above all, antioxidant and antidiabetic properties of *F. deltoidea* in animal models has been shown to be associated with vitexin.[33] Therefore, this study was designed to characterize the changes in behavioral, cortical gyrification and brain oxidative stress markers following *F. deltoidea* and vitexin treatment. Accordingly, we considered it is worthwhile to examine whether these changes are related to hormonal profile, fatty acid composition and the pattern of brain FT-IR spectral.

2. Methods

2.1. Plant materials and extract preparations

The leaves of *F. deltoidea* var. *deltoidea* were collected from Forest Research Institute Malaysia, Kepong, Malaysia in January 2015. The sample was then deposited at the Herbarium Unit, Universiti Kebangsaan Malaysia, Bangi with a voucher number Herbarium UKMB-40315. The leaves were washed thoroughly and over-dried at 37 ± 5 °C. The dried leaves were finely powdered using an electric grinder. For extraction, 100 g of powdered leaves was soaked in 1 L methanol for three days at room temperature.[32] Liquid extracts were concentrated by using a rotary evaporator at 40 °C and subjected to freeze drying for 48 h. The extraction yield calculated was 10.6%. The extracts were kept in tightly closed glass containers and stored at −20 °C until further use.

2.2. Animals

The animal use and experiment protocols involved in the study were approved by the Universiti Putra Malaysia, Animal Care and Use Committee with an approval number: UPM/IACUC/AUP-R090/2014. Thirty four-week-old Sprague Dawley male rats (mean body weight, 80 g) were used. The reason of using younger animals was to minimize the confounding effects of neuronal loss, and other aging changes in rats that might confound the results. It is known that after a period of rapid growth, neuronal loss started to occur as soon as the end of adolescence in rats, or at three months old in normal rats.[34] Furthermore, work done by Wang et al[35] also showed that four months after STZ treatment in 8–10 week old rats, age-related diabetic encephalopathies and cognitive deficits became very evident. The animals were acclimatized upon arrival for a week and were housed at a density of three per cage in a temperature controlled room (22 ± 1 °C and a 12 h light/dark cycle). The rats were identified with a cage card indicating project number, dose level, group, and animal number. They were had access to standard rat chow (Gold Coin Holdings, Kuala Lumpur, Malaysia) and water *ad libitum*.

Diabetes-like hyperglycemia was induced experimentally in rats by STZ injection at a dosage of 60 mg/kg of body weight.[36,37] In order to prevent drug-induced hypoglycemic shock, the rats were given prophylactic 5% glucose water for 2 days following STZ injection.[38] After a week, animals with fasting blood glucose levels >11 mmol/L were considered diabetic.[39]

2.3. Experimental design and procedure

The rats were divided into five groups of six rats per treatment group. The treatment group were normal control rats treated with saline (NC), diabetic control rats treated with saline (DC), diabetic rats treated with 1000 mg/kg of metformin (DMET),[40] diabetic rats treated with 1000 mg/kg of *F. deltoidea* (DFD),[32] diabetic rats treated with 1 mg/kg b.w. of vitexin (DV).[33]

Metformin, methanolic extract of *F. deltoidea* and vitexin were dissolved in saline and treatments were given once daily via oral gavage for eight weeks. At the end of the experiment, all animals were fasted overnight. Animals were then anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg), followed by terminal exsanguinations. Blood samples (10–15 ml) were collected via cardiac puncture from the rats into a plain red-top tube containing no anticoagulants (BD Vacutainer®, USA). The blood samples were then centrifuged at 4000 g for 15 min, and serum was stored in aliquots at −80 °C.

2.4. Morris water maze test (MWM)

MWM was carried out from day 51 and continue each day to day 56 of the treatment period as described by Nagapan et al. and Weitzner et al.[41,42] Latencies to find the platform, swim speed and time in each quadrant were recorded using a ceiling-mounted camera (DSR-SR47; Sony Corporation, Tokyo, Japan) and analyzed with ANY-maze Video Tracking System Software (Stoelting Co., USA). Prior to the maze test, all animals were examined and verified to be free from physical disabilities and motor function deficits that would affect their maze performance. The test was carried out in two phases:

2.4.1. Spatial acquisition

This phase evaluated the spatial learning abilities of the rats. Rats had daily training for five consecutive days with three trials per day per treatment regime. On each trial, the rat was placed in the water, facing the edge of the pool and located at one of four pseudo-randomly determined start positions as described in Table 1. The rats were given 1 min to locate hidden platform which was placed in the centre of SW quadrant. If the animal failed to find the platform within 1 min, it was physically guided to the platform. The escape latency(s) and path length (cm) to find the platform were measured in each trial and averaged over three trials for each rat.

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