บทความวิจัย (Research Article)

Effects of *Moringa oleifera* Leaf Extract on the Acetylcholinesterase and Monoamine Oxidase Activities in Rat Brains with Streptozotocin-Induced Diabetes and Sciatic Nerve Constriction

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Abstract

Cognitive decline in diabetes is related to hyperglycemia-induced cerebral metabolism changes of neurotransmitters that play a role in cognitive function. This study aimed to determine the effect of *Moringa oleifera* (*M. oleifera*) leaf extract on the activities of acetylcholinesterase (AChE) and monoamine oxidase (MAO) in diabetic rat brains with neuropathic pain induced by a sciatic nerve constriction. Type-I diabetes was induced in young adult male Wistar rats (180-220 g) with a single injection of streptozotocin (STZ) at a dose of 65 mg/kg BW (i.p.). The diabetic rats were further subjected to a right sciatic nerve constriction. Then, rats were randomly assigned to three different doses of orally administered *M. oleifera* leaf extract (100, 200 and 300 mg/kg BW) for 21 days. The cerebral cortex, hippocampus, and striatum were collected to determine the activities of AChE and MAO. The results showed that *M. oleifera* leaf extract significantly decreased the activity of AChE and MAO in the studied brain regions.

Keywords: Acetylcholinesterase, diabetes mellitus, monoamine oxidase, *Moringa oleifera*, neuropathic pain

Introduction

Diabetes mellitus (DM), characterized by hyperglycemia and metabolic abnormalities, affects various organs including the brain. [1] Chronic hyperglycemia can induce ischemic brain injury, [2] seizures, [3] and cognitive compromise. [4] The memory impairment was identified in streptozotocin (STZ)-induced diabetic experimental rats. [5] In addition, type I diabetes mellitus patients have been observed to have impaired general intelligence, attention, memory, and executive function as well as slower information processing. [6-8]

Neurotransmitters regulate cognitive function including acetylcholine, norepinephrine, epinephrine, dopamine and serotonin. [9] The cholinergic system plays a role in learning and memory for both human and animals. [10] Parts of the brain that play a role in cognitive function include the cerebral cortex, [11] hippocampus, [12], and striatum. [13] Acetylcholine (ACh) plays a role in parasympathetic regulation, motor, and

cognitive function. It was reported that type I diabetic rodents showed a decrease in the number of acetylcholine (ACh)-containing vesicles. [14] Acetylcholinesterase (AChE) is the main enzyme responsible for hydrolysis ACh into choline and acetate. This enzyme has high concentrations in the neuron located in the brain, nerve, and red blood cells. The alteration of the cholinergic system was observed in the diabetic conditions. In alloxan-induced diabetic rats showed an increase in AChE activity. [15, 16] It was suggested that the normal levels of monoamine in the prefrontal cortex, amygdala, and hippocampus are required for the integration of learning and memory. [17] Thus, the amine neurotransmitters have also been investigated in diabetic condition. Lakhman and Kaur reported that MAO activity significantly increased in discrete brain regions of diabetic rats. [18]

Diabetes has many complications including neuropathic pain. Neuropathic pain is an abnormal pain perception resulted from an impaired sensory signal transmission into the spinal cord and the brain. [19] However, not all diabetic patients develop neuropathic pain. Only 21% of diabetic patients had neuropathic pain. [20] For this reason, some studies only focus on diabetes with neuropathic pain. [21-22] Nerve ligation and nerve constriction are common methods to induce neuropathic pain in animals. [23-25]

Moringa oleifera Lamarck (*M. oleifera*) belongs to the family of Moringaceae. The edible parts of *M. oleifera* are leaves, flowers, young immature pods, root extract, and seed extract. *M. oleifera* leaves contain beta-carotene, vitamins (C and A), and polyphenols which are good source of natural antioxidants. [26] The biological and medicinal properties of *M. oleifera* leaves include antimicrobial, [27] anti-hyperlipidemia, [28] anticancer, [29] anti-diabetic, [30] antioxidant, [21] and neuroprotective effects. [32] In addition, *M. oleifera* leaves also promote the outgrowth of neurites and neuronal differentiation from primary embryonic neurons [33] and show antioxidative activity on the higher brain regions of diabetic rats. [34] However, there is no known data reported about the effect of *M. oleifera* leaf extract on the activities of AChE and MAO, the enzymes that regulate amine neurotransmitters in the brain of STZ-induced diabetic rats with neuropathic pain induced by sciatic nerve constriction. Thus, we set up this experiment to determine the activities of AChE and MOA in the cerebral cortex, hippocampus, and striatum.

Material and Method

Plant material preparation: The leaves of *M. oleifera* were collected from Khon Kaen Province, Thailand. The fresh leaves were immediately cleaned, cut into small pieces and dried at 40°C. The dried leaves were crushed, ground into powder and extracted with 50% ethanol. Then, the extract was filtered and lyophilized until the crude extract was obtained. The percent yield of the extract was 17.49%. The extract was stored at -20°C in a dark container until it was used. The crude extract was suspended in 1% NaCMC (Sodium carboxymethylcellulose) before administration to the rats.

Animals: Young adult male Wistar rats (180–220 g) were obtained from the National Laboratory Animal Center, Salaya, Nakhon Pathom Province, Thailand. Rats were housed at a constant temperature of 20–22°C, with a 12:12-h light:dark cycle. Standard food and tap water were provided ad libitum. All experiments were performed following the approval of the Animal Ethic Committee of Khon Kaen University (AEKKU18/2554).

Experimental Protocol: Diabetes mellitus was induced in the rats by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) (Sigma, USA) in 0.9% cold sterile saline solution at a dose of 65 mg/kg BW. Fasting blood glucose were assessed following the injection for 72 h. Rats demonstrating hyperglycemia (≥ 300 mg/dL) were selected for the operation of sciatic nerve constriction or sham operation. The glucometer with glucose stripes were used (Accu-Chek®) to assess the blood glucose level of the blood sample drawn from the tail vein. Thirty diabetic rats were randomly allocated into five experimental groups: Group I, the sham operation; Group II, sciatic nerve constriction which received NaCMC or vehicle-treated group; Group III-V: sciatic nerve constriction which received *M. oleifera* leaf extract at doses of 100, 200 and 300 mg/kg BW respectively. The doses of the extract were selected based on the study of Jaiswal et al [30] which considered safe according to the report of Awodele et al. [35] STZ-induced diabetic rats with a sciatic nerve constriction were administered the assigned substances each day for 21 days. At the end of the experiment, the rat brains were harvested in order to determine AChE and MAO activities in the cerebral cortex, hippocampus, and striatum.

Sciatic nerve constriction: The diabetic rats were anesthetized by ethyl ether. The right thigh muscle of the rat was opened carefully for separation of sciatic nerve and surrounding connective tissue. Then, the right sciatic nerve was ligated using chromic gut (4-0 silk) at a site just distal to the point at which the posterior biceps semitendinosus nerve branches of the sciatic nerve. The same method was used on the rats that underwent the sham operation, except there was no nerve ligation.

Determination of the acetylcholinesterase (AChE) activity: The activity of AChE was measured according to the method of Ellman and colleagues. [36] In brief, brain homogenate (10 µl) was incubated with a reaction mixture of 200 µl of 0.1 M sodium phosphate buffer (pH 8.0) and 10 µl of 0.01 M DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)) for 5 min at room temperature. The absorbance was measured with a microplate reader (iMark™ Microplate Absorbance Reader) at 415 nm before and after incubating with 10 µl of 0.03 M ACTI (acetylthiocholine iodide) for 3 min. The activity of AChE was expressed as µmol/min/g protein.

Determination of monoamine oxidase (MAO) activity: The activity of MAO was measured according to the protocol described previously by Wattanathorn. [37] The brain homogenate (50 µl) was incubated with a mixture of chromogenic solution (50 µl) and 200 µl of 500 µM of p-tyramine for 30 min at the room temperature. Then, the absorbance was read at 490 nm. The activity of MAO was expressed as U/mg protein.

Statistical Analysis: Data were analyzed by one-way ANOVA, followed by post hoc (LSD) test. The statistically significant difference was set at p value < 0.05. Values are expressed as mean ± S.E.M.

Results

1. Effects of *M. oleifera* leaf extract on acetylcholinesterase (AChE) activity

There was no significant difference of AChE activity between the sham-operated group and vehicle-treated group that received a sciatic nerve constriction. **(Figure 1)** As compared to the vehicle-treated group, *M. oleifera* leaf extract at a dose of 100 mg/kg BW demonstrated a significant lower AChE activity in the cerebral cortex and

hippocampus ($p < 0.01, 0.05$). In addition, the extract at a dose of 300 mg/kg BW significantly decreased AChE activity in the cerebral cortex, hippocampus, and striatum when compared to the vehicle-treated group ($p < 0.05$).

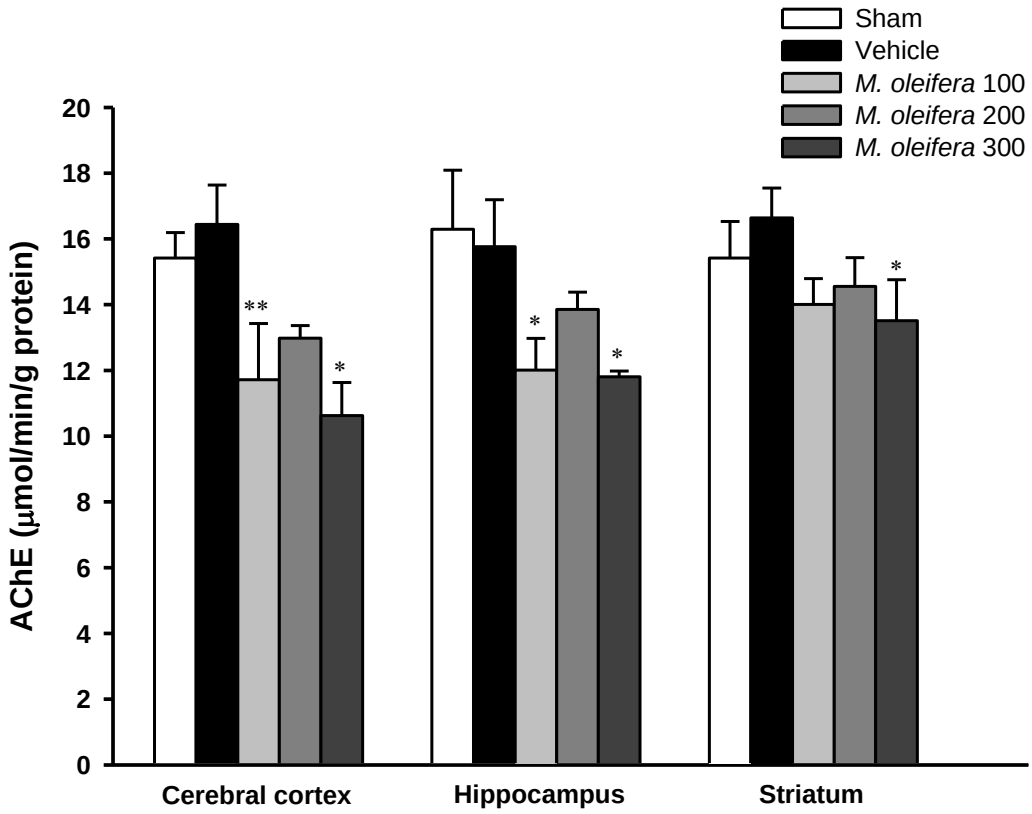

Figure 1 Effect of *M. oleifera* leaf extract on the activity of acetylcholinesterase (AChE) in the cerebral cortex, hippocampus, and striatum. The *M. oleifera* leaf extract at doses of 100 and 300 mg/kg BW significantly decreased the activity of AChE in the cerebral cortex and hippocampus. Only the extract at a dose of 300 mg/kg BW significantly decreased the activity of AChE in the striatum. Data are presented as mean ± S.E.M. (n=6). $^{*}p < 0.05$, $^{**}p < 0.01$ are indicating the comparison to the vehicle-treated group.

2. Effects of *M. oleifera* leaf extract on monoamine oxidase (MAO) activity

There was no significant difference of MAO activity between the sham-operated group and vehicle-treated group that subjected to sciatic nerve constriction. **(Figure 2)** The treatment with all doses of *M. oleifera* extracts exhibited the significant lower MAO activity in the cerebral cortex and hippocampus than the vehicle-treated group ($p < 0.01$). In addition, the group that received *M. oleifera* extract at a dose of 200 mg/kg BW showed significantly lower MAO activity in the striatum than the vehicle-treated group ($p < 0.01$).

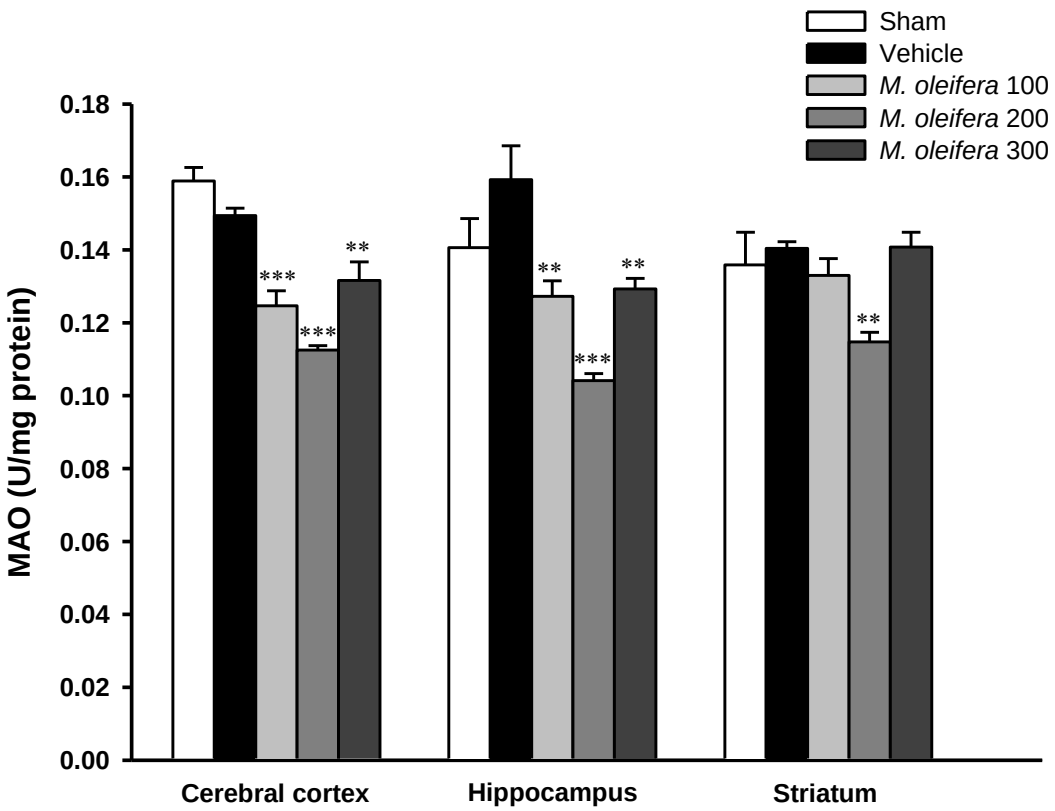

Figure 2 Effect of *M. oleifera* leaf extract on the activity of monoamine oxidase (MAO) in the cerebral cortex, hippocampus, and striatum. The results showed that all doses of *M. oleifera* leaf extract used in this study (100, 200 and 300 mg/kg BW) significantly decreased the activity of MAO in the cerebral cortex and hippocampus. Only the extract at a dose of 200 mg/kg BW significantly decreased the activity of MAO in the striatum. Data are presented as mean ± S.E.M. (n=6). $^{**}p < 0.01$, $^{***}p < 0.001$ are indicating the comparison to the vehicle-treated group.

Discussion

Uncontrolled diabetes mellitus can lead to complications of both peripheral and central nervous systems. [38] One of diabetic central nervous system complications in diabetic experimental animals is an impaired memory and cognitive function. [39,40] It is well established that cognitive function is regulated by various neurotransmitters including acetylcholine (ACh), dopamine (DA), norepinephrine (NE), and serotonin (5-HT). [9] Acetylcholine is oxidized by acetylcholinesterase (AChE) whereas DA, NE, and 5-HT are metabolized by monoamine oxidase (MAO). In this study, we investigated whether peripheral neuropathic pain induced by a sciatic nerve constriction in the diabetic condition alters the activities of AChE and MAO in the cerebral cortex, hippocampus, and striatum of streptozotocin-induced diabetic rats. These specific brain areas play a role in regulating cognitive function.

The results demonstrated that there was no significant difference in the activities of AChE and MAO between the sham-operated group and the vehicle-treated group that received a sciatic nerve constriction. This indicates that neuropathic pain induced by a sciatic nerve constriction had no effect on AChE and MAO in the brain.

Previous studies showed that an alloxan-induced hyperglycemic condition leads to an increase in MAO and AChE activities of various brain areas including the hippocampus. [15, 16, 18] However, the present study only investigated diabetic rats, omitting a comparison to a naïve control group.

The data of the present study revealed that the group of STZ-induced diabetic rats that were subjected to a sciatic nerve constriction and were also treated with *M. oleifera* leaf extract had significantly decreased AChE and MAO in their brain regions as compared to the vehicle-treated group. Specifically, *M. oleifera* leaf extract at a dose of 100 mg/kg BW decreased the activity of AChE and MAO in the cerebral cortex and hippocampus. Administration of *M. oleifera* leaf extract at a dose of 200 mg/kg BW decreased the activity of MAO in the cerebral cortex, hippocampus, and striatum but produced no effect on the activity of AChE. The extract high dose group (300 mg/kg BW) produced a significant reduction of AChE in all three brain regions and also demonstrated a significantly decreased activity of MAO in the cerebral cortex and hippocampus.

Past research indicates that the hippocampus plays an important role in encoding and retrieval of memory. [41, 42] The key components of the neuronal circuitry necessary for these processes is the innervation of the hippocampal cholinergic neuron by basal forebrain cholinergic neuron, which provides modulatory input mediated by ACh. [43] It was reported that administration of *M. oleifera* leaf extract results in a decrease in AChE activity, leading to the improvement of cholinergic function and memory processing. [44] It was also previously demonstrated that the extract from *M. oleifera* leaves reduce the AChE activity in the zebrafish's brain homogenate. [45] Since AChE is inversely related to available acetylcholine, we suggested that *M. oleifera* leaf extract may exert AChE suppression or an anti-cholinesterase effect that enhances cholinergic functions and, as a result, improves cognitive performance.

In the current study, we found that *M. oleifera* leaf extract significantly decreased the activity of MAO in the three brain areas investigated. Previous research demonstrated that the *M. oleifera* leaf extract showed monoamine oxidase type B (MAO-B) suppression activity in stressed rat brains. [46] MAO-B is an indicator to reflect the available dopamine. Therefore, this previous research concluded that *M. oleifera* leaf extract might increase dopamine levels. In addition, MAO inhibitors are a group of drugs used for treatment of psychiatric disorders. The drugs inhibit activity of the MAO enzyme and enhance the transmission of serotonin, norepinephrine as well as dopamine. [47] Since, amine neurotransmission signaling is regarded as one of the key mechanisms for the modulation of mood and emotion as well as the control of motor and cognitive function, [48] it may be that *M. oleifera* leaf extract exerts MAO suppression resulting in the improvement of cognitive function.

The main polyphenols were identified in *M. oleifera* leaf extract including myrecytin, quercetin and kaempferol. [49, 50] It was demonstrated that quercetin significantly decreased AChE activity in the brain homogenates. [51] Quercetin also exhibited a MAO-A inhibitor in the mouse brain. [52] Taking these results together, quercetin seems to be an active component which exerts an inhibitory effect on AChE and MAO. However, it was demonstrated that the different extraction methods and different sources of *M. oleifera* leaves lead to a different amount of the

main constituents in the extract. [53, 54] Nevertheless, we did not investigate the constituents of *M. oleifera* leaf extract in the present work. Therefore, further studies are still required.

In conclusion, the present study suggests that *M. oleifera* leaf extract exerts a suppression effect on AChE and MAO in STZ-induced diabetic rat brains with neuropathic pain induced by a sciatic nerve constriction. However, we suggest further study to evaluate both behavioral cognitive tests and enzymatic activity assessments in the same study as well as the possible mechanism(s).

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