The Antioxidative Effects of *Moringa oleifera* Lam. Leaves in the Higher Brain Regions of Diabetic Rats

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Abstract

Oxidative stress has been implicated in the pathogenesis of diabetes mellitus. This disease affects numerous organs, including the brain which is important for the control of many body functions. Therefore, the present study aimed to determine the effect of *Moringa oleifera* Lam. leaf extract on an oxidative stress marker, malondialdehyde, and the antioxidant enzyme systems in the higher brain regions of diabetic rats. Young adult male Wistar rats were injected with a single streptozotocin at a dose of 65 mg/kg BW, i.p., to induce diabetes. Thirty-six diabetic rats were divided into six groups including a sham group, and five groups with right sciatic nerve constriction. One group received sodium carboxymethylcellulose, and another received vitamin C as a positive control. Additionally, three groups received *M. oleifera* leaf extract orally at doses of 100, 200, and 300 mg/kg BW for 21 days. The rats were sacrificed and the cerebral cortex, hippocampus, and striatum were isolated and prepared as homogenate to determine malondialdehyde (MDA) levels and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The rats treated with 100 mg/kg BW *M. oleifera* leaf extract showed significantly increased antioxidant enzymes activities including SOD, GPx and CAT ($P < 0.05$) in the mentioned brain areas. Although the administration of *M. oleifera* leaf extract reduced MDA levels, the change was not significant. The results suggest that *M. oleifera* leaf extract has the potential to enhance antioxidant enzymes activities in the higher brain regions of diabetic rats.

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Keywords: *Moringa oleifera*, diabetic rats, antioxidative effect, higher brain

Introduction

The global prevalence of diabetes mellitus (DM) is increasing due to population growth, aging, and increasing prevalence of obesity.[1] In 2014, the global number of people with diabetes has increased to 422 million.[2] The mortality rate of diabetic patient is estimated to be about 1.6 million in 2015.[2] Diabetes is a chronic metabolic disease caused by prolonged high blood sugar levels (hyperglycemia).[3] The hyperglycemic condition in diabetes affects various organs, including kidney, retina, small and large blood vessels, and peripheral nervous system (PNS).[4] Additionally, central nervous system (CNS) has structural and functional parts connected to the PNS, and can also be affected by diabetes.[4] The CNS dysfunction is caused by both the vascular and metabolic consequences of diabetes. These consequences lead to brain damage, neurological deficits, cognitive impairment and cerebrovascular diseases.[4]

Diabetic neuropathy is a peripheral nerve injury caused by chronic hyperglycemia, leading to impairments of both sensory and motor control.[5] Neuropathic pain is a frequent and unpleasant consequence of peripheral nerve injury.[5] Chronic neuropathic pain seriously decreases quality of life and is associated with mood and cognitive impairments, as well as variable inability to control movement.[5,6] A previous study has reported a link in diabetic central neuropathy that could be related to hyperglycemia-induced CNS dysfunction.[7] It has also been demonstrated that diabetic complications of the CNS, including cognitive impairment, are caused by diabetic neuropathy.[8] Thus, peripheral nerve injury may well be affected as part of the CNS. Interestingly, diabetes with peripheral nerve injury might interrupt the higher brain regions that regulate pain perception, cognitive function and movement.

The pathogenesis of diabetic neuropathy is complicated; part of the pathogenesis of diabetes is oxidative stress. A previous study has reported that acute and chronic hyperglycemia promote diabetic neuropathy, caused by oxidative stress in the peripheral nervous system.[9] The oxidative stress generation is due to excessive free radical production and reduced antioxidant enzyme systems, that together can cause neuronal damage and brain dysfunction.[10] Furthermore, the increased free radical production causes a decreased ability to synthesize antioxidant enzyme systems in the developing brain.[11] Hence, diabetes with associated neuropathy is

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a strong model demonstrating the effects of increased oxidative stress on both the peripheral and central nervous systems.

Lipid peroxidation is involved in the pathogenesis of diabetes.[12] This refers to a process of free radicals attacking unsaturated fatty acid in the cell membrane, leading to cell damage. The lipid peroxidation product, malondialdehyde (MDA), is widely used for measurement of oxidative stress in the cell.[12] Moreover, the scavenging enzymes expressed in higher brain regions can be used as brain anti-oxidative markers of defense against oxidative stress.[13] Based on this information, there is a need to investigate antioxidants that can scavenge free radicals, by reducing lipid peroxidation and increasing antioxidant enzyme activities, in the higher brain regions using a diabetic rat model.

Moringa oleifera Lamarck, known in Thai as Marum, is a cultivated species of the *Moringa* genus, belonging to the Moringaceae family. The edible parts of *M. oleifera* are leaves, seeds, roots and its extract. Traditional folklore has named *M. oleifera* as a miracle tree because there are many reported benefits in the relief asthma, spasms, ulcers, liver fibrosis, infection, and inflammation.[14-16] Recent studies have also shown the antioxidant and anti-diabetic activities of an aqueous extract of *M. oleifera* in liver,[15] pancreatic,[17] and kidney[18] tissues. *M. oleifera* leaf extract contains antioxidants and is a good scavenger for nitric oxide radicals.[19] It has been shown to increase the rate of wound healing,[20] and is used for the treatment of hyperglycemia and hyper-cholesterolemia.[21] Numerous studies have suggested the presence of antioxidative and antidiabetic effects of *M. oleifera* leaf extract in various tissues. However, the effects of *M. oleifera* leaf extract on malondialdehyde levels and antioxidant enzyme activities in the higher brain of diabetic rats are still unclear with no scientific data available. Therefore, this study aimed to determine the antioxidative effects of *M. oleifera* leaf extract in the higher brain of diabetic rats.

Materials and Methods

Plant material preparation

M. oleifera leaves were collected from Khon Kaen in the northeastern part of Thailand. The fresh leaves were cleaned, cut into small pieces, and dried at a room temperature, about 25°C. Dried leaves were crushed and then powdered in an electrical grinder. The dried powder was then extracted in 50% ethanol. The percent yield of the extract was 17.49 ± 0.58% (n = 3). The extract was stored at -20°C in a brown bottle until use. The crude extract was suspended in 1% sodium carboxymethylcellulose (NaCMC) before administration to the rats.

Animals

Healthy young adult male Wistar rats (weighed 180–220 g; aged 8 weeks) were obtained from the National Laboratory Animal Center, Salaya, Nakhon Pathom, Thailand. The rats were randomly assigned to 3 rats per cage, under a light-dark cycle of 12:12 h and were fed with standard pellets with water *ad libitum*. All experiments were performed in accordance to the ethical norms approved by the Animal Ethic Committee of Khon Kaen University (AEKKU18/2554).

Experimental protocol: Thirty-six diabetic rats were divided into six groups of six animals each as described below:

Group I: A sham group of rats which had surgery but without sciatic nerve constriction.

Group II: Diabetic rats with sciatic nerve constriction and were administered NaCMC; vehicle-treated group.

Group III: Diabetic rats with sciatic nerve constriction and were administered vitamin C at a dose of 100 mg/kg BW.

Group IV-VI: Diabetic rats with sciatic nerve constriction and were given *M. oleifera* leaf extract at doses of 100, 200, and 300 mg/kg BW.

The diabetic rats with neuropathy were treated with the assigned substances for 21 days. At the end of the experiment, the rats were sacrificed and their cerebral cortex, hippocampus and striatum were removed in order to determine the activities of antioxidant enzymes and MDA levels.

Induction of diabetic neuropathy: Experimental rats were given a single intraperitoneal injection of freshly prepared streptozotocin (STZ, Sigma-Aldrich, St. Louis, Missouri, USA), dissolved in 0.9% sterile saline solution, at a dose of 65 mg/kg to induce diabetes mellitus. Three days after STZ injection, rats with marked hyperglycemic condition (fasting blood glucose ≥ 300 mg/dl) were kept for operation with or without sciatic nerve constriction. Seven days after having fasting blood glucose levels higher than 300 mg/dl, the diabetic rats were operated for sciatic nerve constriction. They were anesthetized by using ethyl ether. The right thigh muscle of the rat was opened carefully for separation of the sciatic nerve and surrounding connective tissues. Then, the right sciatic nerve was ligated using chromic gut at the end point of the posterior biceps semitendinosus nerve branches of the common sciatic nerve. The sham-operated rats underwent the same procedure but without sciatic nerve constriction. Three days after surgery, the wound in each diabetic rat appeared to be healed. Rats were orally administered with NaCMC, vitamin C, or *M. oleifera* leaf extract for 21 consecutive days.

Determination of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities, and malondialdehyde (MDA) levels

After the last administration dose, all rats were sacrificed. The cerebral cortex, hippocampus, and

striatum were isolated and prepared as a homogenate in order to determine the MDA levels and the activities of SOD, GPx, and CAT. MDA was determined by the accumulated thiobarbituric acid reactive substances (TBARS),[22] whereas the activities of SOD,[23] GPx,[24] and CAT[25] were determined by the reaction to inhibit cytochrome C, the rate of reduction of hydrogen peroxide (H_2O_2) and the decreasing amount of nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute, respectively.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM) and were analyzed statistically using one-way ANOVA, followed by a *post hoc* test (least significant difference test, LSD). The results were considered statistically significant if P value was < 0.05.

Results

Oxidative stress has an important role in the pathogenesis of diabetic neuropathy. The brain, part of the CNS, is highly vulnerable to oxidative damage, due to its high glucose and oxygen consumption. We selected part of the cerebral cortex, hippocampus and striatum in order to evaluate lipid peroxidation product and antioxidant enzymes activities.

Lipid peroxidation product

The effect of *M. oleifera* leaf extract on MDA levels is shown in Figure 1. Compared to the vehicle-treated group, diabetic rats with sciatic nerve constriction, given *M. oleifera* leaf extract and vitamin C, failed to show any significant change on MDA levels, even though we observed the reducing trend of MDA levels in both groups.

Activities of antioxidant enzymes

Figure 2 shows the effect of *M. oleifera* leaf extract on the activity of SOD in the cerebral cortex, hippocampus, and striatum. Compared to the vehicle-treated group, diabetic rats with sciatic nerve constriction which were subjected to 100 mg/kg BW *M. oleifera* leaf extract, showed a significantly increased SOD activity in the cerebral cortex (P < 0.01). Moreover, the rats subjected to vitamin C and *M. oleifera* leaf extract at doses of 100 and 300 mg/kg BW also showed significantly increased SOD activity in the striatum (P < 0.001, P < 0.05, and P < 0.001, respectively).

The effects of *M. oleifera* leaf extract on the activity of GPx in the cerebral cortex, hippocampus, and striatum are shown in Figure 3. Compared to the vehicle-treated group, the diabetic rats with sciatic nerve constriction, treated with 100 mg/kg BW *M. oleifera* leaf extract, showed significantly increased activity of GPx in the cerebral cortex (P < 0.05). In the striatum, the rats treated with *M. oleifera* leaf extract at all doses, showed significantly enhanced GPx activity (P < 0.05; all doses).

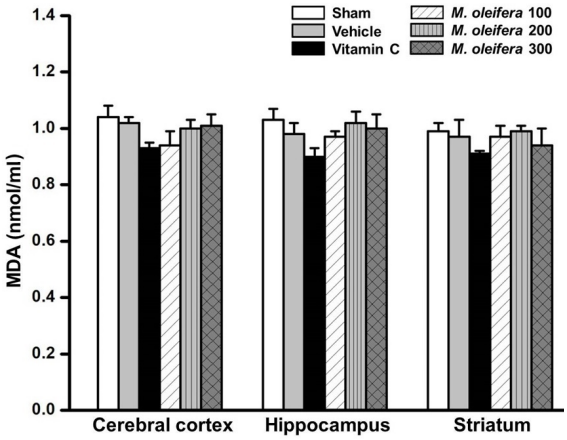

Figure 1 Effect of *M. oleifera* leaf extract on the level of malondialdehyde (MDA) in the cerebral cortex, hippocampus and striatum. No significant differences were observed. Data are expressed as mean ± SEM; n = 6 per group.

Figure 4 demonstrates the effect of *M. oleifera* leaf extract on the activity of CAT in the cerebral cortex, hippocampus, and striatum. Compared to the vehicle-treated group, the diabetic rats with sciatic nerve constriction, subjected to vitamin C and *M. oleifera* leaf extract at doses of 100 and 300 mg/kg BW, showed significantly enhanced CAT in the cerebral cortex (P < 0.001, P < 0.001, and P < 0.01, respectively). In the hippocampus, the rats subjected to vitamin C and all doses of *M. oleifera* leaf extract treatment showed significantly increased CAT activity (P < 0.01, P < 0.001, P < 0.05, and P < 0.001, respectively). Additionally, treatment with vitamin C and all doses of *M. oleifera* leaf extract

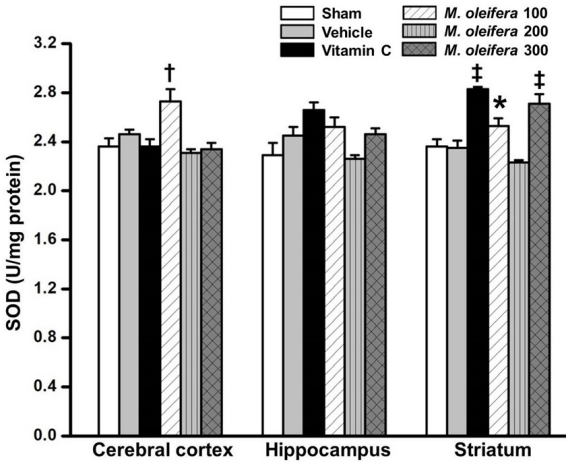

Figure 2 Effect of *M. oleifera* leaf extract on the activity of superoxide dismutase (SOD) in the cerebral cortex, hippocampus, and striatum. The results showed that *M. oleifera* leaf extract at 100 mg/kg BW significantly increased the activity of SOD in the cerebral cortex and striatum whereas *M. oleifera* leaf extract at 300 mg/kg BW significantly increased the activity of SOD only in the striatum. The vitamin C treatment produced a significant increase in the striatum. Data are expressed as mean ± SEM; n = 6 per group. $^{*}P$ < 0.05, $^{\dagger}P$ < 0.01, $^{\ddagger}P$ < 0.001, compared to vehicle-treated group.

significantly increased the activity of CAT in the striatum ($P < 0.001$, $P < 0.001$, $P < 0.01$, and $P < 0.001$, respectively).

Discussion

The present study used STZ because it is a well-known toxin widely used to induce diabetes in experimental rats. This chemical inhibits insulin production and selectively destroys β cells that produce insulin, by causing necrosis.[26] It has been reported that STZ injection causes hyperglycemia and induces free radical generation and oxidative stress.[27,28] However, STZ-induced diabetic rats, producing its complication including diabetic neuropathy, often show only an intermittent effect regarding neuropathic pain. It was indicated that sciatic nerve constriction was a suitable model to mimic prolonged neuropathic pain[5] and promote oxidative stress in the peripheral nervous system.[9] Hence, we made both STZ-induced diabetes and sciatic nerve constriction in this study in order to ensure diabetic condition with neuropathic pain.

Diabetic neuropathy is a serious complication of diabetes mellitus associated with peripheral nerve injury.[5] The damage of the PNS is associated with the CNS in diabetic neuropathy.[8] Thus, peripheral nerve damage in diabetic rats could promote oxidative stress in the CNS.[4] The CNS dysfunction is caused by both the vascular and metabolic consequences of diabetes, leading to neurological and cerebrovascular disorders.[4] The brain, one of the CNS compartments, is an area susceptible to oxidative damage, due to the high oxygen consumption required for its high energy demands. The brain is also vulnerable to lipid peroxidation because it contains high content of unsaturated fatty acids and has low antioxidants.[29] Therefore oxidative stress is an important factor which can cause high mortality in diabetic patients, related to consequent neurological deficits.

Diabetes mellitus has been reported to impair brain function because the latter is the main area of glucose consumption.[30] Hyperglycemia was indicated to relate with impaired sensation,[31] cognitive dysfunction,[32] and movement disorder.[33] The cerebral cortex is the part of the higher brain that perceives sensation, provides awareness of emotions, controls motor skill, and is important for memory, thinking, and language abilities.[34] In STZ-treated mice, the cerebral cortex and hippocampus are highly susceptible to oxidative damage due to decreased glucose uptake into these tissues.[35] Sensory neuropathy, especially loss sensation, is an important complication in diabetes. This impairment leads to skin ulcers and amputation. STZ-induced diabetic mice showed hypoalgesia within five weeks after STZ injection.[31] The hippocampus plays a key role in learning and memory.[36] Ott and coworkers reported that diabetes mellitus affected cognitive function and was associated with dementia, due to changed insulin metabolism, inflammation,

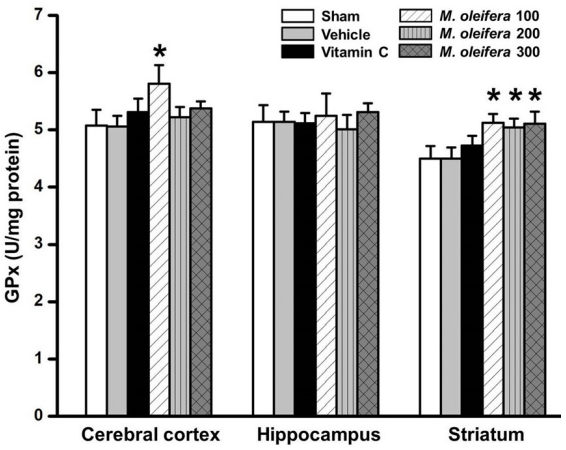

Figure 3 Effect of *M. oleifera* leaf extract on the activity of glutathione peroxidase (GPx) in the cerebral cortex, hippocampus, and striatum. The results showed that *M. oleifera* leaf extract at 100 mg/kg BW significantly increased the activity of GPx in the cerebral cortex and striatum whereas *M. oleifera* leaf extract at doses of 200 and 300 mg/kg BW significantly increased the activity of GPx only in the striatum. Data are expressed as mean ± SEM; n = 6 per group. $^{*}P < 0.05$, compared to vehicle-treated group.

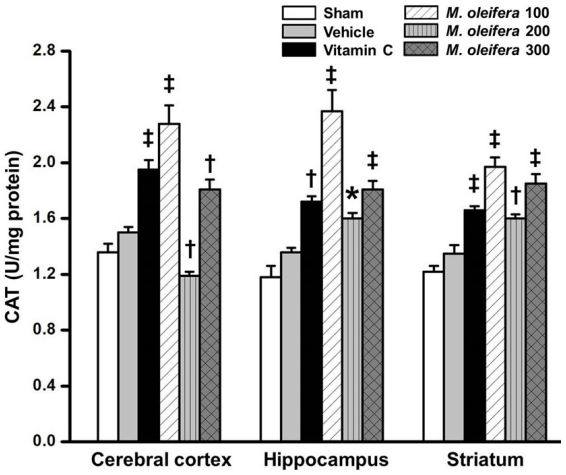

Figure 4 Effect of *M. oleifera* leaf extract on the activity of catalase (CAT) in the cerebral cortex, hippocampus and striatum. The results showed that the treatment with vitamin C and *M. oleifera* leaf extract at doses of 100 and 300 mg/kg BW significantly increased the activity of CAT in all selected brain areas wheras the *M. oleifera* leaf extract at 200 mg/kg BW significantly increased the activity of CAT in the hippocampus and striatum. Data are expressed as mean ± SEM; n = 6 per group. $^{*}P < 0.05$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.001$, compared to vehicle-treated group.

and glucose toxicity.[37] Hyperglycemia was reported to interrupt hippocampal function. STZ-induced diabetic rats exhibited spatial memory deficit in Morris water maze task.[32] In addition, the hippocampal cell proliferation was decreased in diabetic mice leading to cognitive deficit in novel object-placement recognition task.[38] The striatum contains neuronal activity associated with rewards and movements.[39] It is highly vulnerable to neuronal oxidative mitochondrial dysfunction related to neuro-

degenerative diseases.[40] A reduced acetylcholine synthesis in the striatum was reported in STZ-induced diabetic rats leading to memory impairment.[41] Moreover, the activities of dopaminergic neurons and dopamine receptors are also decreased in the striatum and affect locomotor function of diabetic rats.[33] Altogether, the higher brain regions, including the cerebral cortex, hippocampus, and striatum, are the main central control of sensation, cognition and movement. Dysfunction of these brain areas are related to the complications found in diabetes. Therefore, we selected them to determine if their oxidative stress are related to peripheral neuropathy.

We were interested in examining the effects of *M. oleifera* leaf extract on MDA levels, and the activities of the key antioxidant enzymes, including SOD, GPx, and CAT, in the higher brain regions of diabetic rats with sciatic nerve constriction. The present data demonstrated that *M. oleifera* leaf extract treatment at low dosage (100 mg/kg BW) exerted the best results on all measured antioxidant enzymes (SOD, GPx and CAT) in both the cerebral cortex and striatum, but this dose could only increase CAT activity in the hippocampus. The medium dose (200 mg/kg BW) significantly increased the activity of CAT in the hippocampus and striatum but increased the activity of GPx only in the striatum, not in the cerebral cortex and hippocampus. The high dose (300 mg/kg BW) produced a significant increase in CAT activity in all the brain areas and also increased the activity of all antioxidant enzymes in the striatum. The rats received *M. oleifera* leaf extract at 100 mg/kg BW had a higher activity of CAT than those received 200 and 300 mg/kg BW in the cerebral cortex. The results might be due to the crude extract form used in this study. A previous study reported that *M. oleifera* leaf extract contained many chemical constituents, including flavonoids, anthraquinone, saponins, steroids, terpenoids, alkaloids, cardiac glycosides, tannins, anthocyanin, and carotenoids.[42] Moreover, another study also demonstrated that the main active components of *M. oleifera* leaf extract were quercetin, chlorogenic and moringinine. These three compounds were reported to protect against diabetes by normalizing the increased levels of serum glucose, MDA, protein carbonyl, and total antioxidant capacity.[43] Thus, we hypothesized that some ingredients in the extract might have reduced the antioxidant properties of the active compounds that exert its influence on CAT activity. Another observation was that the extract failed to produce a dose-dependent effect, perhaps because many constituents in the extract might affect antioxidant enzymes in various brain areas differently.

MDA is one of the final products of lipid peroxidation in the cells. Excessive free radicals cause MDA to increase. Hence, MDA is well known as a marker of oxidative stress.[12] We did not observe any significant change in MDA levels between untreated and treated groups, possibly because the antioxidant defense mechanism might enhance only a specific pathway of the antioxidant enzyme systems. However, there was no significant change of any antioxidant enzymes between sham and sciatic nerve ligation groups. This suggests the most decisive evidence that there is no relationship between peripheral neuropathy and antioxidant enzyme activity in the higher brain.

The results obtained from the current study showed that *M. oleifera* leaf extract could increase the activity of antioxidant enzymes in the studied brain areas. The current study concurred with previous findings that an aqueous extract of *M. oleifera* leaves could reduce MDA levels in pancreastic tissue.[17] Adeeyo and coworkers also demonstrated that the administration of *M. oleifera* leaf extract could decrease pancreatic MDA levels but increase pancreatic glutathione and SOD concentrations in STZ-induced diabetic rats.[44] Moreover, Jaiswal and colleagues have reported that diabetic rats treated with 200 mg/kg BW *M. oleifera* leaf extract showed increased activities of SOD, CAT, and glutathione-S-transferase, but decreased lipid peroxidation in rat brain tissue homogenates.[45] Therefore, the results of the present study are consistent with these reports. However, the previous studies examined the whole brain tissue, whereas our work focused on some parts of the brain, specifically, the cerebral cortex, hippocampus, and striatum. These selected areas of the brain are the main central controls of body functions. The results of the present study provide a better understanding of the complications of diabetes related to the central control of brain functions, including impaired sensation, movement, and cognitive disorders. These complications might be mainly affected by chronic hyperglycemia, but not peripheral neuropathy. Furthermore, peripheral neuropathy might induce oxidative stress in peripheral nerves rather than in the brain.

Conclusion

M. oleifera leaf extract has a potential for antioxidative effects in the higher brain of diabetic rats. Nevertheless, further studies will be needed to identify the compound in *M. oleifera* leaf extract responsible for the mentioned effects, and to determine the neuro-behaviors relating to these specific brain areas.

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Conflict of Interest

None to declare.

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