Antidepressant-Like Behavioral, Anatomical, and Biochemical Effects of Petroleum Ether Extract from Maca (*Lepidium meyenii*) in Mice Exposed to Chronic Unpredictable Mild Stress

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ABSTRACT Maca has been consumed as a medical food in Peru for thousands of years, and exerts anxiolytic and antidepressant effects. Our present study aimed to evaluate the behavior and anatomical and biochemical effects of petroleum ether extract from maca (ME) in the chronic unpredictable mild stress (CUMS) model of depression in mice. Three different doses of maca extract (125, 250, and 500 mg/kg) were orally administered in the six-week CUMS procedure. Fluoxetine (10 mg/kg) was used as a positive control drug. Maca extract (250 and 500 mg/kg) significantly decreased the duration of immobility time in the tail suspension test. After treatment with maca extract (250 and 500 mg/kg), the granule cell layer in the dentate gyrus appeared thicker. Maca extract (250 and 500 mg/kg) also induced a significant reduction in corticosterone levels in mouse serum. In mouse brain tissue, after six weeks of treatment, noradrenaline and dopamine levels were increased by maca extract, and the activity of reactive oxygen species was significantly inhibited. Serotonin levels were not significantly altered. These results demonstrated that maca extract (250 and 500 mg/kg) showed antidepressant-like effects and was related to the activation of both noradrenergic and dopaminergic systems, as well as attenuation of oxidative stress in mouse brain.

KEY WORDS: • antidepressant • corticosterone • maca (*Lepidium meyenii*) • monoamine neurotransmitters • reactive oxygen species • tail suspension test

INTRODUCTION

DEPRESSION, A CHRONIC AND RECURRENT psychiatric disorder, is the second leading cause of disease worldwide, and its prevalence is expected to increase over the next 10 years.¹ It is closely associated with alterations of many biomarkers, including the anatomical structure of the dentate gyrus in brain, hypothalamic–pituitary–adrenal (HPA) axis, monoaminergic system, and oxidative systems, and some antidepressants work by targeting these markers. Depression may bring on a lower level of proliferating cells in the dentate gyrus. Studies in mice have shown a lower density of the granule cell layer in the dentate gyrus.² The HPA axis is important in the physiological response to stress, and its persistent activation for long periods results in higher blood levels of corticosterone (CORT) and a dysregulated circadian rhythm of CORT secretion.^{3–5} The recovery of HPA axis activity has been suggested as a marker of improvement

in depression. The hypothesis regarding the physiological basis of depression states that depression results from a lack of monoamine neurotransmitters such as serotonin (5-HT), noradrenaline (NE), and dopamine (DA).⁶ On the other hand, there is also evidence that reactive oxygen species (ROS) are increased in the plasma and brain of patients with major depression, indicating that oxidative stress may be a possible etiology of depression.^{7–9}

Present classical antidepressants can lead to many adverse severe effects and clinical problems, including sexual dysfunction, sedation, and sleep disorders.¹⁰ Furthermore, these drugs have no significant effect on a certain proportion of depressed patients.⁶ Thus, it is necessary to identify new effective antidepressants with fewer or no clinical adverse effects, and this has become very important area of medical food research. With fewer side effects, medical food and natural medicines, including Chaihu-Shugan-San, *Echium amoenum*, *Crocus sativus* L., and *Hypericum perforatum*, have been introduced for the treatment of depression.^{11–14} Currently, herbal therapies and medical foods provide prospective alternative/complementary strategies for the treatment of depression. Maca (*Lepidium meyenii*), a plant that grows indigenously at an altitude of more than 4000 m in the central Peruvian Andes,¹⁵ is believed to have great benefits

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for the treatment of depression. It has been used by indigenous Andeans for thousands of years as a foodstuff, and is now used worldwide as a food supplement and medicine. In the past few decades, some of its supposed biological properties, such as nutritional, fertility enhancer properties, energy promotion, and antioxidant effects, have been utilized in medical practice.¹⁶ Recently, the antidepressant-relevant medicinal properties of maca were reported. López-Fando *et al.* reported that maca had antistress activity in a rat model.¹⁷ Rubio *et al.* and Gonzales reported, respectively, that maca reduced the immobility time of ovariectomized mice in the forced swimming test (FST),¹⁸ and that maca and lowered men's scores in the Hamilton Rating Scale for depression after four weeks of use.^{19,20} We recently found that maca lipophilic compounds showed better antidepressant-like activity than water-soluble compounds. Based on the previously limited study of the antidepressant properties, the objective of our present study was to examine further the behavioral, anatomical, and biochemical effects of petroleum ether extract from maca in the chronic unpredictable mild stress (CUMS) model of depression.

MATERIALS AND METHODS

Preparation of petroleum ether extract from maca (L. meyenii)

The maca material was provided by Shao-Feng Zhang (a farmer who lives in Linzhi, Xizang, China). The identity of the plant was authenticated by associate researcher Xiao-Dong Li, Wuhan botanical garden of the Chinese Academy of Sciences. The voucher specimen was placed in College of Life Science and Technology, Huazhong University of Science and Technology. To prepare petroleum ether extract of maca, the dried maca power (20 g) was mixed with petroleum ether (150 mL×3) in a reflux condenser for another 20 min heated by a water-bath heater and then was ultrasonic extracted for 5 min. The preparation was filtered and evaporated using a rotary evaporator at 45°C to remove the liquid and to obtain a dry extract, which was further dissolved with water and Tween-80 to acquire different concentrations. Finally, the dissolved extract was stored at 4°C.

Drugs and chemical reagents

Fluoxetine hydrochloride capsules were purchased from Zhong-Xi pharmaceutical factory (Shanghai, China). NE, DA, 5-HT, ROS, and CORT enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Co. (Minneapolis, MN USA). All other reagents and solvents used in the study were of analytical grade.

Animals

Ninety 60-day-old male Kunming mice were purchased from the Centers for Disease Control and Prevention (Wuhan, China; certificate number SCKX-2008-0005). Animals were housed under controlled conditions (room temperature 22±2°C; relative humidity 50–60%; 12 h/12 h light/dark cycle) and had free access to food and water. All the experiments were conducted between 9:00 AM and 2:00 PM, and the procedures performed in our study complied with NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996). Additionally, the local ethics committee authorized the experimental protocol. We made every effort to reduce the number and suffering of the animals.

CUMS procedure

The CUMS procedure was performed as described by Zhong *et al.*,²¹ with some modifications. Briefly, mice were exposed to the following seven stressors in random order for six weeks: 24 h food deprivation; 24 h water deprivation; overnight illumination; 24 h damp sawdust; 10 min hot environment in oven (45°C); 2 h restraint; and 30 min vibration on rocking device. Table 1 summarizes the detailed stress arrangements. Group 1 animals were not disturbed, except for necessary procedures such as cage cleaning.

Drug administration

After one week of accommodating, baseline measures of immobility in the tail suspension test (TST) were used to separate some of the mice that had large individual differences, and the remaining 72 mice were randomly divided into six groups ($n = 12/\text{group}$). Animals in group 1 (control

TABLE 1. CHRONIC UNPREDICTABLE MILD STRESS PROCEDURE

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Vehicle	Food deprivation	Water deprivation	Overnight illumination	Damp sawdust	Hot environment	Restraint	Vibration
Maca extract (125 mg/kg)	Overnight illumination	Food deprivation	Damp sawdust	Water deprivation	Restraint	Vibration	Hot environment
Maca extract (250 mg/kg)	Vibration	Hot environment	Food deprivation	Restraint	Water deprivation	Damp sawdust	Overnight illumination
Maca extract (500 mg/kg)	Hot environment	Overnight illumination	Damp sawdust	Food deprivation	Vibration	Water deprivation	Restraint
Fluoxetine	Restraint	Damp sawdust	Overnight illumination	Hot environment	Vibration	Food deprivation	Water deprivation

These stressors lasted for six weeks, and the order of the five groups was disrupted randomly to guarantee that each animal randomly received one stress per day.

group) and group 2 (vehicle group) were administrated with distilled water with Tween-80. Animals in group 3 (positive group) were administrated with fluoxetine hydrochloride capsules dissolving in water at a dose of 10 mg/kg. Referencing the usually consumed doses of humans and previous studies in animals, animals in groups 4, 5, and 6 were administrated with maca extract at doses of 500, 250, and 125 mg/kg per day with the volume of 0.2 mL/kg.²² The treatment lasted six weeks, and all drugs were orally administered every day between 9:00 AM and 12:00 AM during the CUMS.

TST

The TST was conducted as previously described by Steru *et al.*²³ Each mouse was individually suspended by its tail with a string (nearly 1 cm from the tip of the end) for 7 min in a homemade box (30 cm × 30 cm × 50 cm), with the animal's head about 5 cm from the bottom. Testing was carried out in a poorly lit room with minimal background noise. A mouse was regarded immobile only when it hung passively and completely motionless with its head perpendicular to the bottom of the box. The immobility represents a failure of persistence in escape-directed behavior.²⁴ The duration of immobility in the six groups was observed during the final five-minute interval of the test using a chronograph.

Open-field test

In order to detect the relationship of immobility in the TST with changes in motor activity, the behavior of mice was tested in an open field. The open-field test (OFT) was performed based on the slightly modified method described by Aragao *et al.*²⁵ Each mouse was placed individually in the center of homemade black boxes (60 cm × 60 cm × 60 cm), which were divided into 25 equal squares by black lines, and they were allowed to explore freely. After one minute of adaption, the mouse was put in the central square and observed for three minutes. Scores were calculated by counting the number of times the mouse stood on its hind limbs (rearing) and the number of times the mouse crossed the sector lines with at least three paws (movement distance). During the experiments, the laboratory room was dark and quiet.

Serum CORT assay

Cardiac blood was collected prior to sacrifice. Samples were kept at room temperature for half an hour, centrifuged at 13,000 g for 10 min at 4°C. Then, the supernatant serum was stored frozen (−80°C) for CORT assay using an ELISA kit.

Pathological histology observation of mouse brain tissue

To observe the effects of the treatments on brain histology, the mouse brain was removed from the ice, and the brain tissue was kept in 4% paraformaldehyde for fixing. After fixing for 24 hours, the tissue was prepared by a series of procedures to complete hematoxylin and eosin (H&E)

staining as follows: dehydrate in the gradient ethanol until transparent, imbed in paraffin wax, cut into 4 μm sections, remove the wax, hydrate the section, apply the hematoxylin nuclear stain, complete the nuclear stain by “blueing,” remove excess background stain, apply the eosin counterstain, rinse, dehydrate, clear and mount (apply cover glass), examine under a microscope, and take photos.

Monoamine neurotransmitters in brain tissue assays

We evaluated whether maca extract was able to change the concentration of monoamine neurotransmitters in the whole brain of mice. The contents of DA, 5-HT, and NE in the brain tissue were estimated using ELISA kits. Briefly, the brains of mice were removed and placed on ice, and the tissue samples were homogenized in 4°C normal saline using a manual glass homogenizer with concentration of 0.01 g/mL. Homogenates were quickly centrifuged at 13,000 g for 10 min at 4°C. Supernatants were reserved for analysis.

ROS in brain tissue assays

We assessed whether maca extract could decrease ROS levels in the brains of mice. In brief, the mouse brain tissue samples were homogenized in 4°C normal saline using a manual glass homogenizer with concentration of 0.01 g/mL. The supernatants of homogenized brain tissue were analyzed using ELISA kits.

Statistical analysis

Data were expressed as means ± standard deviation (SD). Differences in mean values among groups were analyzed by one-way analysis of variance followed by the least significant difference test using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). *P*-values of <.05 or <.01 were considered statistically significant.

RESULTS

After exposure to CUMS for six weeks, the vehicle-treated group showed a significant increase in the duration of immobility (*P* < .01) and significant decreases in movement, rearing times, and duration of sleep compared with the control group. The results of the ethology tests (TST and OFT) revealed that the mouse model of depression was successful.

Effects of petroleum ether extract from maca on duration of immobility in the mouse TST

The effects of treatment with petroleum ether extract from maca and fluoxetine on the duration of immobility in the mouse TST are shown in Figure 1. There were significant differences among the six groups (*F* = 4.835; *P* = .001). The results show that after six weeks of oral administration, maca extract (250 and 500 mg/kg) and fluoxetine significantly decreased immobility time when compared with the vehicle-treated group.

FIG. 1. Effects of maca extract on the duration of immobility in the tail suspension test (TST). Mice were administered vehicle, maca extract (125, 250, and 500 mg/kg bw), or fluoxetine. Values are presented as mean $\pm$ standard deviation (SD; $n=12$). ** $P<.01$ compared with the vehicle-treated group. # $P<.05$, ## $P<.01$ compared with the control group.

Effects of petroleum ether extract from maca in the OFT of locomotor activity

After six weeks of CUMS and oral administration of maca extract, the movement distance ($F=2.218$) and rearing times ($F=4.463$; $P=.002$) were recorded in the OFT. This test excluded the false-positive effects in the TST. The results are shown in Table 2. Maca extract (500 mg/kg) and fluoxetine significantly increased the movement distance, and maca extract (500 mg/kg) also increased the rearing times.

Effects of petroleum ether extract from maca on serum CORT levels

The analysis of CORT concentrations is shown in Figure 2. It can be seen that six weeks of CUMS resulted in a significantly higher levels of CORT in the vehicle-treated group compared with other groups ($F=3.063$; $P=.015$).

TABLE 2. EFFECTS OF MACA EXTRACT IN THE OPEN-FIELD TEST OF MOVEMENT DISTANCE AND REARING TIMES

Group	Movement distance	Rearing times
Vehicle	54 $\pm$ 17 [#]	14 $\pm$ 4 [#]
Control	75 $\pm$ 20 [*]	21 $\pm$ 5 [*]
Maca extract		
500 mg/kg bw	72 $\pm$ 18 [*]	24 $\pm$ 5 [*]
250 mg/kg bw	68 $\pm$ 18	20 $\pm$ 4
125 mg/kg bw	67 $\pm$ 27	14 $\pm$ 3
Fluoxetine	75 $\pm$ 21 [*]	12 $\pm$ 4

Data are expressed as the mean $\pm$ SD ($n=12$). Mice were administered vehicle, maca extract (125, 250, and 500 mg/kg bw), or fluoxetine.

^{*} $P<.05$ compared with the vehicle-treated group.

[#] $P<.05$ compared with the control group.

FIG. 2. Effects of maca extract on serum corticosterone (CORT) levels. Mice were administered vehicle, maca extract (125, 250, and 500 mg/kg bw), or fluoxetine. Values are presented as mean $\pm$ SD ($n=12$). * $P<.05$ compared with the vehicle-treated group. # $P<.05$ compared with the control group.

Compared with the vehicle-treated group, maca extract (250 and 500 mg/kg) significantly decreased CORT levels in mouse serum ($P<.05$ and $P<.05$, respectively).

Effects of petroleum ether extract from maca on pathological histology in mouse brain tissue

After six weeks of CUMS, coronal sections of mouse brain tissue were stained with H&E and examined under an optical microscope. Micrographs of these sections are shown in Figure 3. An obvious difference in the density of the granule cell layer of the dentate gyrus was observed. The thickness of the granule cell layer of the dentate gyrus in vehicle-treated mice was significantly thinner than that in the control groups. Maca extract (250 and 500 mg/kg) and fluoxetine significantly prevented this transformation.

Effects of petroleum ether extract from maca on monoamine neurotransmitters in brain tissue

The content of DA and NE decreased after six weeks of CUMS in the vehicle-treated group compared with the control group ($F=15.025$; $P<.001$, $F=2.565$; Table 3). Maca extract (125, 250, and 500 mg/kg) produced a significant increase in DA levels in the whole brain ($P<.01$, $P<.01$, and $P<.01$, respectively). Maca extract (500 mg/kg) and fluoxetine produced a significant increase in NE levels. 5-HT contents were not different among the groups.

Effects of petroleum ether extract from maca on ROS activity in brain tissue

The activity of ROS increased after six weeks of CUMS in the vehicle-treated group compared with the control group ($F=14.824$; $P<.001$) as shown in Figure 4. In the maca extract (125, 250, and 500 mg/kg) and fluoxetine

FIG. 3. Effects of maca extract on the pathological histology of mouse brain tissue. The dentate gyrus of mice subjected to six weeks of chronic unpredictable mild stress (CUMS) is shown in coronal sections stained with hematoxylin and eosin at 200 $\times$ magnification. As shown by the arrow in (A), the thickness of the granule cell layer was thinner in the vehicle-treated group but was thicker with maca extract (250 mg/kg and 500 mg/kg bw) and fluoxetine treatments. Scale bar=50 μ m. Color images available online at www.liebertpub.com/jmf

treatment groups, ROS significantly declined compared with the vehicle-treated group. ROS levels in the maca extract (125 and 250 mg/kg) groups were significantly lower than even the control group.

DISCUSSION

Stress is involved in the genesis of depression and is considered a paramount factor.²⁶ CUMS, similar to the unexpected stressors of daily life,²⁷ has been widely used in

animal models of depression-like disorders in previous research. It is generally thought to be the most promising model to study depression in animals. The TST has become one of the most widely used models for assessing antidepressant-like activity in mice during the last 20 years.²⁵

TABLE 3. EFFECTS OF MACA EXTRACT ON MONOAMINE NEUROTRANSMITTER LEVELS IN MOUSE BRAIN TISSUE

Group	NE (ng/g)	DA (ng/g)	5-HT (ng/g)
Vehicle	389.5 $\pm$ 10.6 [#]	240.1 $\pm$ 5.7 [#]	605.6 $\pm$ 25.1
Control	405.0 $\pm$ 21.0*	244.4 $\pm$ 4.7*	616.6 $\pm$ 90.2
Maca extract			
500 mg/kg bw	402.6 $\pm$ 12.8*	252.5 $\pm$ 4.9 ^{###}	615.6 $\pm$ 29.8
250 mg/kg bw	396.1 $\pm$ 9.1	263.5 $\pm$ 13.6 ^{###}	621.4 $\pm$ 46.3
125 mg/kg bw	395.0 $\pm$ 8.4	262.1 $\pm$ 9.6 ^{###}	599.1 $\pm$ 32.8
Fluoxetine	403.1 $\pm$ 15.0*	240.1 $\pm$ 5.4	621.8 $\pm$ 42.1

Data are expressed as the mean $\pm$ SD ($n=9$). Mice were administered vehicle, maca extract (125, 250, and 500 mg/kg bw), or fluoxetine.

* $P < .05$, ** $P < .01$ compared with the vehicle-treated group.

[#] $P < .05$ compared with the control group.

NE, noradrenaline; DA, dopamine; 5-HT, serotonin.

FIG. 4. Effects of maca extract on activity of reactive oxygen species (ROS) in mouse brain tissue. Mice were administered vehicle, maca extract (125, 250, and 500 mg/kg bw), or fluoxetine. Values are presented as mean $\pm$ SD ($n=11$). ** $P < .01$ compared with the vehicle-treated group. [#] $P < .05$, ^{###} $P < .01$ compared with the control group.

Various antidepressant medications can relieve immobility and promote the frequency of escape-related behavior.²⁸ We studied the effects of petroleum ether extract from maca on immobility behavior in mice. The maca extract at doses of 250 and 500 mg/kg significantly decreased the duration of immobility, indicating that maca extract had antidepressant activity. This is the first study that verifies the antidepressant effect of maca in CUMS mice. The present data were consistent with the report of Rubio *et al.* that maca can decrease the duration of immobility in the FST in ovariectomized mice.¹⁸ It has been proposed that the TST is less stressful than the FST and has greater pharmacological sensitivity.²⁹

Antidepressant drugs can reduce the duration of immobility, just as the present data show atropine and psychostimulants do. To avoid false-positive results in the TST, it is vitally important to eliminate the possibility that a decrease in immobility time is not merely a result of the psychostimulant effects of the extract.³⁰ The TST combined with the OFT can separate locomotor stimulant drugs from antidepressant drugs.²³ On the other hand, during the OFT, when mice were placed in a novel environment (the black box), an overall decrease in locomotor activity (including movement distance, movement velocity, movement time, and rearing times) was observed in most previous studies using the CUMS model.³¹ Thus, we can determine if the CUMS procedure leads to a decrease in locomotor activity. In our study, the six-week CUMS procedure significantly decreased the movement distance and rearing times, and the 500 mg/kg maca extract significantly prevented this trend. Fluoxetine also increased movement distance compared with the vehicle-treated group, but did not increase rearing times significantly. This may have been due to experimental conditions, sample size, and mouse strain. Compared with the control group, movement distance and rearing times were not increased in any of the drug groups, indicating that maca extract improved the decreased locomotor activity, but had no psychostimulant effects compared with the control group. The aforementioned two behavioral tests provided convincing evidence of the antidepressant activity of maca.

Abnormalities of HPA axis activity have been implicated in the development of depression. The recovery of the axis plays a very important role in depression therapy.²⁵ In the present study, after six weeks of CUMS, CORT levels were significantly increased in the vehicle-treated group. It is possible that there is a close relationship between the hyperactivity of the HPA axis and the increased plasma CORT level. Studies have shown that high levels of CORT contribute to the etiology of depressive symptomatology, and the level of CORT has been identified as a useful diagnostic factor.³² Maca extract (250 and 500 mg/kg) decreased CORT levels to normal values, suggesting that regulation of the HPA axis by maca extract played a very important role in the recovery of depression in the CUMS mice model.

Acute and chronic stress suppresses neurogenesis of dentate gyrus granule neurons.³³ Antidepressants promoted proliferation of subgranular stem cells with a lag time similar to that of their clinical effectiveness.^{34,35} In our study, mouse brain coronal sections were stained with H&E,

and the dentate granule cell layer in the vehicle-treated group was found to be noticeably thinner than that in the control group, indicating that the CUMS procedure negatively affected the granule cell proliferation in the dentate gyrus. Maca extract (250 and 500 mg/kg) and fluoxetine prevented this trend. This phenomenon may play an important role in the beneficial effects of antidepressant treatments.³⁶ Studies showed that increased granule cell proliferation resulting from antidepressant treatments could demonstrate that more competing neurons were available for selection, which might ameliorate the ability of the hippocampus to adapt to emerging environmental challenges.³⁷

It is well known that monoamine neurotransmitters including 5-HT, NE, and DA are important in the pathogenesis of depression. Depression is always accompanied by a decrease in these three monoamine neurotransmitters, which are related to the specific symptoms of a major depressive disorder.³⁸ It is necessary to increase the levels of brain monoamine neurotransmitters to treat depression effectively.^{39,40} In the present study, we found that NE and DA were significantly decreased in the vehicle-treated group compared with the control group. Maca extract (500 mg/kg) and fluoxetine increased NE levels, and DA content was significantly increased by maca extract (125, 250, and 500 mg/kg). Although the region-specific alterations of monoamine neurotransmitters and their metabolites in the brain need to be assessed in further research, at present we can infer that the antidepressant-like effects of maca extract may be closely associated with modulation of the dopaminergic pathway. 5-HT levels were not significantly different among the six groups, indicating that 5-HT levels in the whole brain were not significantly affected, although that may be different in other regions of the brain.

Recent studies have indicated that ROS also play an important role in the pathogenesis of neuropsychiatric disorders, including depression.^{41,42} Excess production of ROS can result in tissue damage and oxidative stress in brain tissue, resulting in nerve damage.⁴³ In our study, we assessed the activity of ROS, and found that ROS activity was significantly increased in the vehicle-treated group under CUMS compared with the control group. Maca extract (125, 250, and 500 mg/kg) and fluoxetine significantly prevented this trend. These results show the excellent antioxidant activity of maca extract, which are consistent with the results of other studies about maca.⁴⁴ In addition, Pino-Figueroa *et al.* recently demonstrated that the lipophilic compounds from maca had potential neuroprotective properties in an *in vitro* crayfish neuronal cell culture.⁴⁵ These findings showed that maca extract prevented ROS damage in the nervous system, thus improving depression.

CONCLUSIONS

In conclusion, the present study confirmed the behavioral, anatomical, and biochemical effects of petroleum ether extract from maca in the CUMS model of depression in mice, and provided convincing evidence supporting the antidepressant-like effects of petroleum ether extract from

maca. These effects were most likely mediated by the noradrenergic and dopaminergic systems, and improved the antioxidant status in brain tissue. These findings indicated that maca could be used as a medical food for patients with depression, although it is necessary to perform further studies to identify the specific compounds that produce these effects.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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