Neurocognitive effects of *Prunus domestica* fruit extract on scopolamine-induced amnesic mice

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**ABSTRACT**

Neuropathology of Alzheimer’s disease comprises cholinergic neuronal loss with an accumulation of amyloid beta (Aβ) protein and oxidative stress. *Prunus domestica* (Rosaceae) has been implicated to treat inflammation, anxiety, obesity, and neurological diseases. This study was designed to determine the antioxidant potential and neurocognitive effects of *P. domestica* fruit extract on scopolamine-induced amnesia. Mice were treated with 200 and 400 mg/kg of ethanolic extract of *P. domestica* (EEPD) for 15 days and induced with amnesia by scopolamine (1 mg/kg). The total phenolic content and free radical scavenging ability on 1,1-diphenyl-2-picrylhydrazyl and 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid radical scavenging assay indicated the potential ability of the antioxidant system by EEPD. Behavioral and habituation memory was assessed using the Y-maze test, open-field test, and traction test, which revealed the potential impact on neurobehavioral improvement in scopolamine-induced amnesia in mice. The brain neurotransmitter metabolic enzyme acetylcholinesterase (AChE) indicated significant reduction and escalated memory performance after the treatment of EEPD. This implies that the EEPD exhibits a significant ameliorating (*p* < 0.05 in 200 and *p* < 0.01 in 400 mg/kg) effect in AChE inhibition on scopolamine-induced amnesia and is proven to have a beneficial effect on treating memory and learning impairment.

**INTRODUCTION**

Amnesia is characterized by memory and learning impairment. Alzheimer’s disease (AD) is one of the most common neurodegenerative diseases affecting the elderly, and to date, there is no treatment capable of reversing, stopping, or significantly slowing down the disease’s progression. Dementia represents a severe public health issue affecting nearly 55 million people worldwide (Prince *et al*., 2016). The number of AD patients is projected to escalate and targets 88 million by 2050 (Alzheimer’s Association, 2019). Oxidative stress in the brain is one of the pathophysiologic conditions for memory and learning impairment. Oxidative stress in the brain accelerates the production of reactive oxygen species (ROS) and impairs brain homeostasis. This leads to increased acetylcholinesterase (AChE) and biogenic amine dysregulation (Li *et al*., 2018). Moreover, in Alzheimer’s type of neurodegeneration, the declined acetylcholine level, especially in the hippocampus of the brain, is also one of the key factors for the cognitive deficit (Souza *et al*., 2020). The level of acetylcholine decreases when there is an increase in the AChE enzyme. It is well known that the increase in AChE is also caused by the generation of free radicals due to the accumulation of amyloid beta (Aβ) peptides (Madav *et al*., 2019). In AD brain, AChE works by initiating the conversion of Aβ peptides to a highly neurotoxic compound which is in the form of neurofibrils (Postu *et al*., 2019). The deposition of neurofibrils induces AD type of learning and memory impairment that is eventually mediated by cholinergic hypofunction. Scopolamine, a nonselective muscarinic receptor antagonist, acts as an amnesic agent by causing blockade in the cholinergic signaling. Thus, the memory and learning ability of an organism will be affected. Scopolamine...
is an experimental tool used to induce AD and associated amnesia in animal models. The exploratory behavior, memory, learning, locomotor activity, spontaneous alternation behavior, biochemical changes (oxidative stress and biogenic amine dysfunction), and the anxiety level in the animal model are affected through the administration of scopolamine (Ucel et al., 2020). The present study evaluated the neuropharmacological effect of Prunus domestica on scopolamine-induced memory impairment in mice. Among the various traditional herbs as nootropic agents, P. domestica fruit is presumed to ameliorate memory and learning in aged rats (Shukitt-Hale et al., 2009) and its efficacy in an AD model is explored in this investigation. The common name for *P. domestica* is European Plum. It is also categorized as stone fruit. The main phytoconstituents that are present in *P. domestica* are vitamin A, vitamin B complex, vitamin K, amino acids, and carbohydrates besides magnesium, zinc, pectin, hemicellulose, cellulose, lignins, boron, dietary fibers, sorbitol, linalool, potassium, benzoic and boric acids, selenium, fructose, benzaldehyde, malic acid, citric acid, ethyl nonanoate, ethyl cinnamate, chlorogenic acid, caffeic acid, coumaric acid, neochlorogenic acid, proanthocyanidin, and melanoidins (Jabeen and Aslam, 2011). Pharmacological activities of *P. domestica* include anti-inflammatory (Hooshmand et al., 2015), anxiolytic (Bouayed et al., 2007), obesity (Noratto et al., 2014), antibacterial and antifungal activity (Pimenta et al., 2012), and antioxidant (Sharma and Sisodia, 2012), which are used to treat hyperhomocysteinemia (Haddadi-Guemghar et al., 2017) and also reveal to improve learning and memory (Shahidi et al., 2013), and *P. domestica* fruits have various other health benefits (Igwe and Charlton, 2016).

**METHODOLOGY**

**Drugs and chemicals**

Scopolamine (scopamine butyl bromide) 10 mg tablets were purchased from Alpro Pharmacy, Nilai, Malaysia, whereas chemicals such as ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulfate, Na₂CO₃, and Ethylenediaminetetraacetic acid (EDTA) were from Merck. Acetylthiocholine iodide and 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from Alfa Aesar.

**Plant material and preparation of fruit extract**

The fruits of *P. domestica* were bought from a commercial fruit vendor and the fruits were authenticated by the Biodiversity Unit, University Putra Malaysia, Malaysia. The collected fruits (1.5 kg) were washed and cut into small pieces and homogenized with alcohol. The fruits were added portion by portion in a blender and blended together with a sufficient amount (700 ml) of double-distilled ethanol to enable it to homogenize coarsely. Once blended, the mixture was subjected to filtration and concentrated using a rotary vacuum evaporator. The concentrated extract was then labeled and refrigerated at 8°C–15°C.

**Determination of total phenolic content**

The polyphenol content of both extracts was determined by using the Folin–Ciocalteu methods with some modification by expressing it as mg of gallic acid equivalent (GAE) per g extract (Zilic et al., 2014). Briefly, an aliquot of 1 ml extract was mixed with 5 ml of distilled water. To this, 1 ml of 2N Folin–Ciocalteu reagent was added and shaken for 3 minutes. Upon shaking, 2 ml of 2% (w/v) of sodium carbonate solution was added and incubated for 3 hours at room temperature. Then, the absorbance of the mixture was measured using a UV-visible spectrophotometer (Shimadzu, Japan) at 760 nm against the solvent blank which was distilled water. The total phenolic content was expressed as mg of GAE per g extract.

**Free radical scavenging ability on DPPH**

The DPPH assay was measured according to the method reported in Blois’s (1958) study, with some modifications. The stock solution was prepared by dissolving 3.94 mg of DPPH with 10 ml of ethanol, whereas the stock concentration of the test samples was prepared and diluted to get five concentrations of 200, 100, 50, 25, and 10 µg/ml, respectively. Then, 2 ml of different concentrations of the test solution (200, 100, 50, 25, and 10 µg/ml) was mixed with 1 ml of DPPH solution. 2 methanol with 2 ml DPPH solution was used as the control and ethanol as the blank (Haddadi-Guemghar et al., 2017). The absorbance of each sample was read at 517 nm by using a UV-visible spectrophotometer (Shimadzu, Japan) after 30 minutes of incubation at room temperature in dark. The scavenging capacity of the extract was compared to L-ascorbic acid and the percentage inhibition was calculated.

**2,2′-azino-bis ABTS radical scavenging**

The free radical scavenging assay was determined by the ABTS method. 7 mM ABTS stock solution was prepared by diluting 9 mM ABTS into water and ABTS•+ was prepared by reacting the stock solution with 2.45 mM potassium persulfate (which was kept in the dark for 16 hours at room temperature). Then, it was diluted in ethanol (1:89 v/v) and equilibrated to 30°C to give an absorbance at 734 nm (Re et al., 1999). 30 µl of the extract was added to 3 ml of fresh ABTS radical action and incubated at room temperature for 15 minutes. The absorbance was read at 734 nm using a UV-visible spectrophotometer (Shimadzu, Japan) (Re et al., 1999). The extract concentration providing 50% inhibition (IC₅₀) was obtained by plotting inhibition percentage versus extract concentration. The lower IC₅₀ value indicates high antioxidant activity. The unit of total antioxidant activity is defined as the concentration of L-ascorbic acid having equivalent antioxidant activity expressed as µg/ml sample extract.

**Pharmacological evaluation**

**Dose selection**

According to Organisation for Economic Co-operation and Development (OECD) guidelines, the acute toxicity study using *P. domestica* in both male and female Swiss albino mice exhibited nontoxic and shown the Median lethal dose (LD₅₀) value of the *P. domestica* to be >2,000 mg/kg (Swaroop et al., 2015). Based on the reported study, two doses were selected. Considering 2000 mg/kg as non-toxic dose, 1/10th of the 2000 mg (200 mg/kg) and 1/5th of the 2000 mg (400 mg/kg) was administered for the experimental animals.

**Experimental design**

**Grouping and induction of amnesia**

A total of 24 Swiss male mice were selected in this study which weighed approximately 20–30 g each, aged 6–7 weeks old.
They were kept in the vivarium of KPJ Healthcare University College, Kota Sereimais, Nilai, Negeri Sembilan, at a temperature of 2 ± 2°C and 12-hours light/dark cycle. The animals were divided randomly into four groups, each consisting of six animals. The animals had water ad libitum and food pellets. They were allowed to acclimatize to the laboratory environment for a week before the experiments. Group I, Control, was administered with normal saline orally and Group II, Negative Control, was administered with scopolamine 1 mg/kg orally. Groups III and IV were treated with a low dose of ethanolic extract of *P. domestica* (EEPD) 200 mg/kg and a high dose of EEPD 400 mg/kg, respectively, and induced with amnesia. The experimental study and the drug (EEPD) treatment duration was 15 days; scopolamine 1 mg/kg body weight (Deng et al., 2019) was administered orally from the 8th day to the 14th day in Groups II, III, and IV. On the day of the behavioral test, the drug was administered 30 minutes prior to the test. The experimental protocol adhered to the ethical guidelines on animal experimentation; the protocol was approved by KPJUC ethics committee (Ref. No. KPJUC/RMC/BPH/EC/2017/107).

### Behavioral study

#### Y-maze test

Y-maze with the specifications of 40 cm long, 13 cm high, and 3 cm wide was used. The arms were constructed in such a way that they are 120°C symmetrically disposed to each other. The floor was made up of dark opaque polyvinyl plastic. On the 15th day of the treatment, 1 hour before the test, EEPD was treated in the treatment group and all the mice were subject to the test. All the three arms of the maze were labeled as A, B, and C, respectively. The mice were placed at the end of the arm labeled “A” and one food pellet was placed at all the three arms in order to motivate the movement of the mice. The number of arm entries and their sequence over 8 minutes were recorded. The ability to alternate requires that the mice know which arm they have already visited. The series of arm entries, including possible returns into the same arm, was recorded. Alteration is defined as the successive entries into the three arms on overlapping triplet sets. The percentage of alteration is calculated as the ratio of actual alterations to possible alterations (Wahl et al., 2017).

#### Open-field test

The field for the test was conducted to measure the locomotor activity, explorative, cognitive, and anxiety levels of the mice. The mice were placed in a 40 cm × 50 cm × 60 cm open field which was painted across with yellow stripes and consisted of 16 quadratic blocks. At the beginning of the test, the animals were placed at the center of the field and the spontaneous ambulatory locomotion of each animal was observed for a duration of 5 minutes. During this period, the number of lines crossed, the number of head dipping, and the number of rearing were measured (Jayasingh Chellammal et al., 2019).

#### Traction test

The effect of scopolamine, as well as the extract of *P. domestica* treatment, on motor coordination of the mice was evaluated using the traction test. The experimental set-up was done using a horizontal bar of 12 mm in size, one 12-inch length fixed in two poles at a height of 40 cm. The height of the setup allows the animal sufficient time and space to land by losing muscle grip on its feet due to the righting reflex (Jänicke and Coper, 1996). The set-up was arranged on the tabletop and the animals were allowed to get a grip with their hind limbs on the horizontal bar. The total motivation time in seconds to hang onto the bar from the aversion to falling is considered as grip index. The motivation time is considered to be proportional to muscle grip strength and balance. Once done with the set-up, the mice were suspended on the static bar and the time taken (in seconds) for the mice to reestablish themselves and retain on the bar was recorded.

### Biochemical studies

#### Determination of AChE

The brains were removed and rinsed with ice-cold normal saline before being dried and weighed. Then, the brain homogenates were prepared at a concentration of 10% (w/v) in an ice-cold medium [1:9 w/v of a 50 mM phosphate-buffered saline, pH 7.0] containing 0.1 mmol/l EDTA. The homogenates were then subjected to centrifugation at 4,000 rpm for 30 minutes at 4°C to prepare clear supernatants (10%). The assay was carried out by taking 0.1 mL of supernatant, added with 6 mL of sodium phosphate buffer (pH 8), 0.2 mL of acetylthiocholine iodide, and 02 mL of DTNB (Ellman reagent). The absorbance of the solution was recorded at 412 nm (Nanaware et al., 2017).

### Statistical analysis

The data obtained were recorded and analyzed using one-way analysis of variance (ANOVA), followed by post hoc Tukey’s test in the GraphPad7 Prism software. The analyzed data were expressed as mean ± SEM and *p* < 0.05 was considered to be statistically significant.

### RESULTS

#### Drug extract and percentage yield

The characteristic nature of the EEPD exhibited as dark brown, creamy, semisolid and percentage yield was noted as 13 compared to the original material.

#### Total phenolic content

The total phenolic content in the EEPD was 0.182 mg GAE/g. The result was obtained from a calibration curve (*y* = 0.003x + 1.105, *R*² = 0.996) of gallic acid and was expressed as GAE.

#### DPPH scavenging capacity

The DPPH radical scavenging (%) activity is shown in Figure 1. *P. domestica* extract exerted the highest inhibition of 88.48% at 200 µg/ml. Ascorbic acid was used as the reference. The IC₅₀ of the EEPD extract was 52.45 µg/ml, while that of the ascorbic acid was 75 µg/ml.

#### ABTS radical scavenging

The ABTS radical scavenging (%) activity is shown in Figure 2. EEPD exerted an inhibition of 85.33 at 200 µg/ml. The absorbance at 734 nm indicated *R*² of 0.8826. IC₅₀ value for EEPD was 23.77 µg/ml compared to that of ascorbic acid at 130 µg/ml.
Behavioral study

Open-field test

The treatment using EEPD (200 and 400 mg/kg) exhibited a significant ($p < 0.001; p < 0.01$) effect, respectively, on the head dips of open-field acquisition of memory compared to the negative control group (Group II). The results obtained indicated that the high dose of *P. domestica* (400 mg/kg) exhibited better exploratory behavior in head dipping (Table 1). The effect of EEPD on line crossings indicated that the administration of 200 and 400 mg/kg EEPD exhibited significant differences of $p < 0.0001$ and $p < 0.01$, respectively. In rearing, the result obtained indicated that the animal treated with 200 mg/kg exhibited a significant effect ($p < 0.05$) compared to the disease-induced group, while the high dose treated animals exhibited an improved behavioral performance ($p < 0.001$); also, a significant ($p < 0.05$) dose-dependent increase in rearing was noted between 200 and 400 mg/kg EEPD.

Y-maze test

The results obtained for the effect of EEPD on the percentage of spontaneous alternation in Y-maze are depicted in Table 2. The control group was compared with the negative control group (scopolamine-induced) and it was found that the induction of amnesia was significant. After the treatment, on comparing the negative group and the negative control group, both the low dose (200 mg/kg) and high dose (400 mg/kg) treated animals showed a significant ($p < 0.001$) improvement in memory and learning ability.
Traction test

In the traction test, the scopolamine-induced amnesiac group (negative control) exhibited loss of muscle coordination, which is considered as a sign of neurotoxicity in general. The animals in the negative control ($p < 0.01$) showed a significant reduction in retention time and downfall in a short duration of time when compared to the normal control group. The administration of EEPD has shown a significant effect on enhancing the motor coordination and balancing ability in mice; however, only the higher dose (EEP 400 mg/kg) exhibited the neuroprotective effect. The animals after treatment with EEPD 400 mg/kg exhibited a significant ($p < 0.001$) difference in time, spending more time balancing when compared to the negative control group. The results are depicted in Table 2.

Biochemical study

Acetylcholinesterase enzyme

The effect of EEPD on AChE inhibitory activity is shown in Table 2 and the results indicate that the treated groups show a significant reduction in comparison to the negative control group. The negative control animals expressed a significantly ($p < 0.001$) higher level of AChE enzyme upon comparison with the control group after the induction of amnesia. In the treatment group, the enzyme levels have reduced as indicative of neuroprotection. The 200 mg/kg EEPD treated group exhibited the inhibition of AChE

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Table 1. Effect of *P. domestica* open field exploration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Head dips</th>
<th>Rearings</th>
<th>Line crossings</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>36.67 ± 4.80</td>
<td>20.00 ± 7.81</td>
<td>77.50 ± 4.03</td>
</tr>
<tr>
<td>II (Scopolamine)</td>
<td>5.33 ± 0.33$^a$</td>
<td>16.00 ± 2.08$^a$</td>
<td>19.17 ± 3.25$^a$</td>
</tr>
<tr>
<td>III (Scopolamine + 200 mg/kg PDEE)</td>
<td>38.33 ± 4.33$^a$</td>
<td>26.67 ± 1.66$^a$</td>
<td>40.17 ± 3.97$^a$</td>
</tr>
<tr>
<td>IV (Scopolamine + 400 mg/kg PDEE)</td>
<td>49.33 ± 5.20$^d$</td>
<td>38.67 ± 2.40$^d$</td>
<td>51.83 ± 5.65$^e$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. Symbol represents the statistical significance done by ANOVA, followed by Tukey’s multiple comparison tests.

$p < 0.001$ and $p < 0.05$ indicates comparison of negative control (Group II) with control Group I, $p < 0.01$, $p < 0.001$, indicates comparison of group III and IV with II (Negative control). $p < 0.05$ indicates the significant dose dependent effect on comparison with group III and group IV.

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Figure 2. Antioxidant effect *P. domestica* ethanolic extract on ABTS.
enzyme level to 11.27 ± 1.19 (µg/minute/mg protein) which was significantly (p < 0.05) lower when compared to the negative control group with the enzyme level, 17.23 ± 2.10 (µg/minute/mg protein). In the high dose (400 mg/kg EEPD) treated animals, the enzyme levels are significantly (p < 0.01) reduced to 9.01 ± 1.05 µg/minutes/mg protein and indicate the protective effect when compared to the negative control group.

**DISCUSSION**

In the present investigation, our studies reveal the antioxidant and neuroprotective potential of *P. domestica* ethanolic extract. The results demonstrated that EEPD has the potential antioxidant properties and anti-amaesic effect on scopolamine-induced amnesia. This can be well correlated with improved behavioral activities and regulated cholinergic transmission through cholinesterase enzyme inhibition.

Oxidative stress is a major contributing factor for cognitive dysfunction and neurodegenerative disease which eventually leads to the progression of amnesia or disruption on memory and learning (Ngoupaye *et al.*, 2017). The deterioration of the cholinergic neurotransmitter system is the pivotal target upon oxidative stress-induced neurodegeneration. The ROS or free radicals are responsible for the destruction of cholinergic producing cells in the basal forebrain area. ROS is the byproduct containing hydroxyl radical and is responsible for the deterioration of biomolecules, such as nucleic acid, protein, and lipids, which act as the main offender in inflammation-related diseases, especially in neurodegeneration (Wang *et al.*, 2020).

It is well known that phytochemicals play a great therapeutic role in oxidative stress-induced neurodegenerative disease. Scopolamine induces amnesia and oxidative stress which eventually lead to Alzheimer’s type of dementia and it was studied with various phytochemicals for neuroprotection (Sawikr *et al.*, 2017). In our investigation, the IC50 of *P. domestica* exhibited significant antioxidant and free radical scavenging activities that were comparable to references of ascorbic acid and the antioxidant potential is dose-dependent as the inhibition percentage increases dramatically with the increase of concentration. The highest concentration of the extract exhibited a high percentage of inhibition with a low number of absorbance. Ethanol extract from *P. domestica* exhibited good reducing power and showed a strong correlation in the antioxidant assay with the presence of phenolic compounds. The antioxidant activity exhibited by these plants is partly ascribed to the phenolic and flavonoids compounds. A previous study showed that free radical scavenging and antioxidant activity strongly correlated with aromatic, phenolic, and flavonoid contents due to redox activity (Cassidy *et al.*, 2020).

*Prunus domestica* exhibits various pharmacological activities and it is reported to be used as a traditional medicine to enhance memory and learning efficiency in normal mice (Shahidi *et al.*, 2013). In our study, we attempted to reveal the effect of *P. domestica* on habitation and behavioral memory with its influence on cholinergic neurotransmission. In neuroprotective evaluation, behavioral studies with Y-maze and open-field habituation memory exhibited promising results on cognitive improvement after scopolamine-induced amnesia. In the Y-maze test, the number of entries in each arm was observed and recorded as ABC, CAB, and BAC. There was a significant improvement, which indicates that there is a significant effect produced by the EEPD on treating memory and learning impairment. The open-field test was conducted followed by the Y-maze test. The purpose of this test was to measure the exploratory behavior of the mice in a new environment, anxiety level, and the level of cognition. In the open-field test, the study showed that the administration of scopolamine exhibited a significant effect on declining the memory and learning level in the mice and it was clearly observable in the line crossing test, rearing, and number of head dipping test. Open-field exploration and habituation memory are considered to be disturbed in age-related disease (Deacon *et al.*, 2009) in animal models and studies suggest that phytoconstituents rich in polyphenols and flavonoids tend to improve (Ramirez *et al.*, 2005). In our study, it is evident from the open-field test that the treatment with EEPD enhanced cognitive performance. In the traction test, the time taken for the mice to retain on the retort bar was recorded and this test was carried out to examine the changes in sensorimotor performance during aging (Jänicke and Coper, 1996). In this test, it was indicated that only the high dose group (EEPD 400 mg/kg) produced a significant effect toward amnesic mice, whereas the low dose group had no significant activity toward the amnesia induced by scopolamine, but an increase in the mean was observed in this group compared to the negative control group. This shows that EEPD is involved in neuroprotection and improves the sensorimotor performance in aging.

Administration of scopolamine for a prolonged period of time causes amnesia and this induction model is associated with Alzheimer’s model. Scopolamine is a nonselective muscarinic acetylcholine receptor which has the tendency to reduce the induction of long-term potentiation in the brain which relates to the decrease in memory and learning ability (Schon, 2005) and additionally the learning impairment is also known to be caused in...
by oxidative stress, followed by a decreased acetylcholine level in the hippocampal region. The decrease of acetylcholine in the brain is due to the increase in the AChE enzyme (AChE) which plays a role in hydrolyzing the acetylcholine present in both cholinergic and non-cholinergic neurons. The influence of oxidative stress is remarkable during neuronal stress which potentiates cognitive deterioration (Vanova et al., 2018). In our study, we evaluated the effect of EEPD on AChE activity and interpreted its neuroprotective activities. EEPD inhibited the enzyme significantly in the mice brain at two doses (200 and 400 mg/kg), which represent the neurocognitive improvement exerted by the P. domestica extract. The results of this research implicate the remarkable therapeutic involvement through AChE inhibition and oxidative stress diminution.

CONCLUSION

In conclusion, it is evident that P. domestica potentially exerts an anti-amnesic effect through regulation of oxidative stress and AChE inhibition. This reveals that P. domestica could be therapeutically active for the treatment of Alzheimer’s type of dementia. Our study suggests further exploration with isolated phytoconstituents in relation to neuroinflammation and oxidative regulation with AChE inhibition in transgenic Alzheimer’s model values for an additional mechanistic impact.

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CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest.

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