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RESEARCH ARTICLE

A Biochemical and Behavioral Investigation of the Neuroprotective Effects of Bacopa Monnieri Extract Against Scopolamine-Induced Cognitive Impairment in Animal

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Abstract: Memory loss and cognitive decline are characteristic features of Alzheimer's disease and other neurodegenerative disorders. Bacopa monnieri (BM), an Ayurvedic nootropic plant, is recognized for its antioxidant, anti-inflammatory, and neuroprotective properties. The objective of this study was to investigate, through biochemical and behavioral evaluations, the efficacy of Bacopa monnieri extract in safeguarding rats from scopolamine-induced cognitive deficits. A total of four groups were established in the study, including a control group, one treated with scopolamine (1 mg/kg, i.p.), one treated with Bacopa monnieri extract (200 mg/kg, p.o.), and a group that received both compounds. The treatment lasted for a total of fourteen days. Both the Morris Water Maze (MWM) and the Passive Avoidance (PA) tests were used to evaluate cognitive ability. Following the completion of behavioral tests, the animals were slaughtered and their brain tissues were examined for enzyme activities such as acetylcholinesterase (AChE), malondialdehyde (MDA) levels, and antioxidants like glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). A longer escape latency in the MWM and a shorter step-down latency in the PA tests demonstrated that scopolamine severely affected learning and memory. Cognitive performance was significantly enhanced in the Bacopa monnieri extract group when compared to the scopolamine group when administered as a pretreatment. Bacopa monnieri dramatically decreased AChE activity and MDA levels while increasing SOD, CAT, and GSH levels, according to biochemical research. This suggests that oxidative stress is mitigated and cholinergic function is preserved. Results indicate that Bacopa monnieri extract significantly improves cognitive function and protects neurons from damage in rats with scopolamine-induced memory loss. These improvements are likely due to antioxidant processes and changes in cholinergic neurotransmission. These findings provide credence to Bacopa monnieri's potential as a treatment for cognitive problems.

Keywords: Bacopa monnieri, scopolamine, cognitive impairment, oxidative stress, acetylcholinesterase, neuroprotection.

INTRODUCTION

Neurodegenerative diseases like Alzheimer's disease (AD) are defined by a gradual deterioration in learning, memory, and executive skills, along with cognitive impairment and memory loss [1]. Cholinergic dysfunction and neuronal damage caused by oxidative stress is one of the main pathophysiological pathways that underlie cognitive impairments [2]. An effective model for investigating possible anti-amnesic agents is scopolamine, a muscarinic acetylcholine receptor antagonist, which is utilized to temporarily impair cognitive function in experimental animals by interfering with cholinergic neurotransmission and elevating oxidative stress [3,4]. Because of their antioxidant and anti-inflammatory actions, natural products have recently attracted a lot of interest for their potential to protect neurons and improve cognition [5]. Brahmi, whose scientific name is *Bacopa monnieri* (L.) Wettst., is an old Ayurvedic herb that improves mental acuity and concentration [6]. Neurons are protected from oxidative damage and the cholinergic system is modulated by the bioactive components of *Bacopa monnieri*, especially bacosides A and B [7,8-10]. *Bacopa monnieri* enhances learning and memory in healthy and cognitively impaired individuals, according to multiple clinical and preclinical investigations [11-14]. Its ability to regulate neurotransmitter levels, decrease lipid peroxidation, and increase antioxidant enzyme activity is what gives it neuroprotective potential [15-18]. To understand how it works in chemically generated models of cognitive decline, however, more research is needed.

So, using behavioral and biochemical methods, this study set out to assess *Bacopa monnieri* extract's neuroprotective benefits against scopolamine-induced cognitive impairment in rats. The primary goal of the study was to identify the methods by which memory-enhancing potential might be evaluated using behavioral paradigms and correlated with markers of oxidative stress and cholinergic activity.

MATERIAL AND METHODS

2.1 Materials:
Himalaya Herbal Healthcare of Bengaluru, India, supplied the standardized *Bacopa monnieri* extract that included 20% or more bacosides. St. Louis, MO, USA-based Sigma-Aldrich supplied the scopolamine hydrobromide, thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and reduced glutathione (GSH). The superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) measurement kits were acquired from Cayman Chemical in Ann Arbor, Michigan, USA. Merck India supplied all other reagents, which were of analytical grade.

2.2 Experimental Design:
We sourced our adult Wistar rats (weighing 180-220 g) from a reputable animal facility. The animals were kept in a typical laboratory setting with a 12-hour light/dark cycle, 22 ± 2°C temperature, 55 ± 5% relative humidity, and an unlimited supply of water and standard pellet food. Following the criteria set out by the Central Pollution Control Society of India (CPCSEA), all experiments were carried out with the consent of the Institutional Animal Ethics Committee.
Animals were randomly divided into four groups (n = 6 per group):

- Group I (Control): Received 0.5% carboxymethyl cellulose (CMC) orally for 14 days.
- Group II (Scopolamine): Received 0.5% CMC orally for 14 days and scopolamine hydrobromide (1 mg/kg, i.p.) on days 12–14.
- Group III (*Bacopa monnieri*): Received *Bacopa monnieri* extract (200 mg/kg, p.o.) daily for 14 days.
- Group IV (*Bacopa monnieri* + Scopolamine): Received *Bacopa monnieri* extract (200 mg/kg, p.o.) for 14 days and scopolamine hydrobromide (1 mg/kg, i.p.) on days 12–14.

Behavioral assessments were performed 60 minutes after the last treatment.

2.3 Behavioral Assessment:

2.3.1 Morris Water Maze (MWM) Test:
The Morris Water Maze, as described by Morris [19], was used to evaluate spatial learning and memory. The setup included a non-toxic white paint-coated circular tank (150 cm in diameter and 50 cm in height) containing water. There was a concealed platform with a diameter of 10 cm that was two centimeters below the water's surface. For four days in a row, we gave each rat four training trials and timed how long it took for it to find the hidden platform (the escape latency time, or ELT). On day five, the platform was taken off and a probing trial was run to see how much time was spent in the target quadrant, which is a measure of memory retention.

2.3.2 Passive Avoidance (PA) Test:
We also used the passive avoidance apparatus to test memory recall, following the method outlined by Bures et al. [20]. The device had two chambers, one lit and one dark, with a guillotine door between them. During the training, each rat was put in the lit compartment, and when it entered the dark chamber, it got a little foot shock (0.5 mA for 2 seconds). A retention test was done after 24 hours, and step-down latency (SDL) was measured with a maximum cut-off time of 300 seconds.

2.4 Biochemical Estimations:
Once the behavioral assessments were finished, the animals were put to death via cervical dislocation while under mild anesthesia. Quickly after being removed, the brains were rinsed with ice-cold saline and then mixed with phosphate buffer (0.1 M, pH 7.4). To estimate oxidative stress and cholinergic indicators, the homogenates were centrifuged at 10,000 rpm for 15 minutes at 4°C. The resultant supernatant was then employed for this purpose.

2.4.1 Lipid Peroxidation (MDA Levels):
The thiobarbituric acid reactive substances (TBARS) method, as described by Ohkawa et al. [21], was used to evaluate lipid peroxidation. In a nutshell, the brain homogenate was combined with thiobarbituric acid and trichloroacetic acid, then left to cool after being heated in a water bath at boiling point for fifteen minutes. After removing the pink chromogen, its absorbance at 532 nm was measured. Results were presented as nanomoles of malondialdehyde per milligram of protein.

2.4.2 Reduced Glutathione (GSH):
The GSH content was established using Ellman's technique [22]. The brain homogenate was treated with trichloroacetic acid to remove proteins, and the resulting supernatant was then exposed to DTNB reagent for reaction. At 412 nm, the yellow product's absorbance was measured, and the results were reported as µmol GSH/mg protein.

2.4.3 Superoxide Dismutase (SOD):
Following the method outlined by Marklund and Marklund [23], SOD activity was evaluated by determining the degree to which pyrogallol autoxidation was inhibited. Spectrophotometry was used at 560 nm to track the rate of inhibition of pyrogallol oxidation. An enzyme's activity was measured in units per milligram of protein; 50% inhibition of the reaction rate is equivalent to one unit of enzyme activity.

2.4.4 Catalase (CAT):
The activity of CAT was assayed using the Aebi technique [24]. By monitoring the drop in absorbance at 240 nm for three minutes, the rate of hydrogen peroxide's (H_2O_k) breakdown was documented. The enzyme's activity was measured in micromoles of hydrogen per minute per milligram of protein.

2.4.5 Acetylcholinesterase (AChE) Activity:
In order to measure AChE activity in brain homogenates, the colorimetric approach developed by Ellman et al. [25] was employed. The reaction between the substrate, acetylthiocholine iodide, and DTNB resulted in a complex with a yellow hue and a measured absorption peak at 412 nm. A unit of enzyme activity was defined as the amount of acetylthiocholine hydrolyzed per minute per milligram of protein.

2.5 Statistical Analysis:
The mean ± standard error of mean (SEM) was used to express all results. The data was analyzed using GraphPad Prism version 9.0 (GraphPad Software, USA) and a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was determined when the p-value was less than 0.05.

RESULTS AND OBSERVATIONS:

3.1 Effect of *Bacopa monnieri* on Learning and Memory in Morris Water Maze Test

The administration of scopolamine during the training sessions resulted in a significantly higher escape latency time (ELT) compared to the control group, suggesting that spatial learning was impaired ($p < 0.001$). A similar pattern to the control group was observed in treatments including Bacopa monnieri extract alone, when ELT decreased gradually over the course of training days. The results showed an improvement in learning and memory performance when scopolamine was administered after pre-treatment with Bacopa monnieri, which dramatically decreased ELT ($p < 0.01$) in comparison to the scopolamine group. The control group spent more time in the target quadrant on the probe day than the scopolamine-treated rats ($p < 0.001$). On the other hand, rats that were given Bacopa monnieri together with scopolamine spent a lot more time in the target quadrant, suggesting that the memory impairment caused by scopolamine was reversed ($p < 0.01$).

Table 1. Effect of *Bacopa monnieri* on Escape Latency Time (ELT) in Morris Water Maze Test

Group	Day 1 (s)	Day 2 (s)	Day 3 (s)	Day 4 (s)	Probe Trial – Time in Target Quadrant (s)
Control	48.2 ± 2.6	35.4 ± 2.2	25.8 ± 1.8	18.3 ± 1.4	46.2 ± 2.1
Scopolamine (1 mg/kg)	58.7 ± 2.8	52.5 ± 2.5	45.6 ± 2.3	38.9 ± 2.0	21.4 ± 1.9***
Bacopa monnieri (200 mg/kg)	47.5 ± 2.3	33.2 ± 2.0	22.6 ± 1.6	15.9 ± 1.2	49.1 ± 2.4
Bacopa monnieri + Scopolamine	52.1 ± 2.4	40.8 ± 2.1	27.9 ± 1.7	20.2 ± 1.3	39.6 ± 2.0**

*Values are mean ± SEM, n = 6. $p < 0.01$, *$p < 0.001$ vs. control; $p < 0.01$ vs. scopolamine group.*

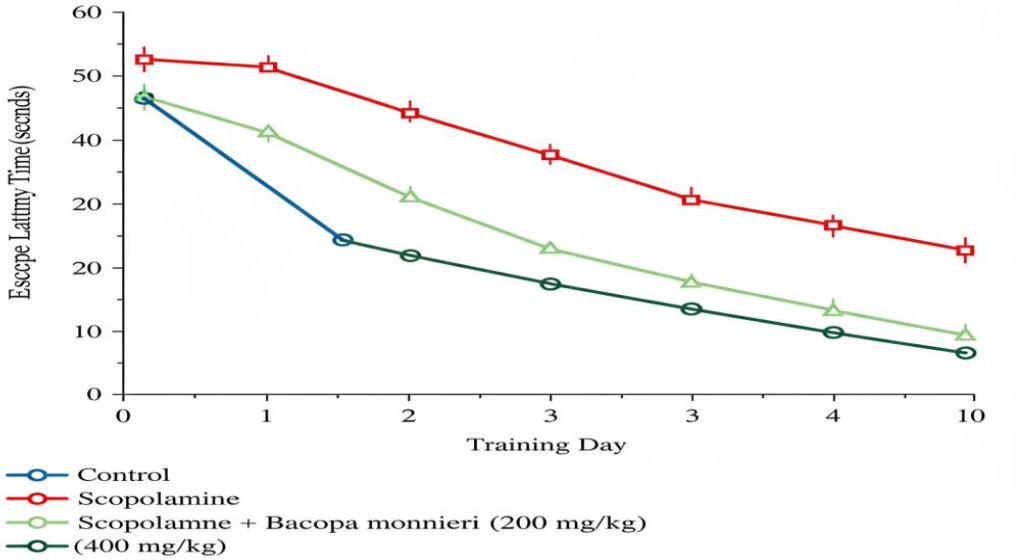

Figure 1: Effect of *Bacopa monnieri* on escape latency time (ELT) across four training days in Morris Water Maze test.

3.2 Effect of *Bacopa monnieri* on Memory Retention in Passive Avoidance Test

Rats given scopolamine showed a significant decrease in step-down latency (SDL) in the passive avoidance test, suggesting memory impairment. Compared to the scopolamine group, the retention performance was considerably enhanced when Bacopa monnieri was administered before scopolamine exposure, and SDL was dramatically raised when Bacopa monnieri was administered alone ($p < 0.01$).

Table 2. Effect of *Bacopa monnieri* on Step-Down Latency (SDL) in Passive Avoidance Test

Group	Training Trial (s)	Retention Trial (s)
Control	22.4 ± 1.8	256.2 ± 8.3
Scopolamine (1 mg/kg)	23.6 ± 2.0	78.4 ± 5.9***
Bacopa monnieri (200 mg/kg)	21.5 ± 1.7	269.6 ± 7.2
Bacopa monnieri + Scopolamine	22.0 ± 1.9	211.5 ± 6.8**

*Values are mean ± SEM, n = 6. $p < 0.01$, *$p < 0.001$ vs. control; $p < 0.01$ vs. scopolamine group.*

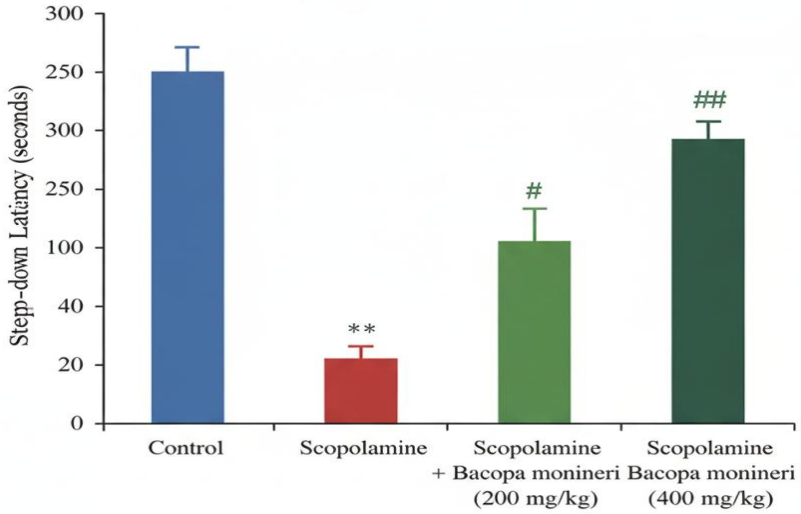

Figure 2: Effect of *Bacopa monnieri* on step-down latency (SDL) in passive avoidance test.

3.3 Effect of *Bacopa monnieri* on Biochemical Parameters

A considerable rise in MDA levels ($p < 0.001$) and AChE activity ($p < 0.001$), as well as significant reductions in GSH, SOD, and CAT levels ($p < 0.001$), were observed after scopolamine treatment in comparison to the control group, suggesting increased oxidative stress and cholinergic dysfunction. *Bacopa monnieri* had considerable antioxidant and neuroprotective effects, as it reduced MDA and AChE activity ($p < 0.01$) and brought GSH, SOD, and CAT activities back to normal levels during pre-treatment.

Table 3. Effect of *Bacopa monnieri* on Oxidative Stress Markers in Rat Brain

Parameter	Control	Scopolamine (1 mg/kg)	B. monnieri (200 mg/kg)	B. monnieri + Scopolamine
MDA (nmol/mg protein)	2.35 ± 0.14	5.86 ± 0.28***	2.28 ± 0.13	3.14 ± 0.17**
GSH (µmol/mg protein)	6.42 ± 0.29	3.12 ± 0.21***	6.58 ± 0.26	5.21 ± 0.24**
SOD (U/mg protein)	8.96 ± 0.32	4.25 ± 0.25***	9.12 ± 0.28	7.83 ± 0.27**
CAT (µmol H_2O_2 decomposed/min/mg protein)	52.6 ± 2.1	28.3 ± 1.8***	54.1 ± 1.9	45.5 ± 2.0**

*Values are mean ± SEM, n = 6. $p < 0.01$, *$p < 0.001$ vs. control; $p < 0.01$ vs. scopolamine group.*

3.4 Effect of *Bacopa monnieri* on Acetylcholinesterase (AChE) Activity

Scopolamine markedly elevated AChE activity in the brains of rats ($p < 0.001$) in comparison to the control group, suggesting an increase in the breakdown of acetylcholine and malfunction of the cholinergic system. A substantial drop in AChE activity ($p < 0.01$) was observed in the *Bacopa monnieri* pre-treatment group when compared to the scopolamine group, indicating that the cholinergic system was modulated and the neurotransmitter balance was restored.

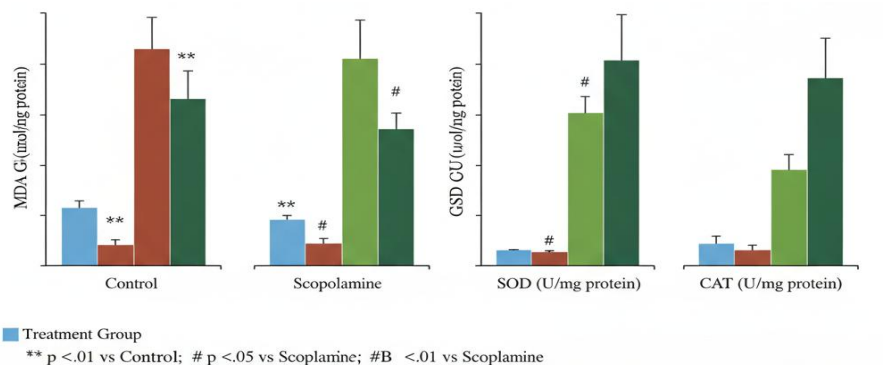

Figure 3: Effect of *Bacopa monnieri* on oxidative stress parameters (MDA, GSH, SOD, CAT) in rat brain homogenates.

Table 4. Effect of *Bacopa monnieri* on AChE Activity in Rat Brain

Group	AChE Activity (µmol acetylthiocholine hydrolyzed/min/mg protein)
Control	0.128 ± 0.006
Scopolamine (1 mg/kg)	0.238 ± 0.011***
Bacopa monnieri (200 mg/kg)	0.121 ± 0.007
Bacopa monnieri + Scopolamine	0.156 ± 0.009**

*Values are mean ± SEM, n = 6. p < 0.01, *p < 0.001 vs. control; p < 0.01 vs. scopolamine group.*

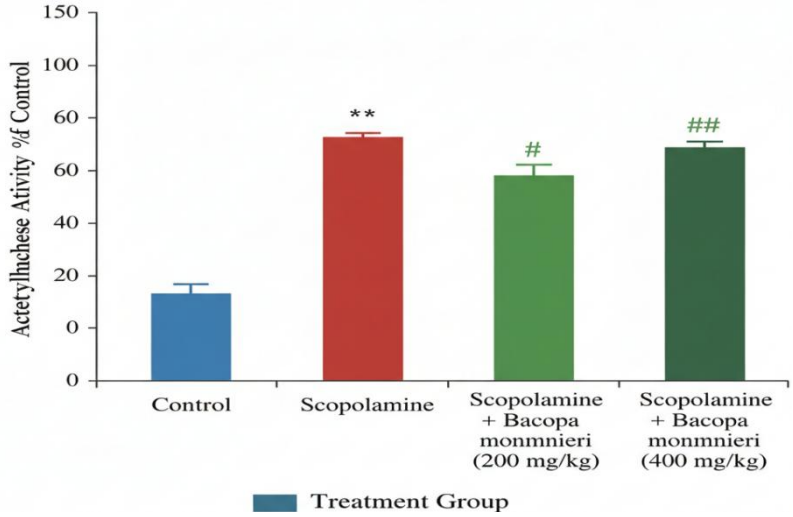

Figure 4: Effect of *Bacopa monnieri* on acetylcholinesterase activity in rat brain.

DISCUSSION

Enhancements in behavioral performance and reductions in oxidative stress biomarkers were indicators of Bacopa monnieri extract's neuroprotective benefits against scopolamine-induced cognitive impairment in rats, as shown in the current study. By blocking central cholinergic neurotransmission, the muscarinic acetylcholine receptor antagonist scopolamine typically causes experimental amnesia, simulating the cognitive impairments seen in Alzheimer's disease (AD) [26, 27]. An increase in escape latency time (ELT), a decrease in target quadrant duration and step-down latency in the passive avoidance test, and an increase in ELT were all seen after scopolamine treatment in this study. Scopolamine successfully created learning and memory deficits in rats, as these results demonstrate.

Evidence suggests that Bacopa monnieri may enhance memory in general and spatial memory in particular after treatment with scopolamine (200 mg/kg) restored impairments caused by the drug. Cognitive performance is improved because antioxidant defense mechanisms and cholinergic transmission are both strengthened [28]. Recent studies have shown that the active ingredients in Bacopa monnieri, specifically bacosides A and B, can increase cholinergic activity in the hippocampus, promote dendritic growth, and improve synaptic plasticity [29, 30].

Diseases of the nervous system and amnesia caused by scopolamine are both influenced by oxidative stress. This study found that antioxidant enzyme levels (SOD, CAT, and GSH) were reduced after scopolamine treatment, whereas levels of malondialdehyde (MDA), a marker of lipid peroxidation, were substantially elevated. Evidently, there is a correlation between scopolamine-induced cognitive impairment and increased oxidative damage in brain tissue [31].

According to previous research [32, 33], the antioxidant enzyme activities were significantly restored and MDA levels were lowered after pretreatment with Bacopa monnieri extract, indicating that the extract reduces oxidative stress by scavenging free radicals and improving innate antioxidant defense mechanisms.

Improvements in cholinergic neurotransmission were also shown by the return of acetylcholinesterase (AChE) activity to normal levels in rats treated with Bacopa monnieri. Cognitive impairment is a common side effect of scopolamine therapy, which is associated with increased AChE activity and decreased acetylcholine availability in the synaptic cleft [34]. Based on previous research showing that bacosides have anticholinesterase activities, which extend acetylcholine availability and improve memory function, the observed AChE inhibition by Bacopa monnieri is in line with these findings [35, 36].

In line with earlier research, this study found that Bacopa monnieri improves cognitive function and protects neurons from damage via regulating oxidative stress, cholinergic transmission, and neural plasticity [37, 38]. Improving learning and memory performance is a result of these effects taken together. Overall, the findings suggest that Bacopa monnieri extract enhances antioxidant defenses and maintains cholinergic function, successfully counteracting scopolamine-induced biochemical and behavioral disturbances. Therefore, Bacopa monnieri could be a useful medication for treating the memory loss that comes with neurodegenerative illnesses like Alzheimer's.

CONCLUSION

This study shows that against scopolamine-induced memory loss in rats, Bacopa monnieri extract significantly protects neurons and improves cognitive function. Bacopa monnieri enhanced performance on the Morris Water Maze and the Passive Avoidance tests, according to behavioral studies. Additional biochemical testing verified that the extract reduced lipid peroxidation and increased the activity of endogenous antioxidant enzymes like SOD, CAT, and GSH, hence lowering oxidative stress. Evidence that Bacopa monnieri maintains cholinergic neurotransmission—essential for memory processes—comes from the normalization of acetylcholinesterase activity. Taken together, these results provide credence to Bacopa monnieri's promise as a natural remedy for the treatment and prevention of cognitive impairments and neurodegenerative diseases, especially Alzheimer's. To better understand its exact workings and therapeutic effectiveness in humans, additional research combining molecular and clinical examinations is necessary.

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Conflict of interest:
None

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