DRUG DISCOVERY

Neurocognitive and neuroprotective potential of *Mimusops elengi* in mice model of scopolamine-induced amnesia

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ABSTRACT

The study was aimed to assess the cognitive and protective potential of methanol extract of *Mimusops elengi* bark (MEME-B) and flowers (MEME-F) against a mice model of the amnesia paradigm. Scopolamine (3 mg/kg i.p) was used to induce amnesia and treatment with MEME-B and MEME-F extract (200mg/kg p.o) was continued for 21 days. To examine cognitive parameters, the elevated plus maze (EPM) and Morris water maze (MWM) tests were used, whilst serum samples and tissue brain homogenate were used to investigate biochemical and antioxidant data along with morphometric factors. The obtained data reveal that scopolamine caused a substantial increase in transfer latency on days 14 and 21 in the EPM model, which was reversed ($p < 0.05$) by both MEME-B and MEME-F. Similarly, in the MWM test, extract-treated mice showed a steady decrease in escape latency time ($p < 0.05$). The findings confirm that scopolamine impairs learning and memory, whereas the administration of MEME-B and MEME-F significantly ameliorated scopolamine-induced amnesia in both the EPM and MWM test, as indicated by significant ($p < 0.05$) reductions in transfer latency (TL) and escape latency (EL). The considerable restoration of biochemical markers such as acetylcholinesterase (AChE) and antioxidants such as super oxide dismutase (SOD) and (CAT) further corroborated the findings. In conclusion, MEME-F has demonstrated a significant neurocognitive property against scopolamine-induced amnesia, as evidenced by significant inhibition of AChE activity to a significant extent with significant reduction of transfer latency and escape latency, whereas MEME-B has demonstrated significant neuroprotective activity, which could be attributed to its significant antioxidant property.

Keywords: *Mimusops elengi*, amnesia, neuroprotection, scopolamine, transfer latency, escape latency.

DISCOVERY SCIENTIFIC SOCIETY

1. INTRODUCTION

Cognition is one of the vital abilities of an individual comprising perception, registration, consolidation, storage, the recollection of various events and happenings. This cognitive process involves numerous direct and indirect complex physiological mechanisms. But, any impairment in memory called amnesia affects the individual's quality of life in terms of occupational and social activities, which leads to a common cause for dementia with decreased neuronal populations in the brain. In this context, only cholinesterase inhibitors are moderately effective in management with a low therapeutic index at certain stages (Dunning and During, 2003).

On the other hand, plants and their extracts may offer a more promising approach and have many active components and over the past decade, plants have been used for cognitive enhancement. The impact of herbal medicaments on dementia has been a hot topic of debate and remains highly controversial. Among all, *Mimusops elengi* is the one type of plant that is more likely to show some beneficial actions toward learning and memory as per the data available in various reviews and research articles.

Mimusops elengi Linn is known to all as Bakul from the Sapotaceae family. This tree is widespread around the globe and generally famous for its usage in ornamental purposes but contains different types of secondary metabolites like quercetin, ursolic acid, D-Mannitol, volatile oil, b-sitosterol have already been reported with diverse pharmacological effects such as cardio tonic, stomachic, hypotensive, antibacterial, antihelmintic, anti-ulcers activities (Baliga et al., 2011; Kadam et al., 2012; Jerline et al., 2009; Raghunathan and Mitra, 2000). Hence, we decided to investigate the cognitive and protective potential of methanol extract of *Mimusops elengi* bark (MEME-B) and flowers (MEME-F) against the experimental amnesia model in mice.

2. MATERIALS AND METHODS

Plants and drugs

The plant materials *Mimusops elengi* bark and flowers were collected and authenticated at the Department of Botany, Sree Venkateshwara University, Tirupati, India and a voucher specimen (Reference No: #45 Dated 07/08/2018) was deposited for further reference. The drugs used in this study, such as scopolamine bromide, donepezil was procured from Sigma (St. Louis, USA) and dissolved in 0.9% physiological saline solution for i.p. injection. All the purchased chemicals were of analytical grade.

Animals and approval process

Adult albino mice of both sexes (weight 25-30g) were employed and kept under regular housing settings such as a light-dark cycle, a temperature of 2310^{o}C and a relative humidity range of 555%. The institutional animal ethics committee (SVCP/IAEC/I007/2017–18) certified the experimental methods in accordance with CPCSEA, New Delhi animal care norms. To eliminate bias, the treatments as distinct experimental groups were assigned to animals using randomized techniques (Zolman, 1993).

Acute toxicity study

The OECD Guidelines 423 were followed in the design of the acute toxicity investigation. Three female mice were given a single oral dose of a methanol extract of *Mimusops elengi* (2000 mg/kg) and each was monitored for any gross behavioral, neurologic, or autonomic toxic signs. These signs include changes in heart rate, respiration, salivation, lacrimation, drowsiness, convulsions and motor coordination, among others (OECD, 2001; Sathiya et al., 2019).

Experimental design

Group I	-	Control (NS 0.9%).
Group II	-	SCOP (3 mg/kg, i.p.)
Group III	-	SCOP (3 mg/kg, i.p.) & Donepezil (4 mg/kg, p.o)
Groups IV	-	SCOP (3 mg/kg, i.p.) & MEME-B (200 mg/kg, p.o)
Groups V	-	SCOP (3 mg/kg, i.p.) & MEME-F (200 mg/kg, p.o)

Morphometric analysis

The body weight of all experimental animals was meticulously recorded at regular intervals to determine the effect of *Mimusops elengi* extracts on experimental animals. Furthermore, at the end of the experiment, the whole brain was separated and weighed to determine any changes in brain weight caused by injecting scopolamine or MEME-B and MEME-F.

Cognitive activity by EPM and MWM tests

In EPM, the number of entries and time spent in each arm, as well as transfer latency (TL), were calculated for each experimental group. Individual animals were put in the plus-maze with their heads toward the open arm. The EL (time necessary to reach closed arm) was measured during a 90-second cut-off period. All the animals were exposed to sufficient trail before the screening day to avoid experimental bias. Similarly, EL was detected in a MWM test with a cut off duration of approximately 120 seconds when conventional protocols were followed (Lavinsky et al., 2003).

Muscle coordination by Rotarod Test

The rotarod is regarded as an essential experimental instrument for documenting a drug's muscular coordination and relaxant characteristics. In this study, the rotarod test automatically records latency to fall animals. Animals were chosen based on their suitability and ability to grip the rotating bar within a time restriction after prior rotating bar exposure (Jayaraman and Lavanya, 2018).

Estimation of Acetylcholinesterase (AchE) Activity and Antioxidant Parameters

By using readily accessible conventional techniques, lipid peroxidation (LPO) and antioxidants like SOD and CAT were assessed in both brain homogenate and serum samples (Ohkawa et al., 1979; Kakkar et al., 1984; Aebi, 1974). AchE was calculated at the completion of behavioral experiments using brain tissue homogenate from sacrificed animals in accordance with the recommended methods using a calorimeter (412 nm for 5 min). The findings were represented as moles/mg protein (Ellman et al., 1961).

Histopathological studies

Following the cervical dislocation, the complete brain was extracted from the sacrificed animals for histological investigations after the experimental period. Each animal's brain was divided in half, preserved in 10% formalin and examined histopathologically with hematoxylin and eosin (H & E). Nuclear chromatin clumping and fragmentation, as well as neuron-like cytoplasmic vacuolation that had undergone degenerative changes, were also assessed.

Statistical Analysis

The results were presented as mean S.E.M. The significance of the difference between the treatment and control groups was assessed using one-way ANOVA followed by Turkey's Multiple Comparison Test. Statistical significance was assessed as $p < 0.05$.

3. RESULTS AND DISCUSSION

Acute toxicity and phytochemical studies

According to the findings of acute toxicity testing, the extracts were not hazardous to the animals up to the maximum-tolerated dose of 2000 mg/kg. There were no reports of morbidity or fatality. Furthermore, the behavior of Mimusops elengi-treated animals appeared normal. Changes in heart rate, respiration, salivation, lacrimation, sleepiness, seizures and motor coordination were all absent throughout the trial (Table 1). Results from phytochemical screening suggest that both MEME-B and MEME-F showed the presence of cardiac glycosides, terpenoids, flavonoids and alkaloids along with carbohydrates, steroids, etc.

Table 1 Parameters recorded during acute toxicity study

S. No	Parameters / Sign	Methanol Extract of *Mimusiops elengi*
1.	Tremors	NO
2.	Motor Activity	YES
3.	Loss of Righting reflux	NO
4.	Sedation	NO
5.	Muscle Relaxation	NO
6.	Lacrimation	NO
7.	Salivation	YES
8.	Diarrhea	NO
9.	Depression	NO
10.	Feed & Water Intake	↑
11.	Mortality	NO

Present: (Yes) Absent: (No) Increase: (↑) Decrease: (↓)

Morphometric assessment

To assess the harmful effect of ME, the weights of all animals were monitored at regular intervals (weeks) during the entire course of study. As indicated in Table 2 and Figure 1, there were substantial weight differences across the groups. Similarly, ME-treated mice's brain weights were not less than those of normal mice's.

Table 2 Effect of *Mimusops elengi* on the body weight analysis of experimental animals

Groups	Changes in body weight (gm)		
	DAY 1	DAY 7	DAY14
CONTROL	22.64 ± 2.34	23.71 ± 3.17	26.26 ± 2.13
SCOP	21.34 ± 2.19	22.78 ± 1.82	25.41 ± 1.57
SCOP + DONEP	21.36 ± 2.19	23.36 ± 1.93	26.53 ± 3.12
SCOP + MEME-B	23.65 ± 2.19	26.02 ± 3.71*	28.10 ± 2.39*
SCOP + MEME-F	23.52 ± 2.57	25.6 ± 2.69	27.8 ± 3.17*

Number of animals (n=5): Values are mean ± SEM: *P < 0.05, **P < 0.01 & ***P < 0.001 When compared to control and scopolamine treated groups; ns: Non-significant.

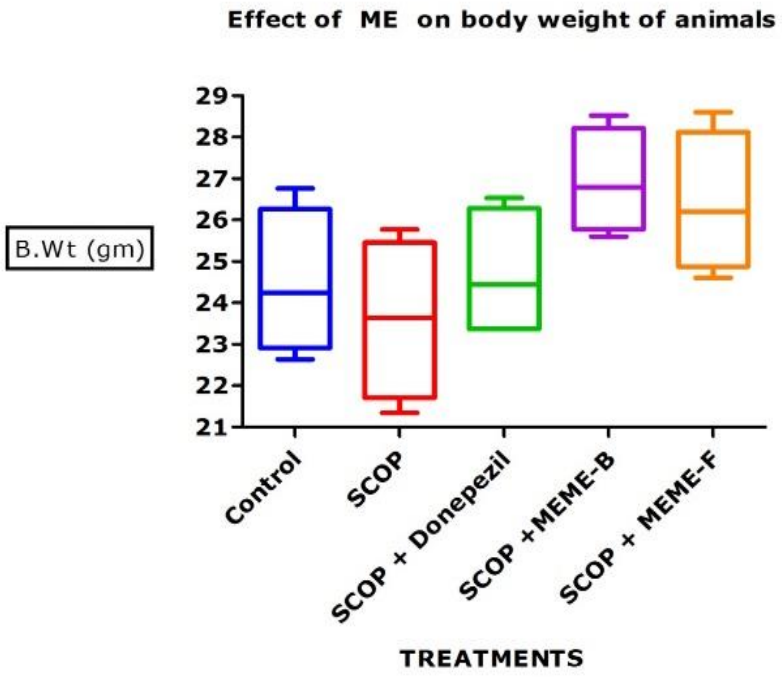
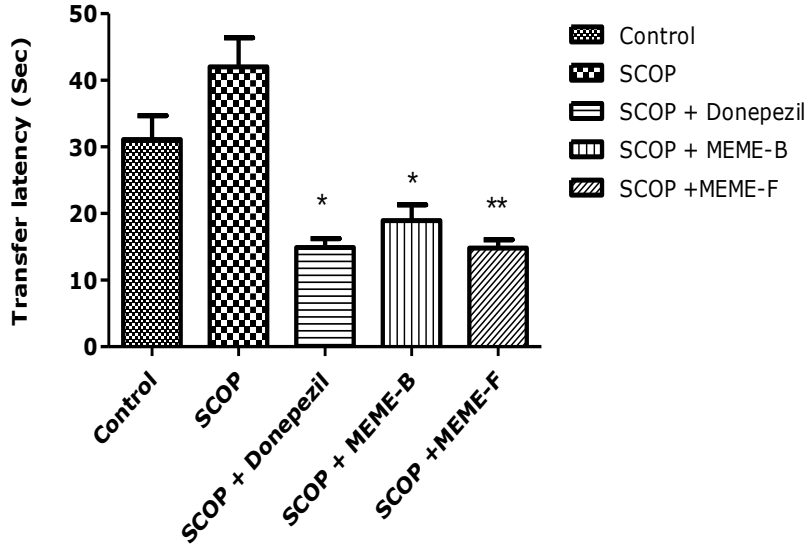


Figure shows the final body weight of all experimental animals: Number of animals (n=5): Values are mean ± SEM; *p < 0.05 (Indicates significance with p-value less than 0.05 when compared to control); **p < 0.01 (Indicates significance with p-value less than 0.01 when compared to control); ***p < 0.001 (Indicates high significance with p-value less than 0.001 when compared to control groups); ns: Non-significant. Significantly noticeable weight differences were observed between the control group and extract-treated groups which reflect the lack of neurotoxic effects of extracts of *Mimusops elengi*.

Figure 1 Effect of *Mimusops elengi* on final body weight of experimental groups

Transfer latency by EPM test

The results of the EPM test in terms of recording transfer latency were depicted in Figure 2. The findings show that SCOP received animals showed a substantial rise in TL, but DONEP and extracts of *Mimusops elangi* showed decline of TL period ($p < 0.05$). As a result, the SCOP hindered the learning process, but *Mimusops eleangi* extracts (200 mg/kg p.o) improved these deficiencies.

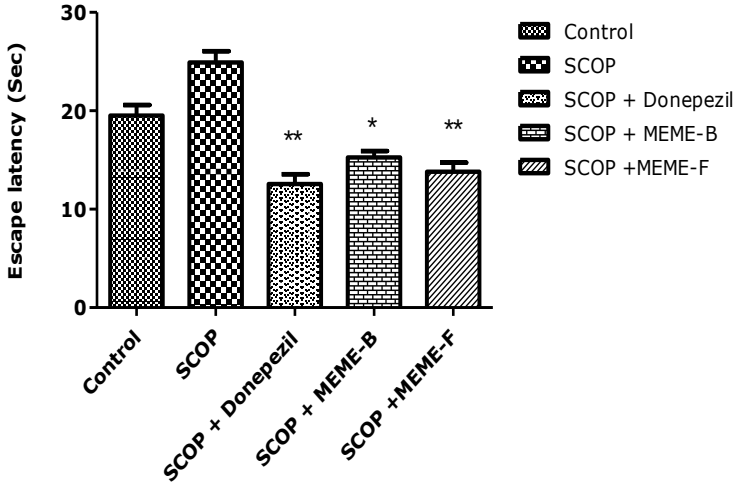


Number of animals (n=5): Values are mean ± SEM; *p < 0.05 (Indicates significance with p-value less than 0.05 when compared to control); **p < 0.01 (Indicates significance with p-value less than 0.01 when compared to control); ***p < 0.001(Indicates high significance with p-value less than 0.001 when compared to control groups); ns: Non-significant.

Figure 2 Effect of ME on transfer latency on EPM test

Escape latency by MWM test

The MWM test results showed that scopolamine-impaired learning and memory processes, as evidenced by an increase in EL, whereas the administration of extracts of both MEME-B and MEME-F significantly ameliorated scopolamine-induced amnesia in mice by lowering the EL, demonstrating its beneficial action toward learning and memory (Figure 3).



Number of animals (n=5): Values are mean ± SEM; *p < 0.05 (Indicates significance with p-value less than 0.05 when compared to control); **p < 0.01 (Indicates significance with p-value less than 0.01 when compared to control); ***p < 0.001 (Indicates high significance with p-value less than 0.001 when compared to control groups); ns: Non-significant.

Figure 3 Effect of ME on escape latency on MWM test

Muscle coordination by Rotarod Test

To examine the muscular coordination, mice were tested for their ability to grip a revolving bar and their time fall. There was no variance in the fall time of the experimental animals observed, as shown in Table 3. The rotarod test revealed that methanol extracts from MEME-B and MEME-F had no effect on the animals muscle coordination properties.

Table 3 Assessment of Muscle coordination by Rota rod Test

Groups	Day 7	Day 14	Day 21
CONTROL	111.75 ± 5.95	114.25 ± 6.81	115.17 ± 8.81
SCOP	112.10 ± 7.41 ns	112.51 ± 8.29 ns	111.25 ± 7.95 ns
SCOP + DONEP	110.29 ± 5.81 ns	112.59 ± 5.29 ns	112.25 ± 6.95 ns
SCOP + MEME-B	111.22 ± 7.81 ns	113.35 ± 7.29 ns	114.15 ± 6.29 ns
SCOP + MEME-F	110.75 ± 6.95 ns	112.49 ± 4.82 ns	115.25 ± 5.5 ns

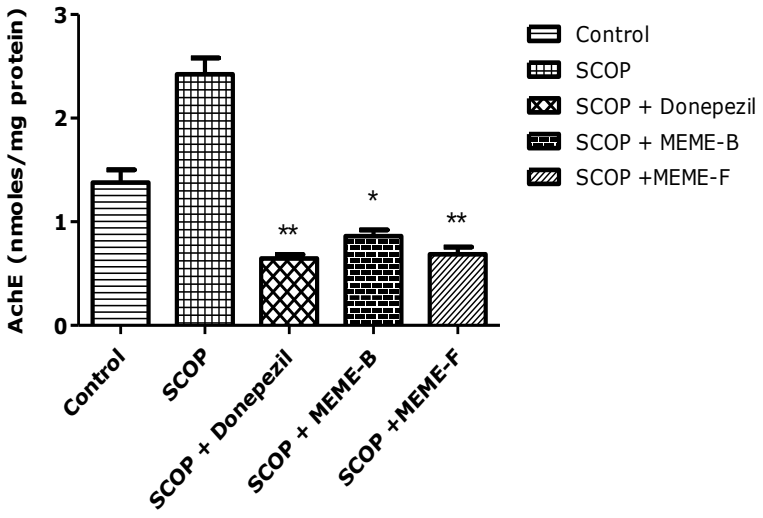
Number of animals (n=5): Values are mean ± SEM: $p < 0.05$, *$p < 0.01$ & **$p < 0.001$ When compared to control and scopolamine treated groups; ns: Non-significant. There was no significant variation in the fall of time of the experimental animals, indicating that MEME-B and MEME-F had no influence on the animals muscle coordination.

Acetylcholinesterase (AchE) Activity

Exposure of experimental animals to scopolamine generated a considerable increase in AChE activity, an enzyme involved in the metabolism of acetylcholine in the hippocampus, as compared to controls Figure 4. From the results, the amount of AchE in brain tissue homogenate in the control group was determined to be 1.38 ± 1.24 nmol/l/min×10−6 /g, but SCOP treated animals showed a larger increase of AchE (2.5 ± 1.24 nmol/l/min×10−6 /g), demonstrating its function in cognitive decline. In comparison to scopolamine-treated mice, donepezil showed a substantial drop-in AchE activity. Similarly, in the treatment groups, the mice were given MEME-B and MEME-F had a substantial lower level of AchE in brain tissue homogenate, indicating a favorable effect in cognitive processes.

Effect of extracts of ME on LPO, antioxidant status

The data depicted in Table 4 reveal that, group II animals who had received SCOP treatment had higher LPO levels in their brain tissue homogenate. Results show that the LPO value for the control group I was 5.27 ± 0.4 nmol of MDA/mg of protein, but it raised to 15.52 ± 1.3 in mice who received SCOP. But when ME was administered, LPO was significantly reduced by MEME-B (7.51 ± 0.5) and MEME-F (11.50 ± 0.3) at a dosage level of 200 mg/kg. In contrast, SCOP-treated mice had lower tissue levels of SOD (66.29 ± 7.64 U/mg protein, $p < 0.005$) and CAT (1.80 ± 0.27 U/mg protein, $p < 0.001$) compared to the animals in the control group. However, supplementing with extracts of *Mimusops elengi* depleted antioxidants to reverse to levels close to normal, indicating its antioxidant and protective potential.



Number of animals (n=5): Values are mean ± SEM; *$p < 0.05$ (Indicates significance with p-value less than 0.05 when compared to control); **$p < 0.01$ (Indicates significance with p-value less than 0.01 when compared to control); ***$p < 0.001$ (Indicates high significance with p-value less than 0.001 when compared to control groups); ns: Non-significant.

Figure 4 Estimation of Acetylcholinesterase (AchE) Activity in brain tissue homogenate

Table 4 Effect of ME on LPO and antioxidants enzymes in brain tissue homogenate

Treatment	LPO nmol of MDA/mg of Protein	SOD U/mg protein	CAT U/mg protein
Control	5.27 ± 0.4	145.22 ± 5.89	4.56 ± 0.59

SCOP	15.52 ± 1.8	66.29 ± 7.64	1.80 ± 0.27
SCOP + DONEP	5.83 ± 0.6 ***	129.34 ± 7.37 ***	3.53 ± 0.25 ***
SCOP + MEME-B	7.51 ± 0.5 **	99.67 ± 5.36 **	2.89 ± 0.28 **
SCOP + MEME-F	11.50 ± 0.3 *	82.44 ± 4.86 *	1.96 ± 0.57 *

Number of animals (n=5): Values are mean ± SEM; *p < 0.05 (Indicates significance with p-value less than 0.05 when compared to control); **p < 0.01 (Indicates significance with p-value less than 0.01 when compared to control); ***p < 0.001 (Indicates high significance with p-value less than 0.001 when compared to control groups); ns: Non-significant.

Histopathological studies

From the results of histopathology, animals treated with both MEME-B and MEME-F showed mild gliosis and lack of neurodegeneration of basal ganglia, which indicates the neuroprotective role of *Mimusops elengi*.

4. DISCUSSION

The neurocognitive and protective potential of MEME-B and MEME-F was evaluated in the current investigation using SCOP-induced cognitive impairment models and the EPM and MWM tests. The plus maze and water maze tests, which are considered the gold standard tests for animal learning and anxiety-related neurobehavioral activity, were used in this work to evaluate the cognitive characteristics (Oh et al., 2009; Terry, 2000). The results obtained in the SCOP-received groups show that in the EPM and MWM tests, both TL and EL were higher than in the control animals. However, treatment with both MEME-B and MEME-F resulted in a substantial favorable reversal of both TL and EL, indicating that *Mimusops elengi* has protective capabilities against SCOP-induced cognitive deficits. Similarly, in MWM, the time required to reach the proper quadrant was decreased, indicating that the extracts had cognitive potential. Additionally, ME extracts performed better in the transfer and escape latency criteria of the EPM and MWM experiments. This finding eventually confirms previous findings on experimental animals' spatial memory (Morris, 1984).

According to existing research, the cholinergic system and acetylcholine levels in the brains of people and animals have been discovered to be associated with functional behavior, including learning and memory. The findings of the present investigation showed that SCOP increased AChE activity, whereas extracts from mice treated with ME significantly lowered the raised level of AChE in SCOP-treated animals. Therefore, AChE activity may be related to the neurocognitive effects of ME extracts. Additionally, the findings of the rotarod test indicated that no discernible alterations in muscle coordination and movement were seen. Therefore, *Mimusops elengi* can enhance cognitive abilities on SCOP-induced deficits in learning and memory without affecting locomotor function.

Due to the oxidative degradation of polyunsaturated lipids, which causes oxidative tissue damage in several biological processes and is thought to be mediated by the production of these free radicals, LPO is one of the primary ROS producing factors. Therefore, lowering the LPO levels may offer a substantial amount of protection from such impacts. A substantial decrease ($p < 0.01$) in LPO levels was noticed with MEME-B and MEME-F extracts, supporting the protective mechanism of the extracts studied. However, methanol extract of ME bark exhibits a more promising antioxidant activity compared to all.

However, it is widely recognized that SOD is a key antioxidant enzyme that regulates many pathogenic processes in various diseases and illnesses. In our investigation, there was a substantial rise in SOD levels ($p < 0.05$), indicating that *Mimusops elengi* bark extract is more protective than MEME-F against SCOP-induced cognitive impairment. The same pattern was seen in CAT. CAT is also regarded as a crucial enzyme composed of hemeproteins that is employed to scavenge generated ROS and protect the tissue from free radical damage (Chance et al., 1952; Yan and Harding, 1997; Kono and Fridovich, 1982). Finally, in addition to this, histopathology studies verified the acquired results (Not shown). According to the findings, brain sections treated with *Mimusops elangi* extracts revealed modest gliosis and a lack of neurodegeneration in the hippocampus and basal ganglia region sections of the brain, which supports and suggests a neuroprotective effect of ME in SCOP-induced amnesia in mice. The current findings show that both MEME-B and MEME-F have potent protective effects against SCOP-induced amnesia in mice, as evidenced by significant reductions in transfer and escape latency in the EPM and MWM models, respectively and significant inhibition of AChE activity in brain tissue homogenates. MEME-B and MEME-F, on the other hand, have more promising neuroprotective action due to their antioxidant properties, as indicated by the fact that both MEME-B and MEME-F decreased LPO levels and raised antioxidants like SOD and CAT.

5. CONCLUSION

The results demonstrate that MEME-F have significant neurocognitive properties against scopolamine-induced amnesia, as demonstrated by significant inhibition of AChE activity to a significant extent with significant reduction of transfer latency, and

escape latency; whereas, MEME-B have significant neuroprotective activity, which may be related to their significant antioxidant property.

Author contribution

JR, DP, HB and NS conducted the study. JR wrote and prepared the research manuscript. NP and AM approved the final version of the manuscript.

Ethical approval

The institutional animal ethics committee (SVCP/IAEC/I007/2017–18) approved and certified the experimental methods in accordance with CPCSEA, New Delhi animal care guidelines.

Informed consent

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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