ORIGINAL ARTICLE

Amelioration of Cognitive Deficit by *Moringa oleifera* flower extract in a Scopolamine-Induced Alzheimer's Disease-Like Condition in a Rat Model

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Abstract

Background: Alzheimer's disease (AD) is the most prevalent form of dementia, marked by cognitive decline, amyloid-β (Aβ) accumulation, cholinergic dysfunction, neuroinflammation, and oxidative stress. *Moringa oleifera* flower (MOF) extract, rich in flavonoids, phenolics, vitamins, and calcium, has been traditionally recognized for its medicinal properties. This study explored the neuroprotective potential of MOF extract against scopolamine (SCO)-induced dementia in rats. **Materials and Methods:** Thirty Wistar rats were divided into five groups: Control (normal saline), SCO (3 mg/kg, i.p., days 15–21), MOF 200 + SCO, MOF 400 + SCO (administered orally for 21 days), and Donepezil (5 mg/kg, i.p., 21 days). Cognitive performance was assessed using the Morris water maze and Y-maze tests. On day 21, rat brains were collected for biochemical estimations (Aβ), acetylcholinesterase (AChE), nuclear factor erythroid 2-related factor 2 (Nrf-2), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA), and histopathological analysis. **Results:** SCO administration significantly impaired memory, increased Aβ and AChE activity, suppressed Nrf-2 expression, and altered antioxidant defense by reducing GSH and SOD while elevating MDA levels. Pretreatment with MOF extract markedly improved cognitive performance, reduced Aβ and AChE activity, restored Nrf-2 expression, and normalized oxidative stress markers. Histopathological findings supported these protective effects. **Conclusion:** MOF extract effectively ameliorated SCO-induced cognitive impairments and oxidative damage, demonstrating its potential as a natural neuroprotective agent for the management of AD.

Key words: Acetylcholinesterase, Alzheimer's disease, dementia, memory, *Moringa oleifera,* scopolamine

INTRODUCTION

Alzheimer's disease (AD) is a slowly progressing neuronal degeneration that particularly affecting the elderly population. It is recognized as the leading contributor to dementia and marked by a gradual loss of memory, behavioral changes, and impaired thinking.[1,2] AD is primarily marked by the buildup of Aβ plaques extracellular and the formation of neurofibrillary tangles intracellular, which are mainly composed of hyperphosphorylated tau proteins.[3,4] These abnormalities are often accompanied by neuroinflammation, oxidative stress, and selective neuronal loss in regions, such as the hippocampus and cortex, which are essential for learning and memory. Among the earliest and most affected neurons are the cholinergic neurons in the basal forebrain, which play an essential role by using acetylcholine (ACh) as a neurotransmitter. Their degeneration is mainly linked to memory loss seen in AD.[5,6] Several risk factors have been associated with its development, including ageing, genetic information, depression, environmental toxins exposure, gut microbiota imbalance, and chronic neuroinflammation. Together, these factors highlight the complex and multifactorial nature of this disorder.[7,8] Several cholinergic drugs have been approved

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through clinical studies for the treatment or management of AD. Acetylcholinesterase (AChE) inhibitors are the most frequently used. Drugs, such as galantamine, rivastigmine, and donepezil, help by inhibiting the breakdown of ACh,[9] which in turn leads to a temporary rise in its levels at cholinergic synapses.[10] However, the availability of approved drugs for managing cognitive decline in AD patients remains limited, largely because of associated side effects, such as nausea, vomiting, and liver toxicity.[11] Therefore, it is important to find out alternative treatment strategies for AD, particularly those based on natural sources that may show minor side effects.

SCO is a non-selective muscarinic cholinergic receptor antagonist. Impairs central cholinergic neurotransmission and is widely used in experimental models to induce cognitive deficits, including learning impairments and memory loss in experimental animals.[12] SCO has been incorporated into the animal model of "cholinergic amnesia" in several investigations since the cholinergic concept of old age memory impairment was first out.[13,14]

Natural products have recently gained significant attention as promising options for managing neurodegenerative diseases, such as Alzheimer's, due to their ability to influence multiple biological targets with minimal side effects.[15] *Moringa oleifera* (MO), often referred to as the drumstick tree, miracle tree, and best friend of mother, is a well-known medicinal plant.[16] Almost every part of the plant, including flowers, bark, leaves, root, oil, and gum, showed very good health benefits and holds promise in a range of environmental uses.[17] MO, a plant native to India, is well-known for its flowers Figure 1. It contains a wide range of bioactive compounds, including alkaloids, flavonoids, phenolics, vitamins that exhibit notable antioxidant, anti-inflammatory, and neuroprotective properties.[18,19] Recent studies suggest that these phytochemicals may contribute in changing key mechanisms implicated in the pathogenesis of AD, particularly by targeting oxidative stress and amyloid toxicity.[20] The Keap1/Nrf2-ARE pathway plays a crucial role in modulating neuroinflammation triggered by central nervous system immune responses and contributes significantly to the regulation of oxidative stress.[21] MOF extract facilitates the activation of the Keap1/Nrf2-ARE pathway, which in turn supports the upregulation of antioxidant enzymes involved in protecting cells from oxidative damage. Under normal physiological conditions, Keap1 binds to Nrf2 and acts as a sensor for oxidative stress, directing Nrf2 for ubiquitination and proteasomal degradation, thereby maintaining its low basal activity within cells.[22,23]

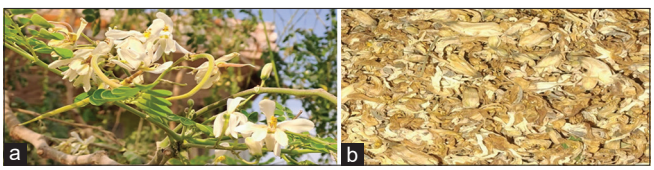

Figure 1: (a and b) Fresh and dried flower of *Moringa oleifera*

The main purpose of this research was to investigate the neuroprotective activity of plant extract from MO flowers (MOF), include enhanced memory and learning following the treatment of the muscarinic receptor antagonist SCO to Wistar rats to assess the cognitive impact of MOF extract in rats. The Morris water maze (MWM) and the Y-maze tests were used to determine the effect of MOF extract on SCO-induced memory disruption and learning. In addition, biochemical estimation using enzyme-linked immunosorbent assay (ELISA) and ultraviolet (UV) spectroscopy methods were conducted for the estimation of Aβ, nuclear factor erythroid 2–related factor 2(Nrf-2), AChE, malondialdehyde (MDA), reduce glutathione (GSH) and superoxide dismutase (SOD) which are critical markers of cholinergic function and oxidative stress. Furthermore, histopathological analysis of rat brain tissue was performed to observe structural alteration and neuronal integrity.

The central aim of this multifaceted investigation is to evaluate the efficacy of MOF extract in mitigating AD. To achieve this, we employed a comprehensive approach that includes behavioral testing, biochemical estimation, and histopathological analysis. The study assesses the extract's impact on memory, cognitive behavior, and cellular pathologies associated with AD. The aim of this research is not only to observation of pharmacological effects. It also focused on understanding the possible mechanism behind those effects. If successful, MO could offer a promising, plant-based alternative for supporting brain health and managing neurodegenerative disorders, such as AD.

MATERIALS AND METHODS

Plant material and sample preparation

MO flowers were collected from Mathura, Uttar Pradesh, India, during February and March months. The plant material was identified and authenticated by the Plant Identification Cell, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India. Fresh MO flowers were washed two times with distilled water to remove any surface impurities or dust particles, then shade-dried at room temperature for 15 days. An electric blender (Sujata Powermatic Plus, 900 W) was used to grind dried MOF, and an electric sieve shaker was then used to sift the mixture through a 100 mm screen. The powdered material was stored in an airtight container.[18] 50 g of MOF powder was macerated in 250 ml of ethanol for 3 days on an orbital shaker at room temperature. The extract was first passed through Whatman filter paper, then centrifuged, and after that then concentrated at 40°C using a rotary vacuum evaporator.[24] The dried extract was weighed. The final concentrated extract was stored at 4°C for further use.[25] The % yield of ethanolic extract of MOF through the maceration method was found to be 34.7%. This revealed that ethanol has very good efficacy to extract

out active constituents from MOF. SCO as an inducing agent and donepezil as a standard drug were procured from Sigma Aldrich.

Experimental animals

Experimentation was conducted on male Wistar rats, weight ranging from 180-220 gram. These experimental animals were procured from the "All India Institute of Medical Sciences (AIIMS)", New Delhi, India. They were kept in groups of 6 animals per cage under temperature-controlled and pathogen-free environment to ensure maintenance of hygiene throughout the study. The rats were housed at a stable temperature of 25 ± 5°C with a 12-h light/dark cycle. They also had unrestricted access to standard food and water. IAEC examined and approved each experimental protocol with approval number IAEC/IPR-12/24-20. After 1 week of acclimatization, rats were divided into 5 groups (n = 6 per group).

Experimental design

To evaluate the neuroprotective efficacy of MOF extract, a preventive approach was adopted using a SCO-induced model of AD-like neurodegeneration. On the basis of the earlier report, the preventive experimental design was chosen.[26] SCO was chosen for its well-documented capacity to induced cognitive impairment and oxidative stress, thereby replicating core pathological characteristics of AD.[27] SCO, MOF extract, and donepezil were prepared in normal saline and administered to animals 30 min after dissolution. The protocol of the experiment is presented in Figure 2. The treatment details for these groups are provided in Table 1.

Rats were carefully trained in a behavioral task before the start of the experiment. Between days 16 and 20, after the final dose of either the drug or vehicle, their cognitive function, particularly learning and memory, was evaluated by behavioral tests. Following the accomplishment of behavioral testing, cervical

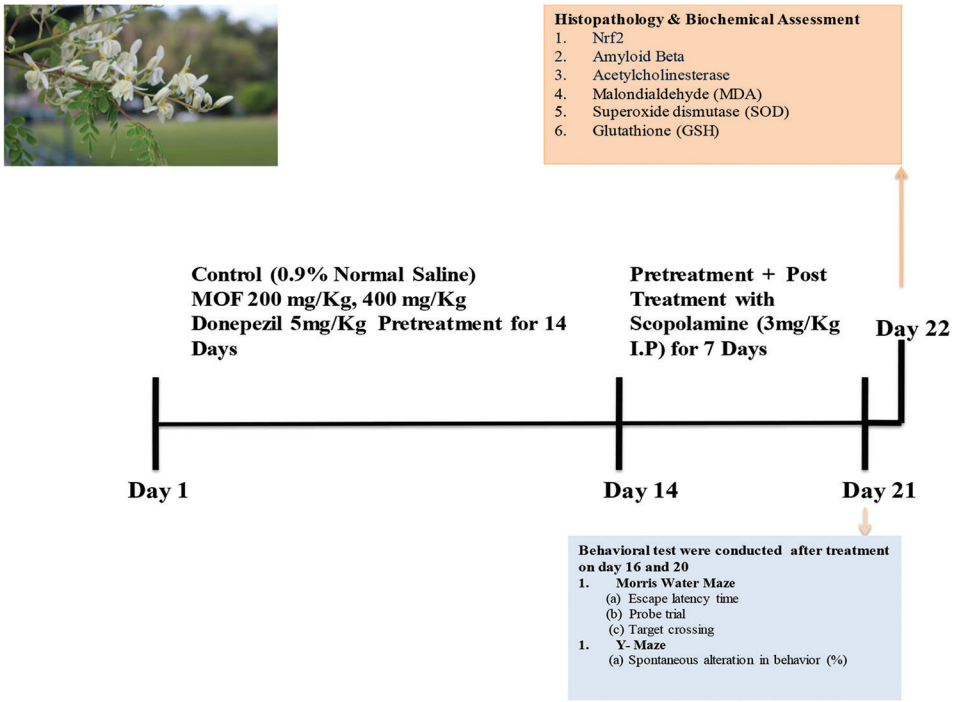

Figure 2: Schematic representation of the experimental and treatment design of Wistar rat

Table 1: Experimental group design

Groups	Treatment	Dose and route	Duration
Group I	Control (normal saline)	0.9% NaCl, i.p.	21 days
Group II	SCO	3 mg/kg, i.p.	(0.9%w/v NaCl) i.p for 14 days SCO: Last 7 days
Group III	MOF extract+SCO	200 mg/kg MOF, oral+3 mg/kg SCO, i.p.	MOF: 21 days, SCO: Last 7 days
Group IV	MOF extract+SCO	400 mg/kg, oral+3 mg/kg SCO, i.p	MOF: 21 days SCO: Last 7 days
Group V	Donepezil+SCO	5 mg/kg Donepezil, i.p+3 mg/kg SCO, i.p.	Donepezil: 21 days, SCO: Last 7 days

MOF: *Moringa oleifera* flower, SCO: Scopolamine

dislocation was used to sacrifice the experimental animals, and their brain tissue was promptly harvested in 10 % formalin for further testing. The rat brains from each group were utilized for biochemical evaluation, including the estimation of AChE activity, MDA, SOD, and GSH levels. Brain tissue was fixed in 10 % formalin for histopathological examination.

Estimation of neurobehavioral parameters

MWM test

This test was performed to assess spatial learning and memory in rats. A water-filled circular tank measuring 120 cm in diameter and 40 cm in height at 26°C was used, with a submerged transparent platform hidden just below the surface. Water was made opaque with milk to conceal the platform. Experimental animals were transferred, facing the tank wall, and pre-trained before testing. Training was done 3 times/day for 5 consecutive days for each rat, with a 120-s maximum per trial. In test trials, rats were given 90 s to locate the platform and allowed to rest on it for 15 s. Transfer latency (TL) was recorded as the duration taken to find the platform. In the probe trial, the platform was removed, and rats swam freely for 60 s. Platform crossing and time spent in the target quadrant were measured. A video tracking system recorded movement and performance data.[6,10]

Y-maze test

Behavioral test Y-maze was conducted based on the protocols detailed by Salama *et al.* (2025) and Hidaka *et al.* (2011). The apparatus formed of three arms, assigned as A, B, and C. In the initial training phase, the rat was permitted to explore the maze for 8 min. After 24 h, a second session was conducted in which the rat was again given 8 min to navigate the maze and its movements were recorded. The number of alterations was defined as successive entries into three different arms in overlapping triplet sequences, for example, in the sequence ABCBACA, there are three alterations. The total arm entries refer to the overall number of entries made into the arms; for instance, ABCBACA equals 7 entries. The % alteration was calculated using the given formula.[28]

$$\text{The percentage alteration} = \frac{\text{Number of alteration}}{\text{Total arm enteries} - 2} \times 100$$

Tissue preparation

All rats were euthanized under anesthesia using Ketamine (50 mg/kg) and Xylazine (10 mg/kg), administered 1 h after the completion of behavioral assessments.[19,22] and perfusion was carried out transcardially using 0.1 M PBS to facilitate the assessment of biochemical and histopathological parameters. The rat brains were quickly isolated and excess blood was rinsed of using PBS. The hippocampus was then isolated and homogenized in 0.1 M phosphate buffer (pH 7.4) containing a protease inhibitor. The obtained supernatant was stored at −80°C for later biochemical tests after the tissue homogenate was centrifuged for 15 min at 4°C at 10,000 rpm.[26]

Biochemical estimation

Amyloid beta

An ELISA kit (Cat: ELK9021, ELK Biotechnology) was used and protocols were followed according to the manufacturer's instructions to measure the levels of Aβ to find out how the MOF extracts affected the neuroinflammation inhibitory index. For the ELISA assay, 100 µL of standard solution, blank, or test sample was placed to every well of a 96-well plate. After covering the plate, it was incubated at 37°C for 80 min after that, each well was washed three times with 200 µL of 1x wash buffer, after removing the contents, allowing each wash to sit for 1–2 min, and the plate was blotted dry. The biotinylated antibody solution was then placed to each well in a volume of 100 µL, and the wells were incubated for 50 min at 37°C. Following three washing cycles, 100 µL of Streptavidin-HRP working solution was added to each well. The plate was then incubated for 50 min, followed by five additional washes. Subsequently, after adding 90 µL of TMB substrate solution, the plate was left in the dark for 20 min at 37°C. When 50 µL of stop solution was added, the color changed from blue to yellow. After gently mixing and ensuring no bubbles or debris, absorbance was measured at 450 nm.

AChE

AChE activity was measured following the procedure proposed by Jasoria *et al*. (2024).[23,29] For the assay, 25 µL of the supernatant derived from tissue homogenate was combined with 100 µL of ACh iodide and 100 µL of DTNB solution in 3 ml of PBS (0.01M, pH 7.4). At 412 nm, absorbance was measured for a duration of 2 min. Results were represented as µM of ACh iodide hydrolyzed per minute/mg protein, following the method described by Ellman *et al.*[30]

Nuclear factor erythroid 2-related factor (Nrf-2)

Nrf-2 levels were quantified using an ELISA kit (MBS752046, MyBioSource, San Diego, USA) following the manufacturer's protocol. A volume of 100 µL from either the prepared standard or test samples was dispensed into the designated wells, and 100 µL of PBS (pH 7.0–7.2) was added to the blank well as a control. For tissue homogenates, 10 µL of balance solution is transferred to each 100 µL sample and combined well. After that, 50 µL of enzyme conjugate is added to every well, excluding the blank control well, and mixed thoroughly. After that, the plate is covered and incubated at 37°C for 1 h. Following incubation, the plate is washed five times with 1× wash solution. After the final wash, any remaining liquid was removed by gently inverting the plate and blotting it on absorbent paper. Next, each well, including the blank, received 50 µL of substrate A and substrate B. The plate was covered and incubated at 37°C in the dark for 15–20 min to prevent light-induced reactions. 50 µL of stop solution is thoroughly

mixed when the color development is complete. At 450 nm, optical density was finally measured.

Estimation of oxidative stress biomarkers

SOD

To initiate this assay, 0.1 mL of tissue homogenate was added to a reaction mixture containing 2 mL of nitro blue tetrazolium and 0.5 mL of hydroxylamine hydrochloride. Subsequently, the absorbance was measured at 560 nm for 2 min using a UV-visible spectrophotometer, with readings taken at 30–60 s intervals. The activity of SOD was quantified in terms of U/mg of brain protein.[31]

Reduced GSH

Among the key antioxidants in aerobic cells, GSH is a major antioxidant, and it is found in micromolar concentration in fluids and millimolar in tissues. 1 ml of supernatant from tissue homogenate was mixed with an equal volume of 4 % sulfosalicylic acid and incubated at 4°C for 1 h for cold digestion. The mixture was then centrifuged at 1200 gyration for 15 min at 4°C. PBS (0.1 M, pH 8.0) and DTNB were placed to 1 ml of supernatant. The resulting yellow coloration was immediately measured at 412 nm using a UV spectrometer. A standard curve was used to calculate the GSH concentration, which was then represented as µM/mg protein.[29]

MDA

MDA is an end product of lipid peroxidation that reacts with two molecules of thiobarbituric acid (TBA) to form a pink colored chromogenic complex, whose absorbance is measured using a spectrophotometer.[32] In this assay, MDA levels were quantitatively measured in brain homogenate. The supernatant (0.1 ml) was added to 0.5 ml of 0.1 M Tris-HCl buffer (pH 7.4), and the mixture was kept at room temperature for 2 h of incubation. Following incubation, 1 ml of 10% (w/v) trichloroacetic acid (TCA) was carefully mixed to the reaction mixture to proceed with the assay, followed by centrifugation at 1000 x g for 10 min. The reaction mixture was then heated in a boiling water bath for 10 min after 1 ml of the supernatant was combined with 1 ml of 0.67% (w/v) thiobarbituric acid (TBA). Once cooled, 1 ml of distilled water was added. A spectrophotometer was used to detect the absorbance of the resultant complex at 532 nm after the reaction between MDA and thiobarbituric acid. The MDA concentration was determined using a standard curve and expressed as nmol/mg protein.[33]

Histopathological studies

The isolated brains from rats were immersed in 10 % formalin for 24 h to ensure proper fixation. Post-fixation, the samples were washed under continuous running tap water and then progressively dehydrated using an ascending concentration of alcohol. After being cleaned with xylene, the tissue samples were embedded in paraffin wax and heated to 56°C for 24 h in a hot air oven. After embedding, paraffin blocks were prepared, and thin sections of approximately 5 µm thickness were cut using a sledge microtome.[34] Tissue sections were fixed on sterile, clean glass slides, deparaffinized, and then stained with hematoxylin and eosin for histological investigation. After that, the stained slides were seen at 100 X magnification using a phase contrast microscope (Cilika BT-E 2021).[35]

RESULTS

Protective effect of MOF extract in MWM test

MWM test was used to assess the ability to learn and spatial memory. Figure 3 shows the impact of MOF extract on

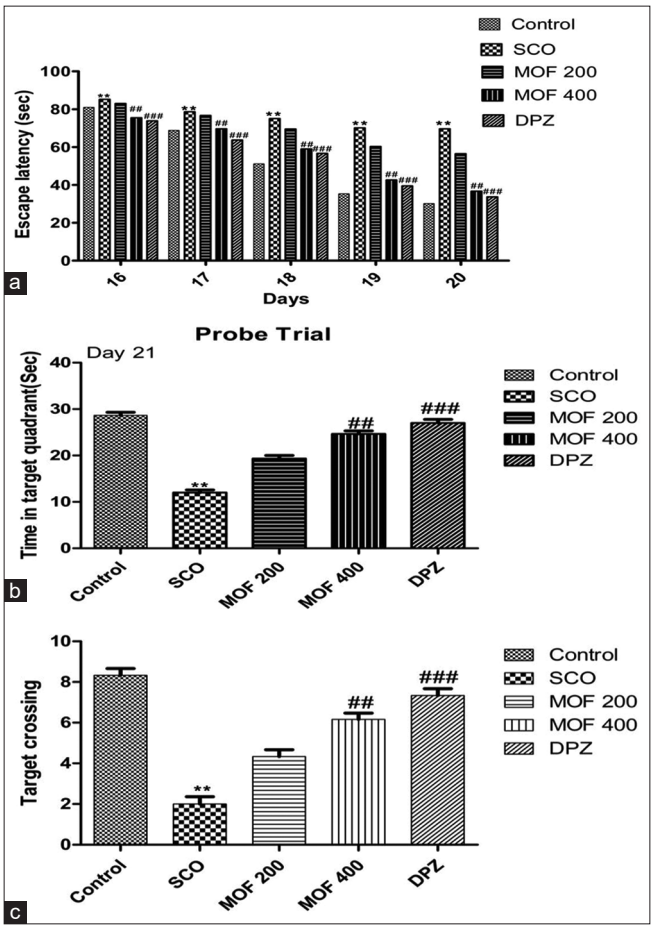

Figure 3: Scopolamine (SCO)-induced deficiencies in spatial learning and memory were mitigated by administering *Moringa oleifera* flower (MOF) extract, accordance to the Morris water maze test. Rats were given MOF extract orally at 200 and 400 mg/kg doses once daily for 21 days, while SCO (3 mg/kg, i.p) dose was administered during the final 7 days to induce memory impairment (a) Escape latency in the hidden platform trials (b) The time spent in the target quadrant (c) number of target platform crossings. Data are expressed as the means±scanning electron microscope. N=6 for each group. The data were analyzed statistically using one-way analysis of variance with Tukey's test for multiple comparisons. $^{**}P<0.05$ versus control group, $^{\#\#}P<0.05$ versus SCO group and $^{\#\#\#}P<0.05$ versus SCO group

escape latency (a), duration spent in the target quadrant (b), and frequency of target crossing (c).

Rats treated with scopolamine (SCO) showed a marked elevation in TL, reflecting impaired cognitive function. In contrast, treatment with MOF and donepezil significantly reduced TL and enhanced cognitive performance as evidenced by increased time spent in the target quadrant and a higher number of target crossing. These findings suggest that treatment with MOF extract had a positive effect on memory in SCO-treated rats, as indicated by their improved ability to locate the platform, reflecting enhanced cognitive performance.

Effect of MOF extract in spatial and learning memory in the Y-maze test

Figure 4 illustrates the effect of SCO on spatial learning and memory, as reflected by changes in spontaneous alteration behavior in rats. A significant decline in spontaneous alteration percentage was observed in the SCO-treated group, indicating cognitive impairment.

The animals' percentage of spontaneous alteration behavior (SAB) decreased, which was indicative of SCO's impact on spatial learning and memory. Significant differences in spontaneous behavioral changes were observed between the various groups of animals. Tukey's multiple comparison test revealed that SCO-administered rats exhibited a significant decline in SAB in the Y-maze test when compared to both control and MOF-treated groups. This reduction in SAB performance indicates that SCO markedly impaired spatial working memory in rats. Comparing the control group to, rats given SCO spent less time in the novel arm, reflecting diminished exploratory behavior and cognitive dysfunction. When the MOF extract was pre-treated before SCO was administered, the duration of time in the new arm was longer than in the group that received SCO alone, indicating a potential protective effect of the MOF extract.

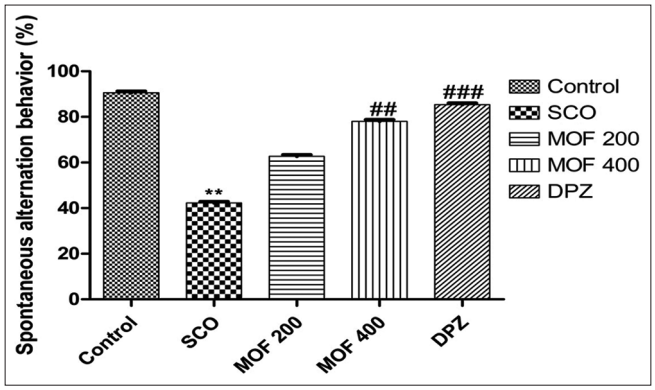

Figure 4: Spontaneous alternation behavior in Y-maze test was used to evaluating short term spatial memory. *Moringa oleifera* flower extract at doses of 200 and 400 mg/kg was given orally for 21 days, while scopolamine (SCO) (3 mg/kg i.p) was administered during the final 7 days to induce memory impairment. The data is shown as means±scanning electron microscope for each group of six. Data were analyzed using one-way analysis of variance, followed by Tukey's multiple comparisons. $^{**}P<0.05$ versus control group, $^{\#\#}P<0.05$ versus SCO group and $^{\#\#\#}P<0.05$ versus SCO group

Biochemical assay

Amyloid-β (Aβ)

Figure 5a showed the activity of the MOF extract on brain amyloid beta levels in SCO-treated rats. One-way analysis of variance (ANOVA) analysis revealed that significant differences in amyloid beta concentrations among the experimental groups. When compared to controls, the brain homogenates of rats treated with SCO showed considerably higher levels of Aβ, suggesting impaired memory performance. Administration of MOF extract and donepezil before SCO administration significantly lowered amyloid

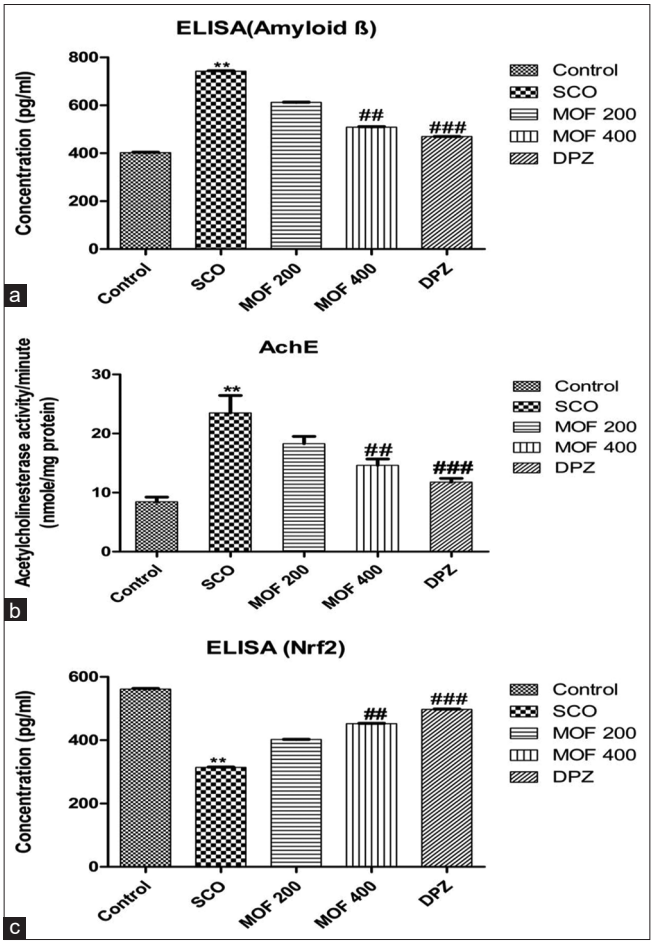

Figure 5: The effect of *Moringa oleifera* flower ectract and donepezil on (a) amyloid-β (b) acetylcholinesterase (c) nuclear factor erythroid 2-related factor 2 levels in the brain of scopolamine (SCO) treated rats. During statistical analysis data were analyzed using one way analysis of variance, followed by Tukey's multiple comparison. Data are expressed as the means±scanning electron microscope. N=6 for each group. $^{**}P<0.05$ versus control group, $^{\#\#}P<0.05$ versus SCO group and $^{\#\#\#}P<0.05$ versus SCO group

beta levels, indicating a potential reversal of SCO induced alteration in brain function.

AChE

The effects of donepezil and MOF extract on AchE activity in the brains of experimental animals treated to SCO were demonstrated in Figure 5b**.** One-way ANOVA statistical analysis verified that there were notable variations in AChE activity between the treatment groups. AChE activity in brain tissue homogenates was significantly higher in rats treated with scopolamine than in the control group, indicating disrupted cholinergic signaling. Pre-treatment with MOF extract or donapezil significantly reduced AChE activity, indicating their protective role against SCO induced impairment of cholinergic function.

Nrf-2

Figure 5c showed the Nrf-2 protein level in rat brain sample of different groups were measured using ELISA. As compared to the SCO group control, MOF and Donepezil group rat brain samples had significantly higher Nrf-2 protein levels. Significant variations in the expression of Nrf-2 were observed among the different experimental groups. The SCO-treated group's Nrf-2 levels were significantly lower than those of the normal control group, showed impaired activation of the antioxidant response pathway due to oxidative stress. The MOF extract-treated group significantly upregulated the Nrf-2 levels as compared to SCO-treated rats group. The group of rats treated with the standard drug, donepezil, also demonstrated a significant increase in Nrf-2 expression. These findings indicated that MOF extract enhances the cellular antioxidant defence system, possibly through activation of the Nrf-2 signaling pathway, thereby contributing to its neuroprotective potential.

Effects of MOF extract on antioxidant markers in SCO-treated rats

SOD

Figure 6a showed how MOF extract affected SOD levels in the brains of SCO-administered rats. One-way ANOVA showed statistically significant differences between the experimental groups. According to Tukey's multiple comparison analysis, SOD levels in the brains of rats treated with MOF 400 were significantly higher than those in the SCO group. Similarly, donepezil administration markedly elevated brain SOD activity compared to SCO-induced animals.

Reduced GSH

Figure 6b illustrates the influence of MOF extract on brain levels of GSH in rats administered with SCO. GSH levels varied significantly among the groups, as determined by one-way ANOVA. Rats treated with SCO had significantly lower levels of GSH in their brains than the normal control group, according to Tukey's multiple comparison test. In contrast,

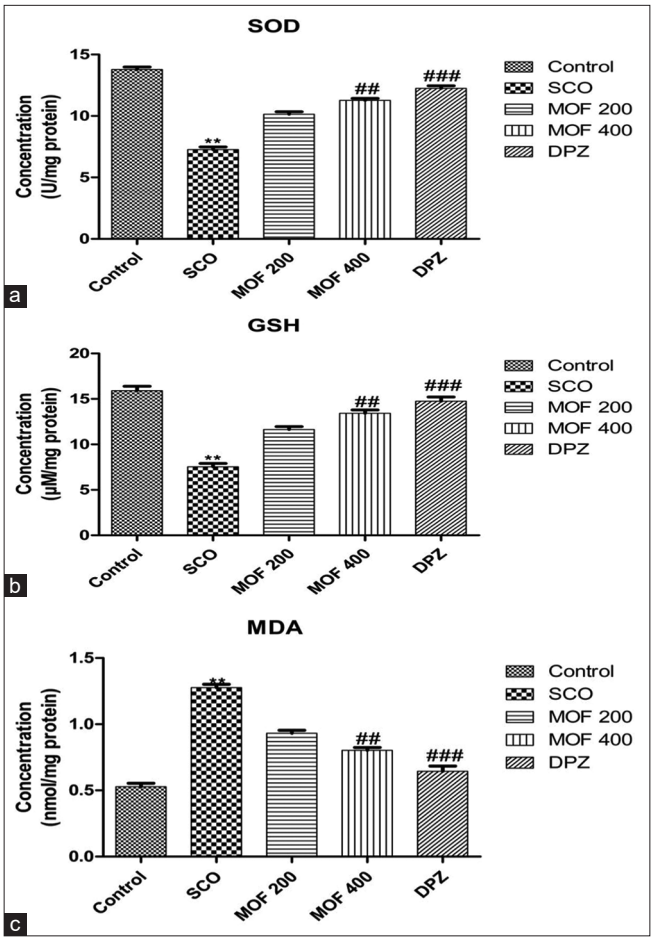

Figure 6: Effect of *Moringa oleifera* flower extract on oxidative stress biomarkers in brain tissue. The graph shows the levels of (a) superoxide dismutase (b) glutathione (c) malondialdehyde across various experimental groups. Data are expressed as the means±scanning electron microscope. N=6 for each group. During statistical analysis data were analyzed using one way analysis of variance, followed by Tukey's multiple comparison. $^{*}P<0.05$ versus control group, $^{\#\#}P<0.05$ versus scopolamine (SCO) group and $^{\#\#\#}P<0.05$ versus SCO group

both MOF extract and donepezil administration dramatically increased GSH levels in SCO-treated rats, countering the reduction seen in the disease group.

MDA

Figure 6c shows the effect of MOF extract on MDA levels in SCO-treated rats. MDA is known as a biomarker of lipid peroxidation. One-way ANOVA analysis showed significant differences in MDA levels among the groups. The result showed a significant elevation in brain MDA levels in SCO-treated disease group. However, treatment with MOF extract and the SCO-induced rise in MDA levels was effectively inhibited by donepezil.

The changes observed in SOD, GSH, and MDA levels in SCO-treated rats indicate that SCO disrupted the antioxidant defense system. Treatment with MOF extract alleviated SCO-administered changes in brain levels of SOD, reduced

GSH and MDA, exhibiting effects in comparison to the standard drug donepezil. These results suggest that MOF extract restored antioxidant defense mechanisms impaired with SCO.

Histopathological assessment of MOF extract's neuroprotective action in the hippocampus of SCO treated rats

Figure 7 showed hippocampal histology from various groups, control rats administered normal saline, SCO-treated rats representing the disease model, and rats pretreated with either MOF extract or donepezil. To check the neuroprotective potential of MOF extract and donepezil, brain section from all groups were examined for morphological alteration linked with SCO-induced neurodegenerative diseases. Compared to the control that received normal saline, the SCO-treated rats exhibited marked histopathological alteration in the hippocampal part of the brain. Neurons in the control group exhibited normal histoarchitecture characterized by basophilic cytoplasm, oval-shaped cell bodies, and euchromatic nuclei. In contrast, the SCO-treated group showed pronounced neurodegeneration, including cytoplasmic shrinkage, nuclear condensation (karyopyknosis), and an increased presence of dark-staining neurons. In the SCO-treated group, evident signs of neuronal damage included microglial activation, swollen astrocytes, and vascular congestion. Conversely, hippocampal sections from rats treated with MOF extract or donepezil displayed substantial histological improvement marked by fewer pyknotic neurons, reduced microglial activity, diminished vascular congestion, and better preservation of overall cellular architecture. These observations suggest that MOF extract has neuroprotective activity, which protects neuronal cells by supporting healthy neurons, lowering microglial activity, and reducing cell death in the hippocampus.

DISCUSSION

The present study gives significant evidence that MOF extract has neuroprotective properties against SCO-administered dementia in Wistar rats, as demonstrated by behavioral and biochemical tests. SCO, a nonselective muscarinic receptor antagonist that acts centrally, has previously been demonstrated to affect memory and learning in rats and humans through muscarinic receptor blockage, cholinergic signaling is impaired, and the enzyme is compensatorily upregulated, SCO increases AChE activity.[36,37] Our research also demonstrated that AChE activity was elevated because of SCO administration. Lower levels of ACh were the consequence of the SCO-induced elevation in AChE activity, which eventually leds to cholinergic dysfunction. Similar to donepezil, MOF extract significantly reduced the rise in AChE activity induced on by SCO**.** According to other findings, MOF extract may have anti-neuroinflammatory properties

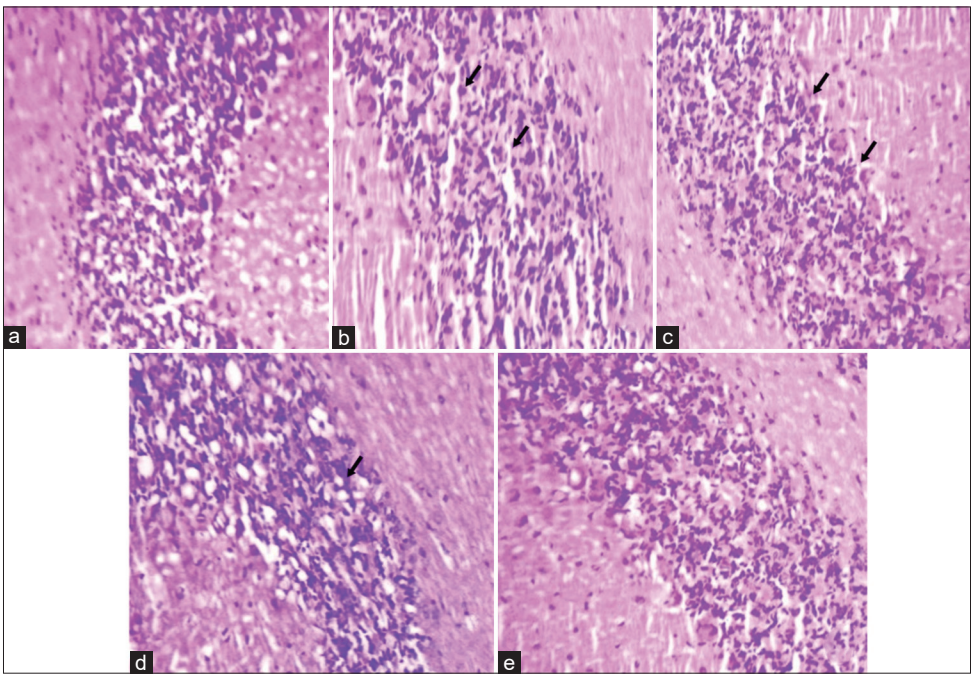

Figure 7: Histopathological evaluation of hippocampal sections from scopolamine (SCO) treated rats brains following *Moringa oleifera* flower (MOF) extract and donepezil administration (H&E) staining at ×100 magnification. (a) Normal control group rat brain showed normal histological image. (b) SCO-treated rats' brain section (3 mg/kg) exhibited severe histological damage, including disrupted cytoarchitecture, microglial activation, pyknosis, necrotic cells and hypertrophied astrocytes. (c and d) Rats pretreated with MOF extract (200 and 400 mg/kg, respectively) showed improved hippocampal architecture with fewer degenerating neurons, reduced glial reactivity and restoration of tissue organization. (e) Donepezil (5 mg/kg) also offer neuroprotection, evident by minimized neuronal shrinkage and attenuated cellular disruption

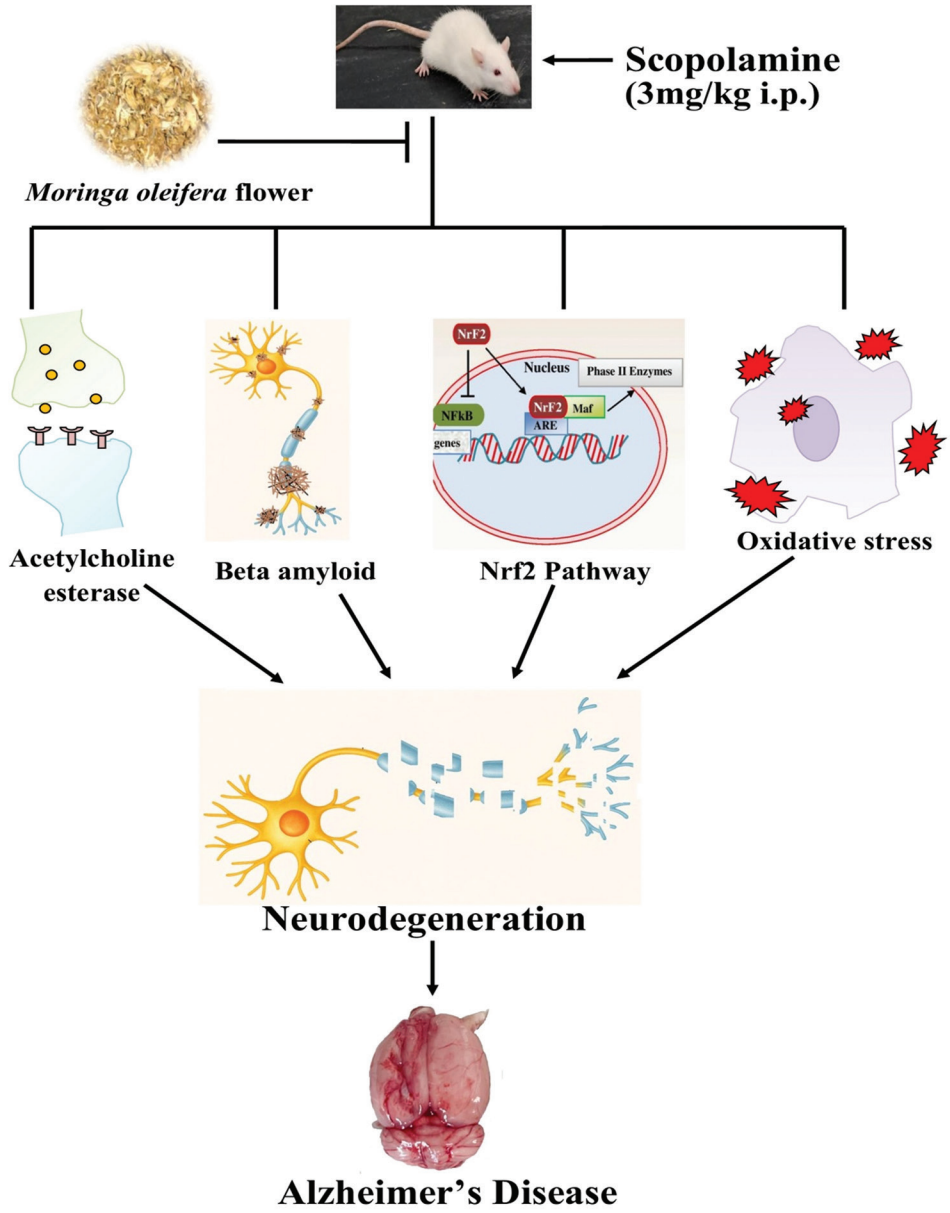

Figure 8: Proposed mechanism and summary of findings; the illustration highlights the potential of *Moringa oleifera* flower extract in Alzheimer's disease by reducing oxidative stress and neuroinflammation along with inhibiting acetylcholinesterase activity

because it exhibited a positive antioxidative property that considerably reduced the rise in ROS species caused by SCO, including MDA levels, while restoring antioxidative mechanisms that had been altered, such as SOD and GSH levels. Using the Y-maze test, short-term spatial memory was evaluated as part of a series of behavioral evaluations examining SCO-induced cognitive impairment and learning deficits.[38,39] We found that the administration of SCO caused cognitive decline in Wistar rats, as showed by their performance on a number of behavioral tests. In SCO-treated rats, MOF extract significantly improved spatial learning and memory in the MWM test by shortening escape latency, increasing time spent in the target quadrant, and increasing the number of target crossings. The duration of time spent in the new arm of the Y-maze was substantially longer after MOF treatment than in the group that was triggered by SCO. Indicating improved spatial working memory. Together, these behavioral results strongly suggest that MOF extract showed neuroprotective activity against SCO-treated cognitive impairments in Wistar rats.

In AD, the breakdown of ACh by AChE contributes to a decrease in cognition, highlighting the therapeutic importance of AChE inhibitors in improving cholinergic signaling.[40,41] Acetic acid and choline are produced when AChE hydrolyzes the ester bond in ACh.[42] Literature survey also indicated that AChE plays a role in the progression of neurodegenerative diseases by influencing both inflammatory response and oxidative stress.[40] AChE effect in the brains of rats administered with SCO was significantly higher than in the

control group, according to the AChE assay. Compared to the SCO group, treatment with MOF and donepezil significantly decreased AChE activity. As a result, MOF treatment inhibited the breakdown of ACh, thereby increasing its synaptic availability. This elevation in ACh levels contributed to the improvement of SCO-induced learning and memory impairments. The administration of SCO caused oxidative stress in rats, as showed by a marked rise in MDA levels, indicating enhanced lipid peroxidation in the diseased group.[37] It seems that the MOF extract effectively lowers MDA concentrations under oxidative stress situations, as both the MOF and donepezil pre-treated groups showed decreased MDA levels in contrast.[43] The GSH and SOD activity of the SCO-treated group was significantly lower than that of the control group. In contrast, treatment of MOF extract and donepezil efficiently increased SOD activity and restored GSH levels. These outcomes highlight the antioxidative role of MOF, supporting its potential in mitigating oxidative stress associated with AD. Hematoxylin and eosin (H & E) staining is a widely used technique for assessing neuronal tissue morphology and pathology. In this study, brain sections from control rats stained with H & E displayed normal histological architecture. In contrast, the SCO-treated group exhibited notable pathological alterations such as necrotic areas, disrupted structural integrity, activated microglia, swollen astrocytes, and nuclear pyknosis. Pretreatment with MOF (400 mg/kg) or donepezil (5 mg/kg) effectively protected brain tissue, as evidenced by the minimal necrosis and preserved structural features observed in the slices, showing that the neuronal damage caused by SCO has been effectively ameliorated by both treatments.

Our study highlights the strong neuroprotective potential of MOF extract against SCO-induced AD-like symptoms in Wistar rats. To avoid hormonal fluctuations that could influence behavioral and biochemical results, only male Wistar rats were taken in the experiments. It is generally known that gender influences neurobiological responses differently. Hormonal factors, particularly the neuroprotective effects of estrogen, may cause female rats to exhibit different baseline sensitivities to SCO-induced dementia.[6,44]

CONCLUSION

The outcomes of this study clearly showed that MOF extract exerts very good neuroprotective potential against SCO-induced cognitive impairments, underscoring its therapeutic efficacy in managing dementia linked with AD. As shown in Figure 8, MOF extract exerts its neuroprotective effects through various mechanisms, consist of the inhibition of AChE activity, reduction of oxidative stress, and enhancement of antioxidant defense systems, such as SOD and reduced GSH. The protective effects of MOF extract counter SCO-induced memory loss and neuronal damage, mimicking AD like pathology. Behavioral, biochemical, and histopathological evaluations further validate the neuroprotective efficacy of MOF extract by revealing its role in preserving neuronal architecture and enhancing overall brain function. Our findings revealed that MOF extract could serve as an effective plant-based strategy for managing Alzheimer's and related neurodegenerative conditions.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

IAEC examined and approved each experimental protocol and utilization of experimental animal for this study with approval number: IAEC/IPR-12/24-20.

CONSENT FOR PUBLICATION

Not applicable.

AUTHORS' CONTRIBUTIONS

Virendra Kumar Kushwaha: Writing original draft, Data curation, Methodology, Software, Resources, Visualization, Formal analysis. Kantrol Kumar Sahu: Review and editing, Supervision, Conceptualization, Validation, Methodology.

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