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ORIGINAL ARTICLE

Black seed oil ameliorated scopolamine-induced memory dysfunction and cortico-hippocampal neural alterations in male Wistar rats



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Ameliorative efficacy

Abstract This study was conducted to evaluate cognitive enhancing effect and ameliorative effects of black seed oil in scopolamine induced rat model of cognitive impairment. These effects were investigated on scopolamine-induced dementia model in Morris water maze test (MWM) and Y maze test. The hippocampal histoarchitectural responses to scopolamine and *Nigella sativa* oil were also examined.

Scopolamine (1 mg/kg ip) was given to induce dementia, followed by oral administration of BSO (1 ml/kg) for 14 consecutive days. MWM and Y-maze paradigms were used to assess hippocampal and frontal dependent memory respectively, thereafter the rats were sacrificed and brains were removed for histopathologic studies.

Scopolamine resulted in memory impairment, by delayed latency in the MWM, reduced percentage alternation in the Y maze that was coupled by alterations in the cortico-hippocampal neurons. Post-treatment of rats with BSO mitigated scopolamine-induced amnesia, by reducing latency period and increasing percentage alternation and histological changes. The observed anti-amnesic effect of BSO makes it a promising anti-amnesic agent for clinical trials in patients with cognitive impairment.

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1. Introduction

Dementia is characterized by a loss or decline in memory and other cognitive functions, and one of the most common sub-

types of dementia is Alzheimer's disease (AD).¹ AD is characterized by a cascade of conditions like neural oxidative stress, deposition of amyloid plaques, neurofibrillary tangles, inflammation and impaired central functions.² The implicated mechanism of AD in the elderly sufferers is linked to the loss of cholinergic neurons in the basal forebrain and hippocampus.³

Scopolamine is a muscarinic receptor antagonist with profound amnesic effects in a variety of learning paradigms and a useful experimental pharmacological model to investigate the pathophysiology of the cognitive deficit in AD.⁴ It has been widely implicated to cause amnesia and a viable model of dementia in humans and animals.⁵⁻⁷ Scopolamine caused its cholinergic deficits via oxidative stress^{8,9} and neuroinflammation.¹⁰ Scopolamine induced memory deficits have been widely implicated for the screening of anti-dementia drugs.^{11,12}

The most common treatment modalities for AD symptoms are the cholinesterase inhibitors, whose primary targets are AChE and BChE for their therapeutic activities. However, these agents cause undesirable side effects, thereby limiting their use. The present therapies produce modest symptomatic improvements in patients; thus, searching for alternative or supplement anti-amnesic agent is an ultimate necessity.

Herbal and natural plant extracts have gained wide attention in their use in the treatment and/or management of neurological, psychiatric and degenerative diseases, due to their no or less side-effects.^{13,14} Black seed oil is a high value medicinal solvent, widely used traditionally in the treatment of many diseases. Compelling evidences have reported that black seed oil (BSO) exhibits protective activities against many diseases depending on its high antioxidant¹⁵ and anti-inflammatory properties.¹⁶ This could suggest its use as a potential remedy for cognitive impairments in AD.

A number of studies have been carried out, acclaimed medicinal properties emphasized on different pharmacotherapeutical efficacies of black seed oil such as antioxidative¹⁷ and neuroprotective,^{15,18} antiasthmatic,¹⁹ anti-inflammatory, immunomodulatory and anti-tumor properties,¹⁶ gastric ulcer healing,²⁰ tumor growth suppression,²¹ men infertility improvement,²² and stimulate milk production.²³ The main active ingredients in BSO are thymoquinone (TQ), alkaloids (nigellidine, nigellimine, and nigellicine), vitamins such as thiamine, riboflavin, pyridoxine, niacin, and folic acid, minerals, and proteins.²⁴

The present study was designed to investigate the ameliorative efficacy of BSO against behavioral alterations in scopolamine demented rats. Their neuroprotective effects on cortico-hippocampal neuronal alterations were also evaluated.

2. Materials and methods

2.1. Drugs

Scopolamine hydrobromide (sigma, USA) was used in the present study. Scopolamine was dissolved in saline (NaCl 0.9%) at final concentrations of 1 mg/kg, and was injected intraperitoneally. The black seed oil (100% pure natural oil) was obtained from Masra warda, Kingdom of Saud.

2.2. Experimental animals

Eighteen adult albino Wistar rats weighting 200 ± 20 g at the time of acquisition and acclimatization were used in this study.

They were housed singly in metabolic cages under standard laboratory conditions in Animal holding of the Faculty of Basic Medical Sciences, University of Ilorin, Ilorin. They had free access to food and water, were housed six in a cage, and kept at controlled temperature ($22 \pm 2^\circ\text{C}$) under a 12/12 h light-dark cycle. All procedures were performed in accordance with institutional guidelines for animal care and use.

2.3. Treatments schedule

The rats were randomly distributed into four groups ($n = 6$) as follows:

- A. received saline for 14 days
- B. received BSO (1 ml/kg) orally for 14 days
- C. received scopolamine (1 mg/kg) intraperitoneally for 14 days
- D. BSO (1 ml/kg) orally, 30 min after scopolamine (1 mg/kg) intraperitoneally for 14 days

All experiments were carried out during the light phase between 9:00 and 15:00. Experimental groups consisted of 6 animals each.

2.4. Ethical approval

All procedures were performed in accordance with institutional guidelines for animal care and use and ethical approvals were received the University of Ilorin ethics review committee.

2.5. Morris water maze test

Morris water maze is one of the most widely used tasks for testing spatial learning and memory in rodents and the procedure used in the present experiment was a modification. In this test, water maze consists of a circular pool (1.6 m diameter and 50 cm height) colored with black nontoxic dye filled to a depth of 44 cm with water. The temperature in pool was maintained at $25 \pm 1^\circ\text{C}$. Four equally spaced points around the edge of the pool were designed as North (N), East (E), South (S) and West (W). A black colored round platform of 9 cm diameter was placed 1 cm below the surface of water. The rats were trained to navigate the submerged platform. The rats were given a maximum time of 120 s (cut-off time) to find the hidden platform and were allowed to stay on it for 30 s. The platform remained in the same position during the training days. Rats that failed to locate the platform within 120 s were put on the platform only in the first session. The animals were given a daily session of five trials. Escape latency time to reach the platform was recorded in each trial.

2.6. Y maze

The behavioral test was conducted in a large quiet room. Y-maze apparatus was used to assess the animals' spatial memory. A stop watch was used to score the behaviors and all events were observed manually. A Y-maze is made up of three equally spaced arms, labeled as A, B, and C which are 120° from each other, 41 cm long and 15 cm high. It was used to assess the spontaneous alternation in the rats. The floor of

the apparatus is 5 cm wide and is leveled with saw shaves. Each rat was stationed in one of the arms and allowed to freely explore the apparatus. The sequence or consecutive entrance of the animals into the arms is termed an alternation.

The total number of arms entered minus two is termed spontaneous alternations, and the percentage alternation was calculated as $\{(\text{actual alternations}/\text{maximum alternations}) \times 100\}$. 5 min was assigned as the test time limit for each of the animals in the Y-maze apparatus.

Recorded data is the total arm entries indicate the total number of a single arm entered (e.g. ABCBCABACBC, contain 11 entries), from which the correct and wrong alternations are recorded.

2.7. Histopathology

The rats were anesthetized 24 h after the behavioral tests, decapitated and their brains were excised and stored in 4% paraformaldehyde preparation. Then, the brains were processed for histo-architectural examination, embedded in paraffin, refrigerated, then the brains were sliced coronally into 8 μm sections (from Bregma 2–4 mm of frontal cortex, –2.5 mm to –4.5 mm of the hippocampal formation and –10 mm to –15 mm of the cerebellar cortex) with a rotary microtome (MK 1110) and the sections were stained with Hematoxylin and Eosine (H&E) for general cytoarchitecture and Cresyl fast violet (CFV) for nissl granulation in accordance with routine laboratory procedures (Bancroft & Gamble, 2008). Representative photomicrograph of each section was produced using an Olympus BX 51 microscope and a DP 12 digital camera.

2.8. Statistical analysis

In this investigation, the results were expressed as mean $\pm$ standard deviation ($n = 6$). The data from Morris water maze and Y maze tests were analyzed statistically by one way analysis of variance (One-way ANOVA) and different group means were compared by Bonferroni's test. $p < 0.05$ was considered statistically significant in all cases. The Graph Pad Prism was used for analysis and graphical representation of data.

3. Results

3.1. Cognitive functions

Scopolamine significantly ($p < 0.05$) impaired hippocampal and frontal-dependent memory tasks, causing a delayed latency to find the escape platform in the MWM paradigm (Fig. 1) and lowered percentage alternation in the Y maze paradigm. From the first (LTM test) and subsequent (STM test) exposure to the MWM paradigm, it was observed that NSO treated rats showed a shorter latency time than saline and scopolamine treated rats ($p < 0.05$, Figs. 1 and 2).

The potential effect of BSO as anti-amnesic agent shows that as a single administration and as intervention following scopolamine significantly ($p < 0.05$) reduced the latency to find the platform (Figs. 1 and 2). Therefore, rats treated with BSO after scopolamine for 14 days had reversed amnesia

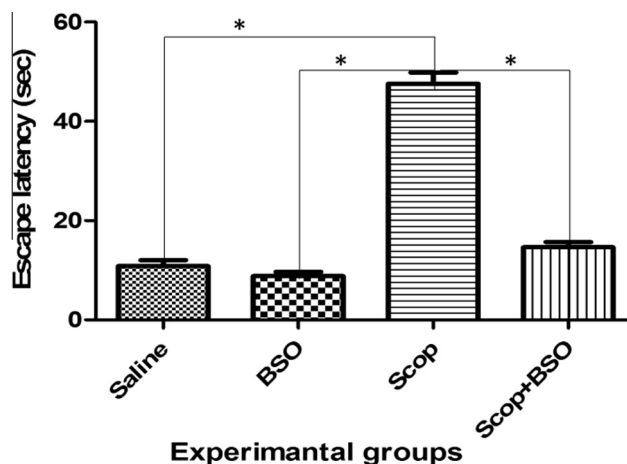


Figure 1 Effects of BSO scopolamine and Scop + BSO on escape latency (LTM) in the Morris water maze paradigm. Data presented as mean $\pm$ SEM, asterisks (*) indicate significant ($p < 0.05$) difference from the scopolamine treated rats.

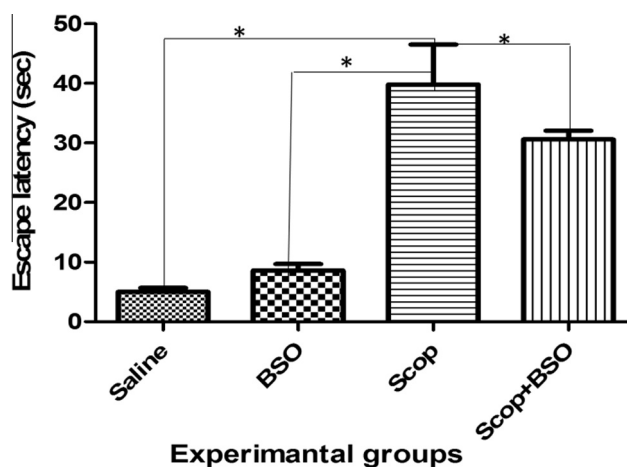


Figure 2 Effects of BSO scopolamine and Scop + BSO on escape latency (STM) in the Morris water maze paradigm. Data presented as mean $\pm$ SEM, asterisks (*) indicate significant ($p < 0.05$) difference from the scopolamine treated rats.

induced by scopolamine. Hence oral administration of 1 ml/kg/b wt of BSO can reverse ($p < 0.05$) the memory impairment induced by scopolamine treatment.

Scopolamine also caused a significant ($p < 0.05$) reduction in the percentage alternation in the Y maze paradigm, an alternation dependent effect, which was significantly ($p < 0.05$) reversed by BSO with increased percentage alternation suggesting its anti-amnesic efficacy against scopolamine induced amnesia (Fig. 3).

3.2. Histopathological results

Sections of the Frontal cortices (FC) of the animals treated with scopolamine, specifically the pyramidal cells at the internal pyramidal cell layer shows some degree of retraction of processes, vacuolation of the surrounding neuropil of the

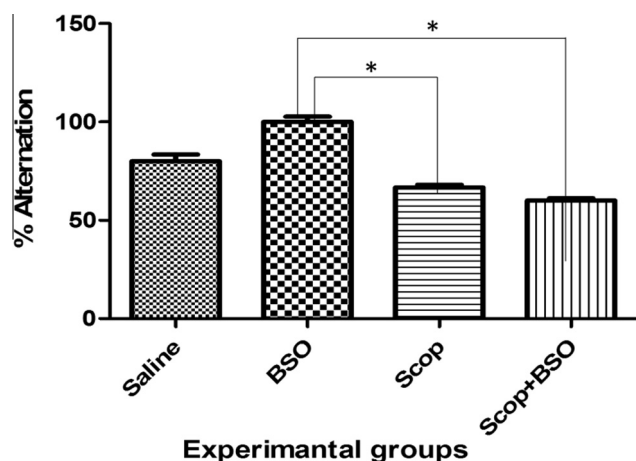


Figure 3 Effects of BSO scopolamine and Scop + BSO on % Alternation (working memory) tasks in the Y maze paradigm. Data presented as mean $\pm$ SEM, asterisks (*) indicate significant ($p < 0.05$) difference from the scopolamine and Scop + BSO treated rats.

pyramidal cells and hyperchromatic and shrunken perikarya (Fig. 4). Also corkscrew-shaped apical dendrites and blending of the membrane into the perikaryal cytoplasm are observed (Fig. 4). BSO was observed in this study to enhance axonal-dendritic connections and also reduce the scopolamine induced vacuolation to nearly normal when compared with the control (Fig. 4). Scopolamine also caused irregular distributions of nissl granulation in the perikaryal, increased dark picnotic nuclei and selective staining of nissl granules (tigroid bodies and nissl bodies), the presence of dark neuron, glial activation and dendritic cytoplasm suggesting protein denaturing and

neural degeneration in the hippocampus, dentate gyrus and frontal cortex (Figs. 8–11). BSO reversed this pathologies to almost normal when compared with the control.

The hippocampus is made up of Cornu Ammonis CA1 and CA2 formed of zone of small pyramidal cells, CA3 and CA4 formed of zone of large pyramidal cells. CA4 projects into the dentate gyrus, made of small granule cells. The neuronal processes (axons and dendrites), glial cells and scattered nerve cells are found in areas between the compact zones of the cells. BSO was able to repair the damages induced by scopolamine exposure, including disorganization and loss of small pyramidal cells (CA1), some of which had pale nuclei while others were dark (Fig. 5). There was also a marked shrinkage in the size of the large pyramidal cells (CA3), the outer layer was more affected, with darkened nuclei and clumped processes (Fig. 6). Some darkened, pyknotic nuclei are observed in the granular cells of the dentate gyrus with contrasting basophilia of some other granular cells nuclei, also marked vacuolation and excess glial cells, effects ameliorated by BSO are observed (Figs. 7–11).

4. Discussion

Scopolamine has been implicated to impair learning and memory in human and rats, as such it is considered to be a good model of AD.²⁵ Scopolamine, a nonselective muscarinic antagonist block cholinergic signaling and produce memory and cognitive dysfunctions and subsequently causes learning and memory deficits including long-term and short term memory impairments.^{26,27} It is thus implicated to exert amnesic effect in rats and therefore an important rat model of cognition.^{28,29}

In this study scopolamine induced dementia model assesses memory function by the MWM and Y maze paradigms.

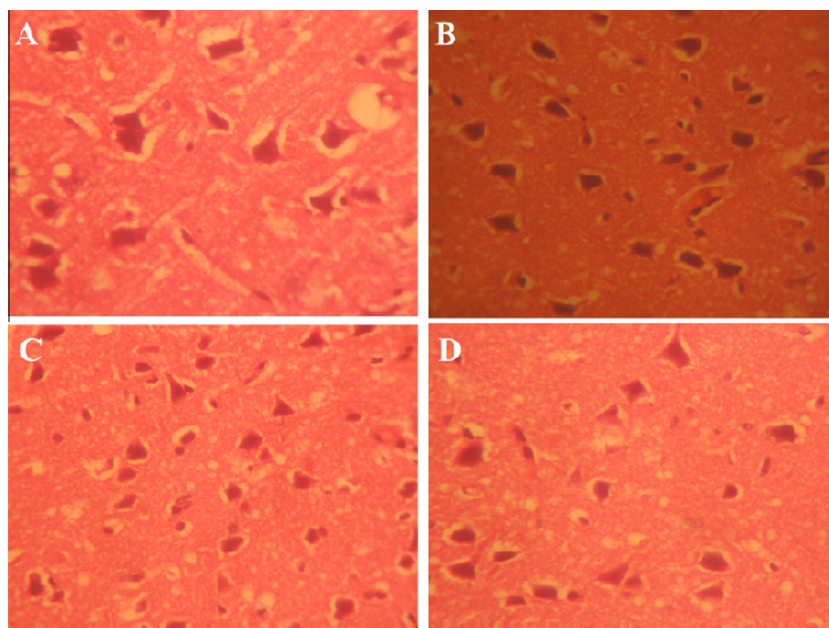


Figure 4 Effects of scopolamine, BSO and Scop + BSO on the Frontal cortices of rats: (A) and (B) normal and unaffected cytology of the pyramidal neuron, with a well outlined soma and unaffected projections; (C) retraction of processes, vacuolation of the surrounding neuropil of the pyramidal cells, shrunken perikarya and blended membrane into the perikaryal cytoplasm; (D) marked regeneration, reduced vacuolation and distinct processes with less blending in the perikarya cytoplasm. (H&E $\times 400$).

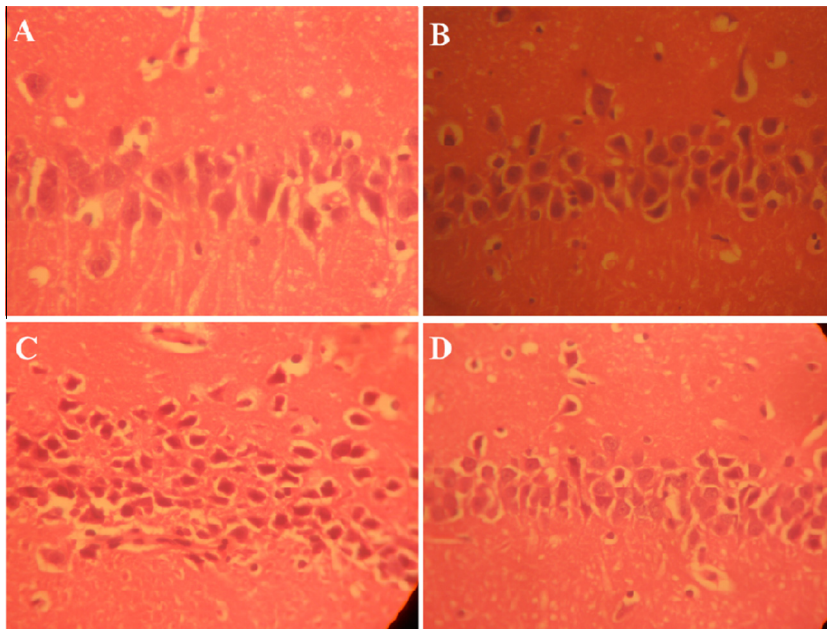


Figure 5 Effects of scopolamine, BSO and Scop + BSO on the hippocampi CA1 of rats: (A) and (B) normal and unaffected cytology of the small pyramidal neurons with well outlined soma and unaffected projections; (C) vacuolation in the surrounding neuropil of the pyramidal cells, shrunken perikarya and blended membrane into the perikaryal cytoplasm. (D) Marked regeneration, reduced vacuolation and neuropil spaces. (H&E $\times 400$).

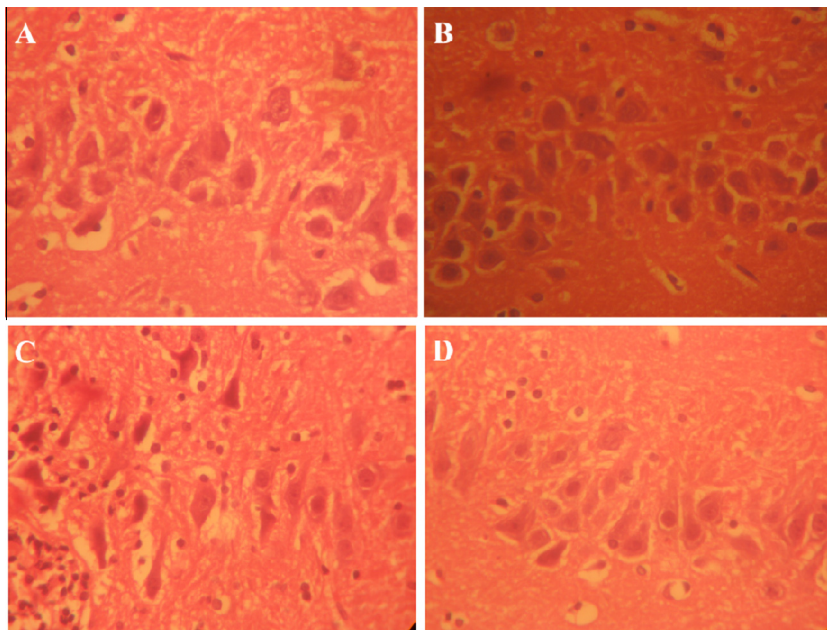


Figure 6 Effects of scopolamine, BSO and Scop + BSO on the hippocampi CA3 of rats: (A) and (B) normal and unaffected cytology of the large pyramidal neurons (CA3) with outlined soma and unaffected projections; (C) shrunken perikarya, blended membrane into the perikaryal cytoplasm and glia activation. (D) Reduced vacuolation and distinct regenerations in the processes. (H&E $\times 400$).

Scopolamine treatment delayed time of latency to find the hidden escape platform submerged in the water in the first and the subsequent exposures after training, indicating impairment of Long Term Memory (LTM) and Short Term Memory (STM) respectively. BSO in this study improved hippocampal-dependent memory tasks and was able to reverse

the cognitive impairment induced by scopolamine by reducing latency period, in both LTM and STM tasks in the MWM paradigm.

These reported activities of BSO can be supported by various workers who also reported BSO reduced escape latency, distance traveled to reach the platform and increased time

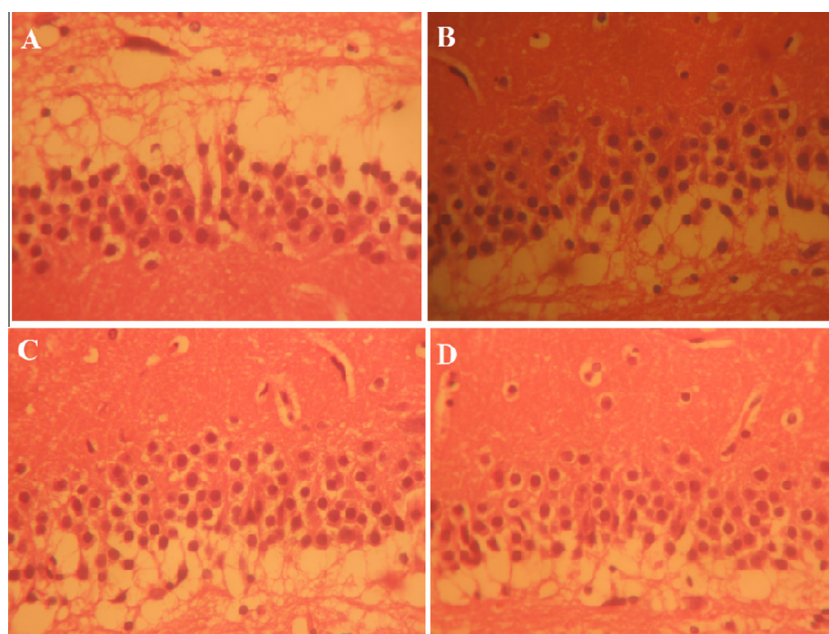


Figure 7 Effects of scopolamine, BSO and Scop + BSO on the dentate gyrus of rats: (A) and (B) normal cytology of the dentate granule cells with well outlined soma; (C) Dense eosinophilia of the granular cells, with hyperchromatic pykaryon. (D) Marked regeneration with reduced hyperchromicity (H&E $\times 400$).

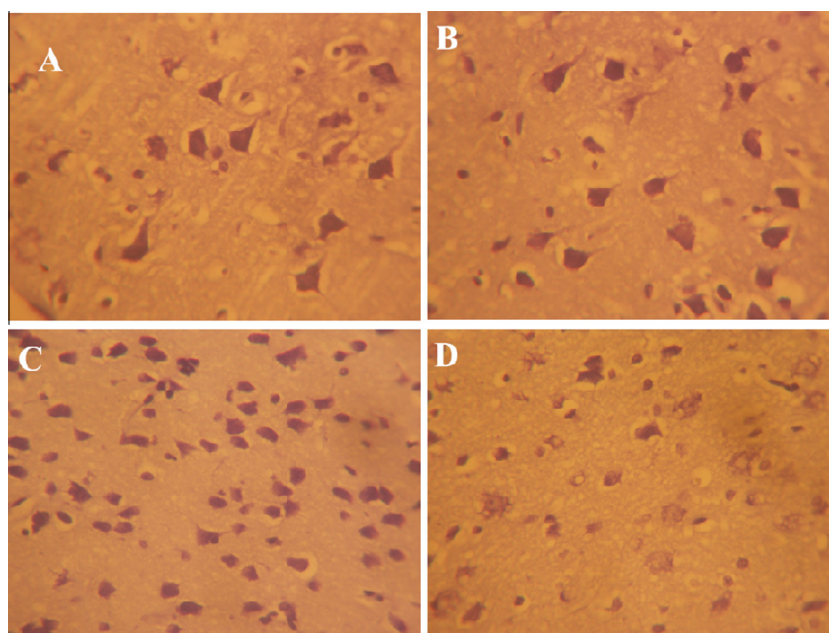


Figure 8 Effects of scopolamine, BSO and Scop + BSO on the Frontal cortices of rats: (A) and (B) normal nissl distributions in the soma and unaffected proximal dendrites of the frontal pyramidal neuron (FC); (C) contrasting stains, pyknotic and darkly stained cell body, presence of dark neurons (D) regeneration, reduced dark neurons (CFV $\times 400$).

spent in the target quadrant, in a MWM paradigm³⁰ and reversed cognitive and novel object recognition induced by scopolamine.³¹ Also, the report of Bin Sayeed and colleagues of the possible modulating impact of BSO on memory, attention, and cognition,^{32,33} memory improvement after scopolamine induced amnesia³⁴ and reversed memory impairment observed in the MWM following pentylenetetrazole (PTZ) –

induced repeated seizures in rats,³⁵ further strengthened the enhanced hippocampal dependent working memory observed in the present study. BSO anti Alzheimer's potential has been reported to improve MWM test scores.³⁶

Spontaneous alteration behavior (SAB) is a viable measure of memory.³⁷ BSO from this study significantly increased percentage alternation when given only and/or after scopolamine,

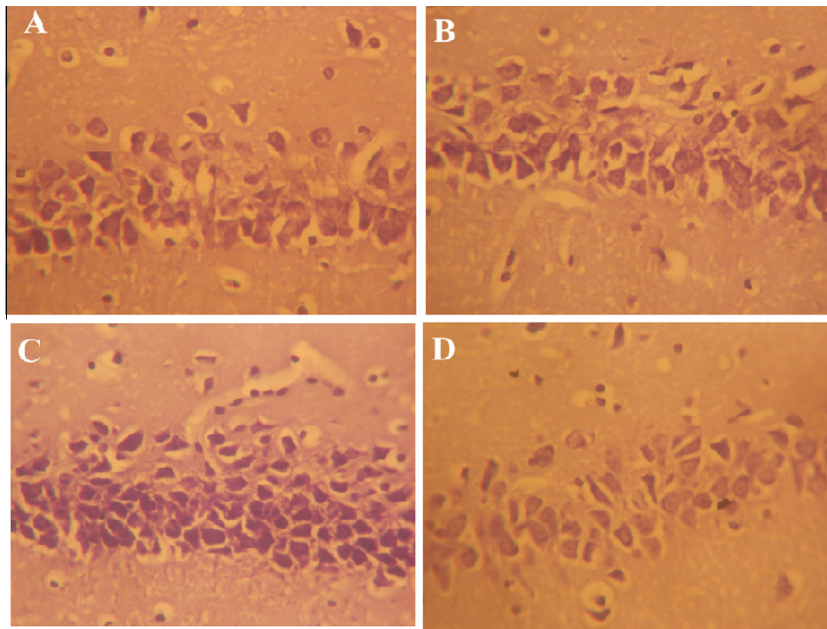


Figure 9 Effects of scopolamine, BSO and Scop + BSO on the hippocampi CA1 of rats: (A) and (B) normal and unaffected nissl granulation in the small pyramidal neuron (CA1); (C) pyknotic and darkly stained cell body, presence of dark neurons and vacuolation in the surrounding neuropil; (D) reduced pyknoticity and disappearance of dark neurons (CFV $\times 400$).

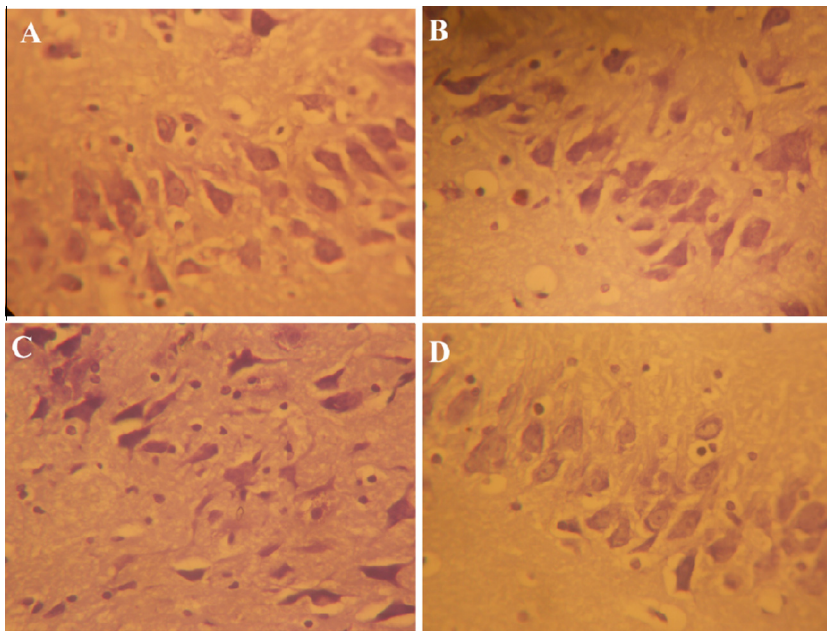


Figure 10 Effects of scopolamine, BSO and Scop + BSO on the hippocampi CA3: (A) and (B) normal nissl granulation in the large pyramidal neurons (CA3); (C) darkly stained cell body, vacuolation in the surrounding neuropil and shrunken perikarya; (D) marked regeneration, less shrunken cells and contrasting soma stain (CFV $\times 400$).

thus confirming its anti-amnesic efficacy and its nootropic property. This effect is strengthened by the report of Rashid and colleagues where a similar increase in percentage alternation was reported of thymoquinone, the active component of BSO against scopolamine³⁸ also its enhancing effect on spatial working memory performance on the radial arm maze.³⁹

The observed effects of BSO in rescuing the deficits in hippocampal neurons following scopolamine insults in the small and large pyramidal cells of the CA1 and CA3 regions, and the observed recovery in the dentate gyral granular cells are strengthened by previous workers who reported that BSO improved neuronal cell viability, protect against

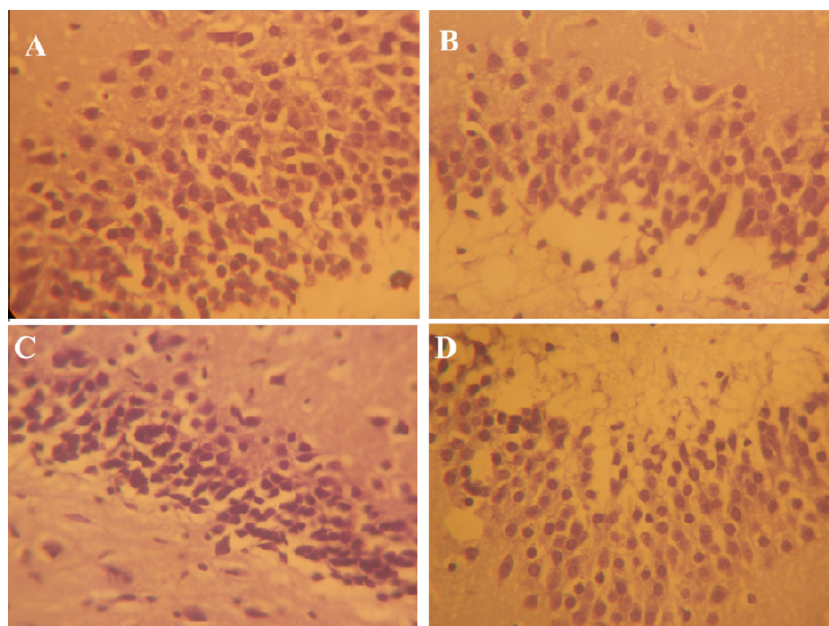


Figure 11 Effects of scopolamine, BSO and Scop + BSO on the dentate gyrus of rats: (A) and (B) normal nissl granulation in the granule cells; (C) pyknotic and darkly stained cell body; (D) marked regeneration, reduced vacuolation and distinct processes with less blending in the perikarya cytoplasm (CFV $\times 400$).

beta-amyloid protein intoxication,^{40,41} protect against cerebral ischemia reperfusion injury in the hippocampus,⁴² hippocampal neurodegeneration⁴² and prevented hippocampal neuron cell death in global cerebral ischemia.⁴³ This study also evidenced the previous wide use of BSO in neurodegenerative diseases like Parkinson and Alzheimer due to its antioxidant potential,^{44–46} a property linked to the presence of thymoquinone which have the potential to prevent neuronal cell death.⁴⁷

5. Conclusion

In this study, black seed oil only improved percentage alternation, reduced escape latencies and ameliorated the delayed latency induced by scopolamine but not alternation. It also improved cortico-hippocampal granulation and histoarchitecture. Hence, BSO could be a potential supplementary in the managements of memory dysfunctions, due to its effects on working memory, hippocampal dependent memory and cortico-hippocampal neuron, against scopolamine induced memory dysfunctions in rats. Exploring further its anti-inflammatory, antioxidant and anti-amnesic efficacy as a potent novel supplements in neurodegenerative diseases.

Conflict of interest

None declared.

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