American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Evaluate the Protective Effect of Bromelain against Scopolamine-Induced Histological Lesions in the Brain Tissues of Albino Rats

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Received: 2024, 28, Apr
Accepted: 2025, 29, May
Published: 2025, 30, Jun

Abstract: The present study aimed to evaluate the protective effect of bromelain against scopolamine-induced histological lesions in the brain tissues of albino rats. For the study, 25 adult female rats were used, which were purchased from the animal house at the Veterinary Medicine College of Tikrit University. Between 10 and 12 weeks of age, the animals weighed an average of 207 grams, with a range of 181 to 204 grams. The experiment was carried out at the animal home of the College of Veterinary Medicine at Tikrit University between August 29, 2024, and September 11, 2024. the results showed in Scopolamine group, the cortex of brain had containing great cavities with diffused Internal pyramidal cells which were surrounded by vacuolar degeneration around its nuclei. the fine micro-blood vessels or capillaries were surrounded by WBCs. in the third group, the internal pyramidal cell layer was enriched with medium sized and small

sized of neurons, few glial cell were demonstrated among the pyramidal cell, micro-blood capillaries were detected in the cortex. in the fourth group, the brain cortex was surrounded by pial membrane of meninges with presence of meningeal blood vessels at the periphery of cortex. the molecular layer was outer most which was formed by few small pyramidal cell surrounded by fine foamy nerve facicle, the external granular layer was containing small medium sized pyramidal cells. in fifth group, the brain cortex was surrounded by meningeal arteries which had blood clot and thick wall, the molecular layer had few pyramidal cell, surrounded with foamy appearance of nerve bundle. It is concluding the effective role of bromelain in improving brain tissue, as it reduces tissue lesions and lymphocyte infiltration caused by scopolamine.

Keywords: bromelain, brain, scopolamine, Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a progressive neurological illness that impairs language, learning, memory, visual-spatial skills, thinking, and conduct, among other cognitive functions. Cognitive abilities can deteriorate to the point that they become disruptive to day-to-day activities. With AD accounting for at least two-thirds of dementia cases in people 65 and older, it is the most common kind of dementia (1). AD was defined as an asymptomatic biological process with AD neuropathologic changes (ADNPC) in the most current version from 2024. As the neuropathologic load rises, AD is said to advance to clinical symptoms (2). The muscarinic acetylcholine receptor is blocked by the muscarinic receptor antagonist scopamine, which also causes electrophysiological abnormalities and temporary cognitive forgetfulness that are similar to those seen in Alzheimer's disease (AD) (3). In fact, a number of investigations have examined neurophysiological alterations linked to scopolamine injection that resemble those seen in AD. Quantitative electroencephalogram resting state investigations have revealed enhanced delta and theta activity and decreased alpha and beta band power following scopolamine treatment. Furthermore, research employing coherence in the resting state has demonstrated a decline in this metric following scopolamine (4). Several studies have demonstrated a decrease in FC among various cortical areas in the alpha and beta bands and an increase in slow band (i.e., delta band)

connection in AD when applying functional connectivity to electrophysiological data (5). To control symptoms, delay the progression of the disease, and enhance the quality of life for those who are impacted, a number of therapeutic and preventive techniques (also known as prevention measures) have been developed. The use of secondary bioactive metabolites (SBMs), specifically polyphenols, anthocyanins, terpenoids, alkaloids, sulforaphane, glucosinolates, vitamins, polyunsaturated fatty acids (PUFA), and fibers—found in fruits, vegetables, grains, and other plant-based foods—in the prevention of NDDs is a growing area of interest in this field (6,7,8). Numerous advantageous applications for bromelain as a phytomedical molecule have been demonstrated (9,10). Bromelain has been shown to promote recovery following drug overdose and surgery (11). Apart from its therapeutic use, bromelain has been used by numerous other businesses, including the food and beverage, apparel, cosmetic, and pharmaceutical sectors (11,12,13). In the treatment of autoimmune diseases, allergies, and inflammation, bromelain offers therapeutic advantages (12,13). The present study aimed to evaluate the protective effect of bromelain against scopolamine-induced histological lesions in the brain tissues of rats.

Materials & Methods

Scopolamine

Scopolamine drug that used in the present experiment was USA (Perrigo company) to induced Alzheimer's disease in albino mice.

The animals

For the study, 25 adult female rats were used, which were purchased from the animal house at the Veterinary Medicine College of Tikrit University. Between 10 and 12 weeks of age, the animals weighed an average of 207 grams, with a range of 181 to 204 grams. The experiment was carried out at the animal home of the College of Veterinary Medicine at Tikrit University between August 29, 2024, and September 11, 2024.

Animal grouping

The rats utilized in this experiment were between the ages of 10 and 12 weeks, and at the start of the trial, their average body weight was 207 grams. The animals were split up into five major groups, each of which had five adult females:

1. The first group (negative control): received normal saline only for 14 days.
2. The second group (positive control): received Scopolamine 0.02 mg/kg (intra peritoneal) during 8–14 days.
3. The third group (Bromelain → Scopolamine + Donepezil): Pre-treated with Bromelain from 1–7 days, then from 8–14 days received scopolamine 0.02 mg/kg + donepezil 4.5 mg/kg concurrently.
4. The fourth group (Bromelain → Scopolamine + Bromelain): Pre-treated with Bromelain from 1–7 days, then from 8–14 days, received Scopolamine 0.02 mg/kg + Bromelain 3 mg/kg concurrently.
5. The fifth group: Pre-treated with Bromelain from 1–7 days, then from 8–14 days, Scopolamine 0.02 mg/kg + combination of Bromelain + Donepezil (5 mg/kg) concurrently.

Histology processing

Rat brain were taken, preserved with 10% formalin, paraffin-processed, sliced using a rotary microtome to a thickness of six micrometers, and stained with Hematoxylin and Eosin (H&E) histological stains [14]. Through the use of an Optica microscope (Italy), sections were investigated.

Results & Discussion

Control group

The histological examination showed that the brain cortex was covered by the pial meninges, the outer layer of cortex was the molecular layer which was formed by few small nerve cell, surrounded by nerve fibers. the external granular layer had multiple pyramidal cell with glial cell, then the outer pyramidal cell with larger pyramidal cell associated with supporting cell (glial cell) and preserve of micro-blood capillaries extended toward the internal granular layer, Internal pyramidal cell layer nearby the medulla (fig: 1).

Scopolamine group

The cortex of brain had containing great cavities with diffused Internal pyramidal cells which were surrounded by vacuolar degeneration around its nuclei. the fine micro-blood vessels or capillaries were surrounded by WBCs (fig: 2). The polymorphic layer was containing great and medium sized pyramidal cells which surrounded by vacuolar cytoplasmic degeneration, certain glial cell was present among the nerve pyramidal cell, the blood capillaries were surrounded by WBCs (fig: 3).

Third group

The internal pyramidal cell layer was enriched with medium sized and small sized of neurons, few glial cell were demonstrated among the pyramidal cell, micro-blood capillaries were detected in the cortex, those cell and capillaries were surrounded with bundle or nerve fascile (fig: 4).

Fourth group

The microscopical examination showed that the brain cortex was surrounded by pial membrane of meninges with presence of meningeal blood vessels at the periphery of cortex. the molecular layer was outer most which was formed by few small pyramidal cell surrounded by fine foamy nerve facicle, the external granular layer was containing small medium sized pyramidal cells (fig: 5).

Fifth group

The brain cortex was surrounded by meningeal arteries which had blood clot and thick wall, the molecular layer had few pyramidal cell, surrounded with foamy appearance of nerve bundle or facicle, the iner most layers of cortex was containing great size of pyramidal cells with glial cella around of most of them, perinuclear transparent zone was demonstrated in large pyramidal cell of polymorphic layer (fig: 6).

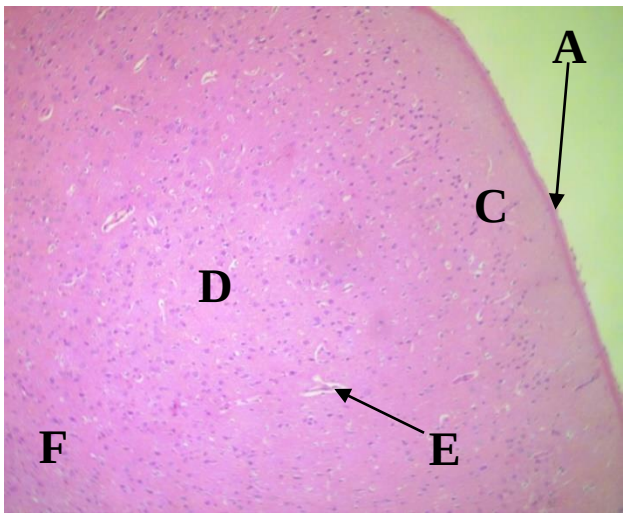

Figure (1): brain cortex, pial meningeal membrane (A), molecular layer (B), external granular layer (C), external pyramidal layer with pyramidal cell (D), micro-blood capillaries (E) , internal granular layer with nerve cell and glial cell (F) (H&E X10).

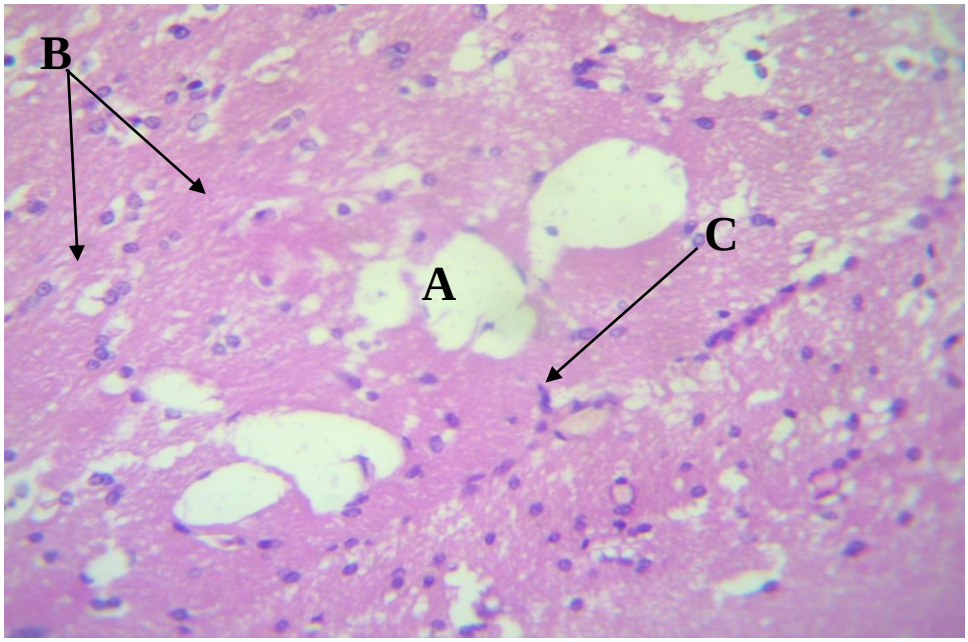

Figure (2): cortex of brain, great cavities (A), pyramidal nerve cells surrounded with vascular degeneration of cytoplasm of nerve cell (B), micro-blood capillaries with WBCs(C) (H&E X40).

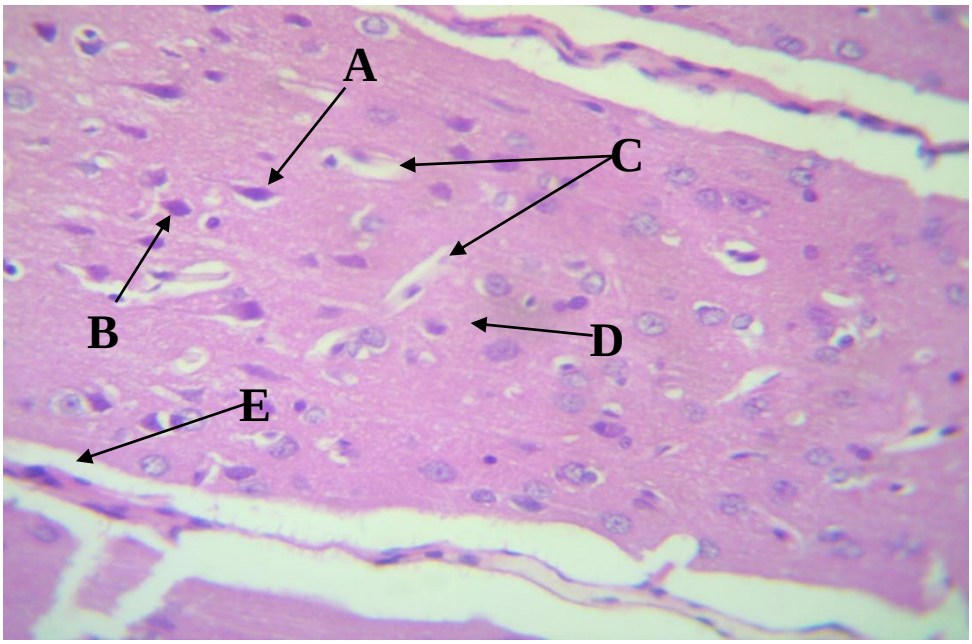

Figure (3): Brain cortex with polymorphic layer, great pyramidal cell (A), medium size (B), vacuolar cytoplasmic degeneration (C), glial cells (D), Blood capillaries surrounded with WBCs(E) (H&E X40).

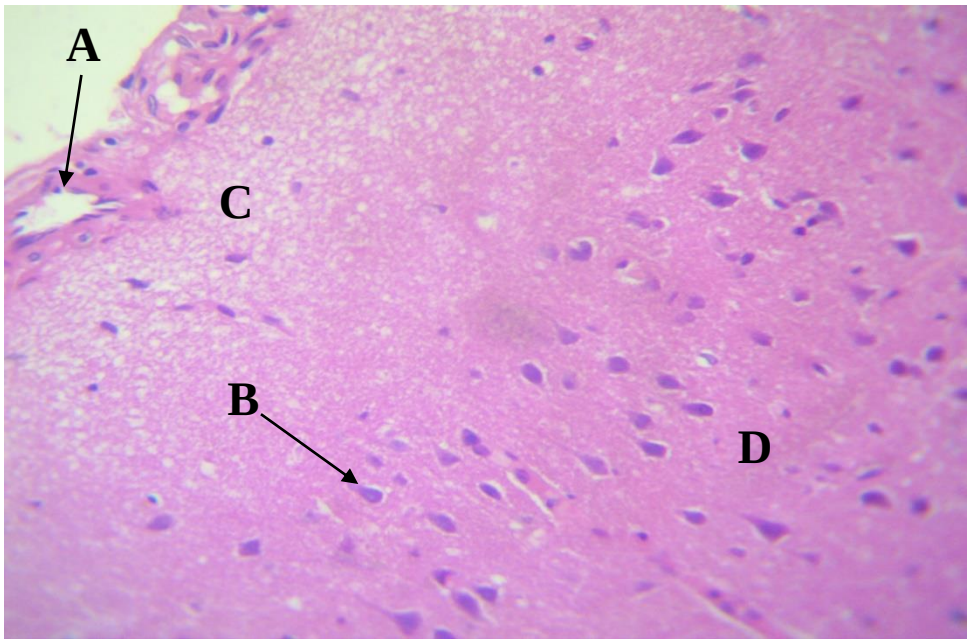

Figure (4-7): Brain cortex, meningeal blood vessels (A), molecular layer with few small pyramidal cells (B), foamy appearance of nerve fibers (C), external granular layer (D) (H&E X10).

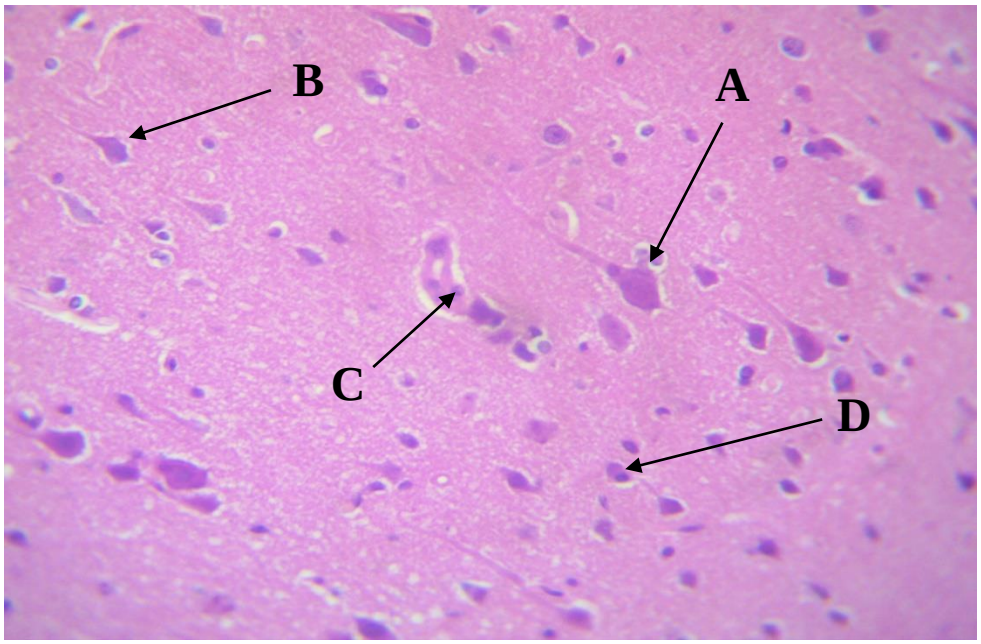

Figure (5): Brain cortex, internalpyramidal layer with large pyramidal cells (A), medium sized pyramidal cells (B), Blood capillaries with protoplasmic (C), glial cell(D) (H&E X10).

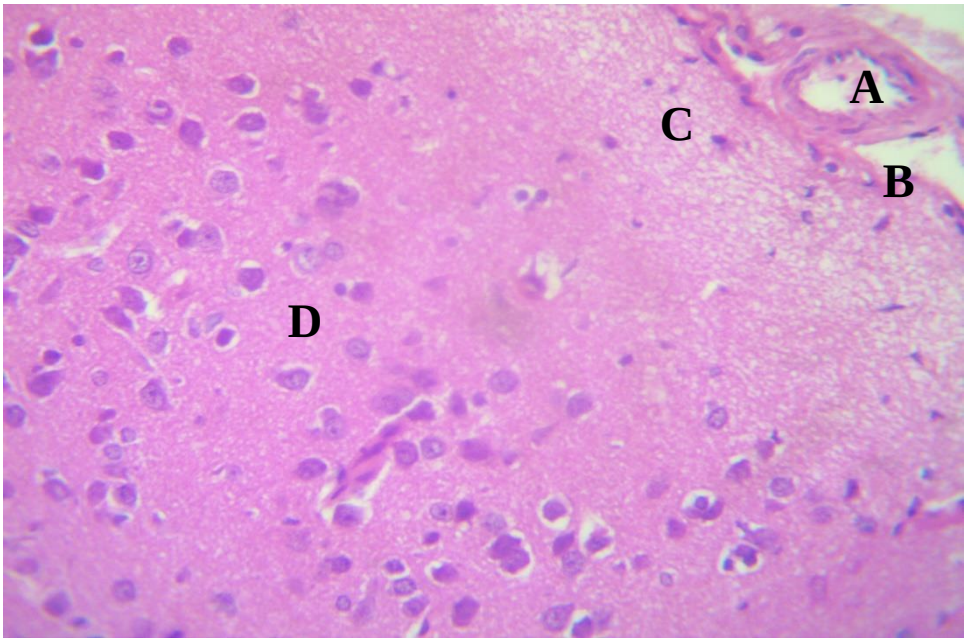

Figure (6): brain cortex, meningeal artery (A), pial meningeal membrane (B), moleculr artery with few nerve cells (C), external granular layer (D), external pyramidal layer with many pyramidal cells (E) (H&E X40).

The results from the current study revealed that histopathological sections of brain tissue from mice induced with scopolamine for 2 weeks showed microscopically changes. Also, findings indicated the validity of AD animal model created in the current study. Similar to those reported in human AD brain tissue. The similarities between human and mouse AD brain tissues obtained in the current study support the validity of the animal model created in the current study and, therefore, suggest that its results can be applied to humans with AD (14). The results of the current study are consistent with the study of Istifo et al. (15) who injected scopolamine intraperitoneally into mice to detect its effects on brain tissue. After examining the brain tissue, they found various histological lesions including multifocal severe deposition of amyloid beta plaques, Granulovacuolar Degeneration, and Hirano Bodies. They indicated that Scopolamine causes cholinergic neuronal damage in the hippocampus by enhancing DNA damage and inhibiting the mRNA expression of many genes encoding neuronal factors that are crucial for cell survival as well as increasing oxidative stress by enhancing lipid peroxidation and decreasing the antioxidant system capacity. On the other hand, Bin-Yoon et al. (16) found in their study, which was designed to reveal the effect of scopolamine on brain tissue in mice, that scopolamine administration and after the study period, a group of different tissue lesions were observed, including neuronal degeneration, apoptosis, and vascular congestion. They indicated that the reason for these tissue lesions is that scopolamine causes oxidative stress in brain tissue, increases free radical levels, and reduces antioxidant enzyme levels, which in turn causes damage to nucleic acids in nerve cells. This is consistent with the results of the current study. In the present study, both bromelain and donepezil were found to have a positive effect against the harmful effects of scopalamine on brain tissues. The current study's findings concurred with a study by Kumar et al. (1) that identified the function of bromelain as a bioactive substance derived from pineapple. When AlCl3 and D-galactose were administered intraperitoneally together for 90 days, albino mice experienced neurotoxicity, memory problems, and cognitive impairment. Nonetheless, the effects of AlCl3 and D-galactose-induced AD animals were significantly reversed after 30 days of intraperitoneal administration of bromelain, donepezil, and bromelain plus donepezil remedies. Hematoxylin and eosin staining of the hippocampus and cerebral cortex revealed hyperchromatic nuclei and eosinophilic lesions in AD animals; however, Brm L, Brm H, Dnpz, and Brm L + Dnpz therapies

reversed these neurodegenerative effects. According to our findings, bromelain enhances cholinergic activity and synaptic plasticity, decreases oxidative damage, neuroinflammation, Aβ 1-42 aggregations, and histopathological damage, and prevents AlCl3 and D-galactose-induced spatial learning and memory deficits and cognitive impairment when taken alone or in combination with donepezil.

Conclusions

The current study concludes the effective role of bromelain in improving brain tissue, as there is a decrease in tissue lesions and lymphocyte infiltration caused by scopolamine. This is due to the role of bromelain as an antioxidant and anti-inflammatory.

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