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Revealing the effect of bromelain and donepezil against scopolamine-induced behavioral and histological changes in the brain

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ABSTRACT

Background & aim: Alzheimer's disease is a progressive neurodegenerative disorder characterized by memory impairment and cognitive dysfunction. Scopolamine is commonly used to induce Alzheimer's-like changes in experimental animals. Therefore, the present study aimed to evaluate the protective effects of bromelain and donepezil against scopolamine-induced behavioral and histopathological alterations in the brain of adult female rats.
Materials and Methods: Twenty-five adult female rats aged 10–12 weeks and weighing 181–204 g were used in this study. The experiment was conducted at the Animal House, College of Veterinary Medicine, Tikrit University, from August 29 to September 11, 2024. The rats were randomly divided into five groups. The control group received normal saline for 14 days. The scopolamine group received 0.02 mg/kg intraperitoneally from days 8–14. The remaining groups were pretreated with bromelain for 7 days, followed by concurrent administration of scopolamine with donepezil, bromelain, or their combination during days 8–14.
Results: Behavioral assessment using the T-maze test demonstrated that scopolamine-treated rats exhibited marked cognitive impairment and reduced exploratory behavior. In contrast, rats treated with bromelain and/or donepezil showed significant improvement in maze performance, exploratory activity, and cognitive function, with the combination-treated group exhibiting the greatest improvement. Histological examination of the scopolamine group revealed severe neuronal degeneration, large cavities in the brain medulla, hyperplastic glial cells, vascular congestion, vacuolation, and inflammatory cell infiltration. Treatment with bromelain or donepezil markedly preserved neuronal architecture, reduced vacuolar degeneration and inflammatory infiltration, and restored the normal organization of the pyramidal cell layers. The combined treatment produced the most pronounced histological improvement.
Conclusions: It is concluded that bromelain exerts a neuroprotective effect against scopolamine-induced behavioral and histopathological changes. Bromelain, particularly when combined with donepezil, improves cognitive performance, preserves brain tissue integrity, and reduces neuronal degeneration and inflammatory responses, suggesting its potential as an adjunctive therapeutic agent for Alzheimer 'disease.

Introduction

Scopolamine, a muscarinic receptor antagonist, produces a blocking of the activity of the muscarinic acetylcholine receptor, and the concomitant appearance of transient cognitive amnesia and electrophysiological changes, which resemble those observed in Alzheimer's Disease (AD) [1]. Indeed, to date, several studies have explored neurophysiological changes associated with scopolamine injection mirroring those observed in AD. After scopolamine administration, quantitative electroencephalogram resting state studies have found decreased power in alpha and beta bands, and increased delta and theta activity. Additionally, studies using coherence during resting-state have shown a decrease in this measure after scopolamine [2]. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes memory loss and other cognitive impairments, such as deterioration in language, learning, memory, visual-spatial abilities, reasoning, and behavior. AD is the most prevalent form of dementia, contributing to at least two-thirds of dementia cases among individuals aged 65 and older [3]. In the most recent update in 2024, AD as described as beginning as an asymptomatic biological process with AD neuropathologic changes (ADNPC), progressing to clinical symptoms as the neuropathologic burden increases. Early-changing Core 1 biomarkers, such as amyloid PET, cerebrospinal fluid, and plasma biomarkers, reflect ADNPC and are sufficient for diagnosis and clinical decision-making. Later changing Core 2 biomarkers provide prognostic insights and increase confidence that AD is contributing to symptoms, with an integrated staging scheme accounting for factors like copathologies and cognitive reserve [4]. Indeed, Alzheimer's Disease prevalence increases significantly with age, and Alzheimer's Disease incidence increases from 2.8 per 1000 person years for people between 65 and 69years to 56.1 per 1000 person years for people who are older than 90 years [5]. various therapeutic and preventive strategies (theravention strategies) have been developed to manage symptoms, slow disease progression, and improve the quality of life for affected individuals [6, 7, 8]. Pineapple is an edible fruit bearing plant and is the most economically significant member in the family of Bromeliaceae [9]. Bromelain's potential therapeutic value stems from its biochemical and pharmacological properties [10]. bromelain was observed to enhance the permeability of brain blood barrier to nutrients [11]. This evidence could represent the therapeutic potential use in AD. Further, In vitro study, bromelain was found to degrade Aβ1–42 monomer and soluble aggregate in cerebrospinal fluids of AD patients that can be potential support for further evaluation of bromelain effects on Aβ1–42 monomer and soluble aggregate in an animal model of AD [12]. So, the present study aimed to reveal the effect of bromelain and donepezil against scopolamine-induced behavioral and histological changes in the brain.

As for 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.

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.

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 [13]. Through the use of an Optica microscope (Italy), sections were investigated.

Results & Discussion

Rat Behavioral Observations with Values

Group 1 - Normal Control

This group represents the normal, healthy rats that did not receive any treatment or Alzheimer's induction. Their behavior and movement patterns were typical, showing consistent exploration of the environment. They moved steadily and methodically, inspecting the maze or area without hesitation or confusion. Their cognitive and motor functions were intact, reflecting a baseline for comparison with other experimental groups.

Group 2 - Scopolamine-Induced Alzheimer's Model

In the T-maze test, the rats in group 2 (Alzheimer's-induced using scopolamine) exhibited noticeable cognitive deficits. Specifically, the animal remained stationary in the central arm of the maze without making any decision to enter either the right or left arm. This lack of exploratory behavior is a clear indication of impaired spatial working memory and decision-making ability. The rat appeared hesitant and displayed prolonged inactivity (freezing), which suggests a disruption in cognitive processing and behavioral motivation. Eventually, instead of choosing either of the goal arms, the rat turned back toward the starting direction, reflecting increased confusion and a possible inability to recall previous spatial cues. This behavior strongly supports the successful induction of Alzheimer's-like symptoms in this group.

Group 3 - Bromelain + Scopolamine + Donepezil

The rat explored the maze by looking around and moving forward, indicating normal exploratory behavior and supported cognitive performance. It entered \empty arm first, suggesting natural curiosity & environmental scanning. Then chose the correct arm with food, reflecting proper learning and memory retrieval through organized, goal-directed behavior.

Group 4 - Preventive & Therapeutic Bromelain

At the beginning of the test, the rat showed clear exploratory behavior, pausing to scan the environment before moving. It gradually advanced into the maze path with cautious but directed movement, examining corners and side paths. The rat did not appear confused or hesitant, suggesting a preserved level of spatial memory. By the end of the video, it correctly chose the arm containing food, avoiding the empty one - indicating that bromelain, administered both as a preventive and therapeutic agent, effectively helped preserve cognitive function and reduced the memory-impairing effects of scopolamine.

Group 5 - Bromelain prophylaxis + Scopolamine + Bromelain & Donepezil treatment

This group received bromelain as a preventive agent before the induction of Alzheimer's-like symptoms using scopolamine, followed by a combined treatment of bromelain and donepezil. The rats in this group exhibited noticeably higher levels of activity and faster movements compared to the other groups. They showed enhanced spatial awareness and navigated toward the target area more quickly and accurately. This suggests a potential synergistic effect between bromelain and donepezil in improving cognitive and motor functions, possibly due to enhanced cholinergic transmission and anti-inflammatory effects.

Histological study

Control group

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). The internal pyramidal layer was formed by medium sized pyramidal cells with glial cell and micro-blood capillaries (fig: 1).

Scopolamine group

The brain medulla was occupied with great cavities with detached nerve cell inside these cavities, these was poly or hyperplastic glial cells which were present in groups, the vacuoles around the blood vessels were engorged with WBCs (fig: 2).

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: 3).

Fourth group

The external pyramidal layer was arranged with small and medium size pyramidal cells with vacuolar zone around few member of nerve pyramidal cell. glial cell also demonstrated among the nerve cells, the blood capillaries were seen pass vertically from outer most zone of cortex toward the medulla and surrounded by protoplasmic astrocyte (fig: 4).

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: 5).

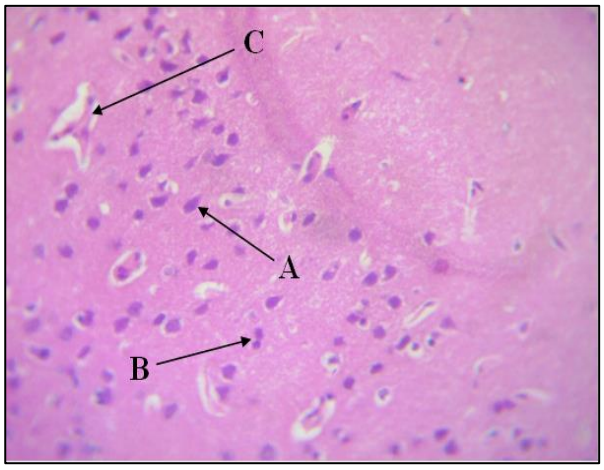

Figure (1): Brain cortex showing the internal pyramidal layer with medium-sized pyramidal cells (A), glial cells (B), and micro-blood capillaries (C) (H&E, ×40).

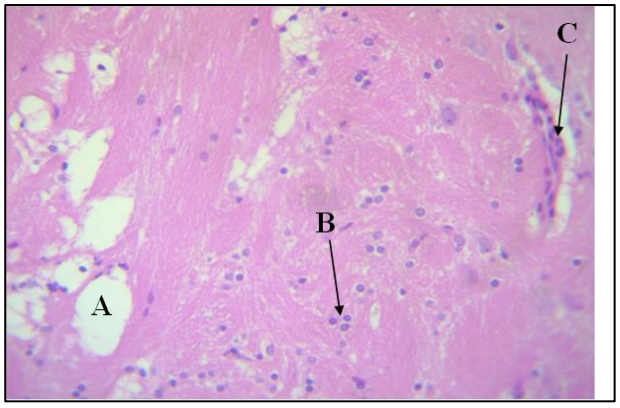

Figure (2): Brain medulla showing extensive cavitation with neuronal debris (A), glial cell hyperplasia (B), and blood capillaries surrounded by a vacuolar zone (C) (H&E, ×40).

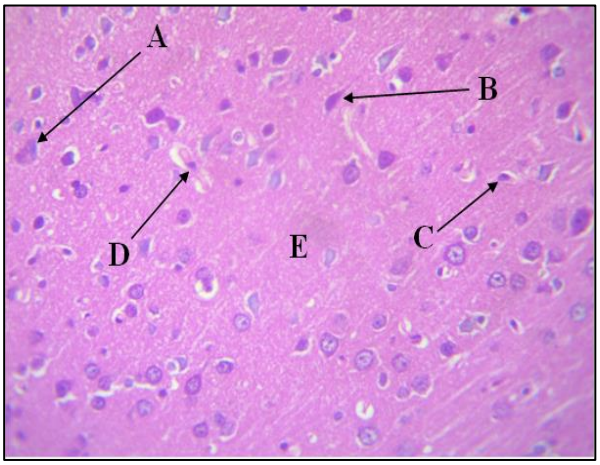

Figure (3): Brain cortex showing the internal pyramidal cell layer with medium-sized neurons (A), small neurons (B), glial cells (C), micro-blood capillaries (D), and nerve fascicles (E) (H&E, ×40).

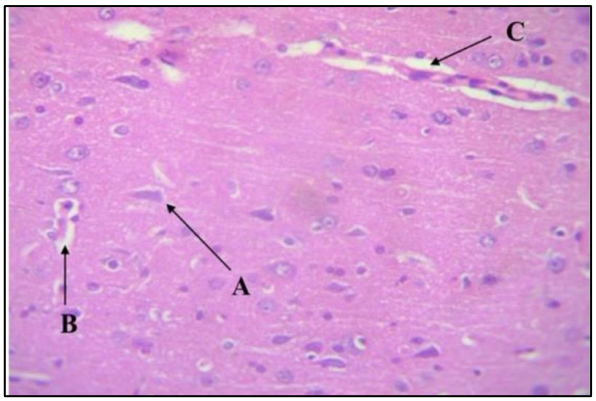

Figure 4. Brain cortex showing the external pyramidal layer with pyramidal cells (A), a vacuolar cytoplasmic zone (B), and a blood capillary associated with a protoplasmic astrocyte (C) (H&E, ×40).

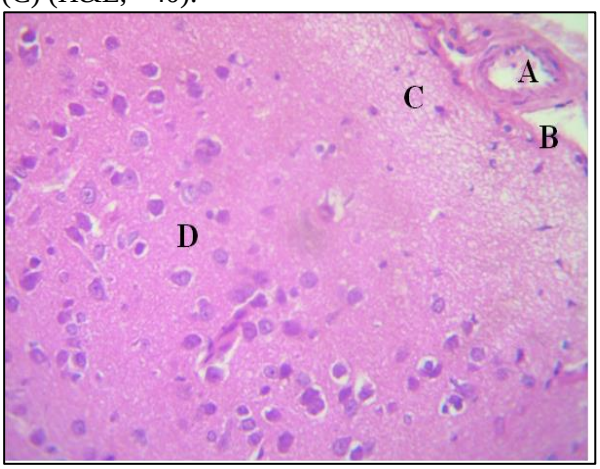

Figure (5): Brain cortex showing a meningeal artery (A), pial meningeal membrane (B), molecular layer with a few nerve cells (C), external granular layer (D), and external pyramidal layer containing numerous pyramidal cells (E) (H&E, ×40).

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 [13]. The results of the current study were also consistent with the study of Kim et al., [14] who indicated that scopolamine administration in rats led to the occurrence of many tissue lesions, including the degeneration of some nerve cells and the explosion of cytoplasm in some, along with degeneration in the blood vessel wall. They indicated that the cause of these tissue lesions is the ability of scopolamine to generate free radicals, which in turn directly affect the young cells and cause the breakdown of fats in the cell membranes. 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 results of the present study were in agreement with a study conducted by Kumar et al., [15] to discovered the role of bromelain as a bioactive compound obtain from pineapple. where, co-administration of AlCl3, and D - galactose via intraperitoneal route for 90 days resulted in cognitive impairment, spatial learning, and memory deficits, as well as neurotoxicity in albino mice. However, 30 consecutive days, treatments via an intraperitoneal route with bromelain, donepezil, and bromelain with donepezil were considerably reversed the effect of AlCl3 and D - galactose induced AD mice. where, hematoxylin and eosin staining of the cerebral cortex and the hippocampus exposed eosinophilic lesions and hyperchromatic nuclei in AD mice, but these neurodegenerative effects were eliminated by Brm L, Brm H, Dnpz, and Brm L + Dnpz treatments. Thus, bromelain alone and in combination with donepezil prevent AlCl3 and D - galactose induced spatial learning and memory deficits, as well as cognitive impairment, by increasing cholinergic activity and synaptic plasticity, as well as reducing oxidative damage, neuroinflammation, Aβ 1 - 42 aggregations, and histopathological damage, according to our findings.

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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Declaration of interests

The authors declare no competing interests.

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Publication consent

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Data and material availability

All data analyzed and generated in this study are included in this published research.

Author contribution

All authors participated in the study design and conception. Data analysis, data collection, performance of the results, and assent to the final version.

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الكشف عن تأثير البروميلين والدونيبيزيل ضد التغيرات السلوكية والنسجية المستحثة بالسكوبولامين في الدماغ

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الملخص.

الخلفية والهدف: يُعد مرض ألزهايمر اضطرابًا عصبيًا تنكسيًا تدريجيًا يتميز بضعف الذاكرة والتدهور المعرفي. ويُستخدم السكوبولامين على نطاق واسع لإحداث تغيرات مشابهة لمرض ألزهايمر في النماذج الحيوانية. لذلك، هدفت الدراسة الحالية إلى تقييم التأثيرات الوقائية للبروميلين والدونيبيزيل ضد التغيرات السلوكية والنسجية المرضية في الدماغ المستحثة بالسكوبولامين لدى إناث الجرذان البالغة.

المواد وطرائق العمل: استُخدم في هذه الدراسة 25 جرذًا بالغًا من الإناث، تراوحت أعمارها بين 10–12 أسبوعًا وأوزانها بين 181–204 غرامًا. أُجريت التجربة في بيت الحيوانات بكلية الطب البيطري/جامعة تكريت للفترة من 29 آب إلى 11 أيلول 2024. قُسمت الجرذان عشوائيًا إلى خمس مجموعات. تلقت مجموعة السيطرة محلولًا ملحيًا طبيعيًا لمدة 14 يومًا، في حين تلقت مجموعة السكوبولامين جرعة مقدارها 0.02 ملغم/كغم داخل الصفاق خلال الأيام (8–14). أما المجموعات الأخرى فقد عولجت مسبقًا بالبروميلين لمدة سبعة أيام، ثم تلقت السكوبولامين بالتزامن مع الدونيبيزيل أو البروميلين أو مزيج منهما خلال الأيام.(8–14)

النتائج: أظهر التقييم السلوكي باستخدام اختبار المتاهة على شكل حرف (T) أن الجرذان المعالجة بالسكوبولامين عانت من ضعف واضح في الأداء المعرفي وانخفاض في السلوك الاستكشافي. في المقابل، أظهرت الجرذان المعالجة بالبروميلين و/أو الدونيبيزيل تحسنًا ملحوظًا في أداء المتاهة والنشاط الاستكشافي والوظائف الإدراكية، وكان التحسن الأكبر في المجموعة المعالجة بالمزيج. وأظهر الفحص النسجي لمجموعة السكوبولامين تنكسًا شديدًا في الخلايا العصبية، ووجود تجاويف كبيرة في نخاع الدماغ، وفرط تكاثر الخلايا الدبقية، واحتقان الأوعية الدموية، وحدوث فجوات نسيجية مع ارتشاح للخلايا الالتهابية. بينما أدى العلاج بالبروميلين أو الدونيبيزيل إلى الحفاظ على البنية الطبيعية للخلايا العصبية، وتقليل التنكس والارتشاح الالتهابي، واستعادة التنظيم الطبيعي لطبقات الخلايا الهرمية، في حين أظهرت مجموعة العلاج المشترك أفضل تحسن نسجي.

الاستنتاجات: يُستنتج أن البروميلين يمتلك تأثيرًا وقائيًا عصبيًا ضد التغيرات السلوكية والنسجية المرضية المستحثة بالسكوبولامين. كما أن استخدام البروميلين، ولا سيما بالاشتراك مع الدونيبيزيل، يُحسن الأداء المعرفي، ويحافظ على سلامة أنسجة الدماغ، ويقلل من تنكس الخلايا العصبية والاستجابة الالتهابية، مما يشير إلى إمكانية استخدامه كعامل علاجي مساعد في تدبير مرض ألزهايمر.

الكلمات المفتاحية: البروميلين، الدماغ، السكوبولامين، مرض ألزهايمر