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Research Article

Long-Term Effects of Scopolamine on Brain Tissue of Mice

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Abstract:

Background: Scopolamine is an anticholinergic drug that disrupts cholinergic transmission in the central nervous system as well as causes cognitive abnormalities and pathological hallmarks that are similar to those seen in Alzheimer's Disease. Therefore, it is used for induction of Alzheimer's Disease in animal models.

Objective: to investigate the effects of long-term induction with scopolamine on the brain tissue of mice.

Methods: Seventy adult mice were divided into 2 equal groups: The first group was the normal control group received distilled water only. The second one was the Alzheimer's Disease induction group received intraperitoneal scopolamine (1mg/kg) for 14 days only after that distilled water was given for the next 6 months. Ten mice were isolated from each group at zero time, after 2 weeks of induction, after 3-month and after 6 months and subjected to the behavioral tests then sacrificed for determination of biochemical factors (including brain-derived neurotrophic factor, total antioxidant status, malondialdehyde, and amyloid β). Data were analyzed using *t*-tests, and ANOVA. All values expressed as Mean±SD and *P* value <0.05 were considered significant.

Result: Scopolamine produced brain histopathological changes similar to those of human Alzheimer's disease. However, it does not produce further statistically significant differences in behavioral tests and biochemical markers during the total period of study.

Conclusion: scopolamine produces brain tissue changes that persist for a long period and it can be used for long-term study of Alzheimer's disease.

Keywords: Alzheimer's disease**;** Antioxidant; Cognitive function; Oxidative stress; Scopolamine**.**

Introduction

Scopolamine (SCM) is an anticholinergic drug that disrupts cholinergic transmission in the central nervous system (CNS) and demonstrates competitive antagonism at muscarinic acetylcholine receptors (mAChRs) (1).

As it easily crosses the blood-brain barrier, scopolamine is frequently utilized in neuroscience research to induce cognitive impairments, similar to those seen in Alzheimer's Disease (AD), in experimental animals (2). These animal models are commonly used to test medications for possible therapeutic usefulness in people with AD-type dementia (1, 3).

Moreover, it is used with different doses in different studies. Some of these studies used it as a single dose and others as multiple doses. Therefore, it is unclear if SCM can induce different intensities and durations of amnesia after single or repeated doses (4).

In addition, many studies indicated that SCM increases the deposition of A*β*, the level of reactive oxygen species, and reduces antioxidant concentration leading to lipid peroxidation that induces oxidative stress.

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Also, it decreases the brain-derived neurotrophic factor (BDNF) and cAMP-response element binding protein (CREB) expression in the brain which are hallmarks of AD disease and ultimately cause memory impairment and synaptic dysfunction (3, 5). Previous studies concerned with the use of SCM for induction of AD in animal models did that over a short induction period. Therefore, the current study aimed to investigate the long-term effects of scopolamine on cognitive and memory functions as well as the pathological hallmark of Alzheimer's disease in mice.

Methods

An experimental study was conducted from 1st, July 2022 to 1st, June 2023.

The current study involved ninety adult female mice (4–8 weeks old) weighing (20–25g) were purchased from the Al-Razi center, Ministry of Industry and Minerals, Baghdad, Iraq. The mice were kept in the experimental area for 2 weeks for the habituation phase and housed as ten mice per cage at an appropriate temperature (25°C) and humidity (30% ± 10%), with a standard 12-hour light/dark cycle and free access to water and standard food (high protein feed and milk powder).

In the beginning, twenty mice were included in a pilot study to detect the effective dose and duration of treatment for Scopolamine Intraperitoneal (IP/SCM) to induce AD (scopolamine hydrochloride, 1mg/kg, was dissolved in distilled water for injection and given IP to induce AD in mice (6). The results of the pilot study showed that IP/SCM (1mg/kg) for 14 days induced AD effectively. Then, seventy adult mice were divided into 2 equal groups; the first group was the normal group that received distilled water only during the total period of the study and was considered a control group, and the second one was the induction untreated Alzheimer group that received IP/SCM (1 mg/kg) for 14 days only after that distilled water was given for the next 6 months.
Mice were subjected to behavioral tests (Barnes Maze (7), Novel Object Recognition (8), and Y-Maze Tests (9). Then, these mice were anesthetized and sacrificed to isolate brain tissue for further determination of biochemical markers such as BDNF, TAS, MDA, and 1-42 *β*-amyloid peptide. This procedure was repeated 3 and 6 months after induction.

Data were analyzed using *t*-tests (paired and unpaired) and ANOVA. All values expressed as Mean±SD and *P* value <0.05 were considered significant (10).

Results

Histopathological changes

Results from the current study revealed that histopathological sections of brain tissue from mice induced with SCM for 2 weeks showed microscopically changes (Figures 1 & 2)

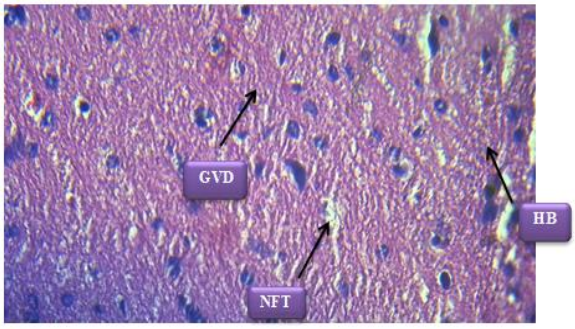

Figure (1): Histological section of mice AD brain tissue after induction with scopolamine for 2 weeks (current study) showing numerous NFT, HB, and GVD (H&E stain) (40X).

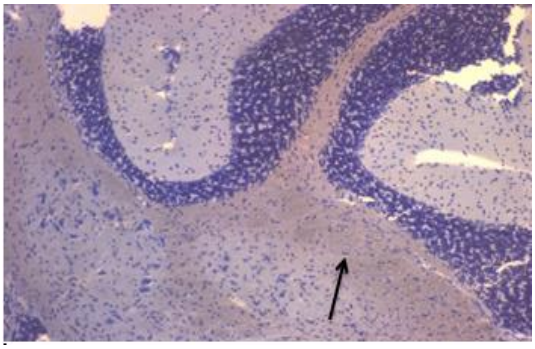

Figure (2): Histological section of mice AD brain tissue after induction with scopolamine for 2 weeks (current study) showing multifocal severe deposition of amyloid beta plaques (orange-red color) (arrow) (Congo red stain) (10X).

Also, findings indicated the validity of AD animal model created in the current study. Similar to those reported in human AD brain tissue (Figures 3, 4, and 5)

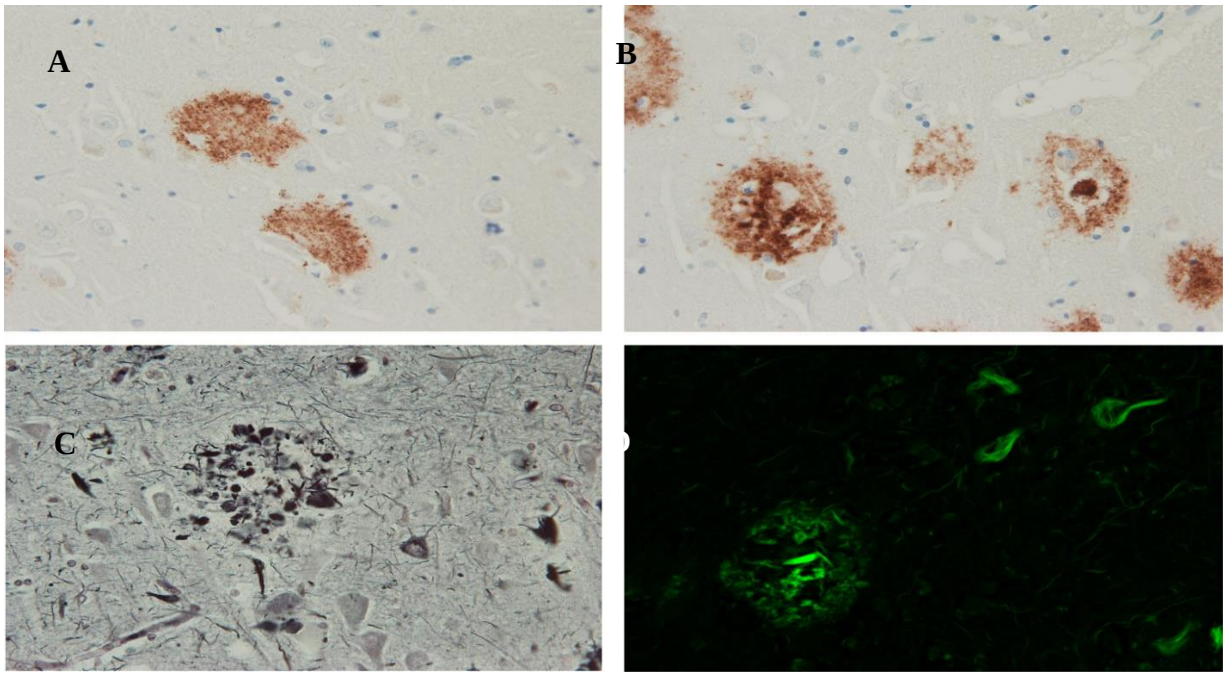

Figure (3): Histological section of human AD brain tissue showing the presence of amyloid *β* Senile Plaques by using antibodies directed against Aβ peptides (A&B), Bielchowsky silver staining (C) or Thioflavin S staining (D) (DeTure & Dickson, 2019).

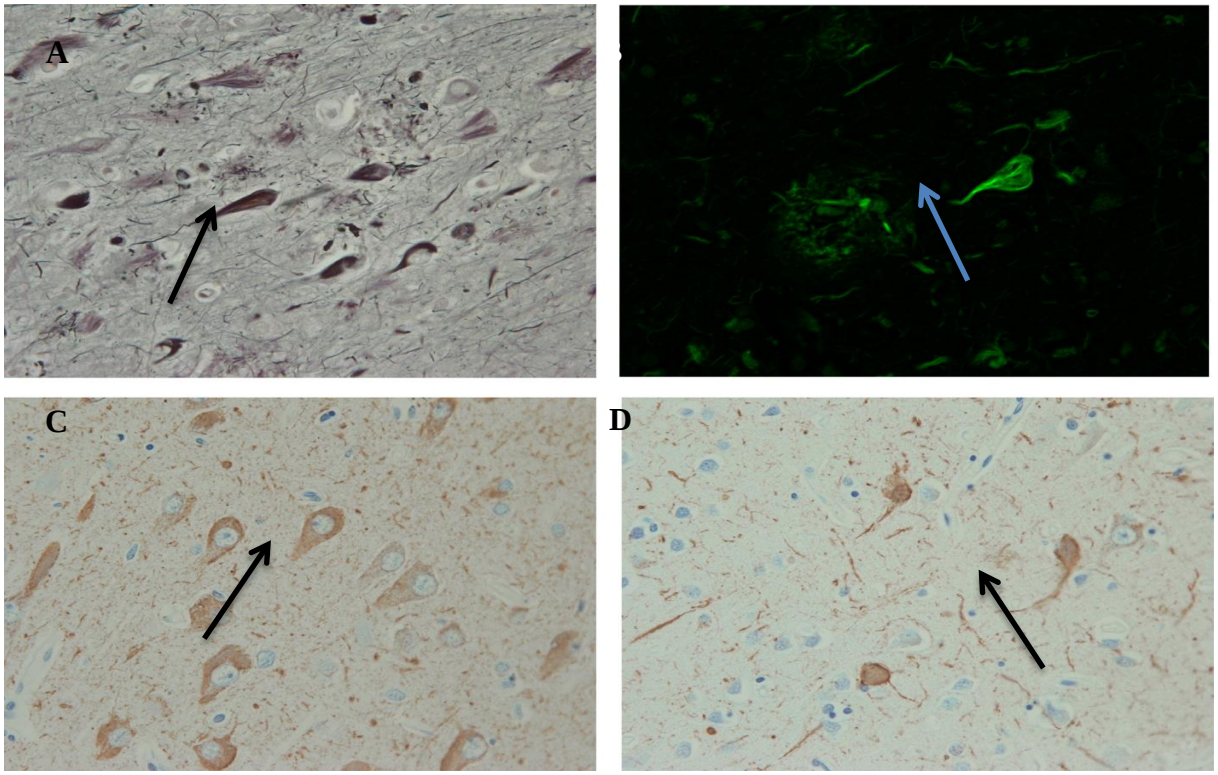

Figure (4): Histological section of human AD brain tissue showing the presence of Neurofibrillary Tangles by using Silverstaining (A), Thioflavin S (B), and tau immunohistochemistry (C, D). (DeTure & Dickson, 2019).

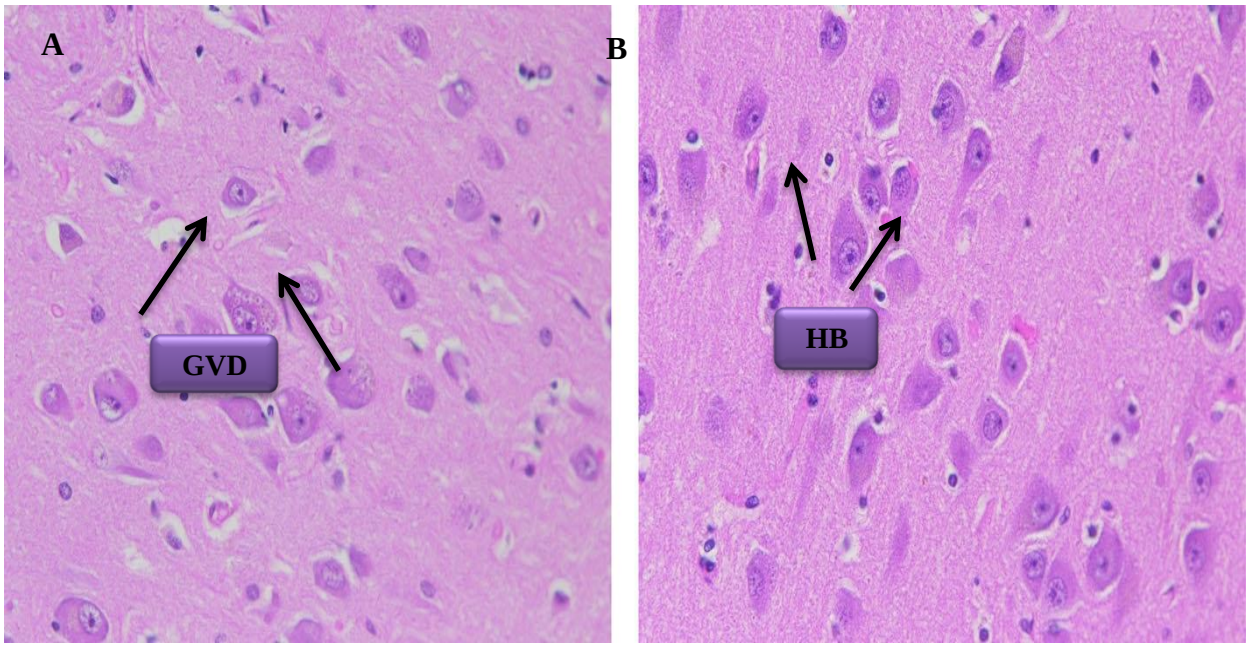

Figure (5): Histological section of human AD brain tissue showing the presence of Granulovacuolar Degeneration (acid phosphatase histochemistry) (A) and Hirano Bodies (H&E stain) (B) (DeTure & Dickson, 2019).

Behavioral tests and biochemical markers along the total period of the study for normal control groups: The current study revealed that there were no statistically significant differences in behavioral tests (Barnes Maze Test, Novel Object Recognition Test, and Y-Maze Test) or biochemical markers (Amyloid *β*, BDNF, MDA, and TAS) in the normal control groups throughout the entire duration of study (6 months) (Tables 1& 2, respectively).

Table (1): Behavioral tests for normal groups at zero time, after 3 months, and after 6 months of treatment (Y-maze Test, Novel Object Recognition Test, and Barnes Maze Test)

Behavioral Test			Mean ± SD			*P* value
			Nor. 0T (n=10)	Nor. 3M (n=10)	Nor. 6M (n=10)	
Y- Maze Test			79.346±3.774	77.554±4.816	77.384±2.215	0.681
NOR Test			80.70±4.446	76.506± 2.073	78.153±6.057	0.341
Barnes Maze test	Acquisition training	Primary latency(s)	45.7±6.056	41.5±4.428	42±5.657	0.196
		Primary error	16±3.367	17±4.522	20±5.888	0.210
		Primary hole distance	8.2±2.699	9.3±2.750	8.875±1.458	0.603
	Probe trial	Primary latency(s)	15.8±3.564	16.6±2.881	16.2±3.899	0.936
		Primary error	6±1.581	5.8±1.924	4.4±1.342	0.278
		Primary hole distance	4.4±1.140	6.2±1.789	8±2.916	0.055
	Reversal learning	Primary latency(s)	50.555±7.859	54.142±11.889	51.4±12.176	0.715
		Primary error	13.111±3.919	10.8±2.932	12±2.748	0.231
		Primary hole distance	10.555±2.455	11.062±2.909	10.2±2.529	0.722

Nor. 0 T=normal group at zero time, Nor. 3M = normal group after 3 months of treatment, Nor. 6M = normal group after 6 months of treatment, s = second, SD = Standard deviation, NOR = Novel Object Recognition Test.

Tables (2): Mean levels of biochemical markers for normal control groups at zero time, normal after 3 months, and normal after 6 months of treatment

Marker	Mean ± SD			*P*- value
	Nor. 0 T (n=6)	Nor. 3 M (n=6)	Nor. 6 M (n=6)	
BDNF (ng/mL)	3.062 ± 0.966	4.092 ± 1.134	4.026 ± 1.125	0.354
Amyloid β (ug/L)	9.930 ± 0.567	12.207 ± 1.683	11.694 ± 1.846	0.076
TAS (pg/mL)	35.192± 3.672	37.378 ± 1.02	41.887 ± 10.994	0.096
MDA (nmol/mL)	0.621 ± 0.018	0.613 ± 0.05	0.594 ± 0.117	0.845

Nor. 0T=normal group at zero time, Nor. 3M = normal group after 3 months of treatment, Nor. 6M = normal group after 6 months of treatment, BDNF= Brain derived neurotrophic factor, TAS = Total antioxidant status, MDA = Malondialdehyde, SD = Standard deviation.

Behavioral tests and biochemical markers for AD induction groups over the total period of the study: Current study revealed that there were no statistically significant differences in behavioral tests (Barnes Maze Test, Novel Object Recognition Test, and Y-Maze Test) or biochemical markers (Amyloid β, BDNF, MDA, and TAS) during the total period of the study for the AD-induction groups (using SCM) after 2 weeks, 3 months, and 6 months (Tables3 and 4, respectively).

Table (3): Behavioral tests for AD induction groups after 2 weeks, 3 months, and 6 months of treatment

Behavioral Test			Mean ± SD			*P*- value
			Induction (SCM 2W) (n=10)	Induction (SCM 3M) (n=10)	Induction (SCM 6M) (n=10)	
Y- Maze Test			46.44±5.185	48.424±3.704	51.204±2.224	0.751
NOR Test			45.744±2.196	47.268±1.846	46.31±2.957	0.605
Barnes Maze test	Acquisition training	Primary latency (s)	158.667±26.585	157.25±21.57	148.111±29.118	0.598
		Primary error	37.833±9.637	38±8.569	36.555±5.659	0.912
		Primary hole distance	17.166±1.642	17.733±1.869	18±2.397	0.597
	Probe trial	Primary latency (s)	58.5±1.914	57.5±3.507	57±5.196	0.846
		Primary error	19±3	22.833±3.544	20.2±2.280	0.137
		Primary hole distance	16.2±2.863	17.667±2.250	15.6±2.701	0.416
	Reversal learning	Primary latency (s)	143.625±17.508	146.8±25.952	165.75±16.568	0.092
		Primary error	34±7.406	31±6.96	30.75±7.648	0.611
		Primary hole distance	16.5±3.070	17.4±2.633	17.75±2.187	0.627

SCM 2W = after 2 weeks of induction by scopolamine, SCM 3M= after 3 months of treatment in the scopolamine induction group, SCM 6M= after 6 months of treatment in the scopolamine induction group, s = second, SD = Standard deviation, NOR = Novel Object Recognition Test.

Table (4): A comparison of the mean levels of biochemical markers in the induction groups throughout the study

Marker	Mean±SD			*P* value
	Induction (SCM 2 W) (n=6)	Induction (SCM 3M) (n=6)	Induction (SCM 6 M) (n=6)	
BDNF (ng/mL)	0.686 ± 0.137	0.624 ± 0.213	0.643 ± 0.161	0.832
Amyloid *β* (ug/L)	57.227± 8.553	61.827± 10.059	49.122± 11.292	0.062
TAS (pg/mL)	10.123 ± 2.949	11.336 ± 1.042	9.450 ± 3.092	0.438
MDA (nmol/mL)	2.764 ± 0.344	2.560 ± 0.377	3.012 ± 0.763	0.354

SCM 2W = after 2 weeks of induction with scopolamine, SCM 3M= after 3 months of treatment in the scopolamine induction group, SCM 6M= after 6 months of treatment in the scopolamine induction group, BDNF= Brain-derived neurotrophic factor, TAS = Total antioxidant status, MDA = Malondialdehyde, SD = Standard deviation.

Comparison of behavioral tests between the AD-induction group after 2 weeks and the normal control group at zero time

Table (5): Comparison between the AD induction group and normal control group at zero time for behavioral (Y-maze, Novel Object Recognition, and Barnes Maze) tests

Behavioral Test			Mean±SD		*P*-value
			Nor. 0T (n=10)	Induction (SCM 2W) (n=10)	
Y maze test			79.346±3.773	46.44±5.185	<0.001
NOR Test			80.70±4.446	45.744±2.196	<0.001
Barnes Maze test	Acquisition training	Primary latency (s)	45.7±6.056	158.666±26.585	<0.001
		Primary error	16±3.366	37.833±9.637	<0.001
		Primary hole distance	8.2±2.699	17.166±1.642	<0.001
	Probe trial	Primary latency (s)	15.8±3.563	58.5±1.914	<0.001
		Primary error	6±1.581	19±3	<0.001
		Primary hole distance	4.4±1.14	16.2±2.863	<0.001
	Reversal learning	Primary latency (s)	50.555±7.859	143.625±17.508	<0.001
		Primary error	13.111±3.919	34±7.406	<0.001
		Primary hole distance	10.555±2.455	16.5±3.07	<0.001

SCM 2W = after 2 weeks of induction by scopolamine, Nor. 0T=normal group at zero time, s = second, SD = Standard deviation, NOR = Novel Object Recognition Test.

Comparison of Biochemical markers between the AD induction group after 2 weeks and the Normal control group at zero time: The current study indicated that there was a highly significant elevation in the mean level of amyloid *β* and MDA, while there was a significant reduction in the mean level of BDNF and TAS in the AD induction group after 2 weeks in comparison with the normal control group at zero time (Table 6).

Table (6): Comparison of mean levels of biochemical markers between AD induction groups and normal group at zero time

Marker	Mean±SD		*P*-value
	Nor. 0 T (n=6)	Induction (SCM 2W) (n=6)	
BDNF(ng/mL)	3.062 ± 0.966	0.686 ± 0.137	0.006
Amyloid *β* (ug/L)	9.930 ± 0.567	57.227± 8.553	<0.001
TAS (pg/mL)	35.192± 3.672	10.123 ± 2.949	<0.001
MDA (nmol/ml)	0.621 ± 0.018	2.764 ± 0.344	0.003

SCM 2W = after 2 weeks of induction by scopolamine, Nor. 0T=normal group at zero time, SD = Standard deviation, BDNF= Brain derived neurotrophic factor, TAS = Total antioxidant status, MDA = Malondialdehyde.

Discussion

Validity of the animal model of AD created in the current study: In the current study, SCM caused accumulation of amyloid (A*β) plaques*, NFT, GVD, and HB by histopathological examination, which is similar to AD of human brain tissue (11).

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 (12). Behavioral tests and biochemical markers for normal control groups: The age of the young mice enrolled in the current study at zero time was (1-2 months), while after 3 months of therapy was (4-5 months), and after 6 months of therapy was (7-8 months). In this study, the cognitive and memory functions of normal control mice were preserved over the total period of the study by measuring behavioral tests including the Barnes maze test, Novel Object Recognition (NOR) Test, and Y-maze test. Thus, these data excluded any effect of aging on the result of the current study (13). These results were in agreement with the results of some previous studies that assessed age-related memory and cognitive function in multiple age groups of normal mice (14-17). Aging is associated with cognitive decline and may be linked to minimal neuronal loss (13). However, mature CNS neurons at a young age are very resistant to cell death. Therefore, neuronal cell death is limited to the adult brain (18).

Additionally, the present study observed that there were no differences in the level of A*β* in the brains of normal mice over the total period of the study and these results agreed with those from previous studies

that measured the level of Aβ in the brains of normal mice (19, 20). Furthermore, for different ages of mice (young, middle-aged, and elderly), the level of the β -secretase (BACE1) enzyme that is necessary for the synthesis of Aβ is consistent (19). Consequently, the Aβ level during the young age is consistent. The present study found that there are no differences in the levels of BDNF in young normal mice of different ages throughout the study. These findings agreed and contrasted those of some previous studies (21-24). The BDNF gene plays a crucial role in neuronal generation, function, and memory. In addition, neuronal BDNF mRNA expression of the hippocampus and cortex is unaffected by aging in normal mice, hence the level of BDNF remains consistent over time (25). Regarding oxidative stress level, the current study has shown that there have been no statistically significant changes in the concentration of MDA and TAS among young normal mice of varied ages. One of the earliest studies has compared the activity of antioxidant enzymes and MDA levels among normal mice of different ages and discovered a contradicter result in which GPx and copper-zinc superoxide dismutase CuZn/SOD activities are higher in 18 and 28 months old mice than younger, 2 month old, mice, while manganese superoxide dismutase (MnSOD), GRD activities, and MDA level do not change with aging (26).

Oxidative damage is considered one of the predominant mechanisms of cellular and tissue damage in aging (27). The oxidative damage during aging occurs due to neuroinflammation, and increased expression of pro-inflammatory factors (28). In the healthy brain of young mice, microglia are "resting" and dormant, which eliminates the impact of neuroinflammation on young mice's normal brain function (29). Thus, OS in young mice is kept at a low level.

Biochemical markers and behavioral tests for AD-induction group over the total period of the study: The current study revealed that the effects of SCM on cognitive and memory function as well as biochemical markers (Amyloid β, BDNF, MDA, and TAS) were consistent throughout the study (6 months). These results suggested that SCM has a prolonged effect, which will strengthen the impact of the study's medications and these findings have not been reported in other previous studies because all other studies focused on the short-term effects of SCM.

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 (30). Additionally, SCM increases Aβ deposition which further enhances oxidative stress (31). Furthermore, SCM interferes with the expression of neurofilaments, which are essential for axonal transport in neurons (32). The loss of cholinergic function in the hippocampus is associated with serious cognitive impairments (32) which may last for a long time.

On the other hand, results obtained from this study found that SCM interfered with, and subsequently caused impairment of, learning and short-term as well as long-term memories as evidenced by conducting Barnes-maze, NOR, and Y-maze tests.

It has been demonstrated that SCM can impair memory and cognitive functions in mice by administering single or multiple injections at various concentrations through assessing many behavioral tests (33-36).

Aykac et al. (2018) found that administering SCM (1mg/kg) IP for 14 days dramatically raised MDA levels, lowered GSH levels, decreased BDNF expression, and lowered short- and long-term memory (36). In addition, Anand et al. (2022) concluded that SCM single injection (2mg/kg) increased oxidative stress in the hippocampus by increasing thiobarbituric acid-reactive substances (TBARS), which reflect lipid peroxidation and decreased GSH as well as CAT levels (37). Also, a recent study conducted by Cheedella *e*t al. (2023) found that SCM (5mg/kg) for 7 days reduced CAT activity and H&E-stained histological sections of the brain showed severe blood capillary congestion with perivascular edema (scars), along with edema and deposition of amyloid plaques in the hippocampus when compared with normal mice (31).

However, a study done by Ban et al. (2020) found conflicting results regarding the impact of SCM on oxidative stress such as that CAT activity was unaffected after SCM injection in mice compared to normal mice (34). Additionally, Lee et al. (2010) found that SCM significantly increased SOD and GSH-Px antioxidant enzyme activities (38).

Despite that, it is well-known that the non-selective muscarinic acetylcholine receptor antagonist scopolamine (SCM) prevents cholinergic signals from traveling through the brain. This SCM-induced dysregulation of cholinergic activity and increased activity of acetylcholinesterase in the hippocampus interferes with mouse learning and memory functions (3, 32). One of the mechanisms causing scopolamine-induced amnesia is oxidative stress through increasing the levels of malondialdehyde, a marker of lipid peroxidation, and lowers the activity of many antioxidant enzymes (4). Additionally, there is a relationship between Aβ and oxidative stress, because prooxidants elevate Aβ formation and Aβ generates oxidative stress (39). Moreover, the degree of synapse loss and cognitive deficits do correlate well with the amounts of soluble Aβ in the brain (19).

Conclusion

In light of results reported by the current study, scopolamine produces histopathological changes in mice brain tissue similar to those reported in human AD brain tissue. In addition, the effects of scopolamine on memory and cognitive functions as well as on pathological hallmark of AD persist for a long period (about 6 months).

Authors' Declarations

Mohammed AH AL- Zobaidy is an Editorial board member but did not participate in the peer review process other than as an author.

We hereby confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the local ethical committee at the Department of Pharmacology/ College of Medicine, University of Baghdad, Baghdad, Iraq, with reference number (PHARMACOMED) in U.vB 23.13 (Appendix I).

Conflict of interest: None

Funding Source: None

Authors' contribution

Study conception & design Mohammed AH JAbarah AL- Zobaidy. Literature search & Manuscript preparation are done by Neven Nihal Hana Istifo. Data acquisition, Data analysis & interpretation are done by Neven Nihal Hana Istifo and Kasim Sakran Abass. Manuscript editing & review are done by Mohammed AH JAbarah AL- Zobaidy.

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الآثار الطويلة المدى للسكوبولامين على أنسجة المخ لدى الفئران

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الخلاصة

خلفية البحث: السكوبولامين هو دواء مضاد للكولين يعطل انتقال الكوليني في الجهاز العصبي المركزي كما أنه يسبب تشوهات إدراكية وعلامات مرضية مشابهة لتلك التي تظهر في مرض الزهايمر. ولذلك، يتم استخدامه لتحريض مرض الزهايمر في النماذج الحيوانية.

الأهداف: كان الهدف من الدراسة الحالية هو دراسة آثار التحريض طويل المدى مع السكوبولامين على أنسجة المخ لدى الفئران.

طرق العمل: تم تقسيم سبعين فأراً بالغاً إلى مجموعتين متساويتين: المجموعة الأولى كانت مجموعة طبيعية ومراقبة تلقت الماء المقطر فقط. أما المجموعة الثانية فكانت مجموعة تحريض مرض الزهايمر حيث تلقت السكوبولامين داخل الصفاق (1 ملجم / كجم) لمدة 14 يومًا فقط بعد ذلك تم إعطاء الماء المقطر لمدة 6 أشهر التالية. تم عزل عشرة فئران من كل مجموعة في وقت الصفر، بعد أسبوعين من التحريض، وبعد 3 أشهر وبعد 6 أشهر، وتم إخضاعها للاختبارات السلوكية ثم تم تشريحها لتحديد العوامل البيوكيميائية (بما في ذلك عامل التغذية العصبية المشتق من الدماغ، وحالة مضادات الأكسدة الكلية، والمالونديالدهيد). والأميلويد β). وقد تم تحليل البيانات باستخدام اختبارات t، وANOVA. مع اعتبار جميع القيم المعبر عنها كقيمة متوسط ± SD وقيمة $P < 0.05$ ذات دلالة احصائية.

النتائج: أنتج السكوبولامين تغيرات نسيجية مرضية في الدماغ مشابهة لتلك التي تحدث في مرض الزهايمر البشري. ومع ذلك، لم تنتج فروق ذات دلالة إحصائية أخرى في الاختبار السلوكي، والعلامات البيوكيميائية، خلال الفترة الإجمالية للدراسة.

الاستنتاجات: يُحدث السكوبولامين تغيرات في أنسجة المخ والتي تستمر لفترة طويلة ويمكن استخدامه لدراسة مرض الزهايمر على المدى الطويل.

الكلمات المفتاحية: الاجهاد التاكسدي؛ الوظيفة المعرفية؛ سكوبولامين؛ مرض الزهايمر؛ مضادات الأكسدة.