Neuroprotective Impact of Baicalein on Scopolamine- Induced Cognitive Deficits Targeting PI3K/Akt /NF-kB Pathway

Suzan A. Khodir[1*], Alaa Osama Nagy[2], Ola A. Mohamed[2], Noha M. Abd El-aziz[3], Fatma Eldesoky Ahmed [4], Mohamed A. Zayed[1,5], Walaa M. Elgheriany[6] , Tarek H. Abd-Elhamid[7,8], Hend R. Omara[9], Asmaa I. Bayomi[10] , Haidy Moustafa Abouhatab[11], Karema Abdelhady Diab[12], Samar M. Hussein[13]

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ABSTRACT

Dementia is a substantial public health concern. Scopolamine (Scop) is a muscarinic acetylcholine receptor antagonist that induces cognitive impairment. The objective of this study was to demonstrate the potential mechanisms by which Baicalein (Baic) might protect against cognitive abnormalities caused by Scop. The thirty male albino rats were divided into three groups: Scop, Scop plus Baic, and control (10/group). After four weeks neurobehavioral assessment was conducted in conjunction with the measurement of hippocampal MDA, SOD, acetylcholine, BDNF, TNF-α, IL-6, IL-1β, and PI3K, Akt, and NF-kB gene expression. The immunohistochemical analysis of Bax, Tau, and GFAP markers was assessed. Scop induced cognitive impairment in rats with a significant increase in the expression of MDA, TNF-α, IL-6, IL-1β, and NF-kB genes. It induced a substantial decrease in the expression of hippocampal SOD, BDNF, acetylcholine, and hippocampal PI3K and Akt genes, as well as an upregulation of GFAP, Tau, and Bax immunoreactions. However Baic dramatically improved Scop-induced cognitive impairment. We can conclude that Baic induced anti-oxidant, anti-inflammatory, neurotrophic, and anti-apoptotic mechanisms in addition to modifying the PI3K/Akt/NF-kB signaling cascade to mitigate the cognitive impairments brought on by Scop.

Introduction

Dementia is a substantial global public health issue that is increasingly prevalent among the elderly and has a lasting impact on memory and cognition (Kosteniuk et al., 2016). The primary treatment for the illness is acetylcholinesterase inhibitors (Hafez et al., 2017). However, these medications have been associated with a variety of adverse impacts due to their nonselective effect on a variety of organ tissues (Mendiola-Precoma et al., 2016).

Scopolamine (Scop) is a muscarinic acetylcholine (Ach) receptor antagonist that induces cognitive impairment. Scop has been shown to elicit oxidative stress spikes, which include an increase in the generation of reactive oxygen species (ROS) in the hippocampus (Thongrong et al., 2024). Furthermore, Scop induces inflammation and apoptosis in the hippocampal region (Demirci et al., 2017).

[1] *Medical Physiology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[2] *Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, Egypt.*
[3] *Anatomy and Embryology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[4] *Clinical Pharmacology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[5] *Medical Physiology Department, Faculty of Medicine, King Abdulaziz University, KSA.*
[6] *Internal Medicine Department Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[7] *Histology and Cell Biology Department, Faculty of Medicine, Assiut University, Assiut, 71515, Egypt.*
[8] *Histology Department, Faculty of Medicine, Aqaba Medical Sciences University, Aqaba, 77110, Jordan.*
[9] *Psychiatry Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[10] *Zoology Department - Faculty of Science, Menoufia University, Menoufia, Egypt.*
[11] *Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[12] *Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin El-Kom, Menoufia, Egypt.*
[13] *Medical Physiology Department, Faculty of Medicine, Suez Canal University, Ismailia 41522, Egypt.*
[*] Correspondence to **Suzan A. Khodir,** Email: suzan.abdalhameed.12@med.menofia.edu.eg., ORCID iD: 0000-0002-2535-9445. Medical Physiology Department, Faculty of Medicine, Menoufia University, Shebin El-Kom, Menoufia, Egypt

Acetylcholine is a neurotransmitter that is involved in both the central and peripheral nervous systems and supports the body's physiological and cognitive processes. Acetylcholinesterase (AChE) is a cholinergic enzyme that impedes neuronal transmission and synaptic signaling. Changes in AChE activity are indicative of cognitive and learning impairments (Jadhav and Kulkarni, 2023).

Brain-derived neurotrophic factor (BDNF) has been shown to have both neurogenic and neuroprotective effects. Recent research in animal models has shown that the depletion of neurotrophic factors leads to AD-related diseases, including tau hyperphosphorylation, Aβ accumulation, and synaptic dysfunction (Tang, 2019).

The phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway is highly expressed during the formation of the central nervous system (CNS). Neurons generate PI3K in response to growth factors such as BDNF. This route appears to be particularly critical for mediating neuronal survival in a diverse array of circumstances. Akt is activated by growth factors and is involved in a pathway with PI3K kinase. It is essential for the regulation of apoptosis and survival. The PI3K/Akt pathway can be used to activate anti-apoptotic proteins and deactivate pro-apoptotic mediators. The PI3K/Akt signaling system's capacity to regulate cell survival, differentiation, and metabolism is widely recognized. Angiogenesis, neurocyte nutrition, learning, and memory are all influenced by these processes. Numerous neuroprotectants function by activating the PI3K/Akt pathway (Shu et al., 2013).

Nuclear factor-kappa B (NF-kB) is a critical transcription factor that regulates the expression of numerous genes that generate inflammatory cytokines (Imam et al., 2015). It is a downstream component of the PI3K/Akt pathway that is initiated by the phosphorylation of the IkB kinase (IKK), which leads to the degradation of IkB (Yu et al., 2017).

The root of Scutellaria baicalensis Georgi is the source of the traditional Chinese herb baicalein (Baic). It is classified as a flavonoid and possesses a variety of biological properties, including anti-inflammatory, antioxidant, and anti-apoptotic properties. It has been shown to enhance memory and learning, and it has the potential to be employed as a medication to prevent and treat CNS issues, as indicated by previous studies (Li et al., 2019a). Baic possesses neuroprotective and AChE-inhibitory properties. The blood-brain barrier is penetrated within 20 to 30 minutes of administration (Hu et al., 2022). In vitro as well as in vivo, Baic protects neuronal cells from the damage produced by amyloid-β (Aβ) (Shi et al., 2021).

The objective of this study was to demonstrate the neuro-protective properties of Baic on Scop-induced cognitive impairments and the potential underlying mechanisms, with a focus on the Pi3k/Akt/NF-kB signaling system.

Materials and Methods

Thirty mature males Wistar albino rats weighing 200 ± 50 g at three months of age were used in the study. They were kept under regular conditions, including natural light-dark cycles. They were allowed full access to water and fed standard rat food. Rats had a week to acclimate before the trial began.

Ethical statement

All procedures followed the rules established by the Committee of Animal Research Ethics at Menoufia University's Faculty of Medicine with registration number: 11/2024BIO13-2.

Grouping

Thirty rats were randomly divided into three equal groups of ten each.

Group I (control): rats received intragastric 0.5% sodium carboxymethylcellulose (CMC-Na) (cat. no. 21904; MilliporeSigma) daily for four weeks, and 0.5 mL 0.9% NaCl by i.p. injection to rats during the final 7 days.

Group II (Scop-induced cognitive deficit group) ***(Scop***): The rats were given CMC-Na intragastrically once daily for four weeks. During the last 7 days of treatment, a daily i.p injection of Scop (3 mg/kg) was given. Scop was dissolved in 0.9% NaCl and administered intraperitoneally to rats (Lin et al., 2016).

Group III (Scop-induced cognitive deficit/Baic treated group) ***[Scop+Baic]:*** Rats received Scop similarly as in group II, but they additionally received 100 mg/kg/d Baic intragastrically for 4 weeks beginning on the first day of the experiment (Song et al., 2024). Baic (Must, Chengdu, China) was dissolved in 0.5% CMC-Na and administered to rats throughout the trial period. In the last 7 days of the study, Baic was administered 1 hour before Scop administration.

The cognitive abilities of the animals were assessed throughout the trial's final five days. After the behavioral tests, the rats were sacrificed via cervical dislocation. To assess neurodegenerative changes, brain hemispheres were dissected. Following dissection, the right hippocampus was immediately frozen at -80°C for future biochemical study. The left hemispheres were processed for hippocampus (CA1) histology and immunohistochemistry assessment.

Cognitive performance assessment

a. *Elevated Plus Maze (EPM) Test* reported by Tchantchou et al. (2018), who used a plus-sign shape device to assess anxiety-like behavior in rats. In summary, rats were placed individually in the center of the contraption and given ten minutes to explore the labyrinth. An above-ground camera tracked the animals' movements. The time spent in the maze's open arms was recorded. An animal's anxiety-like behavior was inversely proportional to the amount of time it spent in the open arms.

b. *Y-Maze Test.* The spatial working memory of rats is assessed using the Y-maze test (Reddy, 1997). Each rat was placed at one end of the arm and permitted to navigate the maze for eight minutes. When all four of the rats' paws were within one of the three arms, it was known as an arm entrance. Entry into any of the three arms on subsequent selections, such as ACB, CBA, or BAC, but not CAC, was deemed an alteration. The entire sequence of arm entrances, including potential returns into the same arm, was filmed with a Sony video camera. The following formula was used to calculate the percentage of alternation:

% of alternation = total number of alternations/(total number of arm entries-2) x 100.

c. *Morris' Water Maze (MWM) Test.* The MWM test was carried out over the last five days of the experiment. The circular pool at MWM was partitioned into four equal portions. During the first four days, a platform was set up 1 cm below the water's surface in one of the quadrants. On each day of the acquisition session, each rat was assigned to one of three randomly selected positions in the pool. The rat was submerged in the pool to start the experiment. The rat was submerged in the pool to start the experiment. When the rat was discovered and climbed onto the platform, the experiment was ended, and the mean escape delay was calculated. The exam had a maximum duration of 60 seconds. The rat was gently placed on the platform, and the time was recorded as 60 seconds if it did not climb up. On the fifth day, a "probe trial" was conducted to determine the rat's ability to recall the position of the concealed platform in less than 60 seconds. The platform was removed from the study pool (Aksoz et al., 2019).

Making a tissue homogenate

The weighted hippocampus tissues were individually homogenized using a tissue homogenizer (MPW120, MPW Medical Instruments, China). The crude tissue homogenate was spun for 15 minutes at 10,000 rpm in an ice-cold centrifuge before being stored at -80°C for testing.

Kits using ELISA were used to measure hippocampal acetylcholine (Cat.: 201-11-0723, Sunred CO., Shanghai, China), TNF-α (Cat.: MBS2507393, MyBioSource, Sandiego, CA, USA), IL-6 (Cat.: MBS269892, MyBioSource, Sandiego, CA, USA), IL-1β (Cat.: ab100768, Abcam, Cambridge, UK), and BDNF (Kit Catalogue Number: SL0131Ra, SunLong Biotech Co., LTD, Hangzhou, China). Calorimetric kits were used to assess hippocampus MDA and superoxide dismutase (SOD) in accordance with the manufacturer's instructions.

Real time PCR (rt-PCR) for detection of Akt, PI3k and NF-kB pathway genes expression

The Akt1, PI3k, and NF-kB were identified using the 7500 real-time PCR equipment (Applied Biosystems, CA, USA). The first PCR step was performed after RNA was extracted from fresh blood using the A direct-zol RNA Miniprep kit (Cat. No. R2051; Zymo Research, USA). After synthesizing complementary deoxy-ribonucleic acid (DNA) using the QuantiTect Reverse Transcription Kit (205311, Qiagen, Applied Biosystems, USA), the second PCR step (rt-PCR step) was carried out using the QuantiTect SYBR Green PCR Kit with ready-made quantities Primer Assay (204143; Qiagen, USA) to measure gene levels.

The forward primer for AKT was (*TCACCTCTGAGACCGACACC*), and the reverse primer was (*ACTGGCTGAGTAGGAGAACTGG*). The forward primer for PI3K was (*AGCTGGTCTTCGTTTCCTGA*), and the reverse primer was (*GAAACTTTTTCCCACCACGA*). The NF-kB forward primer was (TCGACCTCCACCGGATCTTTC). The reverse primer was (GAGCAGTCATGTCCTTGGGT). β-actin gene was endogenous control.

Histological evaluation

The left cerebral hemisphere was prepared for light microscopic evaluation and then stored in 10% neutral formaldehyde for histological examination. Standard histological analysis was performed using 5 μm thick paraffin slices stained with hematoxylin and eosin (H&E) (Kiernan, 2015). After cleaning with PBS, 5 μm slices were deparaffinized and rehydrated before being blocked in a 3% H2O2 compound to limit endogenous peroxidase activity for immunohistochemistry. Following a PBS washing, the microwave antigen retrieval process was performed **(**Suvarna et al., 2019).

Evaluation of hippocampal degeneration

The primary antibodies, Tau (mouse monoclonal antibody, Gene tex company, Cairo, Egypt), GFAP [1:300, mouse monoclonal, Lab vision MS-1376-R7], and caspase-3 [1:1000, rabbit monoclonal, Abcam ab184787], were incubated with brain slices. The sections were treated using different primary antibodies. Secondary antibody binding was detected with 3,3-Diaminobenzoic Acid (DAB). After cleaning the sections with PBS, the slides were counterstained with two drops of hematoxylin.

Morphometric study

A Leica DML B2/11888111 microscope with a Leica DFC450 camera was utilized to gather non-overlapping fields (×400) per section in three distinct serial sections from each rat for morphometric analysis. Image J software, version K1.45, was used to determine the parameters under investigation.

Hippocampal morphometric assessment

Three non-overlapping fields per section were utilized to measure the thickness of the pyramidal layer in H&E-stained sections. Three non-overlapping fields/sections were used to determine the area % of GFAP, Bax, and tau immunopositive cells for quantitative immunohistochemical analysis.

Statistical analysis

The data was processed with SPSS version 23 (SPSS Inc., USA). The Shapiro-Wilk test was applied to all data sets to ensure that they were normally distributed. The data were expressed as mean ± SD. The significance of group differences was assessed using one-way ANOVA and a post-hoc Tukey test. Statistical significance was considered as P values less than 0.05.

Results

Scop-group had significantly shorter mean values for time in open arms of EPM test and % of alternation on Y maze test compared to the control group ($P<0.05$). Scop +Baic had significantly higher values than the Scop group ($P < 0.05$) (Figures 1A, 1B).

On the 2nd, 3rd, 4th, & 5th days of the MWM test, the Scop-group had significantly higher mean escape latency than the control group ($P < 0.05$), whereas the Scop +Baic group had significantly reduced mean escape latency ($P < 0.05$) (Figure 1C).

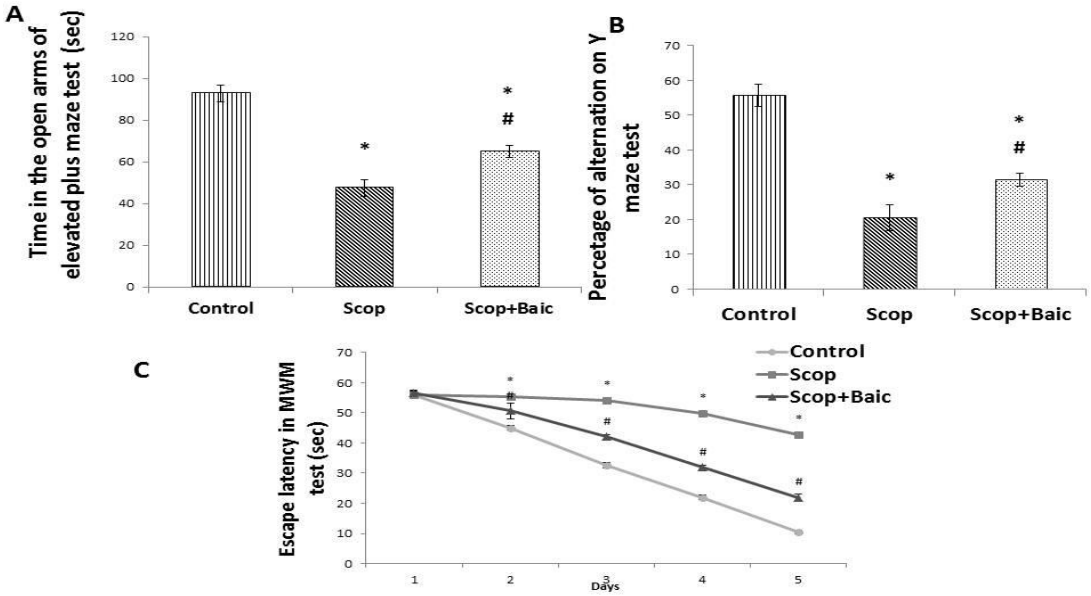

Fig. (1): Baicalein's impact on (A) elevated plus maze, (B) Y maze, and (C) Morris Water Maze tests in all tested groups. * Significant compared to control; # Significant compared to Scop. Data provided as mean ± SD.

The Scop group showed significantly higher levels of MDA, TNF-α, IL-6, IL-1β, and NF-kB gene expression, while Ach, BDNF, SOD, PI3K, and Akt gene expression were significantly lower compared to the control group. The Scop+Baic group had significantly lower levels of MDA, TNF-α, IL-6, IL-1β, and NF-kB gene expression in the hippocampus region compared to the Scop group. However, there was a significant increase in Ach, BDNF, SOD, PI3K, and Akt gene expression **(**Table 1).

Table (1): Hippocampus acetylcholine, MDA, SOD, TNF-α, IL-6, IL-1β, hippocampus PI3K, Akt, and NF-kB gene expression evaluation in the research groups (n: 30 rats, 10 for each group)

	Control group	**Scop group**	**Scop + Baic group**
Hippocampal acetylcholine (U/ml)	66.8±3.45	25.1±1.15*	41.8±1.2 $^{*\#}$
Hippocampal MDA (nmol/ g. tissue)	9.1 ±0.89	38.7± 1.3*	19.5± 1.04$^{*\#}$
Hippocampal SOD (U/g. tissue)	8.5 ±0.93	3.81±0.41*	6.1±0.61$^{*\#}$
Hippocampal TNF-α (pg/ml)	110.3±4.59	318.99±6.91*	242.2±5.1$^{*\#}$
Hippocampal IL-6 (pg/mL)	180.9±8.42	415.12±9.39*	319.85±8.1$^{*\#}$
Hippocampal IL-1β (pg/ml)	108.9±6.42	390.1±9. 59*	281±8.1$^{*\#}$
Hippocampal BDNF (pg/mL)	68.7±2.42	25.5±1.09*	43.9±2.1$^{*\#}$
Hippocampal PI3K gene expression	1	0.34±0.03*	0.62±0.06$^{*\#}$
Hippocampal Akt gene expression	1	0.41±0.07*	0.71±0.08$^{*\#}$
Hippocampal NF-kB gene expression	1	3.9±0.13*	2.47±0.18$^{*\#}$

* Significant compared with control, # Significant compared with Scop. Data represented as mean ± SD. MDA: Malondialdehyde, SOD: superoxide dimutase, TNF-α: tumour necrosis factor alpha, IL-6: interleukin-6, IL-1β: interleukin-1β, BDNF: brain derived neurotrophic factor.

Hematoxylin and Eosin-stained sections

The control group's hippocampus CA1 included a normal distribution of molecular, pyramidal, and polymorphic layers. The pyramidal cells in the SCOP group were grouped in an unsettling pattern; they seemed degraded, with swollen, hyperchromatic nuclei and congested blood vessels. Despite the presence of a few damaged pyramidal cells, the pyramidal cell layer in the SCOP+Biac appeared to be nearly normal and structured (Figure 2A-C).

The thickness of the pyramidal layer reduced significantly (P<0.05) in the SCOP group compared to the control group (22.44±0.72 vs. 52.07±1.88 micrometer). However, the SCOP+Biac group exhibited a significant increase (P<0.05) in comparison to the SCOP group (40.33±1.36 vs. 22.44±0.72 micrometer) (Figure 2D).

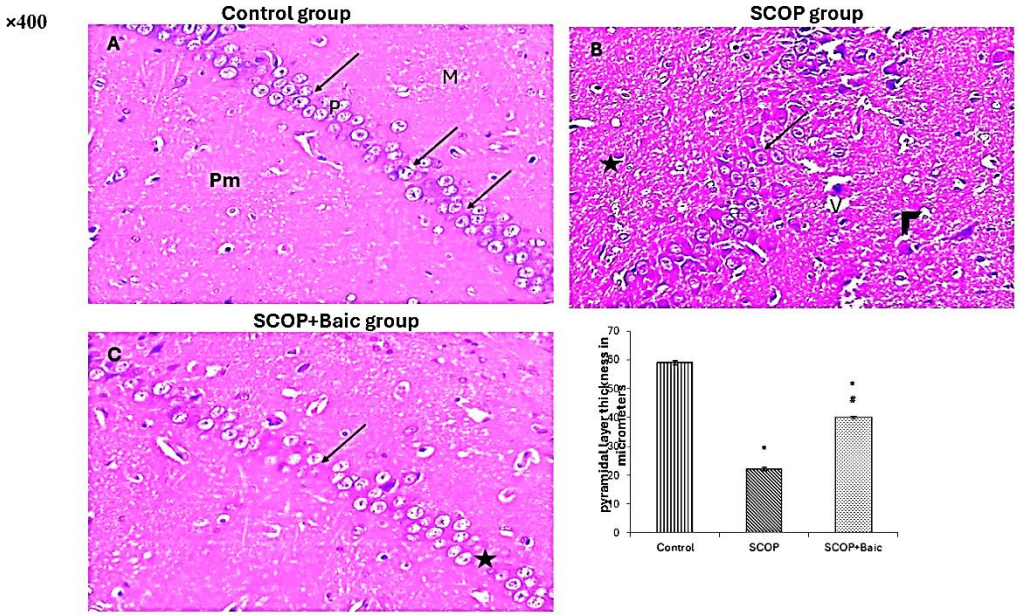

Fig. (2): Photomicrographs of the CA1 area of the hippocampus of the different groups showing the pyramidal (P), molecular (M) and polymorphic (Pm) layers. (A) The control group showed normal small pyramidal cells with large vesicular nuclei (black arrows). (B) The SCOP group showed pyknotic pyramidal cells (black arrow), vacuolations (V), degenerated oligodendroglia (arrowhead) and numerous neuroglial cells (star). (C) SCOP+Biac showing normal pyramidal cells (black arrow) with few degenerated ones (star) (X400), (D) Pyramidal tract thickness in all groups.

Immunohistochemical results:

In Bax stained sections: the SCOP group had a substantially greater (P<0.05) positive Bax area than the control group (54.53±4.15 vs. 8.01±1.07 %). The positive Bax area in the SCOP+Biac group decreased significantly compared to the SCOP group (18.45±1.57 vs. 54.53±4.15 %), but remained higher than the control group (Figure 3d).

In GFAP-stained sections: the SCOP group had significantly higher numbers of astrocytes than the control group (51.66±5.24 vs. 9.16±1.60%, P< 0.05). The SCOP+Biac group had fewer astrocytes than the SCOP group (20.33±1.36 vs 51.66±5.24), but more than the control group (Figure 3h).

In Tau stained sections: the SCOP group showed a significant rise (P<0.05) in

the Tau positive region compared to the control group (72.97±1.95, versus 25.04±5.80). SCOP+Biac had a lower Tau positive area than SCOP (40.04±5.80 vs 72.97±1.95), but still higher than the control group (Figure 3j).

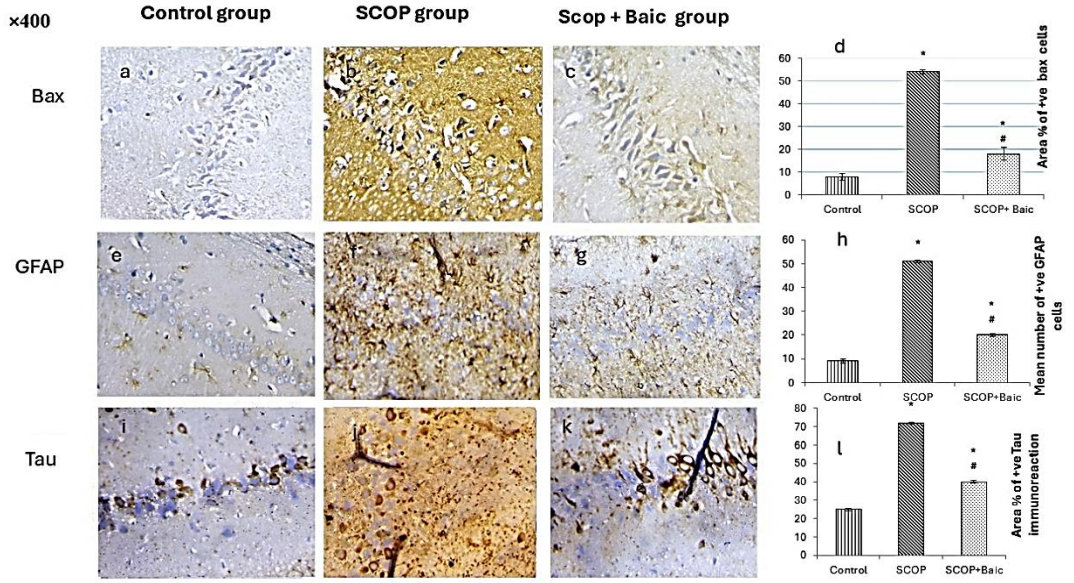

Fig. (3): Representative micrographs of the different experimental groups showing a substantial elevation of the Bax (a-d), GFAP (e-h) and Tau (i-l) immunoreaction in the SCOP group and their decrease in SCOP+Biac (X400).

Discussion

Scop is a validated Alzheimer's disease (AD) model that is employed in neurobehavioral research. It is a neurotoxin that is readily able to traverse the blood-brain barrier. Chen et al. (2020) have observed that it reproduces the primary symptoms observed in AD patients and impairs behavioral assessments of learning and memory in a variety of animal species, which is consistent with the results of our investigation. Scop exhibited cognitive decline, memory impairment, and elevated anxiety in comparison to the control group, as evidenced by significant behavioral alterations in the MWM, Y Maze, and EPM tests. Scop-induces Aβ deposition and oxidative stress are the two inseparable characteristics of AD (Chen et al., 2020; Hancianu et al., 2013). The hippocampal region of the SCOP cohort exhibited numerous degenerative changes. This is consistent with prior research (Yadav et al., 2022).

In agreement with Song et al. (2024) as well as Jadhav and Kulkarni (2023), our results demonstrated that Baic substantially reduced the cognitive abnormalities caused by Scop. The neuro-protective action of baicalein is influenced by its anti-oxidant, anti-inflammatory, and acetylcholinesterase-inhibitory properties (Hu et al., 2022). Baic's neuro-protective properties were demonstrated by its significant enhancement of Scop-induced histological alterations in the hippocampus, as per Song et al. (2024). Baic enhances cognitive impairments by activating the BDNF and decreasing oxidative stress (Oh et al., 2013). Baicalein significantly diminished cortical neuronal damage in diabetic rodents by decreasing inflammation and apoptosis in the central nervous system

and enhancing spatial learning and memory abilities (Li et al., 2019b).

Another characteristic of Alzheimer's disease is an elevated level of oxidative stress in brain neurons. Scopolamine-induced cognitive impairment in animal models is associated with modifications in the brain's oxidative stress condition, as per Yadang et al. (2020). This is consistent with the findings of our study, which showed a decrease in MDA and SOD in Scop in comparison to the control group.

Baicalein significantly mitigated the oxidative stress induced by Scop by increasing SOD activity and decreasing MDA levels. The results of the present study were in agreement with those of a previous study conducted by Jadhav and Kulkarni (2023) and Shata et al. (2020), which emphasize the substance's ability to scavenge free radicals and exert an antioxidant effect. Furthermore, research has demonstrated that baicalein can improve antioxidant properties by restoring antioxidant enzymes and increasing their gene expression (Kang et al., 2012). These results substantiate the results of the current investigation. Additionally, the antioxidant properties of baicalein contribute to the prevention of mitochondrial dysfunction by increasing Nrf2 (Hu et al., 2022).

Frequently, neuroinflammation is observed in the brains of Alzheimer's disease patients. Proinflammatory cytokines produced by brain microglia have been associated with the development of AD (Wang et al., 2015). The results of our research, which demonstrated that Scop administration increased inflammatory cytokines and downregulated NF-kB gene expression in the Scop group, are consistent with a study conducted by Demirci et al. (2017). This implies that scopolamine does, in fact, resemble the AD picture. NF-KB signaling pathways were activated by Scop (Jung et al. 2009). Scop may, therefore, impede cognition by inducing cholinergic neuronal injury that is further exacerbated by inflammation mediated by NF-KB (Shabani and Mirshekar, 2018).

Baic significantly inhibited the inflammatory state induced by Scop in the hippocampal region, as evidenced by a substantial decrease in pro-inflammatory cytokines and downregulation of NF-kB (Jadhav and Kulkarni, 2023). Compare this to the Scop group. Baicalein therapy reduced the production of pro-inflammatory molecules and the activation of glial cells by inhibiting the TLR4/MyD88/NF-κB signaling pathway (Jadhav and Kulkarni, 2023).

Acetyl choline is a neurotransmitter that facilitates the body's cognitive and physiological functions. Changes in AChE activity are indicative of cognitive and learning impairments (Jadhav and Kulkarni, 2023). In accordance with our results, the hippocampal ACh levels of the Scop group decreased, which is in agreement with Thongrong et al. (2024). Scopolamine injection elevates AChE levels, as indicated by research (Thongrong et al., 2024). An increased quantity of AChE results in memory impairment, as it metabolizes a greater amount of acetylcholine, reduces its concentration in synapses, and disrupts cholinergic neurotransmission (Choi et al., 2021).

The enhanced ACh level achieved with Baic is consistent with the findings of Jadhav, et al. (2023). The fact that Baic significantly reduced AChE activity in the cortex and hippocampal regions supports its use in the treatment of AD (Jadhav and Kulkarni, 2023). Baic has exhibited potent AChE inhibitory efficacy in numerous prior in vitro studies (Han et al., 2019).

According to Chen et al. (2017), BDNF plays critical functions in synaptic plasticity, neurogenesis, and neuroprotection.

In rats' hippocampal regions, scop decreased BDNF, which is consistent with Eun et al. (2017), suggesting that scopolamine causes the cognitive impairment via inhibiting the BNDF neurotrophic pathway.

Additionally, BDNF has the ability to alter a number of signaling pathways linked to memory and learning. Neuroinflammation, the development of Aβ plaque, tau protein phosphorylation, and neuronal death have all been connected to the BDNF signaling pathways (Gao et al., 2022). According to Jadhav and Kulkarni (2023), who demonstrated an increase in BDNF expression with Baic's supplementation confirming its neuroprotective impact with improved cognitive function, the Scop+Baic group's BDNF level was significantly higher in this study than Scop's.

In Alzheimer's disease (AD), the levels of phosphorylated Tau protein and amyloid beta (Aβ) are elevated, which is associated with permanent cognitive decline that impairs memory and alterations in the central cholinergic system (Bachurin et al., 2017). The scope group exhibited elevated hippocampal tau immunoreactivity in comparison to the control group. Scop increased the quantity of phosphorylated tau protein in previous research (Safar et al., 2016). Furthermore, Scop was found to enhance tau hyperphosphorylation by enhancing the activity of a tau kinase (Dickey et al., 2008). The current investigation demonstrated a substantial reduction in the tau immunoreaction in rodents that were administered baicalein. The antioxidant action of baicalein and improved cognitive performance were attributed to the close relationship between oxidative stress and tau hyperphosphorylation (Liu et al., 2015).

Inflammation and nerve injury may be monitored through the use of the astrocyte marker, GFAP (Song et al., 2024). Baicalein significantly diminished the GFAB immunoreaction that Scop significantly increased in the rat hippocampal tissue, as it has a potent anti-inflammatory effect against neurodegenerative disorders (Sowndhararajan et al., 2017).

In rats, the expression of the pro-apoptotic protein Bax was elevated by scopolamine, as per Ajami et al. (2012). Our findings illustrated this by exhibiting a significant decrease in the Scop plus Baic group and a sudden rise in the Bax immunoreaction in the hippocampus of the Scop group. The production of proinflammatory cytokines in the hippocampus was downregulated by Baic, which subsequently diminished apoptosis and mitigated the consequences of inflammatory reactions (Li et al., 2019b).

Antioxidants and PI3K and Akt activation are inhibited by increased Aβ, as evidenced by numerous research findings (Matsuda et al., 2018). glycogen-synthase kinase-3 (GSK-3) is a downstream intermediate of P13K/Akt. As a consequence of PI3K/Akt inhibition, the inactive phosphorylated GSK-3β (p-GSK-3β) decreased and GSK-3β was activated in the brains of Alzheimer's disease patients (Giese, 2009). As a result, the active GSK-3β increases the production of Aβ. p-GSK-3β is essential for synaptic plasticity and memory by phosphorylating and activating cAMP-response-element-binding protein (CREB) (Giese, 2009; Jain et al., 2013). Active p-CREB transcriptionally induces the downstream target gene of BDNF, which regulates synaptic plasticity and neurogenesis (Kida and Serita, 2014).

Furthermore, Scop induces modifications in pathways that are involved in the pathophysiology of AD, such as the PI3K/AKT/NF-kB/BDNF pathway, as underscored by our investigations. The rat

hippocampal PI3K/AKT was substantially downregulated in the scope group, which is in accordance with Abuelezz and Hendawy (2023). The anticipated degenerative changes are comparable to those observed in Alzheimer's disease patients and animal models (Choi and Koh, 2016). Scop is therefore a valuable instrument for the examination of the cellular and molecular changes associated with the pathogenesis of Alzheimer's disease, as well as the potential treatment targets for this condition. Baic significantly improved PI3K/AKT/NF-kB/BDNF in comparison to the Scop group, thereby mitigating the cognitive impairments induced by Scop.

Conclusion

Scop has significantly hindered neurocognitive function. Baic improved cognitive deficits. Its neuroprotective effects are attributed to its antioxidant, anti-inflammatory, neurotrophic, and anti-apoptotic qualities through the modulation of the PI3K/Akt/NF-kB signaling pathway.

Conflicts of Interest

The authors declare no conflict of interest.

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References

Abuelezz, S.A. and Hendawy, N. (2023). ‘Spotlight on Coenzyme Q10 in scopolamine-induced Alzheimer’s disease: oxidative stress/ PI3K/ AKT/ GSK 3ß/ CREB/ BDNF/ TrKB’. *Journal of Pharmacy and Pharmacology*, *75*(8), p.p. 1119-1129.

Ajami, M., Eghtesadi, S., Habibey, R. et al. (2012). ‘Effect of short and long-term treatment with omega-3 Fatty acids on scopolamine -induced amnesia’. *Iranian Journal of Pharmaceutical Research*, 11(2), p.p.533.

Aksoz, S.S., Gocmez, T.D., Sahin, D. et al. (2019). “The protective effect of metformin in scopolamine-induced learning and memory impairment in rats.” *Pharmacological Reports*, 71 (5) pp. 818–825.

Bachurin, S.O., Bovina, E.V. and. Ustyugov, A.A. (2017). “Drugs in clinical trials for Alzheimer's disease: the major trends.” *Medicinal Research Reviews*, 37(5) p.p. 1186–1225.

Chen, S.D., Wu, C.L., Hwang, W.C. et al. (2017). ‘More insight into BDNF against neurodegeneration: anti-apoptosis, anti-oxidation, and suppression of autophagy’. *Int. J. Mol. Sci*. 18 p.p.545.

Chen, W.N. and Yeong, K.Y. (2020). ‘Scopolamine, a toxin-induced experimental model, used for research in Alzheimer’s disease’. *CNS Neurol. Disorder Drug Targets*; 19 p.p. 85–93.

Choi, H. and Koh, S.H. (2016). ‘Interaction between amyloid beta toxicity and the PI3K pathway in Alzheimer’s disease’. *J. Alzheimer’s Dis. Parkinsonism*, 6 p.p. 269.

Choi, J.H., Lee, E.B., Jang, H.H. et al. (2021). 'Allium hookeri extracts improve scopolamine - induced cognitive impairment via activation of the cholinergic system and anti-neuro-inflammation in mice'. *Nutrients*. 13 (8) p.p. 2890.

doi: 10.3390/nu13082890.

Demirci, K., Naziroglu, M., Ovey, I.S. et al. (2017). 'Selenium attenuates apoptosis, inflammation and oxidative stress in the blood and brain of aged rats with scopolamine-induced dementia'. *Metab. Brain Dis*. 32 p.p. 321–329.

Dickey, C.A., Koren, J., Zhang, Y.J. et al. (2008). 'Akt and CHIP coregulate tau degradation through coordinated interactions'. *Proceedings of the National Academy of Sciences*, *105*(9) p.p. 3622-3627.

Eun, C.S., Lim, J.S., Lee, J. et al. (2017). 'The protective effect of fermented Curcuma longa, L. on memory dysfunction in oxidative stress-induced C6 gliomal cells, pro-inflammatory -activated BV2 microglial cells and scopolamine-induced amnesia model in mice'. *BMC Complement Altern. Med*. 17 p.p. 367.

Gao L., Zhang Y., Sterling K. et al. (2022). "Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential". *Transl. Neurodegener*.11 p.p. 4. doi: 10.1186/s40035-022-00279-0.

Giese, K.P. (2009). 'GSK-3: A key player in neuro-degeneration and memory'. *IUBMB Life*; 61 p.p.516–521.

Hafez, H. S., Ghareeb, D. A., Saleh, S. R. et al. (2017). "Neuro-protective effect of Ipriflavone against Scopolamine-induced memory impairment in rats." *Psychopharmacology*, 234(20) p.p. 3037–3053.

Han, J., Ji, Y., Youn, K., Lim, G. et al. (2019). 'Baicalein as a potential inhibitor against BACE1 and AChE: Mechanistic comprehension through in vitro and computational approaches'. *Nutrients*, 11 p.p. 2694.

Hancianu, M., Cioanca, O., Mihasan, M. et al. (2013). 'Neuro-protective effects of inhaled lavender oil on scopolamine -induced dementia via antioxidative activities in rats'. *Phytomedicine;* 20 p.p. 446–452.

Hu, Z. Guan, Y. Hu,W. et al. (2022). 'An overview of pharmacological activities of Baicalin and its aglycone baicalein: New insights into molecular mechanisms and signaling pathways'. *Iran*. J. *Basic Med. Sci.* 25 p.p. 14–26.

Imam, N.O., Al-Harbi, M.M., Al-Harbi, M.A. et al. (2015). 'Diosmin downregulates the expression of T cell receptors, pro-inflammatory cyto-kines and NF-κB activation against LPS-induced acute lung injury in mice'. *Pharmacol. Res*. 102 (2015) p.p. 1–11.

Jadhav, R. and Kulkarni, Y.A. (2023). 'Effects of baicalein with memantine on aluminium chloride -induced neurotoxicity in Wistar rats'. *Frontiers in Pharmacology*, *14* p.p. 1034620.

Jain, V., Baitharu, I., Prasad, D. et al. (2013). 'Enriched environment prevents hypobaric hypoxia induced

memory impairment and neuro-degeneration: role of BDNF/PI3K/ GSK3β pathway coupled with CREB activation'. *PLoS One*; 8(5) p.p. e62235.

Jung, K., Lee, B., Han, S.J. et al. (2009). **'**Mangiferin ameliorates scopolamine-induced learning deficits in mice'. *Biol. Pharm. Bull*., 32 (2) p.p. 242–246.

Kang, K. A., Zhang, R., Piao, M. J. et al. (2012). **'**Baicalein inhibits oxidative stress-induced cellular damage via antioxidant effects'. *Toxicol. Ind. Health*, 28 p.p. 412–421.

Kida, S. and Serita, T. (2014). **'**Functional roles of CREB as a positive regulator in the formation and enhancement of memory'. Brain Res. Bull., 105 p.p. 17–24.

Kiernan, J. A. (2015). *Histological and Histochemical Methods: Theory and Practice* (5th ed.). Scion Publishing, p.p. 479-500.

Kosteniuk, J. G., Morgan, D. G., O'Connell, M. E. et al. (2016). "Simultaneous temporal trends in dementia incidence and prevalence', 2005–2013: a population-based retro-spective cohort study in Saskatchewan, Canada." *International Psychogeriatrics*, 28 (10) p.p. 1643–1658.

Li, J., Zhou, Y., Du, G. et al. (2019a). **'**Integration of transcriptomics and network analysis deciphers the mechanisms of baicalein in improving learning and memory impairment in senescence-accelerated mouse prone 8 (SAMP8)'. *Eur. J. Pharmacol*. 865 p.p. 172789.

Li, Y., Chen, Q., Ran, D. et al. (2019b). **'**Changes in the levels of 12/15-lipoxygenase, apoptosis -related proteins and inflammatory factors in the cortex of diabetic rats and the neuroprotection of baicalein'. *Free Radical Biology and Medicine, 134* p.p. 239-247.

Lin, J., Huang, L., Yu, J. et al. (2016). **'**Fucoxanthin, a marine carotenoid, reverses scopolamine -induced cognitive impairments in mice and inhibits acetylcholinesterase in vitro'. *Mar. Drugs* 14 p.p. 67.

Liu, Z., Li, T., Li, P. et al. (2015). **'**The ambiguous relationship of oxidative stress, tau hyperphosphorylation, and autophagy dysfunction in Alzheimer's disease'. *Oxidative Med. Cell. Longev*. 2015:352723 p.p. 1–12.

Matsuda, S., Nakagawa, Y., Tsuji, A. et al. (2018). **'**Implications of PI3K/AKT/ PTEN signaling on superoxide dismutases expression and in the pathogenesis of Alzheimer's disease'. *Diseases*; 6(2) p.p. 28.

Mendiola-Precoma, J., Berumen, L. C., Padilla, K., et al. (2016). **'**Therapies for prevention and treatment of Alzheimer's disease'. *BioMed Research International*, (1) p.p. 2589276.

Oh, S.B., Park, H.R., Jang, Y.J. et al., (2013). **'**Baicalein attenuates impaired hippocampal neurogenesis and the neurocognitive deficits induced by γ-ray radiation'. *Brit. J. Pharmacol.* 168 (2) p.p. 421–431.

Reddy, D.S. (1997). "Assessment of nootropic and amnestic activity of centrally acting agents." *Indian Journal of Pharmacology*, 29 (4) p.p. 208–221.

Safar, M.M., Arab, H.H., Rizk,S. M. S. et al. (2016). 'Bone marrow - derived endothelial progenitor cells protect against scopolamine -induced Alzheimer - like pathological aberrations'. *Mol. Neurobiol*. 53 p.p.1403–1418.

Shabani, S. and Mirshekar, M.A. (2018). 'Diosmin is neuro-protective in a rat model of scopolamine -induced cognitive impairment'. *Biomedicine & Pharmacotherapy, 108* p.p. 1376-1383.

Shata, A., Elkashef, W., Hamouda, M.A. et al. (2020). 'Effect of artesunate vs memantine in aluminum chloride induced model of neurotoxicity in rats'. *Adv. Alzheimer's Dis.* 9 p.p. 1–19. doi:10.4236/aad.2020.91001.

Shi, J., Li, Y., Zhang, Y. et al. (2021). 'Baicalein ameliorates Aβ-induced memory deficits and neuronal atrophy via inhibition of PDE2 and PDE4'. *Front. Pharmacol.*, 12 p.p. 794458.

Shu, Y., Zhang, H., Kang, T. et al. (2013). 'PI3K/Akt signal pathway involved in the cognitive impairment caused by chronic cerebral hypoperfusion in rats'. *PLoS One*, 8 (12) p.p. e81901.

Song, J., Li, M., Kang, N. et al. (2024). 'Baicalein ameliorates cognitive impairment of vascular dementia rats via suppressing neuroinflammation and regulating intestinal microbiota'. *Brain Research Bulletin*, 208 p.p. 110888.

Sowndhararajan. K., Deepa, P., Kim, M. et al. (2017). 'A review on Baicalein as a potent neuro-protective agent: A review'. *Biomed. Pharmacother*., 95 p.p.1021–1032.

Suvarna, S.K., Layton, C., and Bancroft, J.D. (2019). *Immunohistochemical and immunofluorescent techniques.* In: Bancroft Theory and Practice of Histological Techniques (8th ed.). Elsevier Limited, p.p. 337-394.

Tang, K.S. (2019). 'The cellular and molecular processes associated with scopolamine-induced memory deficit: A model of Alzheimer's biomarkers'. *Life Sci.* 15 p.p. 233:116695. doi: 10.1016/j.lfs.2019.116695.

Tchantchou, A. A., Puche, U., Leiste, W. et al, (2018). "Rat model of brain injury to occupants of vehicles targeted by land mines: mitigation by elastomeric frame designs." *Journal of Neuro-trauma*, 35 (10) p.p. 1192–1203.

Thongrong, S., Promsrisuk, T., Sriraksa, N. et al. (2024). 'Alleviative effect of scopolamine-induced memory deficit via enhancing antioxidant and cholinergic function in rats by pinostrobin from Boesenbergia rotunda (L.)'. *Biomedical Reports, 21*(3) p.p. 130.

Wang, W.Y., Tan, M.S., Yu, J.T. et al. (2015). 'Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease'. *Ann. Transl. Med*., 3 p.p. 136.

Yadang, F. S. A., Nguezeye, Y., Kom, C. W. et al., (2020). 'Scopolamine-Induced

memory impairment in mice: Neuro-protective Effects of Carissa edulis (Forssk.) Valh (Apocynaceae) aqueous extract'. *International Journal of Alzheimer's Disease, 2020*(1) p.p. 6372059.

Yadav, Y. C., Singh, A., Kannaujia, S. K. et al., (2022). 'Neuro-protective effect of Citrus limon juice against scopolamine induced amnesia in Wistar rats: Role of cholinergic neurotransmission monitoring and beta-actin signaling'. *Pharmaco-logical Research -Modern Chinese Medicine, 5* p.p. 100191.

Yu, M., Qi, B., Xiaoxiang, W. et al. (2017). 'Baicalein increases cisplatin sensitivity of A549 lung adeno-carcinoma cells via PI3K/Akt/NF-κB pathway'. *Biomedicine & Pharmaco-therapy*, 90, p.p. 677-685.