International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2021.499.505

Research Article
Boswellic Acid Attenuates Scopolamine-Induced Neurotoxicity and Dementia in Rats: Possible Mechanism of Action

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Abstract

Background and Objective: The oleo gum resin of plants belonging to the Boswellia species contains pentacyclic triterpenic acids known as boswellic acid. This acid possesses anti-depressive, anti-anxiety, anti-tumour, neuroprotective, antioxidant and anti-inflammatory activities. This research work was undertaken to assess the neuroprotective effects of boswellic acid in scopolamine-treated rats. **Materials and Methods:** Wistar rats were grouped equally into four groups. Groups I and II received 0.5 mL saline, while animals from Group III and IV received boswellic acid (40 and 80 mg kg^{-1}/day, respectively) for 21 days, intraperitoneally (i.p.). One hour after the respective treatments, daily 0.5 mL of normal saline was given intra-peritoneally to normal control animals and scopolamine (2 mg kg^{-1}/day, i.p.) was given to all other animals. **Results:** On day 21, after 30 min of scopolamine treatment, animals were evaluated for behavioural parameters. Then animals were euthanized and brains were used for biochemical studies and RNA expression analysis. **Conclusion:** Treatment with scopolamine altered the normal behaviours, impaired memory and decreased the mRNA expressions of Brain-Derived Neurotrophic Factor (BDNF), Ca^{2+}/calmodulin-dependent protein kinase(CaMK), Cyclic adenosine monophosphate (cAMP), cAMP-response element-binding (CREB), Extracellular Signal-regulated Kinase (ERK) and Phosphoinositide 3-kinases (PI3K) as well as increased oxidative stress and AchE activity in brain tissue. Whereas, pretreatment with boswellic acid to scopolamine treated animals corrected the altered behaviour, decreased oxidative stress and Acetylcholinesterase (AchE) activity, restored memory, antioxidant capacity and BDNF, CaMK, CREB, ERK and PI3K mRNA expression. These results indicate the protective actions of boswellic acid in scopolamine-induced dementia in rats. Significant free radical scavenging potential points out toward neuroprotective action.

Key words: Alzheimer's disease, dementia, neuroprotective, Boswellia, acetylcholinesterase, basal forebrain, BDNF

Citation: Afzal, M., S. Alzarea, A.M. Quazi, I. Kazmi and A. Zafar *et al*., 2021. Boswellic acid attenuates scopolamine-induced neurotoxicity and dementia in rats: Possible mechanism of action. Int. J. Pharmacol., 17: 499-505.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alzheimer's Disease (AD) is a neurological disorder of the elderly that progress with time and causes dementia[1]. Pathogenesis of AD includes oxidative stress, neuroinflammation, hyperphosphorylation of tau protein and deposition of amyloid plaques, which causes degeneration of cholinergic neurons in the basal forebrain region[2]. Hence, symptoms of AD can be clinically reduced temporarily or delayed by treating with acetylcholinesterase inhibitors. Acetylcholinesterase inhibitors spare the acetylcholine by reducing its enzymatic degradation but the progression of the disease continues[3]. Hence, researchers around the world are searching for new strategies to target AD safely and effectively.

Herbal medicines and synthetic agents with Anti-inflammatory and antioxidant activities have been reported as neuroprotective in preclinical evaluation[4-6]. The Boswellia genus (family Burseraceae) plants include *B. serrata*, *B. sacra*, *B. carterii*, *B. papyrifera*, *B. neglecta*, *B. rivae*, *B. frereana* and *B. ovalifoliolata*, etc. Boswellia species are commonly seen in South Asia, East Africa and Gulf countries[7,8].

Boswellic acid (pentacyclic triterpenic acids) are the products of Boswellia species and are obtained from oleo gum resins. Literature review suggests that boswellic acid possess antidepressive, anti-anxiety, anti-tumour actions in brain tumour patients, neuroprotective effects, antioxidant, anti-inflammatory actions in chronic inflammatory conditions[7-9]. Moreover, boswellic acid is reported to provide neuroprotection against Trimethyltin-induced dementia in experimental rats[7].

This research protocol is planned to assess the neuroprotective potentials of boswellic acid in scopolamine-treated rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Pharmacology, SRL, India from November, 2020-January, 2021.

Chemicals: Boswellic acid was procured from Yucca enterprises, Mumbai, India. Scopolamine hydrobromide (SCOP) Sigma-Aldrich (USA), Acetylcholinesterase (AChE) reagent kit (Sigma-Aldrich, USA)was procured from the authorized distributor of the company, India.

Animals: A total of 24 Wistar rats (160-210 g) maintained in standard laboratory setup were used in the study. The experiments are to be conducted on animals approved by the Local Committee of bioethics (LCBE) and all experiments are conducted as per standard procedures and guidelines of CPCSEA, Govt. of India.

Experimental design: Accurately weighed quantities of boswellic acid and scopolamine hydrobromide were dissolved in saline (pH 7.4) and these solutions were prepared fresh daily just before administration to the animals. Scopolamine 2 mg kg^{-1} administered intraperitoneally for induction of dementia. Boswellic acid at (40 and 80 mg kg^{-1}) dose chosen based on the reported scientific literature[7].

A total of 24 Wistar rats (n = 6) were randomly assigned to one of four groups. Animals from Groups I and II have received 0.5 mL of saline (i.p.). Group III and IV were treated with boswellic acid (40 and 80 mg kg^{-1}/day, i.p.), respectively, the total duration of the treatment protocol was 21 days.

Daily one hour after the offer-mentioned treatments, a group I animals were administered with (0.5 mL kg^{-1}) normal saline (i.p.) and groups II-IV animals were administered with scopolamine (2 mg kg^{-1}, i.p.) for 21 days.

On day 21, after 30 min of scopolamine treatment, rats were tested for behavioural parameters later euthanized and the brain from each animal wascollected for further biochemical estimations and RNA isolations.

Behavioural tests: To avoid the circadian influences, behavioural parameters were evaluated at daytimes between 10:00 AM and 4:00 PM[5]. The behavioural tests were carried as per published protocols in a sequence of locomotor activity in open field apparatus[10,11], memory dysfunction was evaluated using a plus-maze test[4,5] and Morris water maze test[12].

Biochemical estimation: After evaluation of behavioural parameters, animals were sacrificed, brains were collected and a portion of brain tissue homogenized (10% w/v) chilled phosphate-buffer saline, centrifuged at 12000×g in cold temperature for 20 min. The supernatant estimated for lipid deoxidation, glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD) quantifications using colorimetric methods.

Estimation of tissue protein: The amount of protein per gram of protein was estimated [13].

Estimation of Lipid peroxidation as tissue malondialdehyde (MDA): Test tube containing (0.1 mL) tissue homogenate added with 1 mL of TCA (10% w/v),1 mL of TBA(0.67% w/v) and kept in boiling water for half an hour. Later test tubes were cooled in an ice bath for 10-15 min and centrifuged at

4000×g for 10 min. The pink coloured clear supernatant separated and the absorbance recorded at 532 nm[14].

Estimation of GSH: Proteins were precipitated by the addition of 100 µL (25%) TCA to 500 µL of homogenate and which was later centrifuged for 5 min at 4000 rpm, followed by supernatant collection. 300 µL of supernatant added with 500 µL of phosphate buffer (0.1-M, pH 7.4) and 200 µL of DTNB (10 mM) mixed and incubated for 10 min. Later absorbance noted at 412 nm against a blank[15].

Estimation of CAT: To a cuvette added with 0.95 mL of H_2O_2 (10 mM) in 60 mM phosphate buffer (pH 7.0) the 50 µL supernatant of tissue homogenate was added. The H_2O_2 degradation rate was documented at 240 nm for 60 sec[16].

Estimation of SOD: SOD reagent constituting of xanthine (0.1 mmol L^{-1}), of EDTA (0.1 mmol L^{-1}), BSA (50 mg), NBT (25 mmol L^{-1}) and Na_2CO_3 (40 mmol L^{-1}) was prepared. A total of 50 µL of tissue homogenate was mixed with 0.9 mL of SOD reagent and 25 units of xanthine oxidase and at room temperature, this solution was incubated for 20 min. And the ongoing reaction was terminated by adding 1 mL of $CuCl_2$ (0.8 mmol L^{-1}), finally, the absorbance recorded at 560 nm[17].

Estimation of AchE: Aliquoted brain tissue homogenized in 0.1 M (pH 7.5) PBS and centrifuged at 14,000×g for 5 min. The clear supernatants separated for estimation of AchE activity and assessed using the kit as a manufacturer's instructed. Briefly, the fresh working reagent was prepared by dissolving weighed quantity of reagent in assay buffer.

The freshly prepared (190 µL) working reagent was mixed with 10 µL of sample in a 96-well plate by briefly tapping the plate. For reference, 200 µL water (assay blank) and 200 µL calibrator poured to separate wells. Samples incubated at normal temperature for about 2 min and initial absorbance at 412 nm noted. Incubation of the plate incubated at room temperature continually and final absorbance recorded after 10 min of reaction. The AChE activity calculated and expressed (U mg^{-1}/protein).

Isolation of total RNA and RT-PCR: From brain tissue,total RNA was extracted with ice-cold Trizole reagent under cold temperature as manufacturer's protocol. Total RNA indices estimated at 260 nm utilizing a standard curve. First-strand cDNA generated (using high-capacity cDNA reverse transcription kit) as per manufacture's instructions. Shortly, 1.5 µg isolated RNA added to 3.2 µL nuclease-free water containing 2.0 µL of 10× reverse transcriptase buffer, 0.8 µL (100 mM) of 25× dNTP mix, 2.0 µL 10× reverse transcriptase random primers and 1.0 µL of multi-scribe reverse transcriptase. The reaction mixture was then incubated at room temperature for 10 min, later temperature elevated to 37°C for the next 120 min and cooled to 4°C[18].

qRT-PCR carried out using ABI Prism 7900 HT (Applied Biosystems, USA) for determining the mRNA expressions. The cDNA kept for amplification in the 96-well plates. The reaction mixture (25 µL) of 0.1 µL (10 µM) forward primer, 0.1 µL (10 µM) reverse primer (40 µM final concentration of each primer), 12.5 µL of Universal Master Mix (SYBR Green) reagent, 11.05 µL nuclease-free water and 1.25 µL cDNA sample. To test contamination of assay reagent, assay controls were also added to the same well plates. RT-PCR data analyzed using relative gene expressions protocol (Applied Biosystems User Bulletin No. 2). The data presented as the fold change in gene expressions normalized to a reference gene (endogenous GAPDH) and relative to a calibrator[18].

Statistical analysis: The results are presented as mean±SEM and analyzed statistically using GraphPad Prism software. Except for the Morris water maze test, all other parameters were analyzed using One-way ANOVA followed by Tukey's post hoc test. Morris water maze test results wereanalyzed by two-way ANOVA followed by Bonferroni test, $p<0.05$ considered as statistically significant.

RESULTS

Behavioural tests

Open field test: Animals treated with scopolamine alone spent more time in the peripheral area of the open field when compared with normal control animals and the numbers of line crossings were counted less in those animals. Administration of boswellic acid to the animals treated with scopolamine spent more time (15-20%) in the central square of the open field and crossed the line more frequently (12-18%) compared to scopolamine alone treated animals. Animals treated with scopolamine alone showed raring behaviour in the open field. Administration of boswellic acid reduced the raring behaviour of the animals. Statistical analysis revealed that none of the treatments had a significant influence on locomotor activity.

Elevated plus-maze: Administration of scopolamine-induced 47.17±2.41% memory loss in rats, compared to normal

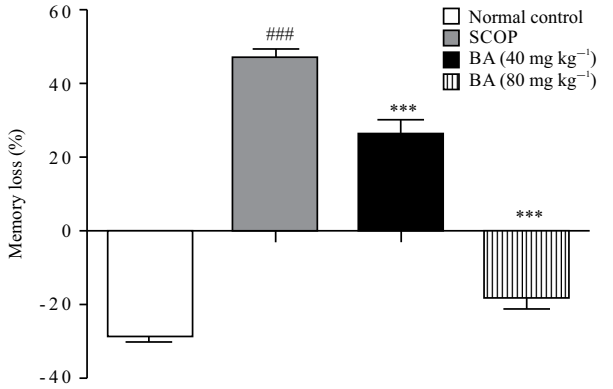

Fig. 1: Elevated plus maze examination for assessment memory and cognitive performances

Values are expressed as Mean±SEM (n = 6), ###p<0.001 vs normal control and *p<0.05, **p<0.01 and ***p<0.001 vs scopolamine alone

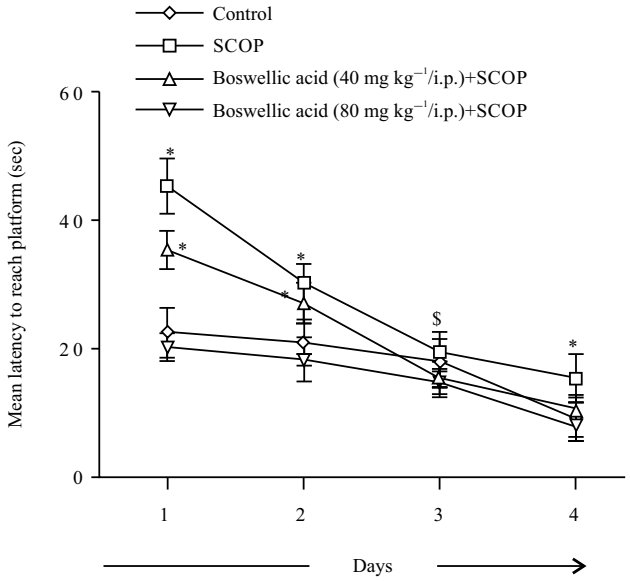

Fig. 2: Morris water maze study for assessment of cognition and memory

Values are expressed as Mean±SEM (n = 6), *p<0.05 vs scopolamine alone

control animals this was found to be statistically significant (p<0.001). Treatment with a lower dose of boswellic acid significantly (p<0.001) reversed the scopolamine-induced memory loss (26.33±3.85%) compared to scopolamine alone in treated animals. However, treatment with a higher dose of boswellic acid completely reversed scopolamine-induced memory loss (Fig. 1).

Morris water maze study: Spatial learning and memory were recorded significantly different among treatment groups, which was measured as escape latency in the training

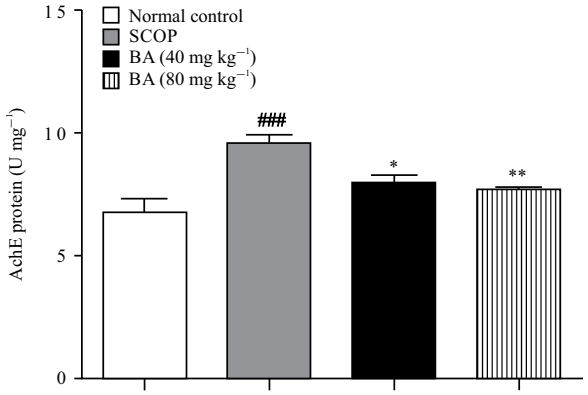

Fig. 3: Acetylcholinesterase activity.

Values are expressed as Mean±SEM (n = 6), ###p<0.001 vs normal control and *p<0.05 and **p<0.01 vs scopolamine alone

sessions of the Morris water maze. Progressively decreased escape latency was recorded over the training sessions. But compared to the normal control more prolonged escape latency was recorded in scopolamine alone treated animals. Administration of boswellic acid to scopolamine treated rats shortened the escape latency significantly. The detailed observations of the Morris water maze are shown in Fig. 2.

Biochemical estimations

MDA, GSH, CAT and SOD levels: Administration of scopolamine was found to elevate the MDA 41.25±7.465 (43.47%) and decline GSH 5.45±1.05 (34.49%), CAT 10.8±0.58 (78.48%) and SOD 2.5±0.12 (62.68%) levels when compared with normal control animals. Treatment with boswellic acid to scopolamine-treated animals significantly reversed the levels of antioxidant enzymes and reactive oxygen species, which was witnessed by declined intracellular levels of MDA18.50-15.50 (55-62%) and an increase in GSH 6.70-7.80 (22-43%), CAT 34.5-41.5 (200-400%) and SOD 5.0-6.5 (100-160%) activities, when compared to scopolamine alone, treated animals (Table 1).

AChE activity: Scopolamine alone treated rat brain tissue showed 41.02% (p<0.001) of higher content of AchE as compared to normal control animals. Boswellic acid treatment at tested doses (40 and 80mg kg^{-1}, i.p.) significantly decreased (8.21±0.80, 7.50±0.50, p<0.05, p<0.01), respectively the elevated levels of AchE(9.80±1.00) in scopolamine treated animals (Fig. 3).

Total RNA levels in the brain: Brain tissue qRT-PCR report suggests that scopolamine reduced significantly the mRNA expressions of BDNF, CaMK, CREB, ERK and PI3K proteins.

Table 1: Effect of boswellic acid on brain MDA, GSH, CAT and SOD levels in scopolamine treated rat brain

Group	MDA	GSH	CAT	SOD
Normal control	28.75±6.250	8.32±0.90	50.2±1.41	6.7±0.3
SCOP alone	41.25±7.465###	5.45±1.05###	10.8±0.58###	2.5±0.12###
Boswellic acid 40 mg kg^{-1}	18.50±1.936*	6.70±0.30**	34.5±0.80	5.0±0.5**
Boswellic acid 80 mg kg^{-1}	15.50±2.102*	7.80±1.50**	41.5±1.31**	6.5±0.5***

Values are expressed as Mean±SEM (n = 6), ###p<0.001 vs normal control and *p<0.05, **p<0.01 and ***p<0.001 vs scopolamine alone. malondialdehyde (MDA), superoxide dismutase (SOD), Glutathione (GSH) and catalase (CAT)

Table 2: Effect of boswellic acid on mRNA expressions in scopolamine treated rat brain tissues

Group	BDNF	PI3K	Akt	ERK2	CaMK IV	CREB
Normal control	1.02±0.14	1.01±0.12	1.00±0.11	0.99±0.12	1.01±0.10	1.01±0.13
SCOP alone	0.39±0.12##	0.31±0.12##	0.36±0.10##	0.30±0.09##	0.30±0.10##	0.34±0.10##
Boswellic acid 40 mg kg^{-1}	0.71±0.12*	0.65±0.11*	0.78±0.12*	0.68±0.14*	0.72±0.12*	0.62±0.13*
Boswellic acid 80 mg kg^{-1}	0.61±0.10*	0.45±0.11*	0.58±0.11*	0.58±0.08*	0.55±0.11*	0.51±0.09*

Values are expressed as Mean±SEM (n = 6), ##p<0.01 vs normal control and *p<0.05 vs scopolamine alone. Brain-Derived Neurotrophic Factor (BDNF), Phosphoinositide 3-kinases (PI3K), Protein kinase B (PKB) (Akt), Extracellular Signal-regulated Kinase (ERK), Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and cAMP-response element-binding (CREB)

Whereas treatment with boswellic acid to scopolamine treated animals restored tmRNA expressions as compared to scopolamine alone treated animals (Table 2).

DISCUSSION

In the present study administration of scopolamine resulted in altered behavioural changes, memory loss, spatial learning ability and increased oxidative stress in the animals. Treatment with boswellic acid to scopolamine-treated animals significantly reversed behavioural changes, memory loss, spatial learning ability and reduced the oxidative stress in rats and it was evident by 55-62% decreased levels of MDA and increased levels of endogenous antioxidant levels such as GSH (22-43%), CAT (200-400%) and SOD (100-160%) to compare with scopolamine alone, treated animals. Moreover, Boswellic acid significantly attenuated scopolamine-induced elevated levels of AchE activity. In addition treatment with boswellic acid to scopolamine treated animals restored the tmRNA expressions of BDNF, CaMK, CREB, ERK and PI3K proteins as compared to scopolamine alone treated animals. These findings suggest the protective effect of boswellic acid against scopolamine-induced neurotoxicity and dementia in rats.

A muscarinic receptor inhibitor scopolamine disrupts the cholinergic system in the basal forebrain and induces dementia which simulates AD-like pathology. Hence, scopolamine-induced dementia in an animal is commonly used as an experimental model for investigating anti-AD drugs[2, 12].

Scopolamine is a neurotoxin that has been used for a long for deciphering various agents in the treatment of impaired memory[19]. An open field test was performed to assess the changes in general behavioural and locomotor activity in the animals. The altered behaviour of scopolamine treated animals was evident from the more time spent in the peripheral area and decreased line crossings in the open field apparatus compared to the normal animals. Administration of boswellic acid reversed scopolamine-induced behavioural and locomotor activity changes in the animals.

Elevated plus maze and Morris water maze test were used to investigate spatial learning and memory in animals. Treatment with scopolamine affected the memory and learning ability of the animals when compared with normal control animals. Pretreatment with boswellic acid to scopolamine-treated rats reversed the scopolamine-induced spatial learning and memory. This is in agreement with a previous study that reported improved cognition upon boswellic acid treatment in rats[7].

The administration of scopolamine increased oxidative stress in the rat brain. Increased oxidative stress is evident by increased MDA levels and decreased antioxidant enzymes such as GSH, CAT and SOD levels. Pretreatment with boswellic acid to scopolamine-treated animals attenuated the levels of MDA and restored the antioxidant enzyme status in the brain tissue. These results support earlier reports that describe antioxidant properties of boswellic acid-containing plants[20,21].

Previous studies by Ebrahimpour *et al.*[7] reported that boswellic acid inhibits AchE activity significantly in Trimethyltin-treated Wistar rats. The present study findings are in agreement with this f as treatment with boswellic acid significantly inhibited the elevated AchE activity in scopolamine treated rats.

Earlier reports confirmed that scopolamine administration to experimental rats down-regulates the neuronal expressions of BDNF, CaMK, CREB, ERK and PI3K[22]. BDNF is considered essential neutrophils to play a role in cognition and memory enhancement. By activating enzyme-linked receptors BDNF transduces the signal to produce intracellular molecules which

are involved in cognition and memory[23]. BDNF leaded activation of these ERKs accelerates two different ERK/MAPK pathways and PI3K/Akt pathway[24]. Instigation of these pathways transduces the genetic expressions of various proteins which are involved in memory and learning[25]. Down regulation of BDNF/ERK signalling potentiates the transcription of CREB[24]. Further, it has also been confirmed that Impaired phosphorylation of CREB is a possible cause of an AD-like state, although pharmacological phosphorylation of CREB is suggested by the scientific community for AD treatment[26].

BDNF has an important role in the survival of a variety of CNS neurons[27]and increased mRNA expression of BDNF is linked with slower cognitive decline in humans[28]. CREB is the mediator of BDNF transcriptional autoregulation in CNS. Treatment with boswellic acid restored the scopolamine-induced decline in mRNA expression of BDNF and CREB indicating its anti- Alzheimer's actions.

CaMK is well known for its action on neuronal growth, learning, memory and synaptic plasticity[29]. Administration of boswellic acid improves the scopolamine-induced decline in mRNA expression of CaMK suggests its protective role against scopolamine-induced dementia in rats.

In the present study, the administration of scopolamine to the rats decreased the ERK mRNA expression. ERK is important in learning, memory and addiction pathways[30]. Treatment with boswellic acid in the scopolamine-treated rats improved the mRNA expression of ERK when compared with the scopolamine alone treated group. This indicates the protective action of boswellic acid against scopolamine dementia in rats.

The PI3K signalling pathway is vital in both normal brain development and human neurological disease. Reduced expression of PI3K in the brain is correlated with brain lesions, epilepsy and cognitive impairment in humans and animals[31]. Improvement in mRNA expression of PI3K in scopolamine treated animals by boswellic acid further confirms its protective action against scopolamine dementia in rats.

CONCLUSION

Pretreatment with boswellic acid to scopolamine treated animals corrected the altered behaviour, restored memory and antioxidant capacity, attenuated the AchE activity and increased the BDNF, CaMK, CREB, ERK and PI3K mRNA expression. These results indicate the protective actions of boswellic acid due to its significant anti-oxidative properties against scopolamine-induced dementia and neuronal damage in rats. However, further research is warranted to identify and confirm the exact mechanism of action and its clinical applications.

SIGNIFICANCE STATEMENT

This study discovers the possible neuroprotective effects of boswellic acid that can be beneficial for scopolamine-treated rats. This study will help the researcher to uncover the critical area of memory loss and spatial learning ability that many researchers were not able to explore. Thus, a new theory on boswellic acid against scopolamine-induced dementia in rats may be arrived at.

ACKNOWLEDGMENTS

The authors acknowledge Deputyship for Research & Innovation, Ministry of Education, Saudi Arabia for all sorts of financial support via the grant number ''375213500". The authors also tribute their sincere gratitude to the central laboratory of Jouf University- Aljouf-Saudi Arabia.

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