Exploring the nootropic effect of *Juniperus recurva* extract: Possible involvement of acetylcholinesterase inhibition

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Abstract

Background: Currently, available therapy for the treatment of memory impairment is far from satisfactory. Therefore, the agents of natural origin may serve as potential therapies. **Objective:** The present study was designed to evaluate the memory-enhancing effect of *Juniperus recurva* extract. **Materials and Methods:** The methanol extract of *J. recurva* was prepared by Soxhlet extraction and characterized by high-performance liquid chromatography (HPLC). The *in vitro* antioxidant activity of the extract was corroborated by diphenyl picryl hydrazine scavenging, nitric oxide scavenging, metal chelating, and reducing power activity. Memory impairment was induced by the administration of scopolamine (1 mg/kg i.p) on 3 consecutive days to mice and assessment of memory acquisition and memory retention was done using Morris water maze test, passive avoidance test, elevated plus maze test, and light and dark box test, motor coordination was evaluated using the rotarod test and inclined plane test; and depression was evaluated by forced swim test. Serum acetylcholinesterase (AChE) activity was quantified by Ellman's method. **Results:** The HPLC analysis of *J. recurva* extract revealed gallic acid as a prominent peak. The extract was found to have an excellent antioxidant effect in all the tests employed. The *in vivo* studies revealed memory enhancing and improved motor coordination activity of the extract in mice. Serum AChE activity was decreased on the administration of the extract. **Conclusion:** The inhibition of the AChE enzyme contributes to the memory-enhancing activity of *J. recurva* extract.

Key words: Acetylcholinesterase, antioxidant, *Juniperus*, memory, scopolamine

INTRODUCTION

The process of acquisition and deciphering information to develop logical thoughts is termed as cognition, and it extends to building attention, perception, developing motor skills, judgment, decision making, etc.[1] Dementia is symptomatized as loss of memory, impaired objective thinking, difficulties in problem-solving or language impairment often accompanied by changes in mood and behavior. Brain damage due to trauma or disease also leads to dementia.[2] Alzheimer disease is reported to be one of the common causes of dementia that accounts for 50–70% of all reported cases. Other causes may include Lewy body dementia (15%), vascular dementia (25%), and frontotemporal dementia.[3]

Medicinal plant species represent a large source of novel molecules that help in new drug development to address a variety of disease states. India has a rich diversity of medicinal plants which have been used as sources of medicines for the treatment and mitigation of several diseases. *Juniperus recurva* which belongs to family Cupressaceae is a commonly grown ornamental plant.[4] Plant constitutes a diverse source of secondary metabolites, most of which are phenolic compounds. These include flavones, monoterpenoids, and sesquiterpenoids.[5] Essential oil from *Juniperus virginiana* is also a source of podophyllotoxin.[6,7] It is valuable bioactive lignin and is a precursor to different anticancer drugs including tenopside, etoposide, and etopophos phosphate.[8] Furthermore, *Juniperus* species is extensively used in folk medicine

for different ailments, such as kidney infections, common colds, and dermatological diseases.[9]

The pharmacological activities attributed to the *Juniperus* species include anti-inflammatory,[5,9] diuretic, antiseptic and antimicrobial,[10-12] anthelmintic,[13] hypoglycemic,[14] hypotensive, abortifacient, antinociceptive,[9,15] antiviral,[15] anticancer,[13] and antioxidant.[5,16] The plant was found to be beneficial in the treatment of neurosis and psoriasis.[17]

The protective effect of different plant-derived secondary metabolites in the neuronal system is well documented.[18] Antioxidant potential of phenolic compounds is well known. Nucleophilic and metal chelating nature of phenolic compounds contribute to their radical scavenging ability and retardation of lipid peroxidation has also been documented.[19] The polyphenol-rich fractions from different species of *Juniperus* such as *Juniperus oxycedrus badia*, *Juniperus navicularis*, *Juniperus phoenicea,* and *Juniperus turbinate* are found to possess *in vitro* acetylcholinesterase AChE, antioxidant, and neuroprotective activity.[17,20] However, the effect of *J. recurva* on the *in vivo* AChE activity and memory impairment has not been documented so far. Therefore, the current study was designed to explore the effect of *J. recurva* on scopolamine-induced memory deficit in mice.

MATERIALS AND METHODS

Drugs and Chemicals

Scopolamine, A23187 and veratrine were purchased from Sigma-Aldrich, India. The plant *J. recurva* was collected from Botanical Garden of Guru Nanak Dev University, Amritsar, and Punjab. Plant material was authenticated by Dr. Saroj Arora, Professor, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab (Ref no. 1828 Bot Sc & Env. Sc.). All other solvents and chemicals used were of analytical grade and procured from local suppliers.

Experimental Animals

Swiss albino male mice (25–30 g) were procured from Central Research Institute, Kasauli, Himachal Pradesh and maintained in well-ventilated air-conditioned room (25 ± 5°C) with a 12 h light and dark cycle in the animal house of Guru Nanak Dev University, Amritsar. Free access to food and water was provided to mice. They were allowed to acclimatize for 2 weeks before experimentation. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (226/CPSCEA2016/07). Guidelines given by the Committee for the purpose of control and supervision of experiments on animals were followed during animal experimentation.

J. recurva Extract Preparation

The leaves of the plant were properly cleaned and shade dried. Then leaves were crushed to make powder. The extract was prepared by Soxhlet extraction at 60°C temperature using methanol as solvent. The extract was concentrated in rota evaporator and lyophilized.

Phytochemical Screening

J. recurva extract was tested for the presence of various phytoconstituents, i.e., alkaloids, saponins, flavonoids, and cardiac glycosides using standard tests.[21]

High-Performance Liquid Chromatography (HPLC) Standardization of Extract

In the extract, phenolic compounds were detected with the help of HPLC. The analysis was done using HPLC Agilent Technology, 1260 infinity system coupled with EZ Chromelite Software using Agilent column XDB-C18, 250 mm × 4.6 mm long, with 5 µM diameter. Mobile phase A was composed of potassium dihydrogen orthophosphate solution. 0.5 ml orthophosphoric acid was added and diluted to 1000 ml with water, whereas acetonitrile constituted mobile phase B. The gradient elution was done with mobile phase A and B as following: 95% A and 5% B phase, 0–10 min; 80 % A and 20% B phase, 10–25 min; 78% A and 22 % B phase, 25–30 min; 60 % A and 40% B phase, and 30–35 min; 95% A and 5% B phase, 35–40 min. The analysis was performed at a flow rate of 1.2 ml/min, injection volume 20 µl, and UV detection at 270 nm.

In vitro Antioxidant Activity of Extract

Total phenolic content determination

Total phenolic content in the extract was determined using the method described by Folin–Ciocalteu.[22] Briefly, solution 0.5 ml Folin–Ciocalteu reagent was added to 1 ml of extract solution. After 3 min, 1.5 ml of Na_2CO_3 (20%) was added, followed by the addition of 2 mldistilled water. The mixture was kept at 30°C for 40 min and the absorbance (abs) was noted at 765 nm. Results were expressed as mg of gallic acid equivalents/g of extract (GAE/g of extract).

Diphenyl Picryl Hydrazine (DPPH) Radical Scavenging Activity

DPPH scavenging activity was determined by the method described earlier.[23] Briefly, to 1 ml of each dilution of *J. recurva* extract, 1 ml of 0.1 mM DPPH was added and kept in the dark at room temperature for 30 min. The absorbance (abs) was noted at 517 nm using a spectrophotometer. Sample without extract was taken as control.

% DPPH scavenging activity = $(Abs_{contol} - Abs_{test})/Abs_{control} \, 100$

Azino-bis3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Radical Cation Scavenging Activity

The assay is based on the potential of different substances to scavenge ABTS radical cations (ABTS•+). The test was performed according to the method given in literature.[24] ABTS stock solution (7 mM) was added to potassium persulfate (2.45 mM) to form blue-green ABTS•+ and kept in the dark for 12–16 h until a steady absorbance of the resulting solution was achieved. The resulting solution was diluted using ethanol to achieve the absorbance of 0.70 ± 0.02 at 734 nm that forms a working ABTS solution. Briefly, 1 ml of different dilutions of extract were added to 1 ml of working ABTS•+ solution, stirred for 1 min, and absorbance was measured at 734 nm.

$$\%\text{ABTS scavenging activity} = (Abs_{contol} - Abs_{test})/Abs_{control} \times 100$$

NO Scavenging Activity

NO scavenging activity was quantified by Griess reaction.[25] Briefly, to 1ml of each dilution of *J. recurva* extract, sodium nitroprusside (500 µl) was added, followed by incubation at 37°C for 3 h. Sulfanilamide (1%) and naphthylethylene diamine dihydrochloride (0.1%) in 2.5 % v/v phosphoric acid were mixed in the ratio of 1:1 to prepare Griess reagent. Absorbance was noted at 540 nm after the addition of 1ml of Griess. The sample without extract was taken as control.

$$\%\text{NO scavenging activity} = (Abs_{contol} - Abs_{test})/Abs_{control} \times 100$$

Metal Chelating Activity

Metal chelating activity was determined by the method described in literature.[26] Briefly, 1 ml of each dilution of extract samples was added to 50 µl of 2 mM $FeCl_2$ and incubated at 37°C for 30 min. 2 ml of 5 mM ferrozine was added, shaken and left at 37°C for 10 min. Absorbance was measured spectrophotometrically at 562 nm.

$$\% \text{Metal activity} = (Abs_{contol} - Abs_{test})/Abs_{control} \times 100$$

Reducing Power Assay

The assay was performed according to the method reported in literature.[27] Briefly, to 1 ml of each dilution of extract, 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1 % w/v potassium ferricyanide were added followed by incubation at 50°C for 20 min and then brought to room temperature. 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm. Upper layer (2.5 ml) was separated and mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%). The abs was noted at 700 nm. Absorbance is directly taken as a measure of reducing power.

PHARMACOLOGICAL STUDIES

Schematic protocols are presented in Figure 1.

The mice were divided into five different groups, each comprising six animals.

Group 1: Control (0.1% CMC i.p)

Group 2: Scopolamine (1 mg/Kg i.p)

Group 3: *J. recurva* Extract (200 mg/kg p.o) + Scopolamine (1 mg/Kg i.p)

Group 4: Veratrine (125 µg/Kg i.p) + Extract (200 mg/kg p.o) + Scopolamine (1 mg/Kg i.p)

Group 5: A23187 (20 µg/Kg i.p) + Extract (200 mg/kg p.o) + Scopolamine (1mg/Kg i.p).

Test for Cognition and Memory

Morris water maze (MWM)

MWM model was used to assess memory acquisition and retention as described in literature.[28] A circular platform (10 cm diameter) was kept 2 cm below the water that was made opaque by adding nontoxic color. The training was given for 6 consecutive days; mice received 4 consecutive trials per day. The mice were individually placed in a water tank. The time taken to reach the platform was taken as latency time.

Passive avoidance paradigm

In the passive avoidance paradigm, the animal was placed in a chamber containing a grid to which electric current was supplied with a wooden shock free platform in the center.
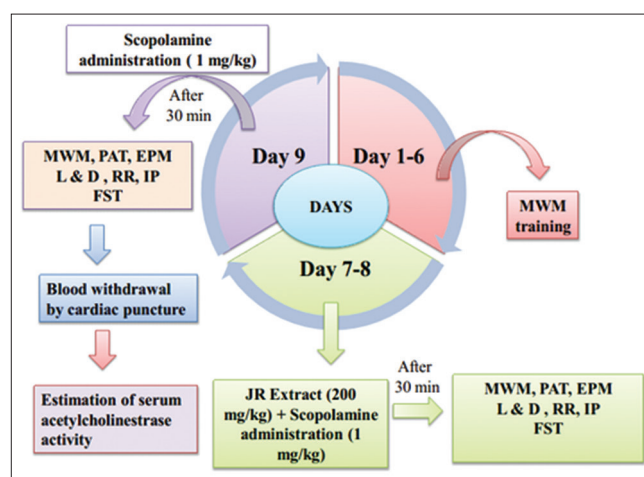
Figure 1: Schematic representation of protocol (MWM: Morris water maze, PAT: Passive avoidance task, EPM: Elevated plus maze, L and D: Light and dark box, RR: Rotarod, IP: Inclined plane, FST: Forced swim test)

The animal was trained for the shock free zone by allowing it to explore for a period of 10 s. Each time when the animal descended from the platform, foot shock (50 Hz; 1.5 mA; 1 s) was applied. After 24 h, the animal was again kept on the platform. The passive avoidance reflex time was measured as the time taken to reach the shock free zone. The cutoff time was 60 s.[29]

Elevated plus maze (EPM)

Memory retention and acquisition were tested using EPM apparatus (cutoff time is 5 min). Maze consisted of two open and two closed arms. The mouse was placed in the open arm, facing outward. As soon as it entered the closed arm, a buzzer with shrilling sound was pressed. The animal was trained until it learned to correlate buzzer with the closed arm. The time spent in the open arm was recorded. Memory retention was measured after 24 h.[30]

Light dark box

The apparatus consisted of two chambers; one light and one dark chamber 44 cm × 21 cm × 21 cm in dimensions, connected by 13 cm long × 5 cm high door. The mouse was placed in the light compartment. When animal moved to a dark compartment, a buzzer with shrilling sound was pressed. Time spent inside the light compartment was noted. A cutoff time of 10 min was observed.[29]

Test for Motor Coordination

Inclined plane

In the inclined plane test, two rectangular ply boards were connected to each other at an angle of 65°. The mouse was placed on an inclined plane which was covered with a rubber mat with ridges 0.2 cm in height. Fall of time was noted.[29]

Rotarod

Rotarod is a rotating horizontal metal rod of 3 cm diameter. The length of rotarod was 75 cm and motor speed 20 rotations per minute. The animal was placed for 5 min and fall of time was noted.[31]

Test for Depression

Forced swim test (FST)

In FST mice were placed into glass chamber 25 cm high, 10 cm in length and 5 cm wide containing water at a temperature of 25°C for 5 min. Immobility time, i.e., when the animal shows no signs of struggle was noted as a sign of depressive behavior.[32]

Serum AChE activity

The method is based on the principle that AChE acts on a substrate to produce thiocholine that reacts with Ellman's reagent to form a colored complex.[33,34] The standard procedure included 3 ml phosphate buffer (0.1 mol/l, pH = 7.4) added to 0.1 ml of DTNB 10 nmol/l (dithio-bis-2-nitrobenzoic acid) and after which serum was added, equilibrated at 37°C for 10 min and followed by the addition of acetylthiocholine iodide (0.05 ml). Absorbance was measured at 436 nm using a colorimeter, and the standard curve was plotted using neostigmine in a series of dilutions. The enzyme activity was expressed as µmol/L/min.

Enzyme activity (µM/L/min) = $(\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{blank}})/10.6$

Statistical Analysis

All results are expressed as mean ± standard error mean (SEM). Statistical analysis was done by one-way analysis of variance followed by Tukey's multiple comparisons using GraphPad Instat Software version 3.0. The statistical data were considered significant at $P < 0.05$ value.

RESULTS

Preliminary Phytochemical Screening of *J. recurva*

The preliminary phytochemical studies indicated the presence of alkaloids, cardiac glycosides, saponin glycosides, anthraquinone glycoside, steroids and triterpenoids, flavonoids, tannins, and phenolic compounds in *J. recurva* extract.

HPLC Standardization of Extract

The HPLC analysis revealed the presence of gallic acid in the methanol extract of *J. recurva,* as indicated by the retention time in chromatogram [Figure 2].
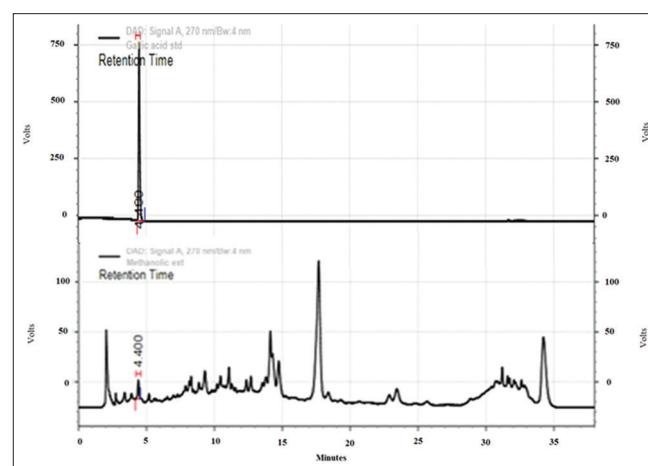
Figure 2: High-performance liquid chromatography chromatogram of standard gallic acid, and methanol extract of *Juniperus recurva*

Percentage Yield of Extract

The percentage yield of methanol *J. recurva* extract was found to be 13.5% and total phenolic content of methanol extract was 78 GAE/g of extract.

In vitro Antioxidant Activity of the Extract

DPPH and ABTS cation scavenging activity

The *J. recurva* extract possess appreciable DPPH and ABTS cation scavenging activity that was found to increase linearly with increase in the concentration of extract [Figure 3].

Metal Chelating and Nitric Oxide (NO) Scavenging Activity

The *J. recurva* extract was found to exhibit appreciable NO scavenging and metal chelating activity [Figure 4].

Reducing Power Assay

The reducing power of *J. recurva* extracts increases as an increase in the concentration of *J. recurva* extract [Figure 5].

Pharmacological Studies

Effect of J. recurva extract on escape latency in MWM test

In MWM test, scopolamine increased the escape latency 91.3% as compared to normal control. The *J. recurva* extract treated group exhibited a significant nootropic effect as evidenced by a significant decrease (71.3%) in escape latency as compared to scopolamine treatment. No significant alteration was observed on pretreatment with A23187 and veratrine [Figure 6a].

Effect of J. recurva extract on time taken to reach the platform in passive avoidance task (PAT)

Scopolamine was found to enhance the time taken to reach the platform by 83.7% as compared to control. Administration of *J. recurva* extract was found to significantly decrease the time taken to reach the platform (82.3%) as compared to scopolamine treatment. No significant alteration in the effect of *J. recurva* was observed on pretreatment with A23187 and veratrine [Figure 6b].

Effect of *J. recurva* extract on time spent in open arm in EPM

Scopolamine was found to cause loss of memory as evidenced by a decrease in time spent in the open arm by 12.2% as compared to normal control. *J. recurva* extract treated group
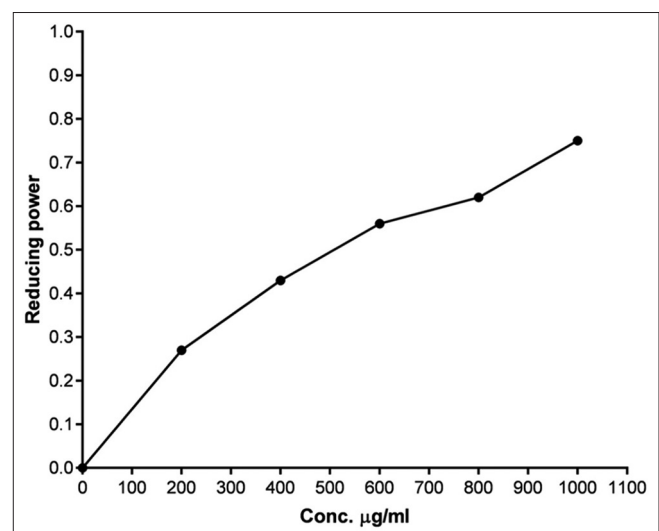
exhibited a significant increase in time spent in open arm (11.2%) as compared to scopolamine-treated group [Figure 7a].
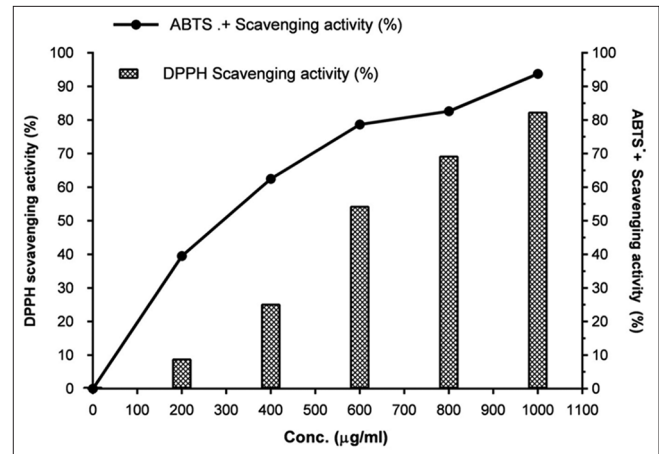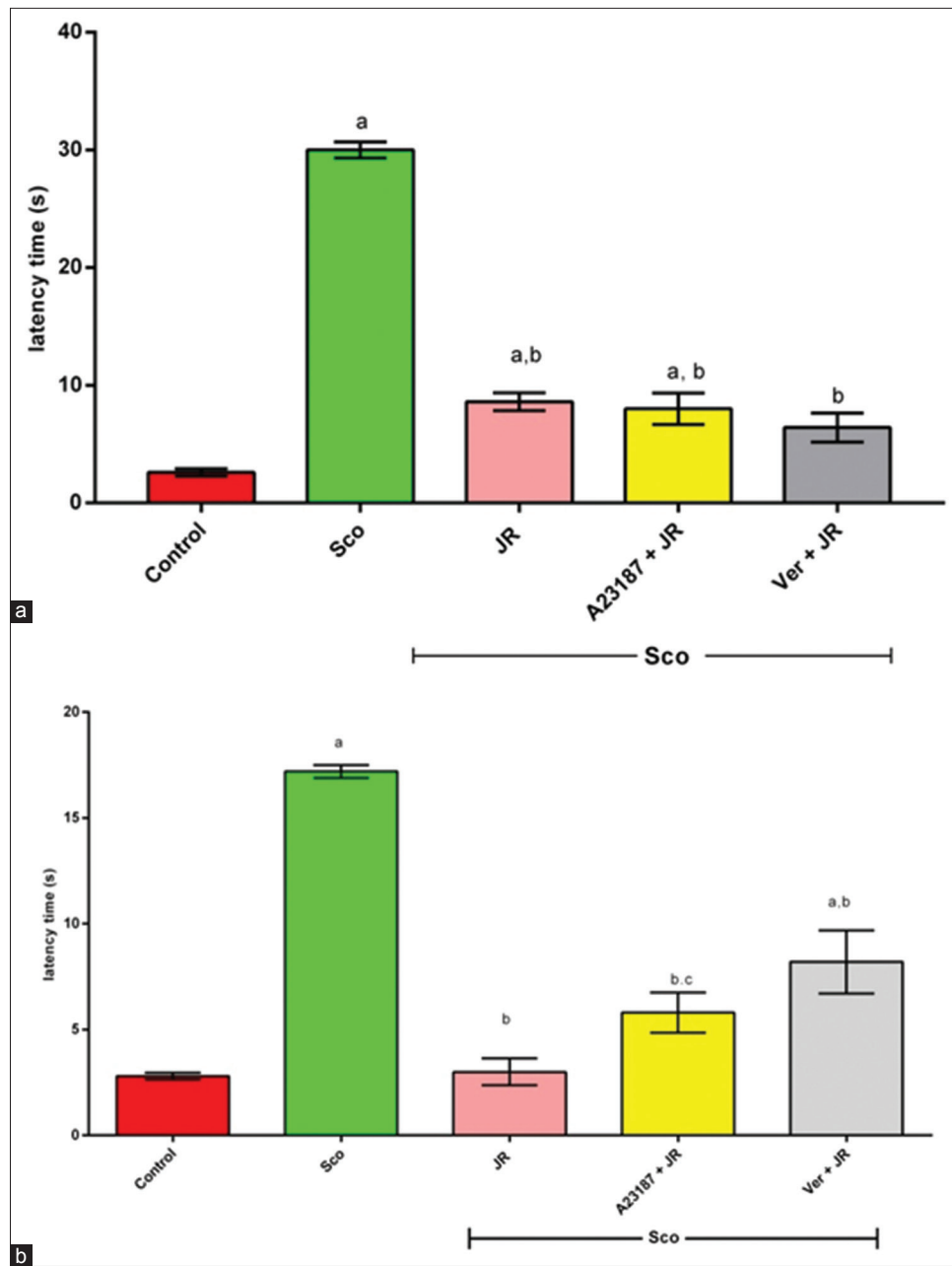

Figure 3: *In vitro,* DPPH and ABTS scavenging activity (%) of the methanol extract of *Juniperus recurva*
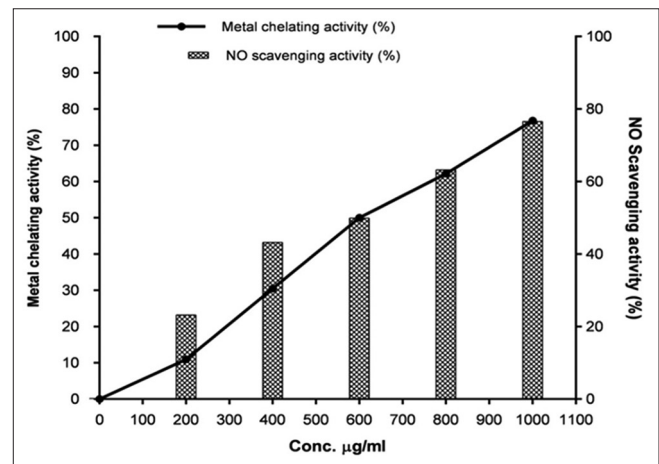
Figure 4: *In vitro,* metal chelating (%) and NO scavenging activity (%) of the methanol extract of *Juniperus recurva*

Figure 5: *In vitro,* reducing the power of the methanol extract of *Juniperus recurva*
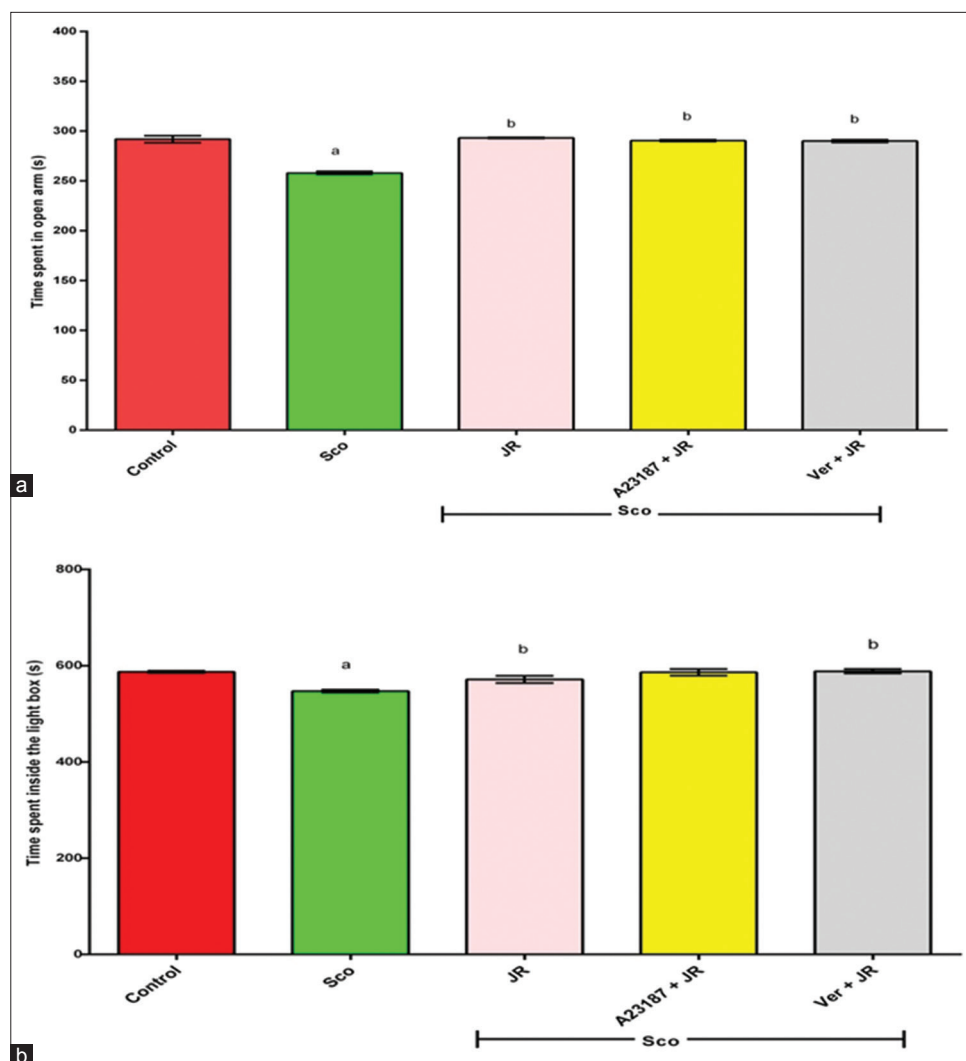
Figure 6: Effect of various pharmacological interventions on escape latency in Morris water maze (a) and on escape latency in passive avoidance task (b) All values are expressed as mean ± standard error mean; data were analyzed by one-way analysis of variance followed by Tukey's test. a = $P < 0.05$ as compared to control; b = $P < 0.05$ as compared to Sco. Sco, JR, Ver indicate Scopolamine, *Juniperus recurva*, Veratrine, respectively

Effect of *J. recurva* Extract on Time Spent in a Light Compartment in Light-dark Box

A significant decrease in time spent inside the light compartment of the light and dark box (6.8%) on scopolamine administration was observed as compared to normal control. The time spent inside a light compartment on the administration of *J. recurva* extract was increased, but it was not significant (3%). However, no significant effect was observed with *J. recurva* on pre-treatment with A23187 and veratrine [Figure 7b].

Effect of *J. recurva* Extract on Fall Off Time in an Inclined Plane

Scopolamine administration was found to reduce the fall off time on the inclined plane by 47.8% as compared to normal control. Administration of *J. recurva* was found to increase the fall off time significantly (27.08%) as compared to scopolamine treated group. Pre-treatment with A23187 and veratrine did not alter the effect *of J. recurva* extract [Figure 8a].

Figure 7: Effect of various pharmacological interventions on time spent (s) in open arm in the elevated plus maze (a) and time spent (s) in light compartment in light and dark box (b). All values are expressed as mean ± standard error mean; data were analyzed by one-way analysis of variance followed by Tukey's test a = $P < 0.05$ as compared to control; b = $P < 0.05$ as compared to Sco. Sco, JR, Ver indicate Scopolamine, *Juniperus recurva,* and Veratrine, respectively

Effect of *J. recurva* Extract on Fall Off Time in the Rotarod

Scopolamine was found to reduce the fall off time on the rotarod by 80.78% as compared to normal control. Administration of *J. recurva* was found to increase the fall off time significantly (79.3%) as compared to scopolamine treatment. However, no significant effect was observed with *J. recurva* on pre-treatment with A23187 and veratrine [Figure 8b].

Effect of *J. recurva* Extract on Immobility Time in FST

The immobility time was increased on scopolamine administration by 58.07% as compared to normal control in the FST. Administration of *J. recurva* extract was found to significantly decrease the immobility time (35.5%) as compared to scopolamine-treated group. However, no

significant effect was observed with *J. recurva* on pre-treatment with A23187 and veratrine [Figure 9a].

Effect of *J. recurva* Extract on Serum AChE Activity

Scopolamine was found to increase the activity of enzyme significantly by 57.1% as compared to normal control. Administration of *J. recurva* extract was found to decrease the activity of the enzyme (42.3%) as compared to scopolamine treatment. Pretreatment with A23187 and veratrine did not alter the effect of *J. recurva* treatment on enzyme activity [Figure 9b].
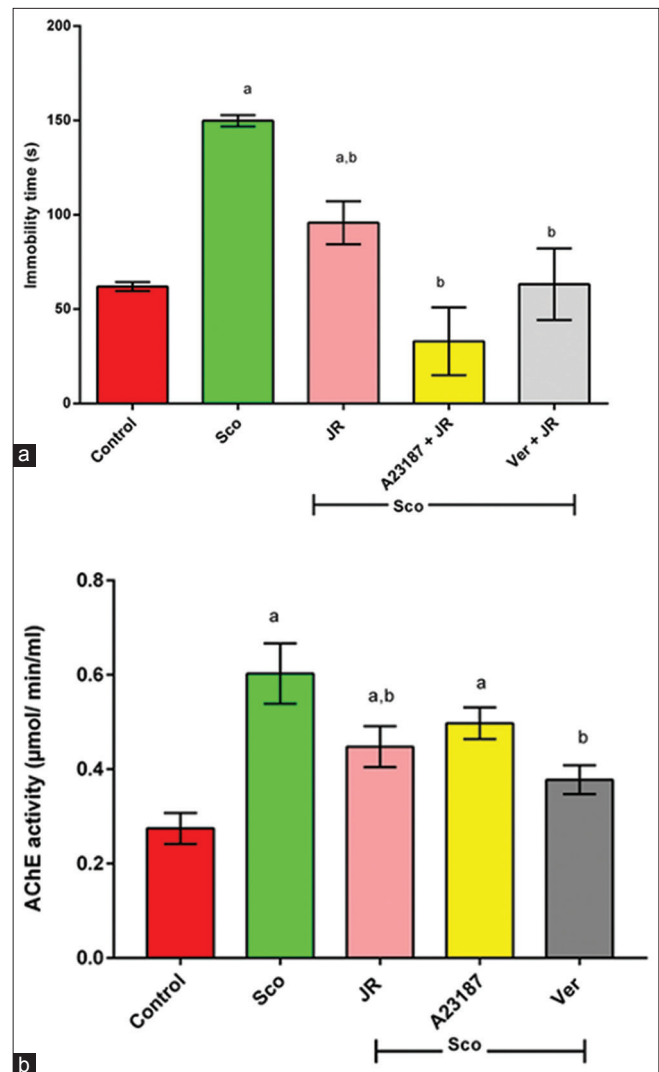
DISCUSSION

The current study was designed to characterize *J. recurva* extract and explore its *in vitro* antioxidant potential and
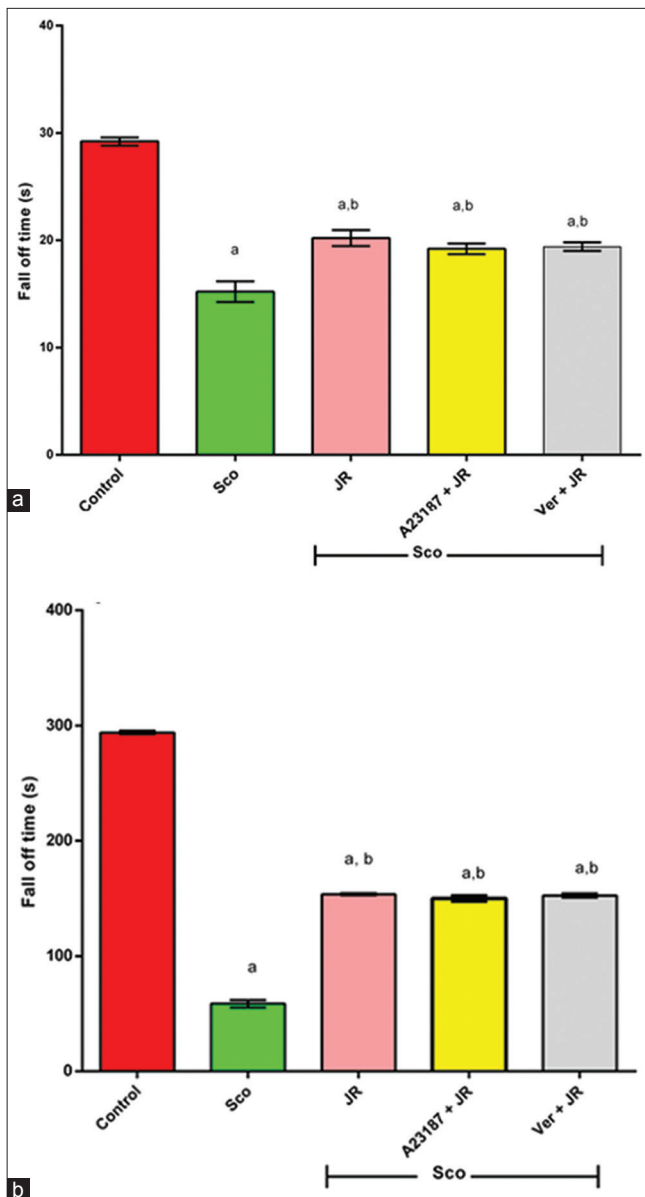
Figure 8: Effect of various pharmacological interventions on fall of time (s) in the inclined plane (a) and on fall of time (s) in rotarod (b). All values are expressed as mean ± standard error mean; data were analyzed by one-way analysis of variance followed by Tukey's test, a= $P < 0.05$ as compared to control; b = $P < 0.05$ as compared to Sco. Sco, JR, Ver indicate Scopolamine, *Juniperus recurva*, Veratrine, respectively

Figure 9: Effect of various pharmacological interventions on immobility time (s) in forced swim test (a) and on serum acetylcholinesterase activity (b). All values are expressed as mean ± standard error mean; data were analyzed by one-way analysis of variance followed by Tukey's test, a = $P < 0.05$ as compared to control; b = $P < 0.05$ as compared to Sco. Sco, JR, Ver indicate Scopolamine, *Juniperus recurva*, Veratrine, respectively

effect on scopolamine-induced memory deficit in mice. The role of ion channels, i.e., voltage-gated sodium channel and calcium channel has been also explored. The HPLC analysis of *J. recurva* extract revealed the presence of gallic acid. *In vitro* analysis showed the appreciable antioxidant potential of the extract. Scopolamine (1 mg/kg, i.p) administration was instituted to induce memory impairment. Acquisition and retention of memory was evaluated using MWM test, EPM test, step down latency test, and light dark test, while inclined plane test, FST, and rotarod test were used to evaluate the depression and muscle coordination after dementia. Scopolamine was found to cause significant memory

impairment as evidenced by an increase in the escape latency time in MWM and in PAT. Furthermore, stimulus-induced animal behavior in EPM and light and the dark box also showed the amnesic effect of scopolamine as animals failed to correlate the sound stimulus with previous training. Scopolamine was also found to decrease motor coordination as shown by a decrease in fall off time on inclined plane and rotarod.

The current investigation revealed the presence of various phytochemicals such as alkaloids, glycosides, tannins, flavonoids, steroids, triterpenoids, and phenolic compounds. The *Juniperus* species have been reported to contain flavonoids, tannins, lignans, etc., that exhibit neuroprotective

property.[19] HPLC-diode array detection/mass spectrometry profile of methanol extracts of *J. recurva* leaves revealed a prominent peak similar to that of gallic acid. The antioxidant activities of methanol extract *J. recurva* were investigated in various *in vitro* models, and it was found that the *J. recurva* extract possesses appreciable antioxidant potential. The DPPH and NO scavenging activity, metal chelating activity and reducing the power of *J. recurva* extract increases linearly with increase in concentration. Free radicals are the highly toxic chemical species that contain unpaired electron and are produced continuously in our body. It has been known that the excess production of free radicals in body could be one of the etiologic factors for neurodegenerative diseases such as Alzheimer's disease, Parkinsonism, and also for cardiovascular and hepatic disorders.[2] The scavenging of DPPH and ABTS by extract indicates the free radical scavenging effect of the extract. NO is another reactive molecule that has been implicated in various disease models such as neurodegenerative diseases.[35] The scavenging activity of extract could help to arrest the toxic reactions initiated by the reactive species within the cellular system and may be considered good in preventing ailments. The appreciable antioxidant potential of *J. recurva* extract could be attributed to the presence of gallic acid which is known to the very effective antioxidant in both *in vitro* and *in vivo* experiments. It has been documented that the antioxidant activity of phenolic compounds is due to their ideal structure to donate the hydrogen atom to free radicals.[36]

The result of the current investigation revealed that *J. recurva* extract decreased the escape latency in MWM and PAT. Furthermore, the extract treatment also increased the time spent in the open arm of EPM and light compartment in L&D box model. The *J. recurva* extract also significantly increased fall off time in rotarod and inclined plane test. These findings corroborate to the fact that *J. recurva* has nootropic activity and improves muscle coordination in mice.

J. recurva is a medicinal plant that is being used in the treatment of various diseases. The *Juniperus* species have been reported to contain terpenoids, cardiac glycosides, alkaloids, and many phenolic compounds.[5] The plant has various biological properties such as anti-inflammatory,[5,9] diuretic, antiseptic,[10-12] hypoglycemic,[14] antinociceptive,[9] antiviral,[15] anticancer,[12] antioxidative, [5] and anticholinesterase.[37]

Therefore, the current study was designed to explore the effect of *Juniperus* on scopolamine-induced memory deficit in mice. Veratrine and A23187 pretreatment did not change the effect of *Juniperus*. A23187 is a calcium ionophore which increased the intracellular calcium influx while veratrine is a voltage-gated sodium channel opener.[38] To study the modulation of the nootropic effect of the extract by Na^+ and Ca^{2+}, veratrine and A23187 pretreatment were instituted before extract treatment. However, both the pharmacological interventions did not alter the effect of *J. recurva* extract,

indicating the non-involvement of sodium and calcium channels in the antiamnesic effect of *J. recurva* extract.

Scopolamine-induced memory impairment has been documented as a standard and widely employed model to investigate the drugs affecting cognition.[39] Scopolamine-induced deleterious changes in the hippocampus are possibly due to the impaired binding of acetylcholine to muscarinic receptors that affect memory and learning processes.[40] Cholinergic neurons are well distributed in the basal forebrain and hippocampus, that participate in learning and memory. Both nicotinic and muscarinic cholinergic receptors contribute to memory consolidation. The nicotinic receptors play a crucial role in memory consolidation by regulating the level of acetylcholine and help in cognitive processing.[41] Scopolamine is a cholinergic receptor antagonist and blocks the acetylcholine binding to muscarinic receptors which lead to memory impairment.[40] AChE degrades acetylcholine and leads to cholinergic deficiency and subsequently, memory impairment. Thus, anticholinesterase therapy is used for retrieval of memory in Alzheimer disease.[42]

The involvement of neurotransmitter acetylcholine and enzyme acetylcholinesterase in memory impairment is well documented. It has been found in the current investigation that the administration of scopolamine increased the serum AChE activity. The finding is consistent with the previous reports which suggest that in a rodent model of scopolamine-induced cognition impairment, there is a cholinergic deficit, characterized by reduced acetylcholine at synaptic cleft due to enhanced AChE activity.[43] Treatment with *J. recurva* extract decreased the AChE activity in mice serum. This may increase the acetylcholine activity and its binding to cholinergic receptors. Thus, the antiamnesic effect of *J. recurva* extract may be through the mediation of the cholinergic nervous system. Moreover, various phenolic compounds such as ferulic acid, gallic acid, vanillic acid, and plant extract containing these compounds are known for their AChE activity.[44]

Thus, it is suggested that antioxidant activity and anticholinesterase activity may be the tentative mechanism of antiamnesic effect of *J. recurva* extract in scopolamine-induced memory deficit in mice.

ACKNOWLEDGMENT

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