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Research Study *Medical research*

Evaluate the memory enhancement activity of ethanolic extract of the leaves of the citharexylum spinosum

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ABSTRACT

The present study suggest that ethanolic extract of *A. pyrethrum* increased brain cholinesterase level and hence it possess memory enhancing activity in scopolamine induced amnesia model by enhancing central cholinergicneurotransmission.

Keywords: Memory Enhancement Activity, Citharexylum Spinosum

INTRODUCTION

Memory is one of the most essential roles of the brain. Memory is vital for survival because it is the process by which organisms are able to record their experiences and use this information to adapt their responses to the environment. Loss of memory and cognitive impaired functions are the major features of Alzheimer's disease (AD). Presence of acetylcholine within the neo cortex is sufficient to ameliorate learning deficits and restore memory. Decreased cholinergic firing in brain, rise in oxidative stress, hypercholesterolemia, and neuro inflammatory reactions have been demonstrated to play an etiological role in memory decline. The central cholinergic system is involved in cognitive functions and plays an important role in learning and memory for humans and animals[1,2,3,4].

Alzheimer's disease (AD) is progressive irreversible neurodegenerative disorder that was first identified and written by Dr. Alois Alzheimer in early 1900s. It occurs gradually and results in cognitive impairment, unusual behavior, personality changes, an ultimately death. It is the most common form of adult onset dementia. Presently, it is the 4th leading cause of death in western countries, preceded only by heart disease, cancer and stroke[5,6].

AIM AND OBJECTIVE

AIM:

To evaluate the memory enhancement activity of ethanolic extract of the leaves of theCitharexylum spinosum.

OBJECTIVE:

The objective of present study is-

- To evaluate the memory enhancement activity by herbal extract to treat dementia in mice.
- In - vivostudies

CITHAREXYLUM SPINOSUM

Citharexylum spinosum has a wide distribution in the Caribbean. It is most common in the Florida Keys

and has a scattered presence in pinelands and hammocks along Florida's east coast, north to Brevard County and to Manatee County on the west coast. It is a nuisance plant in Bermuda, Australia and on several Pacific Islands including Inflorescences, early January.

Taxonomical classification Kingdom :Plantae,
Subkingdom :Tracheobionta,
Division : Magnoliophyta orAngiospermae,
Class :Magnoliopsida,
Subclass : Asteridae,
Order : Lamiales,
Family : Verbenaceae,
Subfamily : Verbenoideae,
Genus :*Citharexylum*
Species :spinosum.
Binomialname:Citharexylumspinosum

PLAN OF WORK

Collection of *Citharexylum spinosum* leaves and authenticated

↓

Powdered

↓

Subjected to extraction with non- polar (ethanol) solvent using Soxhlet apparatus

↓

Drying

↓

Preliminary phytochemical analysis

↓

Screening for nootropic activity in Alzheimer's disease

↓

Results and discussions

MATERIALS AND METHODS

Collection & Authentication of Plant Material

The leaves of *Citharexylum spinosum* were authenticated by Dr.P.Satyanarayana Raju (Plant taxonomist) of department of botany and microbiology in Acharya Nagarjuna University, Guntur.

Preparation of Extract

The *Citharexylum spinosum* leaves are powdered in a mechanical grinder. The collected powder was successively, extracted with ethanol using Soxhlet apparatus. The extraction was carried out for 72 hrs at a temperature not exceeding the boiling point of the solvent. Excess solvent was removed by the solvent evaporation to obtain the dry weight of the plant

extracts.

Procedure and process of extraction

Procurement of Plant Material:

For the present investigation of *Citharexylum spinosum* was collected around from Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh.

Extracts

The commonly employed technique for separation of active substance from crude drug is called as 'Extraction' which involves the use of different solvents. The plant material used for extraction should be properly authenticated or identified. The choice of the plant Material for extraction depends upon its nature and the components required being isolated. The dried powdered plant material is commonly used for extraction. The solvent used for extraction is called menstruum and the residue is known as marc.

- The basic parameters influencing the quality of an extract are:
- Plant part used as startingmaterial
- Solvent used forextraction
- Extractionprocedure
- Effect of extracted plant phytochemicals depends on:
- The nature of the plantmaterial
- Itsorigin
- Degree ofprocessing
- Moisturecontent
- Particlesize

The variations in different extraction methods that will affect quantity and secondary metabolite composition of an extract depend upon:

- Type ofextraction
- Time ofextraction
- Temperature
- Nature ofsolvent
- Solventconcentration
- Polarity

Experimental Animals

Albino mice of either sex (20-30) were maintained for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard conditions temperature (24 ± 10 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard mice pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. 5 mice/group were used in all sets of experiments.

Ethical Approval

All the protocols were approved by Institutional Animal Ethical Committee (IAEC) and conducted according to Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) registered no: 1048/PO/Re/S/07/CPCSEA at Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, Guntur.

IN-VIVO STUDIES

- Y-maze
- Group I- Control (vehicle)
- Group II- Induced (Scopolamine- 0.4 mg/kg) Group III- Standard (Donepezil- 1mg/kg) Group IV- Test (CS extract 100mg/kg)

The Y-maze is designed for studying shock motivated brightness discrimination response, i.e., simultaneous brightness discrimination in rats [7,8]. The Y-maze has been designed to make the animal to learn to discriminate between two arms- one illuminated without shock and other non- illuminated with shock and learn to reach the correct 'arm', the illuminated one. The spatial working memory was measured through the spontaneous alteration of behavior in Y-maze (INCO). The Y-maze consists of three identical removal sun mica lined chambers arranged in Y- shape connected to the central chamber. Each arm has a working dimension of approx. 30 ×15× 15 cm with rat presence indicator and hinged top. Each mouse is placed in the central chamber and allowed to move freely through the maze during an 8-minute session. The mouse trend to explore the maze systematically, entering each arm in turn. When mouse enters one arm the rat presence indicator glows. The series of arm entries was recorded. Alteration is defined as the number of successive entries in to the three arms on overlapping triplet sets. The percentage alteration was calculated as the ratio of actual to possible alterations [9-10].

- Y-maze is divided into 4 parts. It consists of
- Three identical removable sun mica lined chambers arranged in Y shape connected to centralchamber.
- The central chamber is about 20 cm height with three openings which allow the other three arms to beconnected.
- Each arm has its own power plugs and is connected to the semiautomatic power controllertool.
- Each arm has a working dimension of approximately 30X15X15 cms with electriable grill and has chamber light or cue light with indicator, grill charge indicator, rat presence indicator and hinged top. The central compartment also contains a wiregrill.
- Each chamber along with central compartment contains grills which gives electricshock.

Procedure

- A group of mice are trained to provide colonies for pharmacologicalstudies.
- Every mice, in turn is placed in one of the chambers for a period of 30 seconds without any stimulus to allow an accommodation to thestimulation.
- The naive animal is allowed to explore Y-maze apparatus for 5 min at the start of their training.
- The mice is then put into central compartment and set the program selectoraccordingly.
- After 5 sec (approximately) shock is applied by switching the unit ON and by using the program selector set the position to eitherA/B/C.
- Observe the indicators till the animal reaches the goal in the illuminatedarm.
- Shift the positions of the program selector by rotating and note the observations as follows
- First preference of the mice to illuminated or non-illuminatedarms
- Number of entries in illuminated arm and non-illuminated arm. (An arm entry is defined as the entry of four paws into thearm)
- Average time each animal spends in each arm (Average time = Total duration in arm/Number ofentries)
- Inject standard or test drug to the animal group, after 30 mins, place the animal individually in the center of the maze and note allparameters.
- Compare the preference of animal to illuminated or non-illuminated arms, average time spent in illuminated or non-illuminated arms and number of entries in both thearms.

Evaluation

Errors are scored. An error means that the animal enters the wrong arm with all four legs. During retention the numbers of trials until the animal makes correct choices are counted.

Statistical analysis

All the values are expressed as mean ± SD. Statistical significance was determined using One Way-ANOVA, followed by Dunnet's test. $P<0.05$ was considered to be significant.

LABYRINTH MAZE:

- Group I- Control (vehicle)

- Group II- Induced (Scopolamine- 0.4 mg/kg) Group III- Standard (Donepezil- 1mg/kg) Group IV- Test (CS extract 100mg/kg)
- Labyrinth maze is another best known device for studying the learning, remembering (memory) and reasoning in animals.
- Labyrinth maze contains channel or tunnel which is formed by number of Y figures for exploring by the animals like mice and rats the mazes are connected alternatively opposite to each other in a continuous line and ending of maze is like coneshape.
- To a maximum 5 stages of mazes are present and are arranged in a **zigzag** position.
- Each stage was about 30cm long and 15cm wide. The top roof of apparatus which covers the mazes is about 60cm wide & 55cm long.
- As similar to that of the rectangular maze, the labyrinth maze consists of 3 chambers.
- Chamber-A in which rat is placed. It has a sliding door that is opened to allow rat to enter maze.
- The maze (chamber-C) the animal has toexplore.
- Chamber-B at other end of the maze in which the reward iskept.

All these divisions of maze are hinged separate. Top lids 50 as to maintain a uniform environment inside the maze and prevents any land of outside stimulus or clue to be delivered to theanimal.

An electrical system provides indication when the rat is placed in chamber. The chambers A and B has tapping sensor plates and when a rodent enters into it the time not will be observed on the digital displays and light blinks at the indicator showing the presence of animal.[11,12,13]

Procedure

- Group of rats are trained on equipment to perform the pharmacologicalactivity.
- First close the slide door connecting to chamber A tomaze.
- Place the animal in chamber A, then the A indicator glows and display showsready.
- Close the top lid of all three components; leave the apparatus as such to let the animal acclimatize to environment inside themaze.
- After allowing sufficient time to the animal to get used to environment, open the slide door.
- The "A" light will go out as soon as the animal leaves the chamber and moves into the maze. Simultaneously the "C" light will start to glow and counter will start counting the time elapsed inseconds.
- The counting will stop and the C indicator light will go out as soon as the animal enters the end compartment i.e. chamber B and the B indicatorglows.
- The B indicator indicates that the animal has reached the end compartment and the completion of the experiment (the reading

recorded on timer will be total time taken in seconds, by the animal in traversing).

Evaluation

The reading recorded on timer will be total time taken in seconds by the animal in traversing the maze.

Statistical analysis

All the values are expressed as mean ± SD. Statistical significance was determined using One Way-ANOVA, followed by Dunnet's test. P<0.001 was considered to be significant.

MORRIS WATER MAZE:

- Group I- Control (vehicle)
- Group II- Induced (Scopolamine- 0.4 mg/kg) Group III- Standard (Donepezil- 1mg/kg) Group IV- Test (CS extract 100mg/kg)

The procedure, technique, and end point for testing memory were followed as per the parameters described earlier [13,14]. Briefly, Morris water maze-(MWM) for mice consisted of a circular pool (60□cmindiameter,25□cminheight)filledtoadepthof2 0□cmwithwatermaintainedat25℃.The water was made opaque with nontoxic white colored dye. The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (with top surface 6□cm × 6□cm and painted in white) was placed inside the target quadrants (Q4 in present study) of this pool 1□cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animalwas subjected to four consecutive trials each day with a gap of 5□min for four consecutive days (starting from 6th day of drug administration to 9th day), during which they were allowed to escape on to the hidden platform and to remain there for 20□s. During the training session, the mouse was gently placed in the water between quadrants, facing the wall of pool with drop locationchangingforeachtrial,andallowed120□sectolo catesubmerged platform.Ifthemouse failed to find the platform within 120□s, it was guided gently on to the platform and allowed to remain there for 20□s. Escape latency (EL) is the time taken by the animal to move from the starting quadrant to find the hidden plateform in the target quadrant. EL was recorded on the 6th day to 9th day for each animal. Each animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the trainingperiod.[15,16]

On the fifth day (i.e., 10th day of drug administration), the platform was removed and mouse was placed in any of the three quadrants and allowed to explore the target quadrant for 300□s. Meantime spent in all the three quadrants that is, Q1, Q2, and Q3 was recorded. The mean time spent in the target quadrant in search of the missing platform was noted as index of retrieval or memory. The observer always stood at the same position. Care was taken not to disturb the relative location of water maze with respect to other objects in thelaboratory [17,18]

RESULTS AND DISCUSSION

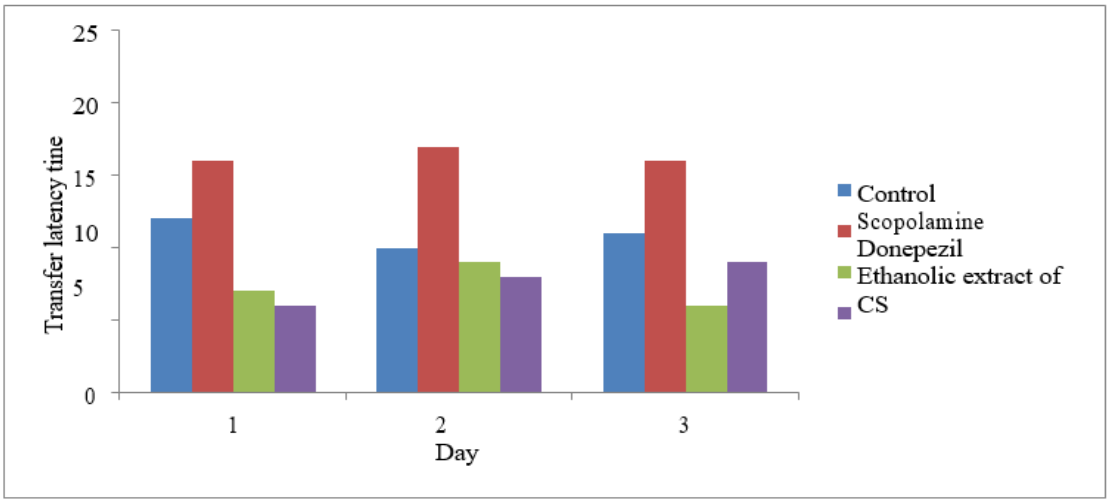
Spatial working memory activity

Spatial working memory activity of leaf extract of *Citharexylumspinosm*using Y- maze method

S. No	Groups	Treatment	Programme set			Transfer Latency Time (in sec)	Mean ± SEM
			A	B	C		
1	I	Control	L S S	S S L	S L S	12 10 11	11±0.577
2	II	Scopolamine	L S S	S S L	S L S	16 17 16	16.3±0.333
3	III	Donepezil	L S S	S S L	S L S	7 9 6	7.3±0.881
4	IV	Ethanolic extract of CS	L S S	S S L	S L S	6 8 9	7.6±0.881

Ethanolic leaf extract showed significant nootropic activity when compared with inducedand control.

Shock motivated response in mice using Y maze

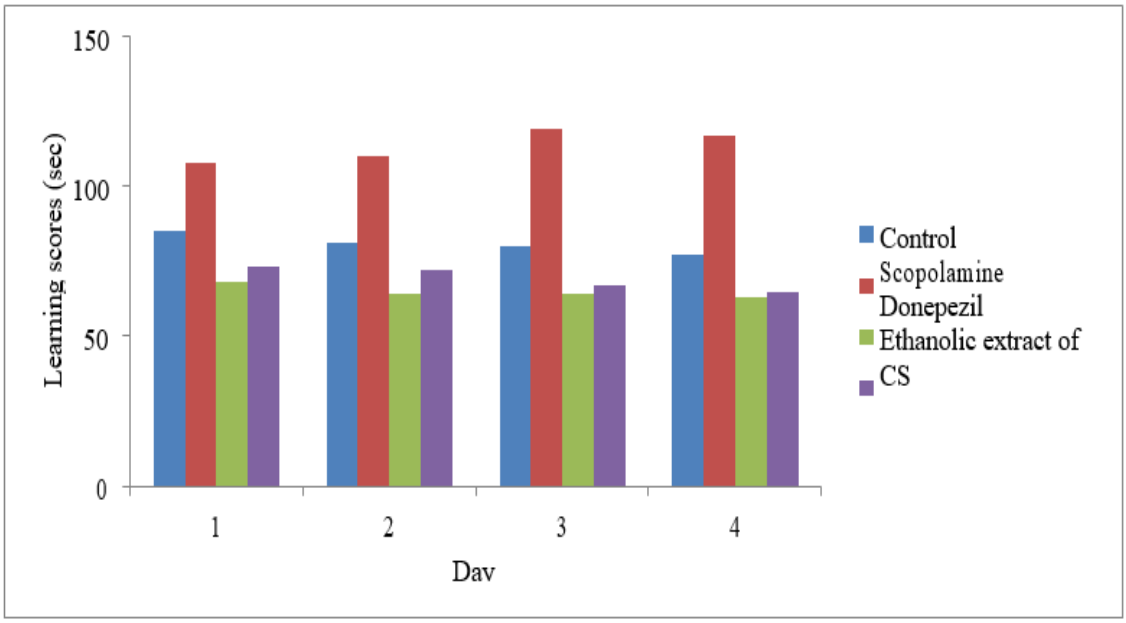


Spatial working memory Y maze method

The learning and memory enhancement activity of leaf extract of *Citharexylum spinosum* using labyrinth maze.

Days	LATENCY TIME/ LEARNING SCORES (SEC)															
	Control				Scopolamine				Donepezil + Scopolamine				Ethanolic extract of CS + Scopolamine			
1	80	89	84	88	110	99	105	119	75	70	62	66	76	74	70	75
2	85	80	77	83	120	111	106	102	69	65	60	63	74	70	75	69
3	81	78	75	86	112	118	120	125	68	66	60	61	70	69	65	62
4	80	75	72	80	119	116	122	120	65	60	62	65	67	65	64	66

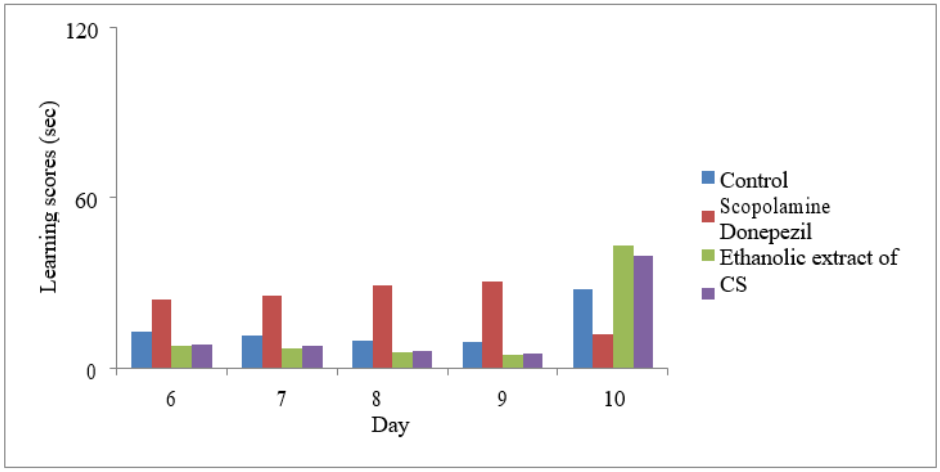
Ethanolic extract showed significant learning and memory enhancement activity when compared with control and induced treatment groups using labyrinth maze.



Learning and memory enhancement activity by labyrinth maze

The learning and memory enhancement activity of leaf extract of *Citharexylum spinosum* using Morris water maze.

S.No	Group	Treatment	Transfer Latency (In seconds)				
			Day6	Day7	Day8	Day9	Day10 (Without platform)
1.	I	Control	13±0.912	11.5±0.645	9.7±0.478	9±0.408	28±1.080
2.	II	Scopolamine	24.2±0.853	25.7±0.853	29.2±1.108	30.7±2.868	12±1.080
3.	III	Standard+ Scopolamine	8±0.707	6.7±0.478	5.7±0.478	4.7±0.478	43.2±1.201
4.	IV	Test+ Scopolamine	8.2±0.478	7.7±0.629	6.2±0.75	5±0.707	39.7±0.577



Learning and memory scores by Morris water maze

DISCUSSION

Y Maze: The latency time was taken as evaluation parameter in case of Y maze. For scopolamine group it takes more time to identify the correct choice where as the donepezil and test group have assessed the right choice and results in less latency time.

Labyrinth Maze: In case of labyrinth maze also, the latency time was taken as an evaluation parameter as the animal takes time to cross the maze from one side to other. As the scopolamine on chronic administration induces amnesia in mice, the group of animals that administered with scopolamine takes more time to cross from one point to other. This results in increased latency time. Similarly the group of animals that are treated with donepezil and test showed better results in decreasing latency time.

Morris Water Maze: As the mechanism of morris water maze is different when compared with other mazes, the time taken to select the site of platform is considered as evaluation parameter. Thus the learning scores are enhanced in test and donepezil treated group when compared with control and scopolamine induced group.

Ach esterase levels: As the Acetylcholine esterase enzyme acts by decreasing the concentration of Ach levels, identification of presence of Acetylcholine esterase through UV absorbance is a simple chosen method to evaluate the Acetylcholine esterase levels. DTNB/Ellman's reagent acts by highlighting the Acetylcholine esterase level and can be identified in absorbance levels and calculated by % inhibition. Based on results, the test and donepezil treated group acts in a good way to inhibit the concentration of Acetylcholine esterase levels when compared with control and scopolamine inducedgroup[19,20].

CONCLUSION

Screening models for studying drugs or conditions that affect memory enhancement were standardized and evaluated by using extracts of leaves of *Citharexylum Spinosum.* The ethanolic extract of *Citharexylum Spinosum* has shown significant activity flavonoids.

The evaluation of Ach esterase levels also showed that the ethanolic extract of *Citharexylum Spinosum* has shown significant % inhibition and leads to enhance in Ach level which resukts in active transformation of nerve impulses in Brain.

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