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Phytochemical and Pharmacological Screening of Salvia Hispanica Seed Extracts against Alzeimer's Disease in Rodents

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Abstract **Original Research Article**

The objective of this study was to identify the potential of *salvia hispanica* as protective and therapeutic agent against Alzheimer's disease. The learning and memory enhancing activity of saliva hispanica seed extract were investigated in rats by using the ethanol induced cognitive impairment and diazepam induced amnesia and its effects on learning and memory were examined by using Morris water maze (MWM) test and 8-radial arm maze (8-RAM) test. For Morris water maze test all groups showed significantly (P value is < 0.01 &< 0.05) decreases transfer latency time at all periods as compared to ethanol and diazepam inducing group & for radial arm maze test all groups showed significantly (P value is< 0.0001) decreased in the time taken to reach the paired arm and number of entries in baited arm and non-baited arm as compared to ethanol inducing group. Therefore seed extracts of *salvia hispanica* exhibited significantly learning and memory activity in Alzheimer's disease.

Key words: Salvia hispanica, learning and memory activity, morris water maze, radial arm maze and Alzheimer's disease.

INTRODUCTION

Alois Alzheimer and Augusto D

The German psychiatrist and neuropathologist Dr. Alois Alzheimer is credited with describing for the first time a dementing condition which later became known as AD. In his landmark 1906 conference lecture and a subsequent 1907 article, Alzheimer described the case of Auguste D, a 51-year-old woman with a 'peculiar disease of the cerebral cortex,' who had presented with progressive memory and language impairment, disorientation, behavioural symptoms (hallucinations, delusions, paranoia), and psychosocial impairment. Remarkably, many of the clinical observations and pathological findings that Alzheimer described more than a century ago continue to remain central to our understanding of AD today [1]. Alzheimer's disease is a form of brain degeneration in which abnormal particles called neurofibrillary tangles and neuritic plaques form in the brain and destroy healthy neurons (brain cells). These abnormalities tend to settle in brain areas that control the ability to learn a new fact and remember it 30 minutes, or a day later, a skill we refer to as "memory"[2].

The word 'chia' derives from the Náhuatl word 'Chian', which means 'oily'. The other part of the name Salvia hispanica was given to the plant by Carl Linnaeus (1707-1778), who discovered the wild growing plant in the new world and confused it with a native plant from Spain. However, chia comes from Mexico and it was imported to Spain by Hernán Cortés [3]. Salvia, a member of the mint family 'Lamiaceae' comprises the largest genus of the family. The genus has complex and rich diversity with healing qualities of different species occurring throughout the world. The genus Salvia is derived from the Latin word "Salvare" meaning "to heal" or "to be safe and unharmed" referring to the medicinal properties of the genus. It encompasses about 900 species, wide spread throughout the world with three distinct region of diversity: central and South America (500 species), Central Asia/Mediterranean (250 species) and Eastern Asia (90 species). The center of origin of this genus has been reported to be Afghanistan and Soviet Central Asia. Although mexico has the highest number of species (about 250) [4].

Fig-1: *Salvia hispanica* seeds

MATERIALS AND METHODS

Collection of plant material

The seeds of *Salvia hispanica* was identified and purchased from local market of Nuzvid.

Preparation of extract

The *Salvia hispanica seed* are powdered in a mechanical grinder.The collected powder was successively, extracted with water & ethanol by using Soxhlet apparatus.The extraction was carried out for 72 hrs at a temp not exceeding the boiling point of the solvent.Excess solvent was removed by the solvent evaporation to obtained the dry weight of the plant extracts.

Preliminary phytochemical screening [5-7]

The preliminary phytochemical investigation was carried out with both aqueous & ethanolic extracts of *Salvia hispanica seed* for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods.

Experimental animals

SD rats of either sex (200-300g) 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 rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. 5 rats/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, and Guntur.

Drugs and chemicals

Inducing agents -Ethanol - 60% (Ethanol was prepared as a 60% solution in distilled water and administered i.p at a dose of 2.5 mg/ kg) [Ethanol (20%) is used to induce dementia like condition in the dose 4.5 mg/kg administered s.c for 21 days) & Diazepam- was diluted in normal saline and administered i.p at a dose of (1 mg/kg).

Standard drug- Donepezil hydrochloride (2.5 mg/kg, p.o)

Investigation of learning and memory activity in alzheimer's model by using morris water maze (mwm)

Morris water maze (MWM)

To assess hipocampal dependent spatial learning and memory, all rats were trained in a standard Morris water maze task (Morris *et al.*, 1982; Stackman *et al*., 2002) [8]. Maze consisted of large circular pool (75cm &30cm) filled with water at a depth of 20cm. The pool was divided into four quadrants. A circular platform was placed at the centre of one quadrant. The rats performed four trials per day for four consecutive days. In the swimming trials, each individual rat was released gently into the water at a randomly chosen quadrant. The rats swim and learned how to find the hidden platform within 60 s. After reaching the platform rat was allowed to stay on the platform for 15 s and was then taken back into the cage. The rats were placed on the platform by hand for 15 s, if they could not escape to the platform within 60 s by themselves, and their escape latency was accepted as 60 s. During the inter-trial intervals, animals were kept in a dry home cage for 60 s. The time to reach the platform (latency) was recorded. 24h after the last day of training, subjects were tested on a probe trial, during which the escape platform was removed and the time spent in the correct quadrant was measured for a 60 s trial [9].

Table-1: The sequence of trials during the study period of MWM test [10]

1st day	2nd day	3rd day	4th day
Q1	Q2	Q3	Q4
Q2	Q3	Q4	Q1
Q3	Q4	Q1	Q2
Q4	Q1	Q2	Q3

Fig-2: MWM

Evaluation parameters

- Transfer latency in sec

Ethanol- induced cognitive impairment [10]

Ethanol is neurotoxin that able to alter behavioural and cognitive performance in experimental animals in addition to humans. It mainly impairs hippocampus-dependent learning and memory functions. The mechanism of ethanol-induced neurotoxicity is not well understood. Several studies show that free-radical mediated oxidative stress play an imperative role. The brain is extremely susceptible to oxidative stress due to high level of polyunsaturated fatty acids (PUFAs) and catecholamines, large amounts of oxygen (O_2) in relatively small mass and in conjunction with low antioxidant activities. Furthermore, certain regions of the central nervous system (CNS), especially hippocampus and cerebellum, may be more sensitive to oxidative stress because of their low endogenous antioxidant, in relation to other brain regions. Study showed that acetaldehyde dehydrogenase is responsible for the generation of reactive oxygen species (ROS) by converting cytotoxic acetaldehyde produced from oxidation of ethanol to acetate. It has been confirmed that ethanol induces the synthesis of CYP2E1 that lead to oxidative stress. It also increases the ratio of NADH/NAD, responsible for reduction of ferric ion (Fe3+) to ferrous ion (Fe2+)) which causes lipid peroxidation by generating hydroxyl radical.

Selection of dose and treatment period

The learning and memory enhancing activity of the aqueous and ethanolic seed extracts of *Salvia hispanica* was investigated using the ethanol- induced cognitive impairment [Ethanol (60%) is used to induce dementia like condition in the dose 2.5 mg/kg administered i.p for 15 days) [9]. The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group- Ethanol (2.5 mg/kg was administered i.p for 15 days).

Group II: Standard Group -Donepezil hydrochloride [10] (2.5 mg/kg was administered orally for 15days) + Ethanol.

Group III: Test-I -Aqueous seed extract of *Salvia hispanica* [SHAE- 100mg/kg was administered orally for 15days) + Ethanol.

Group IV: Test -II -Ethanolic seed extract of *Salvia hispanica* [SHEE- 100mg/kg was administered orally for 15 days) + Ethanol.

Diazepam induced amnesia

Diazepam 1mg/kg, i.p was administered to rats and TL was noted after 45 min of injection on 8th day and after 24hrs. Extracts and standard Donepezil hydrochloride were administered for successive 8 days. After 60 min of administration of the last dose on 8th day, Diazepam 1mg/kg i.p was administered. TL was noted after 45 min administration of diazepam and after 24 hrs [11].

Selection of dose and treatment period

The learning and memory enhancing activity of the aqueous and Ethanolic seed extracts of *Salvia hispanica* was investigated using the [Diazepam is used to induce amnesia like condition in the dose 1 mg/kg administered i.p for 8 days) [11]. The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group-Diazepam (1mg/kg was administered i.p for 8 days).

Group II: Standard Group-Donepezil hydrochloride [10] (2.5 mg/kg was administered orally for 8days).

Group III: Test-I- Aqueous seed extract of *Salvia hispanica* [SHAE- 100mg/kg was administered orally for 8days).

Group IV: Test –II- Ethanolic seed extract of *Salvia hispanica* [SHEE- 100mg/kg was administered orally for 8 days).

Investigation of learning and memory activity in alzheimer's model by using 8-arm radial maze (8-arm/8-ram)

Behavioral study

Before starting the behavioral studies 1 week training was conducted. Only food and water was administered during this period [10].

Radial arm maze

The radial arm maze was developed by Olton and Samuelson (1976) and has become an essential tool for testing memory in rats [12]. The spatial memory was evaluated by the instrument. Open type radial arm maze was used in the study. It had a circular central arena and 8 equally sized arms. (20 x 60 cm). Small dishes with animal food was kept at far end inside each arm was mounted. Initially animals were habituated to the environment [13]. In the present study, baited and unbaited arms were fixed throughout the tests. The 1st, 3rd, 5th, and 7th arms were baited while the 2nd, 4th,

6th, and 8th arms were unbaited. The rats was placed in the centre of the maze and allowed to freely explore the maze for 10 minutes on the first day. The rats were required to take the food pellets from each arm without making a re-entry into the arm already visited. The trail was terminated when the animal takes the food reward from all the eight arms or after 10 minutes if all the eight arms were not visited. Correct score was give when the visits an arm and collects the food reward and a maximum score of '8' can be attained per trail. The first entry into the baited arm was recorded as a correct choice. An entry into an unbaited arm was considered a reference memory error (RME) .When a rat reenters an already visited arm it was taken as a working memory error (WME) [14].

Fig-3: 8-RAM

Evaluation parameters

- No. of entries in baited arms and non-baited arms.
- Time taken to reach the paired arm.

Ethanol- induced cognitive impairment [10].

Ethanol is neurotoxin that able to alter behavioral and cognitive performance in experimental animals in addition to humans. It mainly impairs hippocampus-dependent learning and memory functions. The mechanism of ethanol-induced neurotoxicity is not well understood. Several studies show that free-radical mediated oxidative stress play an imperative role. The brain is extremely susceptible to oxidative stress due to high level of polyunsaturated fatty acids (PUFAs) and catecholamines, large amounts of oxygen (O_2) in relatively small mass and in conjunction with low antioxidant activities. Furthermore, certain regions of the central nervous system (CNS), especially hippocampus and cerebellum, may be more sensitive to oxidative stress because of their low endogenous antioxidant, in relation to other brain regions. Study showed that acetaldehyde dehydrogenase is responsible for the generation of reactive oxygen species (ROS) by converting cytotoxic acetaldehyde produced from oxidation of ethanol to acetate. It has been confirmed that ethanol induces the synthesis of CYP2E1 that lead to oxidative stress. It also increases the ratio of NADH/NAD, responsible for reduction of ferric ion (Fe3+) to ferrous ion (Fe2+)) which causes lipid peroxidation by generating hydroxyl radical.

Selection of dose and treatment period

The learning and memory enhancing activity of the aqueous and ethanolic seed extracts of *Salvia hispanica* was investigated using the ethanol- induced cognitive impairment [Ethanol (20%) is used to induce dementia like condition in the dose 4.5 mg/kg administered s.c for 21 days) [10].. The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group-Ethanol (4.5 g/kg was administered subcutaneously for 21 days).

Group II: Standard Group -Donepezil hydrochloride [10]. (2.5 mg/kg was administered orally for 21 days) + Ethanol.

Group III: Test-I -Aqueous seed extract of *Salvia hispanica* [SHAE- 100mg/kg was administered orally for 21 days) + Ethanol.

Group IV: Test -II -Ethanolic seed extract of *Salvia hispanica* [SHEE- 100mg/kg was administered orally for 21 days) + Ethanol.

All the treatment group animals received respective control, standard and test treatment 30 minutes prior to the ethanol administration for 21 days of experimental period.

STATISTICAL ANALYSIS

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between haloperidol group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

RESULTS AND DISCUSSION

In this study, we found that aqueous & ethanolic seeds extract of *Salvia hispanica* Possess the following chemical constituents (Table 2).

Table-2: Phytochemical screening of SHAE & SHEE

Phytochemical constituents	Aqueous seed extract of *Salvia hispanica*	Ethanolic seed extract of *Salvia hispanica*
Alkaloids	-	-
Carbohydrates	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Terpenoids	-	-
Sterols	+	+
Tannins	+	+
Proteins	+	+
Amino acids	-	-
Glycosides	-	-
Fixed oils and fatty acids	-	-

+ indicate the compulsory present and – indicate the absent.

SHAE – *Salvia hispanica* aqueous extract.
SHEE- *Salvia hispanica* ethanol extract.

Effect of seed extracts of salvia hispanica on behavioural parameters i.e. Mwm:

Animals treated with ethanol [2.5 mg/kg] alone for15 days showed a increase in transfer latency in seconds on 1st,7th& 15th days as well as diazepam [1mg/kg] alone for 8 days showed a increase in transfer latency in seconds on 8th day & after 24 hrs i.e. 9th day.

Table-3: Effect of seed extracts of *Salvia hispanica* on transfer latency (ethanol- induced cognitive impairment)

S.No.	Group	Treatment	Transfer latency (In seconds)		
			1st DAY	7th DAY	15th DAY
1.	I	Ethanol	24.2±2.36	27.6±3.99	27.8±4.05
2.	II	Standard+ethanol	4.8±1.75	4.6±1.20	2.2±0.74
3.	III	SHAE+ ethanol	7.4±1.70	6.6±2.42	3.2±0.97
4.	IV	SHEE+ ethanol	6.6±1.17	4.4±1.54	2.4±0.75

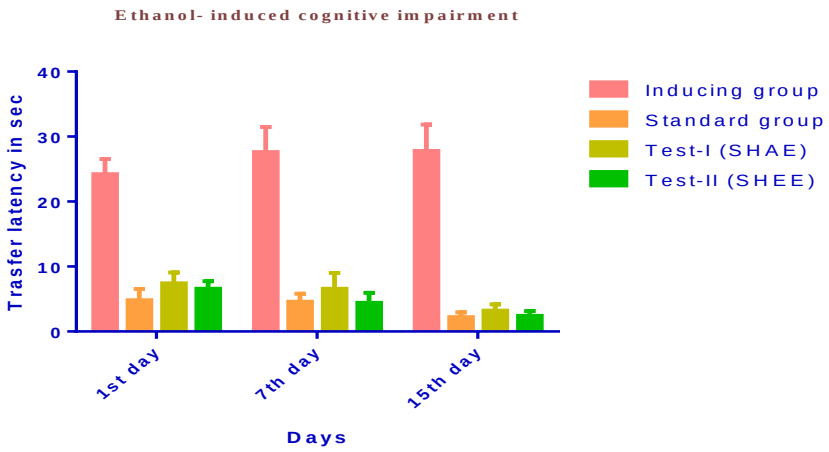

Fig-4: Effect of seed extracts of *Salvia hispanica* on ethanol- induced cognitive impairment. Values are expressed as Mean ± SEM, P < 0.01 vs. control (n = 5 animals).

Table-4: Effect of seed extracts of *Salvia hispanica* on transfer latency (diazepam induced amnesia)

S.No.	Group	Treatment	Transfer latency (In seconds)	
			8TH DAY	After 24 hours (i.e.9TH DAY)
1.	I	**Diazepam**	26.2±3.09	24.2±4.71
2.	II	Standard+ **diazepam**	4.4±0.82	2.0±0.32
3.	III	SHAE+ **diazepam**	9.6±2.40	6.6±1.13
4.	IV	SHEE+ **diazepam**	5.6±0.40	2.0±0.64

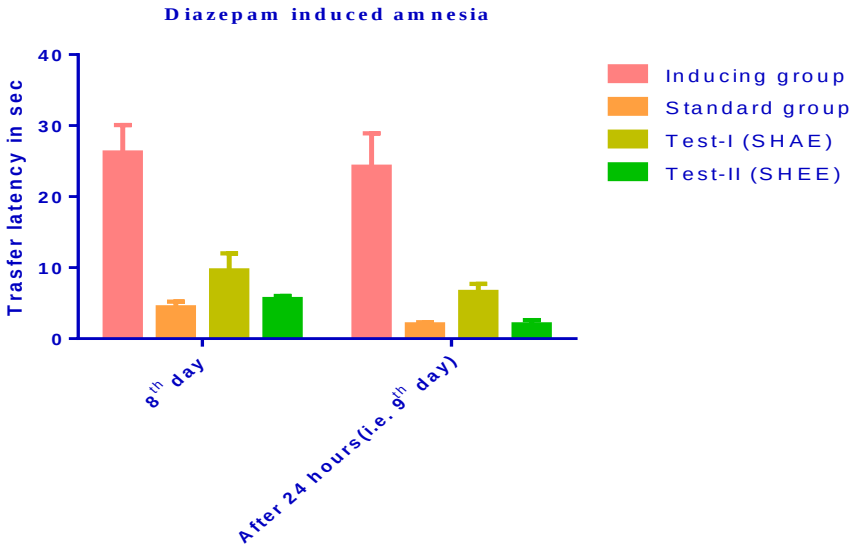

Fig-5: Effect of seed extracts of *Salvia hispanica* on diazepam induced amnesia. Values are expressed as Mean ± SEM, P < 0.05 vs. control (n = 5 animals).

Effect of seed extracts of salvia hispanica on behavioural parameters i.e. 8-ram

Animals treated with ethanol [4.5 mg/kg] alone for 21 days showed an increase in time taken to reach paired arm & number of entries in baited arms and non-baited arms in 1st,7th , 15th & 21 st days.

Table-5: Effect of seed extracts of *Salvia hispanica* on time taken to reach paired arm (ethanol- induced cognitive impairment)

S.No.	Group	Treatment	Time Taken to reach Paired arm (Sec)			
			1st day	7th day	14th day	21st day
1	I	Ethanol	152±1.32	141.6±0.75	124±2.78	105±2.10
2	II	Standard+ethanol	79.4±0.93	70.4±2.21	62±2.63	49.8±2.63
3	III	SHAE+ ethanol	131.6±1.21	126±1.0	104.6±0.93	97.2±1.66
4	IV	SHEE+ ethanol	103.4±0.51	95.8±0.74	90.8±1.16	76.2±1.53

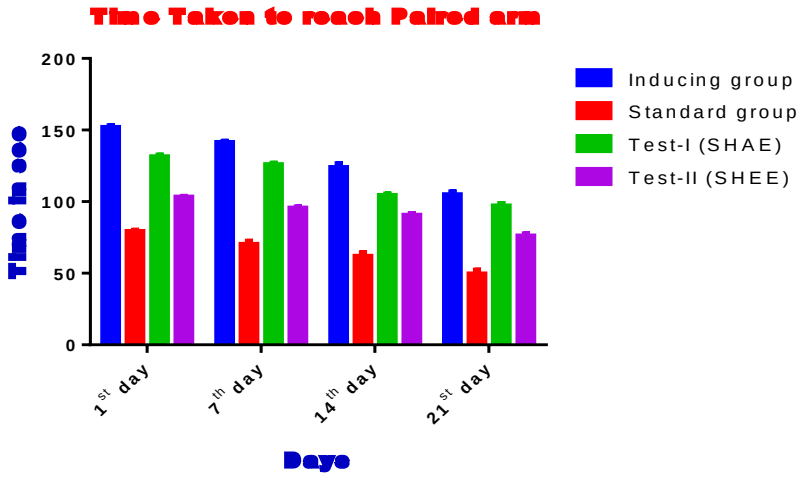

Fig-6: Effect of seed extracts of *Salvia hispanica* on **Time taken to reach Paired arm.** Values are expressed as Mean ± SEM, P< 0.0001 vs. control (n = 5 animals).

Table-6: Effect of seed extracts of *Salvia hispanica* on number of entries in baited arms and non-baited arms (ethanol- induced cognitive impairment)

Group	Treatment	Number of entries in baited arms and non-baited arms							
		1st day		7th day		14th day		21st day	
		B.A	N.B.A	B.A	N.B.A	B.A	N.B.A	B.A	N.B.A
I	Ethanol	2.4±0.25	2.2±0.20	3.2±0.20	3.4±0.40	3.2±0.20	3.6±0.25	2.6±0.40	1.8±0.20
II	Standard+ethanol	5.6±0.25	5.4±0.25	5.2±0.38	4.2±0.59	7.8±0.38	10.8±0.38	11.4±0.51	14.8±1.16
III	SHAE+ ethanol	4.2±0.38	4.4±0.40	4.6±0.25	6.4±0.51	7.4±0.51	8.8±0.80	7.8±1.02	9.2±1.16
IV	SHEE+ ethanol	8.4±0.51	9.0±1.31	9.0±1.31	13.0±0.90	12.4±0.93	10.8±0.97	10.8±1.16	12.2±1.40

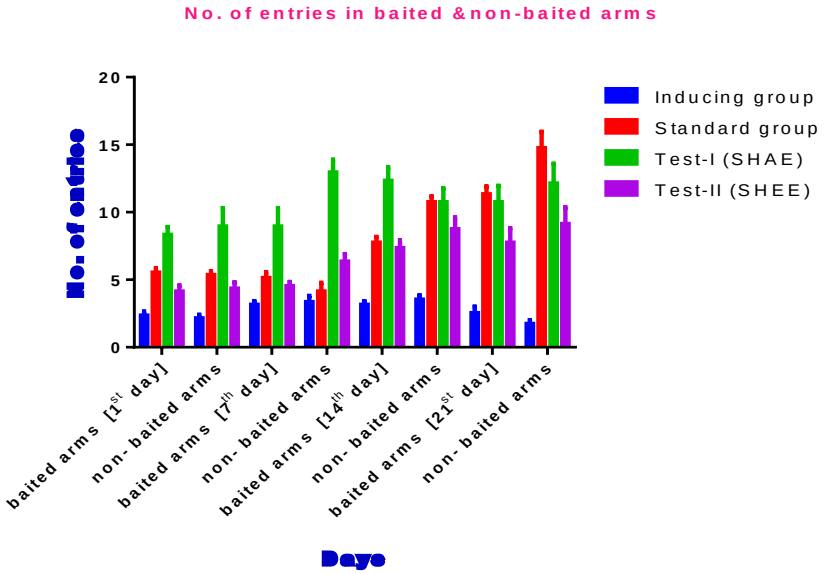

Fig-7: Effect of seed extracts of *Salvia hispanica* on number of entries in baited arms and non-baited arms. Values are expressed as Mean ± SEM, P < 0.0001 vs. control (n = 5 animals).

CONCLUSION

In the present investigation, *Salvia hispanica* posses the presence of carbohydrates, tannins, flavonoids, phenols, sterols and proteins. *Salvia hispanica* showed cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol-induced cognitive impairment & diazepam induced amnesia in rats. *Salvia hispanica* ethanolic extract showed comparatively significant effect exerted to standard drug donepezil hydrochloride in the finding of transfer latency in sec (i.e. learning and memory activity). Transfer latency was recorded after administration of ethanol & diazepam at different days and graphs were ploted according to the results obtained. This effect is attributed to its ability to improve the levels of the acetylcholine that are decreased in the Alzheimer's disease. *Salvia hispanica* showed cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol- induced cognitive impairment. *Salvia hispanica* ethanolic extract showed comparatively significant effect exerted to standard drug donepezil hydrochloride in the finding of time taken to reach paired arm (sec) & number of entries in baited arms and non-baited arms (i.e. learning and memory activity). Time taken to reach paired arm (sec) & number of entries in baited arms and non-baited arms was recorded after administration of ethanol at different days and graphs were ploted according to the results obtained. Therefore finally suggests ethanolic extract of *Salvia hispanica* show same effects exerted to the standard drugs.

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