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Polyherbal Maceration Methanolic Extraction for Nootropic Activity

J.Anantha Lakshmi*[1], D. Satyavati[2]

1. Sri Venkateswara College of Pharmacy,Madhapur. Hyderabad.

2. Sri Datta College of Pharmacy, Ibrahimapatnam. Hyderabad.

ABSTRACT

Nootropic drugs used as a memory enhancer can improve thinking, memory, and alertness in people with Alzheimer's disease and other disease that affect the mind. Memory is perhaps the most vital of all aspects that differentiates human beings from other animals. However, memory can become faulty due to several reasons, and in that case the person is not able to make full use of his or her potentials. Since ages, drugs and natural remedies have been prescribed to enhance memories in people. 4 million people are thought to be suffering from age related memory and increased risk of developing Alzheimer's disease. The present study investigate the nootropic activity of poly herbal methanolic extraction (*Rhodiola Rodantha* Root, *Blepharis Maedeterpensis* Root, *Celastrus Paniculatus Plant* seed, *Brassica Caulorapa* Bud) by using Active avoidance paradigm, Passive avoidance paradigm, Scopolamine-induced amnesia, Sodium nitrite intoxication method to assess the nootropic activity.

Keywords: Nootropic activity, Alzheimer's disease, *RhodiolaRodantha, Blepharis Maedeterpensis, Celastrus Paniculatus, Brassica Caulorapa*

*Corresponding Author Email: anu_manas0108@yahoo.com

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INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The indigenous traditional knowledge of medicinal plants of various ethnic communities has been transmitted orally or in manuscripts for centuries and reached us[1]. The pharmacological treatment of disease began long ago with the use of herbs[2]. Methods of folk healing throughout the world commonly used herbs as part of their tradition. Alzheimer's disease is a neurodegenerative disorder that destroys cells in the brain, leading cause of dementia, a condition that involves gradual memory loss, decline in the ability to perform routine tasks, disorientation, difficulty in learning, loss of language skills, impairment of judgment and personality changes. As the disease progresses, people with Alzheimer's are unable to care for themselves and the loss of brain cells eventually lead to the failure of other systems in the body. The rate of progression of Alzheimer's varies from person to person. The time from the onset of symptoms until death ranges from 3 to 20 years and the average duration is about 8 years. The greatest known risk for developing Alzheimer's is increasing age as 10% of people with 65 years of age and nearly 50 % of people with 85 years of age have the disease. A family history of the disease is another known risk and having a parent or sibling with the disease increases chances of developing Alzheimers in individuals[3]. In AD basal forebrain cholinergic neurons innervating cortex, amygdale and hippocampus degenerate, and is characterized by the presence of senile plaques and neurofibrillary tangles in the brain with a loss of cholinergic neurons in the basal nucleus[4]. Currently there is no cure and the traditional system is directed at managing the symptoms of AD. However there are avenues for the development of new therapies that will hopefully be directed towards improvements of cognitive functions. Biochemical abnormalities such as reduction of Acetyltransferase, biosyntheses of Acetylcholine and increase in Acetyl cholinesterase (AChE), Ach metabolism are strongly associated with the degree of cognitive impairment. The cholinergic hypothesis is based on the loss of cholinergic neurons in brain and the degree of cognitive impairment in AD. Other neurotransmitters such as Noradrenaline, Dopamine and Serotonin may play a role in AD, but the effect of these on cognitive function remains unclear[4].

MATERIALS AND METHODS

Collection and authentication of plant materials:

The selected plants *Rhodiola Rodantha* Root, *Blepharis Maedeterpensis* Root, *Celastrus Paniculatus Plant* seed, *Brassica Caulorapa* Bud materials are collected from in and around of thirupathi, Andhra pradesh. And authenticated by Dr. MadhavaShetti Department of Botany, Sri Venkateswara University, Tirupati

Preparation of Poly Herbal Extract

A wide range of solvents with increasing polarity were chosen.

Step.1:

In a 250ml round bottomed flask, weighed quantity of different plant powdered macerated with the respective solvents in the ratio of 1:2 (i.e. 50gm in 100ml) and kept with occasional shaking for a period of 72 hrs. After the maceration process, the active ingredients present in the supernatant solvent were collected in petri dishes and concentrated under reduced pressure.

Step.2:

These extracts were labeled and its chemical constituents were identified, among the different solvent extracts, the extract possessing more number of active compounds were selected and prepared for bulk extraction similar as step 1.

Acute Oral Toxicity Studies

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic class method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality and /or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance (Figure.1).This procedure results in the use of a minimum number of animals while allowing for acceptable data-based scientific conclusion. The method used defined doses (5, 50, 500, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of the chemical, which cause acute toxicity. Six female Wistar rats weighing between 150-175 gm were used for study. The starting dose Poly herbal methanolic extraction was 2000mg/kg body weight p.o as most of the crude extracts possess LD_{50} value more than 2000mg/kg in b.w.p.o. Dose was administered to the rats, which were fasted over night with water *ad libitum*, food were with held for a further 3-4 hrs after administration (p.o) of drugs & observed for another 14 days. Body weight of the rats before and after treatment were noted and any changes in skin and fur, eyes and mucous membranes and also autonomic, central nervous systems, somatomotor activity and behavior pattern were observed and also signs of tremors, convulsions, salivation, diarrhea,

lethargy sleep and coma were noted. The onset of toxicity and signs of toxicity was also to be noted.

Experimental Animals

Adult Wister rats and mice of both sexes weighing 150-175gms&30 to 35 gms were used in the pharmacological and toxicological studies. The inbred animals were taken from the animal house in Sri Venkateshwara college of Pharmacy. The animals were maintained in well-ventilated room temperature with natural 12h ± 1h day-night cycle in the propylene cages .They were fed balanced rodent pellet diet from Poultry Research experimental period . The animals were housed for one week, prior to the experiments to acclimatize to laboratory temperature. The experimental protocol was proved by the Institutional Animal Ethics Committee IAEC Ref No: IAEC NO IS IAEC/SVCP/2012/01

Determination of nootropic activity[5, 6, 7, 8]

Exteroceptive Behavior Models

Active avoidance paradigm: Four Groups of adult male albino rats 100-150 g each consisting of 6 animals were divided in to the following groups and animals are fasted overnight prior to the test but water was supplied ad libitum.

Group I: Normal control (distilled water 10ml/kg, p.o.)

Group II: Piracetam (200 mg/kg, p.o.)

Group III: PHME (200 mg/kg, p.o.)

Group IV: PHME (300 mg/kg, p.o.)

Experimental procedure:

All groups of rats were trained up to 100% learning criterion of active avoidance response. During the training period, each rat was placed in one of the two chambers of the Sidman box, and after 5 sec the buzzer (conditioned stimulus, CS) was sounded for 2 sec followed by an electric shock (unconditioned stimulus, UCS; 30v, 0.5 sec) through the grid floor. Thereafter, a rest pause of 180 sec was allowed. If the rat jumped within the CS duration to the unelectrified safe box, so as to avoid the USC, it was allowed to rest there for next 30 sec. However, if the rat did not show the avoidance response, it was removed from the shock chamber after 180 sec and was initiated for the next trial. The rats were given 10 trials daily until they reached the 100% criterion of active avoidance response. After an interval of 15 days the rats were subjected to a repeat the test with treatment of different doses of the PHME in order to assess the retention of the previously learned active avoidance response. Similarly nootropic activity of standard drug was evaluated.

Treatment schedule:

Group I was maintained as normal control, which was given with distilled water only daily once for 14 days. Group II with piracetam (200 mg/kg, p.o.), which served as standard. Groups III and IV were treated with different doses of PHME (200 and 300 mg/kg p.o.) respectively for 14 days.

Passive avoidance paradigm:

Groups of adult Swiss male albino mice 24-32 g, each consisting of 6 animals was divided into following groups and animals are fasted overnight prior to the test but water was supplied ad libitum.

Group I: Normal control (distilled water 10ml/kg, p.o.)

Group II: Phenytoin alone (25 mg/kg p.o.)

Group III: Piracetam (200 mg/kg, p.o.)+ Phenytoin (25 mg/kg p.o.)

Group IV: PHME (200 mg/kg, p.o.)

Group V: PHME (300 mg/kg, p.o.)

Experimental procedure

Passive-avoidance task is a method widely used for screening drugs affecting learning and memory. The method described by Papazova et al (1994) was modified as follows. An inverted petridish placed in the centre of the grid floor of a continuous avoidance apparatus (Techno, Lucknow) was used. The petridish served as the shock-free zone (SFZ). Mice were placed in the SFZ and up on stepping down from the SFZ were given an electric shock (20 V) through the grid floor. Animals were trained to remain on the SFZ for at least 60 sec and mice which did not meet these criteria in 5 trials were rejected. Observations were made for acquisition i.e. the number of trials required to reach the learning criteria and for retention of learning for 10 min at 2 h and 24 h post-training. The following retention parameters like step-down latency (SDL) in seconds, step-down error (SDE) as the number of times the animal stepped down from the SFZ and the time spent in the shock zone (TSZ) in seconds are noted.

Treatment schedule:

The memory- impairing dose of phenytoin 25 mg/kg was administered daily for 14 days and the selected doses of PHME for 7 days i.e. on 8^{th} to 14^{th} day and the parameters mentioned above were noted. Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Groups II phenytoin (25 mg/kg p.o.) daily once for 14 days. Additionally group III with piracetam (200 mg/kg, p.o.) which served as standard, Groups IV, V were treated with different doses of PHME (200 and 300 mg/kg p.o respectively daily once for 7 days as mentioned above.

Interoceptive Behavior Models

Scopolamine-induced amnesia

Group of adult Swiss male albino mice 18-25g, each consisting of 6 animals was divided into following groups and animals are fasted overnight prior to the test but water was supplied ad libitum.

Group I: Normal control (distilled water 10ml/kg, p.o.)

Group II: Scopolamine (1.0 mg/kg, i.p.)

Group III: Piracetam (200 mg/kg, p.o.)+ Scopolamine (1.0 mg/kg, i.p.)

Group IV: PHME (200 mg/kg, p.o.) + Scopolamine (1.0 mg/kg, i.p.)

Group V: PHME (300 mg/kg, p.o.) + Scopolamine (1.0 mg/kg, i.p.)

Experimental procedure

All groups were treated accordingly as mentioned above for a period of 14 days and Scopolamine was given 1 mg/kg, i.p. 90 minutes after last dose of standard and different doses of PHME to induce impairment of memory through muscuranic system. Transfer latency (TL) was recorded using Elevated Plus maze (EPM) 45 minutes and 24 hrs after injection of scopolamine. The apparatus used in this model consists of two open arms (16 cm ×5 cm) and two closed arms (16 cm×5 cm×12 cm), extended from a central platform (5 cm ×5 cm) and the maze is elevated to a height of 25 cm from the floor. Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.) only daily once for 14 days. Group II with Scopolamine (1.0 mg/kg, i.p.) alone. Group III with piracetam (200 mg/kg, p.o.) Which served as standard, Groups IV, V were treated with different doses of PHME (,200 and 300 mg/kg p.o.) respectively daily once for 14 days. On the 15^{th} day, 90 min after above treatment, each mouse was placed at the end of an open arm of EPM facing away from the central platform. TL (Transfer latency) was recorded i.e. the time taken by mouse to move into one of the enclosed arms with all its four legs. If the animal did not enter into one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for next 10 s and then returned to its home cage. Retention of this learned-task was examined 24 h after the 15^{th} day trial. The inflexion ratio was calculated by the formula as follows.70.

Inflexion ratio (IR) = (L0 −Lt)/ L0,

where

L0is the initial TL (s) on 15^{th} day and Lt is the TL (s) on the 16thday.

Treatment schedule:

Group I was maintained as normal control which was given with distilled water (10ml/kg, p.o.), Group II with Scopolamine alone (1 mg/kg, i.p) only on 14^{th} day, Group III with piracetam (200 mg/kg, p.o.) which served as standard, Groups IV, V were treated with different doses of PHME

(200 and 300mg/kg p.o.) And after 90 min of the last dose for all the groups III, IV, V, VI, VII, VIII, IX were given with Scopolamine (1 mg/kg, i.p.).

Metabolic influence

Sodium nitrite intoxication

Groups of adult Swiss male albino mice 18-25 g, each consisting of 6 animals were divided into groups and animals are fasted overnight prior to the test but water was supplied ad libitum.

Group I: Normal control (distilled water 10ml/kg, p.o.)

Group II: Sodium nitrite alone (250 mg/ kg s.c)

Group III: Standard drug (Piracetam) + Sodium nitrite (250 mg/ kg s.c)

Group IV: PHME(200 mg/kg, p.o.)+ Sodium nitrite (250 mg/ kg s.c)

Group V: (300 mg/kg, p.o.) + Sodium nitrite (250 mg/ kg s.c)

Experimental procedure

All the groups were treated according to the protocol as mentioned above for a period of 7 days and sodium nitrite 250 mg/kg was given s.c 60 minutes after last dose of standard/PHME to induce chemical hypoxia. Sodium nitrite reduces the oxygen carrying capacity of the blood by converting hemoglobin to methemoglobin and cessation of respiration time in each group of mice was recorded.

Treatment schedule:

Group I was maintained as normal control which was given with distilled water (10 ml/kg, p.o.), Group II with Sodium nitrite alone (250 mg/ kg s.c) daily once for 7 days Group III with piracetam (200 mg/kg, p.o.) which served as standard, Groups IV, V, were treated with different doses of PHME(200 and 300 mg/kg p.o.) respectively daily once for 7 days as mentioned above.

RESULTS AND DISCUSSION:

Table 1: Incremental dose finding experiment and its Signs of Toxicity

No	Treatment	Dose level (mg/kg)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	I	50	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	II	100	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3	III	150	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
4	IV	300	+	-	-	-	-	+	+	-	-	-	-	-	_	+	-	-	-	-	+	-
5	V	600	+	+	+	-	-	+	+	-	-	-	-	-	_	+	-	-	-	-	+	-
6	VI	1000	+	+	+	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	+	-
7	VII	1500	+	+	+	-	-	+	+	-	-	-	-	+	+	+	-	-	-	-	+	-
8	VIII	3000	+	-	+	-	-	+	+	-	-	+	-	+	+	+	-	+	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Increased Motor Activity 8.Tremors 9.Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18.Writhing 19.Respiration 20. Number of Deaths (Mortality)

Table 2: Effect of PHME on Active Avoidance and learning Retention model in mice (Mean ± SEM)

Treatment	Dose	No of Shocks in 10 trails			Time spent in shock zone foe 10 trials (Sec)		
		Learning (Acquisition)	Relearning	Retention	Learning (Acquisition)	Relearning	Retention
Control (Vehicile)	10ml p.o	5.50±0.22	5.33±0.21	2.66±0.21	26.66±1.45	23.16±1.53	16.33±1.17
Piracetam	200mg p.o	4.00±0.25	1.50±0.34**	0.83±0.16**	15.83±1.53**	8.66±0.61	3.66±0.33**
Group 3 PHME	200mg	4.66±0.21	2.50±0.22**	1.66±0.21**	19.83±2.10**	15.83±1.66	10.83±8.06**
Group 4 PHME	300mg	4.33±0.33	2.16±0.16**	1.16±1.66**	16.33±1.20**	9.00±1.03	5.83±0.70**

Table 3: Effect of PHME on Passive avoidance learning and retention on (Shock box model) in mice

Animals	Step down latency (SDL) in Sec									
	Normal control		Toxic control		Standard (Piracetam)		PHME (200mg/kg)		PHME(300mg/kg)	
	Retention At		Retention At		Retention At		Retention At		Retention At	
	2h	24hr	2h	24hr	2h	24hr	2h	24h	2h	24h
H	40	140	10	60	120	283	45	140	50	120
B	60	120	20	90	82	245	60	180	45	240
T	30	80	10	140	75	232	40	195	75	200
HB	40	95	20	120	119	262	51	190	50	240
HT	50	130	30	60	46	255	65	120	55	120
BT	30	110	20	90	45	234	20	180	46	300
Mean ± SEM	41.66±4.7	112.05±9.10	18.33±3.07	93.33±	81.33±13.66	251.33±7.83	46.83±6.56	167.5±12.36	53.5±4.53	203.33±29.40
Step down error (SDE) in no										
H	4	2	4	4	1	1	4	3	3	2
B	2	1	5	2	1	0	3	2	2	2
T	3	2	3	3	2	1	2	2	2	1
HB	4	2	4	4	1	1	3	2	1	2
HT	3	2	5	3	2	1	3	3	3	1
BT	3	1	4	3	1	0	4	2	2	0
Mean ± SEM	3.16±.0.38	1.66±0.21	4.16±0.30	3.16±0.30	1.33±0.21	0.66±0.21	3.16±0.33	2.33±0.21	2.16±0.30	1.33±0.33
Time spent in Shock zone (TSZ) in sec										
H	26	14	29	10	11	5	11	7	12	5
B	17	10	19	9	10	6	11	10	13	8
T	18	12	24	13	12	4	16	8	15	7
HB	21	8	19	12	8	4	23	16	14	6
HT	18	9	22	8	12	4	24	8	8	5
BT	21	11	12	15	10	6	8	10	11	8
Mean ± SEM	21.6±1.35	10.66±0.88	20.18±2.33	11.16±1.07	10.5±0.61	4.83±0.40	15.5±2.74	9.83±1.32	12.16±1.01	6.5±0.56

H: Head, B: Body, T: Tail, HB: Head & Body, HT: Head & Tail, BT: Body &Tail

Table 4: Effect of PHME on Passive avoidance learning and retention on (Shock box model) in mice (Mean ± SEM)

Treatment	Dose/Kg	Step- Down latency Trail	Step down latency (SDL) in Sec		Step down error (SDE) in no		Time spent in Shock zone (TSZ) in sec	
			Learning	Retention	Learning	Retention	Learning	Retention
Normal Control	10ml p. o	2.66	41.66±4.77	112.5±9.10	20.16±1.35	10.66±0.88	3.16±0.30	1.66±0.21
Toxicant Control (Phenytoin)	25mg p. o	3.3	18.33±3.07	93.33±13.08	20.83±2.33	11.16±1.07	4.16±0.30	3.16±0.33
Standard (Piracetam)	200mg p. o	2.33	81.33±13.66**	251.83±7.83**	10.55±0.61**	4.83±0.40**	1.33±0.21**	0.66±0.21**
PHME	200mg p. o	3.0	50.33±3.37**	174.33±15.58**	14.16±0.91**	7.83±0.40**	2.66±0.055*	2.00±0.25*
PHME	300mg p.o	3.83	55.33±2.99**	211.6±13.73**	12.33±1.02	6.00±0.44**	2.33±0.33**	1.16±0.30**

Table 5: Effect of PHME on inflextion ratio in scopolamine induced amnesic model in mice(Interceptive behavior model)

Animals	Normal control			Toxic control			Standard (Piracetam)			PHME (200mg/kg)			PHME(300mg/kg)		
	Transfer Latency (Sec) After		IR=(Lo-Lt)/Lt	Transfer Latency (Sec) After		IR=(Lo-Lt)/Lt	Transfer Latency (Sec) After		IR=(Lo-Lt)/Lt	Transfer Latency (Sec) After		IR=(Lo-Lt)/Lt	Transfer Latency (Sec) After		IR=(Lo-Lt)/Lt
	45min (Lo)	24h (Lt)		45min (Lo)	24h (Lt)		45min (Lo)	24h (Lt)		45min (Lo)	24h (Lt)		45min (Lo)	24h (Lt)	
H	43	24	0.441	54	44	0.185	52	12	0.769	58	40	0.310	42	19	0.547
B	60	41	0.316	74	58	0.216	85	32	0.623	49	32	0.346	70	27	0.610
T	80	42	0.475	44	38	0.316	80	14	0.825	80	49	0.387	55	25	0.545
HB	58	30	0.482	72	40	0.305	57	15	0.736	80	53	0.337	80	36	0.550
HT	72	32	0.555	80	62	.225	34	11	0.676	65	43	0.338	62	30	0.516
BT	47	27	0.425	68	50	0.265	39	14	0.641	83	52	0.373	52	18	0.653
Mean ± SEM			0.449±0.032			0.222±0.024			0.711±0.032**			0.347±0.011			0.554±0.020*

Determination of Nootropic activity

Exteroceptive Behavior Models (Shuttle box)

Effect of PHME on active avoidance learning and retention in rats Effect of PHME on learning and retention was studied using Active avoidance paradigm (Shuttle box) apparatus. In learning period i.e. on the 1st day of the study the number of shocks received and time spent in the shock zone with the standard drug (piracetam) treated group animals was 4.00 ± 0.25 sec and 15.83 ± 1.53 sec respectively. When compared with the control group on the 15th and 16th days of the study there was a significant decrease in the above parameters i.e. the number of shocks received was 1.50 ± 0.34 and 0.83 ± 0.16 respectively and time spent in the shock zone was 8.66 ± 0.61 sec and 5.50 ± 0.61 sec respectively. When compared to control group PHME treated groups have shown significant nootropic activity in relearning and retention phases of the activity i.e. the number of shocks received and time spent in the shock zone was significantly decreased on the 15th and 16th days of experimental study. The results are tabulated in the Table-1. Effect of PHME on passive avoidance learning and retention in mice Effect of PHME on learning and retention was tested using passive avoidance paradigm apparatus. When compared to normal control group Phenytoin intoxicated animals have noted with decreased SDL and increased SDE and TSZ. Piracetam treated group has shown significant increase in the SDL and significant decrease in the SDE and TSZ. On 14th day PHME treated groups have shown dose dependant nootropic action at 2 h and 24 h after the last dose of extract. Piracetam, PHME treated groups have shown significant nootropic activity at both 2nd and 24th h after the last dose of extract and Piracetam on 14th day.

Interoceptive Behavior Models

Effect of PHME on inflexion ratio in mice (scopolamine-induced amnesic model) Scopolamine treated group exhibited with impairment of memory and has shown decrease in IR as compared to normal control group which indicates the induction of amnesia. When compared to IR of normal control group 0.449 ± 0.032, scopolamine treated group was noted with impairment of memory as depicted by decrease in IR 0.222 ± 0.024. Piracetam, PHME with Low medium and high dose treated groups have shown significant increase in the IR as recorded by 0.711 ± 0.032 and 0.366 ± 0.035, 0.576 ± 0.030 and 0.637 ± 0.026 respectively. The results are tabulated in the Table 5.

CONCLUSION

The nootropic activity of poly herbal methanolic extraction (*Rhodiola Rodantha* rhizome, *Blepharis Maedeterpensis* Root, *Celastrus Paniculatus whole Plant* , *Brassica Caulorapa* Bud) by using Active avoidance paradigm, Passive avoidance paradigm, Scopolamine-induced amnesia, Sodium nitrite intoxication method to assess the nootropic activity. Effect of PHME on active

avoidance learning and retention in rats Effect of PHME on learning and retention was studied using Active avoidance paradigm (Shuttle box) apparatus. Effect of PHME on inflexion ratio in mice (scopolamine-induced amnesic model) Scopolamine treated group exhibited with impairment of memory and has shown decrease in IR as compared to normal control group which indicates the induction of amnesia.

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