

## Evaluation of nootropic activity of *Limonia acidissima* against scopolamine-induced amnesia on rats

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### Abstract

**Introduction:** The present study was aimed to evaluate nootropic activity of *Limonia acidissima* on rats.

**Materials and Methods:** Methanolic extract of *Limonia acidissima* was used to evaluate nootropic activity, piracetam (200 mg/kg, i.p.) was used as standard and scopolamine (1 mg/kg, i.p.) was used to induce amnesia. Effect of drugs on learning and memory of rats was evaluated by using Y-maze task and elevated plus maze on scopolamine induced amnesia models. Locomotor activity was performed using actophotometer. Also, levels of acetylcholinesterase enzyme including histopathological study of rat brain were carried out.

**Results:** Methanolic extract of *Limonia acidissima* showed increase in the alternation of behaviour response and percentage spontaneous alteration with Y-maze task. While in elevated plus maze scopolamine induced amnesia model, methanolic extract of *Limonia acidissima* showed decreased in transfer latency, which is an indicative of cognition improvement. Methanolic extract increases locomotor activity in rats and decreases the levels of acetylcholinesterase enzyme significantly. The histopathological study with both low and high dose of extract showed effective regenerative score as compared to other group.

**Conclusion:** The results suggested that the administration of methanolic extract of *Limonia acidissima* enhances learning and memory in different experimental model. The histopathological study reveals neuro-protective property of extract. The study indicates that the extract may be used in the treatment of Alzheimer disease.

**Keywords:** Nootropic activity, *Limonia acidissima*, Alzheimer disease, piracetam, scopolamine.

## INTRODUCTION

According to the world health organization, approximately 450 million people suffer from a mental or behavioral disorder<sup>[1]</sup>. Dementia is one of the age-related mental problems and a characteristic symptom of Alzheimer's disease (AD)<sup>[2-4]</sup>. AD is a progressive neurodegenerative and cerebrovascular disease<sup>[2,5]</sup>. It destroys cell in the brain causes problems with memory, un-usual behavior<sup>[6]</sup>, thinking, personality changes<sup>[7]</sup> and finally ultimately death<sup>[8-10]</sup>. AD characterized by the presence of excessive amounts of neurotic plaques containing amyloid  $\beta$  protein loss of cholinergic markers in brain<sup>[7,11]</sup>. The central cholinergic system play important role in learning and memory process<sup>[12]</sup>. Acetylcholine (Ach) is one of the brain neurotransmitters involved in learning, memory and attention processes<sup>[13]</sup>. A decrease in acetyl choline in the brain of patients with AD appears to be a critical element in producing dementia<sup>[7]</sup>. The centrally acting anti- muscarinic drugs like scopolamine impairs learning and memory in rodents and human being<sup>[4,13]</sup>. Age, stress and emotion are conditions that may lead to memory loss, amnesia, dementia, anxiety, schizophrenia and Alzheimer's diseases<sup>[14,15]</sup>.

There are few nootropic medicines which are used in treatment of AD, called nootropic drugs belong to the class of psychotropic Agents<sup>[16]</sup>. The term nootropic was coined by Giurgea in 1972, from Greek noon (mind) and Greek tropos (turn)<sup>[17]</sup>. Nootropics are also referred as smart drugs, improve mental function such as memory, also increase blood circulation to brain and improves oxygen supply to brain<sup>[18]</sup>. Synthetic medicines like tacrine, donepezil<sup>[19]</sup>, aniracetam, piracetam and rivastigmine are used for treatment of cognitive dysfunction and memory loss associated with AD<sup>[20]</sup>. But these drugs possess adverse effects including gastrointestinal disturbances and problems associated with their bioavailability<sup>[21]</sup>. To overcome these problems researchers are looking for herbal formulation which can overcome the adverse effect of synthetic drugs.

A number of herbs have been extensively studied and reported to have memory enhancing property<sup>[22]</sup>. The herbal plant *Limonia acidissima* is one of the important herbal drugs

used in Ayurvedic systems of medicine<sup>[23]</sup>. It is also used against variety of conditions such as antimicrobial, hepatoprotective, antidiarrhoeal, anticancer, diuretic, hepatoprotective, antispermatogenic, antioxidant, anti diabetic and wound healing activities<sup>[24,25]</sup>.

The present study seeks to determine whether the methanolic extract of leaves and fruits pulp of *Limonia acidissima* shows nootropic activity in animal model.

## **MATERIALS AND METHODS**

### **Experimental Animals**

Wistar rats of either sex weighing between (150-200g) body weights were used for the present study. They were housed under standard laboratory conditions (temperature  $25 \pm 1^\circ\text{C}$ ), relative humidity  $55 \pm 5\%$  and 12.00:12.00h dark: light cycle) with standard pellet diet and water ad libitum.

The experiment was conducted as per the standard procedure prescribed by CPCSEA, India. The study protocol was approved by the IAEC of Yashoda Technical Campus, Satara (YSPM/YTC/PHARMA/20/2017).

### **Drugs and chemicals**

Piracetam (Dr. Reddy's, India), Scopolamine hydrobromide (APP Pharmaceuticals, India) and normal saline were used for study. All other reagents and chemicals were of analytical grade and procured from Loba Chemie, Mumbai, India.

### **Plant material and preparation of extracts**

The plant material was collected from Phaltan and was identified and authenticated by Department of Botany, Yashwantrao Chavan Institute of Science, Satara, Maharashtra, India. A voucher specimen (No-57) of plant material was kept in Department of Pharmacognosy, Yashoda Technical Campus, Satara. The fully ripe fruits and leaves of plant were kept for shade drying for one week. Then fruits were subjected to break open and rind, seed, pulp was separated and it was shade dried with leaves at room temperature more than two weeks, after drying rind, pulp and seeds were separately coarse powder and leaves was also separately coarse powder for further extraction. The coarsely powdered material was extracted with methanol by using Soxhlet extraction method for four days at  $45^\circ\text{C}$ , at the ratio 1:1:1 (seeds, pulp and leaves). The obtained extract was filtered and concentrated (yield 8.8%).

### **Preliminary phytochemical investigation**

The methanolic extract was subjected for phytochemical test like alkaloids, flavonoids, glycosides, saponins, carbohydrate and tannins<sup>[26]</sup>.

Mayer's test, Wagner's test, Dragendroff's test and Hager's test was used to confirm presence of alkaloids. In brief, extract was treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow colored precipitate indicates the presence of alkaloids. The extract was treated with Wagner's reagent (iodine in potassium iodide). The formation of brown or reddish precipitate indicates the presence of alkaloids. Then extract was treated with Dragendroff's reagent (solution of potassium bismuth iodide). The formation of red precipitate indicates the presence of alkaloids. The extract was treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of yellow colored precipitate.

Presence of flavonoids was confirmed by performing alkaline reagent test and lead acetate test. In alkaline reagent test, extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. In lead acetate test, extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Molisch's test and Benedict's test was used to confirm presence of carbohydrate. *Limonia acidissima* extract was dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. In Molisch's test filtrate was treated with two drops of alcoholic  $\alpha$ -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates. In Benedict's test, filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

The presence of glycosides in extract was confirmed by modified Borntrager's test. The methanolic extract was hydrolyzed with dilute HCl and treated with ferric chloride solution. The resultant reaction mixture was immersed in boiling water for five minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Forth test and foam test was used to confirm presence of saponins. In Forth test, the extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. In Foam test, the 500 mg extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins

Biuret's test, Million's test, Xanthoprotein test and Ninhydrin test was performed to detect proteins in extract. In Biuret's test, extract was treated with 4 % w/v sodium hydroxide and 1% w/v copper sulphate. The change in colour of the solution to violet or pink indicates the presence of proteins. In Million's test, extract was treated with Million's reagent and heated. The appearance of white precipitate which changed to brick red on heating indicates the presence of proteins. In Xanthoprotein test, extract was treated with concentrated sulphuric acid. The appearance of white precipitate which turns to yellow on boiling and orange on addition of ammonium hydroxide indicates the presence of proteins containing tyrosine tryptophan. In Ninhydrin test, extract was treated with lead acetate solution and boiled on water bath for 10 min. The change in colour of solution to purple or blue indicates the presence of amino acids.

For presence of steroids, extract was treated acetic anhydride and sulphuric acid. The change in colour from violet to blue or green in sample indicates presence of steroid. Ferric chloride test was performed to detect phenols from extract. The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

#### **Acute toxicity study**

The acute toxicity study was carried out according to OECD-423 guidelines. Six swiss albino female mice, weighing in the range of 20-25 gm, were administered the test solution at a dose of 2000 mg/kg. The mice were critically observed for clinical signs, gross behavioral changes and mortality following the administration of the test formulation at different time intervals like 30min, 1h, 2h, 4h, 24h, 48h and 72h up to a period of 14 days<sup>[27]</sup>.

#### **Behavioral study**

A rat was trained for behavioral study by conducting 1week training during which they did not received any plant extract or drug. The completely trained rat was selected for the study. An experiment was carried out in the light period between 8.00 am and 03:00 pm in a sound proof room.

### Y maze task

The five groups of animals were made, each group comprises 6 animals. Group I: Control group (normal saline), Group II: Negative group (Scopolamine 1 mg/kg i.p), Group III: Standard treatment (Piracetam 200 mg/kg i.p + Scopolamine 1 mg/kg i.p), Group IV: Low dose of extract + Scopolamine (200 mg/kg p.o + 1 mg/kg i.p), Group V: High dose of extract + Scopolamine (400mg/kg p.o+1mg/kg i.p).

Y-maze task is a simple method to evaluate memory enhancing activity in laboratory animal, it is generally used to check behavioural pattern in animal. Wooden Y-maze was used for study. It consists of three arms with an angle of 120° between each of the two arms. The each arm was 8cm wide, 30cm long and 15cm deep. The arms were designated as start arm (A), novel arm with food stimuli (B) and other arm (C)<sup>[28]</sup>. In first trial, rat was placed just inside arm and allowed to move freely through apparatus for 5-10 min. In second trial, rat was placed in maze and explored the arm A, B and C systematically rat entered in each arm. The Ability to alternate requires that the rat know which arm they have already visited, total 13 entries were recorded of each rat visually and at last percentage of spontaneous alteration is calculated by using following formula<sup>[29,30]</sup>:

$$\text{Percentage alteration} = \frac{\text{Number of positive entries}}{(\text{Total number of arm entries} - 2)} \times 100$$

### Scopolamine induced amnesia in Rats

The grouping of animals was done as per Y maze task. All the animals were treated for 14 days and at the end of treatment period all the extract treated animals were subjected to scopolamine (1mg/kg i.p) 60 minutes after administration of extract, except the first group which served as vehicle control.

In this method, scopolamine is used as inducer which causes memory impairments in rats, which is calculated by using elevated plus maze (EPM) apparatus. EPM is made up of wooden material. It consists of two arms closed arm and open arm. In EPM task transfer latency was recorded. Rat was placed in arm and allowed to explore the maze for 1 min, if rat is not transferred into other arm within 90 sec gently pushed into other arm and transfer latency was considered as 90 sec. All groups of animals were treated for 14 days and at the end of treatment transfer latency is recorded served as parameter transfer latency on 14<sup>th</sup> day (acquisition day) and 15<sup>th</sup> day (retention day) <sup>[31]</sup>.

### **Locomotor activity**

Grouping of animals were done as per the Y maze task. Before test all rats were fasted for 4h and treated with as per the grouping. One hour after treatment each rat was placed individually in actophotometer for a period of 10 minutes and locomoter activity was measured in terms of score. The difference in the Locomotor activity of all groups were recorded<sup>[6,32]</sup>.

### **Estimation of acetylcholinestrase enzyme**

Animals were grouped as per the Y maze task. All rats were treated as per the groups with saline, scopolamine, piracetam and extract. After 60 minutes of treatment rats were decapitated, brains were removed quickly and stored in ice cold saline. Frontal cortex, hippocampus and septum were quickly dissected out on a petri dish chilled on crushed ice. The tissues were weighed and homogenized in 0.05M phosphate buffer (pH 7.2). The homogenate (0.4ml) was added test tube containing 2.6ml phosphate buffer and 100 $\mu$ l of 5,5-dithiobisnitrobenzoic acid (DTNB), and mixed. The absorbance of resulting mixture was measured at 412nm using spectrophotometer. Stable value of absorbance was recorded. Then, acetylthiocholine iodide (20 $\mu$ l) was added and change in absorbance per minute was determined. The mean change in absorbance was considered for calculation using following formula and acetylcholinestrase activity is measured as  $\mu$ M/l/min/gm of tissue<sup>[33]</sup>.

$$R = (\delta \text{ OD volume of assay} / E) \times \text{mg of protein}$$

Where, R is the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein,  $\delta$  OD is the change in absorbance per minute and E is the extinction coefficient ( $1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ )<sup>[34]</sup>.

### **Brain histopathology**

After the treatment and behavioral studies, two animals from each group were sacrificed by excessive CO<sub>2</sub> anesthesia and the brains were isolated and were kept in 10% formaldehyde solution. The brain was stained with crestyl violet, cerebellum and basal ganglia was studied under light microscope<sup>[35]</sup>.

### **Statistical analysis**

The statistical analysis was carried out by using Graph pad prism software version 5.0 and results were compared by one-way ANOVA followed by Tukey's Multiple Comparison Test. A *p* value less than 0.05 was considered as statistically significant.

## RESULTS

### Preliminary phytochemical investigation

The results of the preliminary phytochemical screening of the methanolic extract of *Limonia acidissima* is given in table 1. The *Limonia acidissima* extract gave positive results for alkaloids, flavonoids, carbohydrates, glycosides, saponin, and absence of proteins, steroids, phenols.

**Table 1: Phytochemical analysis of extract *Limonia acidissima***

Sr. No.	Phytochemical test	Result
1	Test for Alkaloids	+Ve
2	Test flavonoids	+Ve
3	Test for carbohydrates	+Ve
4	Test for Glycosides	+Ve
5	Test for Saponin	+Ve
6	Test for proteins	-Ve
7	Test for steroids	-Ve
8	Test for Phenols	-Ve

+Ve indicates the presence of compounds,  
-Ve indicates the absence of compounds.

### Acute toxicity test

As female mice are more sensitive to drugs than male, in acute oral toxicity study female mice were taken as per OECD-423 guideline. Methanolic extract *Limonia acidissima* did not show any toxic effects up to 2000 mg/kg oral dose. CNS stimulation parameters such as hyperactivity, irritability, tremors and convulsions were found to be negative in mice. CNS depressant parameters such as hypoactivity, narcosis and ataxia were found to be negative in mice.

### Y-maze task

Y maze model proved to be a sensitive measure of spatial recognition memory. The effect on alteration behavior was studied on one parameter such as % alteration (Table 2). The negative control group showed significant ( $p < 0.001$ ) decrease in the alternation of behavior when compared with the Normal control. The results of the standard treatment groups showed significant ( $p < 0.001$ ) increase in the alternation of behavior with respect to methanolic extract of *Limonia acidissima* 400mg/kg.



### Scopolamine induced amnesia in rats

Scopolamine induced amnesia in rat model was carried out by using elevated plus maze. The Scopolamine treated group showed a significant ( $p < 0.001$ ) increase in transfer latency and memory retention on (acquisition 14<sup>th</sup>) and (retention 15<sup>th</sup>) day when compared with Normal control, standard treatment and a high dose of extract groups respectively. Whereas the Piracetam standard treatment group when compared methanolic extract of *limonia acidissima* at the dose of 200 mg/kg showed significantly ( $p < 0.01$ ) i.e., decrease in transfer latency and memory retention on (acquisition 14<sup>th</sup>) and (retention 15<sup>th</sup>) day respectively. The result is given in Table 2.

**Table 2: Effect of methanolic extract on alteration behavior and transfer latency in rats**

Group	Treatment	Alterations (%)	Acquisition day 14 (sec)	Retention day 15 (sec)
I	Normal Control: (Normal saline, p.o.)	66.31 ± 2.45	41.00 ± 1.67	38.33 ± 1.22
II	Negative Control:	35.80 ± 3.19 <sup>c#</sup>	74.14 ± 3.37 <sup>c#</sup>	71.50 ± 3.50 <sup>c#</sup>
III	Scopolamine (1 mg/kg, p.o.)	80.50 ± 3.61 <sup>a#c†</sup>	25.67 ± 1.22 <sup>c#c†</sup>	21.33 ± 1.22 <sup>c#c†</sup>
IV	Standard treatment: Piracetam (200 mg/kg, i.p.) + Scopolamine (1mg/kg, i.p.)	46.45 ± 2.71 <sup>c#c††</sup>	34.83 ± 1.24 <sup>c†a††</sup>	31.50 ± 1.35 <sup>c†a††</sup>
V	Low dose of extract (100 mg/kg, p.o.) + Scopolamine (1mg/kg i.p.)	70.27 ± 2.29 <sup>c†</sup>	27.50 ± 1.17 <sup>c#c†</sup>	23.67 ± 1.17 <sup>c#c†</sup>
	High dose of extract (400 mg/kg, p.o.) + Scopolamine (1 mg/kg, i.p.)			

Values represent mean ± SEM; n= 6. Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. <sup>a</sup> p<0.05, <sup>b</sup> p<0.01, <sup>c</sup> p<0.001. <sup>#</sup>Data compared with Normal control. <sup>†</sup>Data compared with negative control. <sup>††</sup>Data compared with standard treatment.

### Locomotors activity

The locomotor activity in rats was carried out using actopotometer. The methanolic extract of *Limonia acidissima* showed nootropic activity by increasing locomotors activity (Table 3). The negative group showed decrease in locomotor activity as compared to normal control while extract treated groups showed significantly ( $p < 0.001$ ) increased in locomotor activity as compared with negative treated group.

### Estimation of acetyl cholinesterase enzyme

The result of levels of acetyl cholinesterase (AChE) is given in table 3. In normal control level of AChE was very low while in negative control it was found to be high. In case of

piracetam treated group level of AChE was significantly reduced as compared to negative control group. The methanolic extract of *Limonia acidissima* (200 and 400 mg/kg) significantly ( $P < 0.001$ ) lowered AChE activity as compared to negative control. The significant decrease in level of AChE indicated that *Limonia acidissima* is a potential anti-cholinesterase agent and possesses nootropic activity.

**Table 3: Effect of methanolic extract of on locomotor activity and brain acetylcholinesterase levels in rats**

Group	Treatment	After treatment	AChE ( $\mu\text{M/l/min/mg Protein}$ )
I	Normal Control: (Normal saline, p.o.)	$562 \pm 30.12$	$13.03 \pm 1.55$
II	Negative Control: Scopolamine (1 mg/kg, p.o.)	$251.2 \pm 42.61^{c\#}$	$24.09 \pm 1.56^{c\#}$
III	Standard treatment: Piracetam (200 mg/kg, i.p.) + Scopolamine (1mg/kg, i.p.)	$515 \pm 52.65^{c\ddagger}$	$15.52 \pm 1.13^{c\ddagger}$
IV	Low dose of extract (100 mg/kg, p.o.) + Scopolamine (1 mg/kg i.p.)	$324.5 \pm 48.5^{c\#c\ddagger\ddagger}$	$20.97 \pm 1.20^{c\#c\ddagger\ddagger}$
V	High dose of extract (400 mg/kg, p.o.) + Scopolamine (1 mg/kg, i.p.)	$451.3 \pm 48.18^{b\#c\ddagger}$	$18.00 \pm 1.19^{c\#c\ddagger}$

Values represent mean  $\pm$  SEM; n= 6; Analysis was performed using one way ANOVA followed by Tukey's multiple comparison test; p value less than 0.05 was considered as statistically significant. <sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.01$ , <sup>c</sup>  $p < 0.001$ . <sup>#</sup>Data compared with Normal control. <sup>†</sup>Data compared with negative control. <sup>††</sup>Data compared with standard treatment.

### Histopathological analysis of scopolamine-induced amnesia in rats

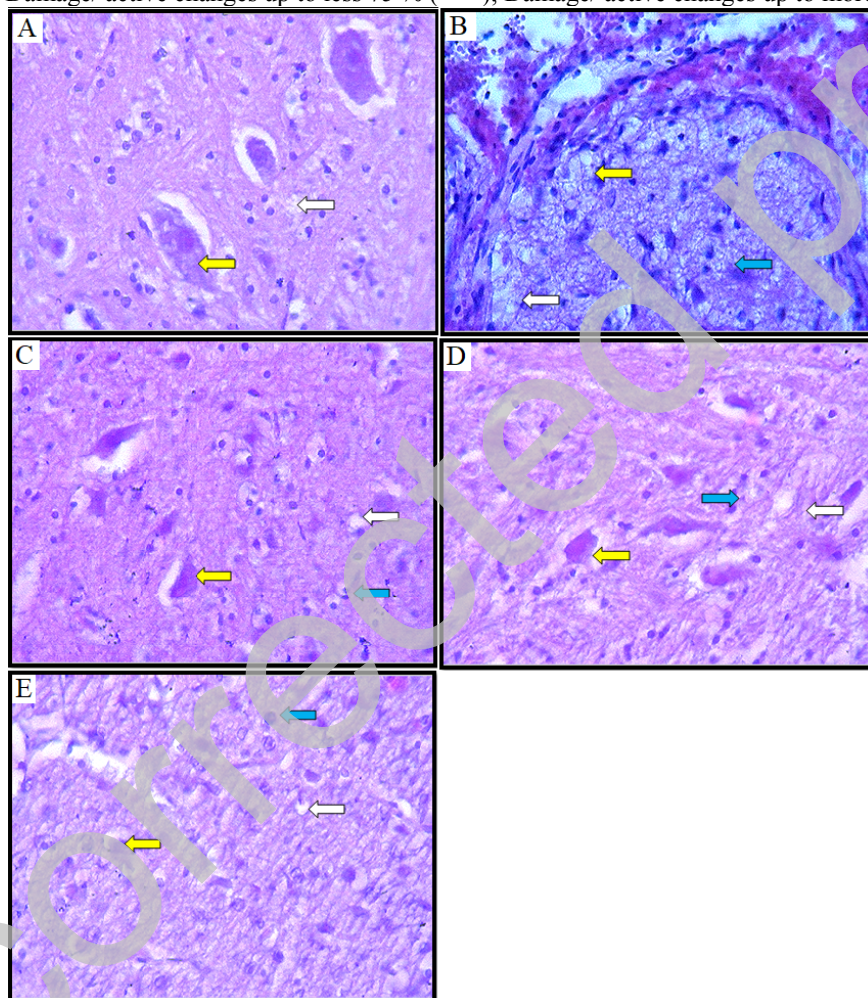
The results of histopathological analysis are given in figure 1 and table 4. Figure 1A shows the histopathological section of normal control rat showed neuronal degeneration without vascular degeneration and gliosis, while negative control group (Figure 1B) showed vascular degeneration, neuronal degeneration and gliosis. In case of standard treatment group (Figure 1C), low dose group (Figure 1D) and high dose group (Figure 1E), vascular degeneration, neuronal degeneration and gliosis was found to be less as compared to negative control group. The group II treated with scopolamine showed maximum pathological changes as compared to rest of the groups. Both low dose and high dose of methanolic extract of *Limonia acidissima* showed good regenerative score as compared to other group.

**Table 4: Histopathology of the brain tissues in scopolamine induced amnesia in rat**

Group	Vacuolar degeneration	Neuronal degeneration	Gliosis
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Normal Control: (Normal saline, p.o.)	0	+	0
Negative Control: Scopolamine (1 mg/kg, p.o.)	+++	+++	++
Standard treatment: Piracetam (200 mg/kg, i.p.) + Scopolamine (1mg/kg, i.p.)	++	++	+
Low dose of extract (100 mg/kg, p.o.) + Scopolamine (1mg/kg i.p.)	++	++	+
High dose of extract (400 mg/kg, p.o.) + Scopolamine (1 mg/kg, i.p.)	+	++	+

No abnormality detected (0); Damage/ active changes up to less than 25 % (+); Damage/ active changes up to less than 50 % (++); Damage/ active changes up to less 75 % (+++); Damage/ active changes up to more than 75 (++++)



**Figure 1:** Histopathological observation of brain tissues in scopolamine-induced amnesia in rat.

**A-Normal control:** Section showing only stress amount of neuronal degeneration (yellow arrow)

**B- Negative control:** Section showing vascular degeneration (white arrow), neuronal degeneration (yellow arrow) and glial cell infiltration (blue arrow).

**C-Standard treatment:** Section showing vascular degeneration (white arrow), neuronal degeneration (yellow arrow) glial cell infiltration (blue arrow) less as compared with negative control.

**D-Low dose of extract:** Section showing vascular degeneration (white arrow), neuronal degeneration (yellow arrow) glial cell infiltration (blue arrow) less as compared with negative control.

**E-High dose of extract:** Section showing vascular degeneration (white arrow), neuronal degeneration (yellow arrow) glial cell infiltration (blue arrow) less as compared with negative control.

#### 4. DISCUSSION

Number of patients suffering from AD's are resign steadily day by day all over the world<sup>[36]</sup>, AD is characterised by degenerative changes in the brain accompanied by loss of memory<sup>[37,38]</sup>. The main cause of AD is death of cholinergic neurons in basal forebrain area which results in the deficit of acetylcholine (Ach)<sup>[36]</sup>. Scopolamine is antimuscarinic agent, after administration it produces memory deficit<sup>[39]</sup>. Scopolamine induced memory loss was well reported animal model for screening anti-amnestic molecule<sup>[40]</sup>. It is a non-selective muscarinic receptor antagonist. It competitively inhibits muscarinic receptor for acetylcholine and reduces the level of acetylcholine and further, it causes impairment of learning acquisition short-term memory as it produces depression of cerebral cortex, especially of motar areas<sup>[41]</sup>.

Piracetam is a standard drug used to treat amnesia, dementia and other health problem like brain stroke, AD, vascular dementia, DLB and Huntington disease. Piracetam have ability to bind to receptor and increases Ach level in brain. It acts on cholinergic receptor and increases synthesis of Ach. It increases oxygen supply to brain. It has positive therapeutic effect which is useful in clotting, coagulation and thrombosis disorder. It also acts as antioxidant/neurotonic. It also enhances number of acetylcholine receptor thus it might cause for enhancement of learning and memory<sup>[42]</sup>.

In this study, we have investigated the effect of methanolic extract of *Limonia acidissima* on spatial memory and neurodegeneration in animal model of Alzheimer's disease. The results clearly demonstrated that *Limonia acidissima* significantly improved spatial memory and neurodegeneration.

Methanolic extract of *Limonia acidissima* at doses 200mg/kg and 400mg/kg was administered orally for 7 days, which have improved learning and memory of rat significantly in the Y maze task. It was seen that when negative control group was compared with that of the

standard treatment and extract group, both standard and extract treated groups showed significant ( $p<0.001$ ) increase in percentage alteration behaviour.

In elevated plus maze model methanolic extract of *Limonia acidissima* possess nootropic activity by scopolamine-induced amnesia. The extract treated group showed significantly decrease in transfer latency and memory retention with  $p<0.001$  when compared with negative and standard group at (acquisition 14<sup>th</sup>) and (retention 15<sup>th</sup>) day of the study, which is an indicative of cognition improvement.

Locomotor activity in patients suffering from AD is absent. Actophometer is model which is used to see the locomotor behaviors of animals. So, in present study actophometer model was used to know about the locomotor activity of animals treated with methanolic extracts. The methanolic extract of *Limonia acidissima* passes nootropic activity by increasing locomotor activity. The significant ( $p<0.001$ ) locomotor activity was observed in rats treated with low dose and high dose treated groups.

Acetylcholine is considered as the most important neurotransmitter involved the regulation of cognitive functions. Cholinergic neurons plays important role in cognitive deficit associated with AD and neurodegenerative disease<sup>[31]</sup>. It has been demonstrated that impairment in learning, memory and behavior observed in the patients with dementia are caused at least by change within cholinergic system. In present study *Limonia acidissima* inhibited acetylcholinesterase enzyme, there by elevating acetylcholine concentration in the brain. The findings suggested that the possible neuroprotective role of *Limonia acidissima*. Thus, it seem that *Limonia acidissima* may prove to be useful in treatment of AD. However further investigations is warranted to explore the possible involvement of other neurotransmitters responsible for nootropic property of *Limonia acidissima*.

The histopathological study have also shown that both low dose and high dose of methanolic extract of *Limonia acidissima* were showed good regenerative score as compared to other group, which is good sign towards neuro-protective property to treat AD.

In few of the studies, the use of anti-inflammatory drugs showed reduction in symptoms of AD<sup>[43]</sup>. Epidemiological studies also confirmed suitability of non steroidal anti-inflammatory drugs to reduce incidence of AD<sup>[44]</sup>. *Limonia acidissima* have been reported to produce anti-inflammatory action in rodents<sup>[45]</sup>. This anti-inflammatory property of *Limonia acidissima* might certainly helps to treat Alzheimer patients.

## 5. CONCLUSION

In the conclusion, present study suggested that methanolic extract of *Limonia acidissima* provided significant protection in lowering AD. The methanolic extract of *Limonia acidissima* increased locomotor activity in rats and inhibited acetylcholinesterase enzyme, thereby elevating acetylcholine concentration in the brain. The histopathology of brain had also showed good regenerative score, hence methanolic extract of *Limonia acidissima* can be used in the management of AD. An extensive study on these lines is required in future to strengthen the methanolic extract of *Limonia acidissima* as novel and natural nootropic/memory enhancing agent.

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