EVALUATION OF NOOTROPIC EFFECTS OF AQUEOUS EXTRACT OF TRIDAX PROCUMBENS LINN ON COGNITIVE FUNCTIONS IN MICE

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ABSTRACT: Alzheimer’s disease (AD) is characterized by the presence of excessive amounts of neurotic plaques containing amyloid β protein loss of cholinergic markers in the brain. Loss of cholinergic cells particularly in the basal forebrain is accompanied by loss of the neurotransmitter acetylcholine. Scopolamine-induced amnesic animal models are used to screen for agents that are claimed to have cognition-enhancing activity through stimulation of the cholinergic system, thus making them candidates for the treatment of AD. In the present study was undertaken to investigate the Nootropic effects of Tridax Procumbens L. on cognitive functions in mice. The aqueous extract of Tridax procumbens Linn was extracted by Percolation method. Piracetam 100 mg/kg body weight used as a standard drug and different dose of aqueous extract of Tridax Procumbens L. are used to evaluate its Nootropic effect on Elevated plus Maze and Pole Climbing apparatus. From the result, it indicates that in EPM model Tridax Procumbens L. (12.6 mg/ kg bw) and in Pole climbing model Tridax Procumbens L. (12.6 mg/ kg bw) significantly shows Nootropic effect and also reverse scopolamine-induced amnesia.


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1.INTRODUCTION
Memory is the ability of an individual to record the event, information and retains them over short or long periods of time. The different conditions such as age, stress, and emotion may lead to memory loss, amnesia, anxiety, high blood pressure, dementia to more threat like schizophrenia and
Alzheimer’s disease [1, 2]. Alzheimer’s disease (AD) is characterized by the presence of excessive amounts of neurotic plaques containing amyloid β protein loss of cholinergic markers in the brain. Loss of cholinergic cells particularly in the basal forebrain is accompanied by loss of the neurotransmitter acetylcholine [3]. Scopolamine-induced amnesic animal models are used to screen for agents that are claimed to have cognition-enhancing activity through stimulation of the cholinergic system, thus making them candidates for the treatment of AD [4]. AChE inhibitors from general chemical classes such as physostigmine, tacrine, galantamine, and heptylphysostigmine have been tested for the symptomatic treatment of AD [3]. Medicinal plants are playing a significant role in the management of AD and memory deficit. The important traditional therapeutic methods are Ayurvedic, homeopathy, Unani and Siddha systems of medicine. Unani system of medicine offers traditionally a highly scientific healthcare therapy as a divine gift and as a result, the global interest of the medical profession is focused on medicinal plants. The traditional system of medicine is fundamentally preventive, protective, nutritive and curative. Therefore, traditional medicines are safe and harmless which treat the patients with fewer or no side effects [5]. The plant *Tridax Procumbens* L. belonging to the family Asteraceae is popularly known as Coatbuttons – Mexican Daisy in English, Gavpattha in Hindi, and Dagdipala in Marathi. The chemical constituents present are alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. Mineral composition present in leaves is calcium, magnesium, potassium, sodium, and selenium. Leaf mainly contains crude proteins 26%, crude fiber 17% soluble carbohydrates 39% calcium oxide 5%, Luteolin, glucoluteolin, quercetin, and isoquercetin. Whereas the oleanolic acid, fumeric acid, β-sitosterol, and tannin is present in good amounts. *Tridax Procumbens* is known for several potential therapeutic activities like anti-inflammatory [6], Immunomodulatory [7], Anti-diabetic [8], antiviral, antibiotic efficacies [9], antiparasitic [10], Antiobesity [11], Anticancer [12], wound healing and antioxidant activity [13]. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding, as a hair tonic. In southern Orissa, a paste prepared from the whole plant is taken orally to relieve diarrhea. A fine paste of the leaves is applied externally to reduce swelling of hemorrhoids by the Urash in southern Bihar [14]. In the light of above, the present study was undertaken to investigate the Nootropic effects of *Tridax Procumbens* L. on cognitive functions in mice.

2. MATERIALS AND METHODS

*Tridax procumbens* leaves were collected from the campus of the college in a month of June and July and shade dried. The plant was identified Agharkar institute of Pune. Piracetam UCB India Ltd, India, Scopolamine Buscopan, German Remedies, India. All drugs were dissolved and/or diluted with distilled water (vehicle). *Tridax procumbens Linn* was dissolved in distilled water and administered intraperitoneally.
Animals
Male/Female albino mice weighing 20-25 g were obtained from National Institute of Toxicology, Pune. Animals were housed in groups of five per cage under standard laboratory conditions with food and water continuously available. A 12 h: 12 h (light: dark) cycle was used with the light on from 7:00 to 19:00 h. All behavioral testing was done during the daylight period between 10:00 and 17:00 h. Animals were tail marked and handled daily for 5 min during the last 3 days before the experiment.

Extraction [15]
The aqueous extract of *Tridax procumbens* Linn was extracted by Percolation method. Moisten 1 kg powdered leaves of *Tridax procumbens* with a sufficient amount of the prescribed menstrum (solvent). After 24 hours lower orifice is opened and menstrum is collected with a controlled speed until ¾ of menstrum is collected. Then more menstrum is added and collected from the lower orifice so that marc does not become dry. Then Marc is pressed to get extract which is combined with previous liquid. Then it is allowed to stand and then it is filtered. Recover the menstrum from the remainder of the percolate and concentrate to a soft extract in a vacuum apparatus at a temperature not exceed 45°C.

Elevated plus maze [16-21]
The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm ×12 cm). The arms extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of the open arm, facing away from a central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day for each animal. The mouse was allowed to explore the maze for another 2 min. and returned to its home cage. Retention of this learned task was examined 24 h after the first-day trial. The TL was expressed as inflexion ratio (IR) using the formula earlier by Jaiswal and Bhattacharya [22].

\[ IR = \frac{(L1 - L0)}{L0} \]

Where \( L0 \) = TL after 24 h or on the ninth day and \( L1 \) = initial TL(s).

Treatment Schedule
Mice were divided into 10 groups and each group consisted of a minimum of 5 animals separate animals were used for each experiment.

**Group I:** It represented the control group for young mice. The vehicle was administered orally for nine successive days and transfer latency was measured after 90 min of administration on the second day and ninth day.

**Group II:** It represented the disease control group for young mice. Scopolamine (0.3 mg/kg i.p.)
was injected into young mice for nine successive days and transfer latency was measured after 90 min of administration on the second day and ninth day.

**Group III:** It represented the positive control group for young mice. Piracetam (100 mg/kg i.p.) was injected into young mice for nine successive days and transfer latency was measured after 90 min of administration on the second day and ninth day.

**Group IV:** Piracetam (100 mg/kg i.p.) was injected for nine successive days to young mice. At 60 min after the injection of Piracetam on the nine-day, scopolamine 0.4 mg/kg, i.p. was administered. TL was noted after 45 min of administration of scopolamine on the second day and ninth day.

**Group V, VI and VII:** Aqueous extract of *Tridax procumbens* Linn (4.2, 8.4, 12.6 mg/kg, p.o.) was administered orally to the young mice for nine successive days and transfer latency was measured after 90 min of administration on the second day and ninth day.

**Group VIII, IX, and X:** Aqueous extract of *Tridax procumbens* Linn (4.2, 8.4, 12.6 mg/kg, p.o.) was administered orally to the young mice for nine successive days and Scopolamine (0.3 mg/kg) was injected i.p. to young mice at 90 min. after administration of extract on day nine. TL was noted 45 min. after injection on the second day and ninth day.

**Cook’s Pole Climbing [23-30]**

The basic operational mode of this method is that following an auditory warning stimulus, the animal learns to avoid the foot – shock delivered through the cage floor by jumping to the pole. This method has long been accepted as a reliable technique to evaluate learning and memory in experimental animals. In this method, Cook’s pole climbing apparatus was used for inducing stable baseline behavior. The rats had to learn to jump on a pole to avoid foot shock. A tone 50 Hz. Was used as a conditioned stimulus and foot shock of 1 mA was the unconditioned stimuli. In the training procedure, the animal was initially allowed to adopt in the chamber for 1 min. this was followed, in succession, by conditioned and unconditioned stimuli, for a period of 15 sec each. The trial ended either after the animal responded by jumping on the pole or after 30 sec, whichever was earlier the animal was given such trial every day for 10 days. A trained animal either responded spontaneously or to buzzer without waiting for the shock. Retention of the memory of the painful stimuli established in a learning procedure was tested before and after drug treatment. It was quantified as the percentage of animals avoiding shock by jumping on the pole. The data of different groups were tested for statically significance.

**Treatment Schedule**

Mice were divided into 10 groups and each group consisted of a minimum of 5 animals separate animals were used for each experiment.

**Group I:** It represented the control group for young mice. A vehicle was administered orally for nine successive days and jumping on pole response was measured after 90 min of administration on first, second, fifth and ninth day.
Group II: It represented the disease control group for young mice. Scopolamine (0.3 mg/kg i.p.) was injected to young mice for nine successive days and jumping on pole response was measured after 90 min of administration on first, second, fifth and ninth day.

Group III: It represented the positive control group for young mice. Piracetam (100 mg/kg i.p.) was injected to young mice for nine successive days and jumping on pole response was measured after 90 min of administration on first, second, fifth and ninth day.

Group IV: Piracetam (100 mg/kg i.p.) was injected for nine successive days to young mice. At 60 min after the injection of Piracetam on the nine-day, scopolamine 0.4 mg/kg, i.p. was administered. Jumping on pole response was noted after 45 min of administration of scopolamine on first, second, fifth and ninth day.

Group V, VI and VII: Aqueous extract of *Tridax procumbens* Linn (4.2, 8.4, 12.6 mg/kg, p.o.) was administered orally to the young mice for nine successive days and jumping on pole response was measured after 90 min of administration on first, second, fifth and ninth day.

Group VIII, IX, and X: Aqueous extract of *Tridax procumbens* Linn (4.2, 8.4, 12.6 mg/kg, p.o.) was administered orally to the young mice for nine successive days and Scopolamine (0.3 mg/kg) was injected i.p. to young mice at 90 min. after administration of extract on day nine. Jumping on pole response was noted 45 min. after injection on first, second, fifth and ninth day.

3. RESULTS AND DISCUSSION

In the traditional system of medicine the leaves of *Tridax procumbens* Linn. (Family: Asteraceae) are employed for bronchial catarrh, dysentery, and diarrhea and for restoring hair. An aqueous extract of the plant produced reflex tachycardia and showed a transient hypotensive effect on the normal blood pressure of dogs. It has also a marked depressant action on respiration (The wealth of India). *Tridax procumbens* Linn has various pharmacological effects, antimicrobial activity against both gram–positive and gram–negative bacteria, Hepatoprotective, Immunomodulatory, Antihypertensive and stimulate wound healing. The central nervous system is complex, regulating/controlling various body functions through the balance of a variety of stimulating and inhibitory neurotransmitters. Any drug that alters the action of any of the neurotransmitters may affect various neurobehavioral and neuroendocrinal functions. In the present study leaf extract of ‘*Tridax procumbens*’ (TP) Linn. Family Asteraceae was screened for its pharmacological action on Central Nervous System to access the Nootropic activity (learning and memory) by using Elevated plus maze and Pole Climbing Model. Nootropics represent a class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capacity and memory. A number of drugs, including Piracetam, Aniracetam have been introduced in therapy to ameliorate cognitive deficits [22]. The neurochemical basis of learning and memory remains controversial, despite extensive experimental and clinical studies. Although the role of the central cholinergic system is fairly well established, its
deficiency being implicated in memory deficits, the role of the other neurotransmitter systems cannot be ignored. Several studies have indicated that an increase in serotonergic neurotransmission can interfere with learning acquisition and memory consolidation. Numbers of experiments have presented evidence suggesting that learning and memory can be modified by stimulation of the central dopaminergic system. Many other studies also suggest the involvement of the mesolimbic cortical dopaminergic system in cognitive effects. Increased noradrenergic activity has been shown to improve memory.

**Table 1: Effect of aqueous extract of *Tridax procumbens* Linn (TP) on learning and memory using Elevated Plus Maze**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg)</th>
<th>Day I</th>
<th>Day II</th>
<th>Day IX</th>
<th>Inflexion ratio Day II</th>
<th>Inflexion ratio Day IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8.29±0.69</td>
<td>4.79±0.71***</td>
<td>7.75±0.66</td>
<td>0.59±0.02</td>
<td>0.15±0.03</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine (0.3)</td>
<td>7.73±1.51</td>
<td>8.83±1.05</td>
<td>8.39±0.29</td>
<td>-0.12±0.10</td>
<td>-0.40±0.63**</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam (100)</td>
<td>9.39±2.40</td>
<td>6.45±1.06***</td>
<td>6.21±0.06***</td>
<td>0.25±0.01</td>
<td>0.26±0.01*</td>
</tr>
<tr>
<td>IV</td>
<td>Piracetam (100) + Scopolamine (0.3)</td>
<td>8.30±1.41</td>
<td>7.33±0.80</td>
<td>5.26±1.41***</td>
<td>0.05±0.08</td>
<td>0.28±0.04**</td>
</tr>
<tr>
<td>V</td>
<td>TP (4.2)</td>
<td>14.51±2.61</td>
<td>7.69±1.34***</td>
<td>6.58±0.07***</td>
<td>0.41±0.01</td>
<td>0.49±0.02*</td>
</tr>
<tr>
<td>VI</td>
<td>TP (8.4)</td>
<td>10.66±1.18</td>
<td>8.50±1.68***</td>
<td>7.13±0.05***</td>
<td>0.22±0.02</td>
<td>0.34±0.03**</td>
</tr>
<tr>
<td>VII</td>
<td>TP (12.6)</td>
<td>10.54±1.74</td>
<td>5.86±1.04***</td>
<td>5.20±0.07***</td>
<td>0.38±0.01</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td>VIII</td>
<td>TP (4.2) + Scopolamine (0.3)</td>
<td>15.21±1.97</td>
<td>12.03±2.58***</td>
<td>2.20±0.08***</td>
<td>0.46±0.01</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>IX</td>
<td>TP (8.4) + Scopolamine (0.3)</td>
<td>10.38±2.06</td>
<td>5.99±0.59***</td>
<td>5.46±0.07***</td>
<td>0.35±0.02</td>
<td>0.37±0.03**</td>
</tr>
<tr>
<td>X</td>
<td>TP (12.6) + Scopolamine (0.3)</td>
<td>13.91±2.92</td>
<td>6.81±1.46***</td>
<td>5.04±0.06***</td>
<td>0.47±0.07</td>
<td>0.55±0.96**</td>
</tr>
</tbody>
</table>

n=5, Values are Mean ±SEM, *p<0.05, **p<0.01, ***p<0.001, as compared with control group (ANOVA followed by Tukey Kramer multiple comparison test).

The elevated plus maze is used to measure transfer latency i.e. the time elapsed between the movement of the animal from an open to an enclosed arm was markedly shortened if the animal had previously experienced entering open and closed arms, and this shortened transfer latency has been shown to be related with memory processes. In EPM, acquisition (learning) can be considered as transfer latency on first-day trials and the retention/consolidation (memory) is examined 24 hr later.
In the present study, *Tridax procumbens* at 4.2 mg/kg (i.p.) showed significant (p<0.05) increase in the inflexion ratio at day two, also 8.4 mg/kg (i.p.) showed significant (p<0.01) increase in the inflexion ratio at day two. Scopolamine significantly (p<0.01) decreases the inflexion ratio at day two. Piracetam significantly (p<0.05) increase in the inflexion ratio at day two. The combination of Scopolamine and *Tridax procumbens* shows significant (p<0.01) increase in the inflexion ratio at day two (Table 1).

### Table 2: Effect of aqueous extract of *Tridax procumbens* Linn (TP) on learning and memory using Cook’s Pole climbing

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg)</th>
<th>Day I</th>
<th>Day II</th>
<th>Day V</th>
<th>DAY IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.8 ±0.05</td>
<td>0.76 ± 0.04</td>
<td>0.84 ± 0.04</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>Scopolamine (0.3)</td>
<td>1 ± 0.03*</td>
<td>1.16 ± 0.05***</td>
<td>1.06 ± 0.02</td>
<td>0.96 ± 0.06***</td>
</tr>
<tr>
<td>III</td>
<td>Piracetam (100)</td>
<td>0.24 ± 0.02***</td>
<td>0.44 ± 0.02***</td>
<td>0.34 ± 0.04***</td>
<td>0.58 ± 0.08***</td>
</tr>
<tr>
<td>IV</td>
<td>Piracetam (100)+ Scopolamine (0.3)</td>
<td>0.76 ± 0.02</td>
<td>0.84 ± 0.02</td>
<td>0.68 ± 0.05</td>
<td>0.48 ± 0.03</td>
</tr>
<tr>
<td>V</td>
<td>TP (3)</td>
<td>0.54 ± 0.05**</td>
<td>0.54 ± 0.05**</td>
<td>0.46 ± 0.05***</td>
<td>0.46 ± 0.05**</td>
</tr>
<tr>
<td>VI</td>
<td>TP (6)</td>
<td>0.84 ± 0.02</td>
<td>0.84 ± 0.02</td>
<td>0.46 ± 0.05***</td>
<td>0.40± 0.1</td>
</tr>
<tr>
<td>VII</td>
<td>TP (9)</td>
<td>0.84 ± 0.05</td>
<td>0.84 ± 0.02</td>
<td>0.40 ± 0.04***</td>
<td>0.58 ± 0.08</td>
</tr>
<tr>
<td>VIII</td>
<td>TP (3) + Scopolamine (0.3)</td>
<td>1.04 ± 0.05**</td>
<td>0.78 ± 0.03</td>
<td>0.78 ± 0.03</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>IX</td>
<td>TP (6) + Scopolamine (0.3)</td>
<td>0.62± 0.03</td>
<td>0.56 ± 0.02</td>
<td>0.32 ± 0.06***</td>
<td>0.52 ± 0.05*</td>
</tr>
<tr>
<td>X</td>
<td>TP (9) + Scopolamine (0.3)</td>
<td>0.76± 0.05</td>
<td>0.50 ± 0.04 **</td>
<td>0.60± 0.05*</td>
<td>0.52 ± 0.05***</td>
</tr>
</tbody>
</table>

n=5, Values are Mean ±SEM, *p<0.05, **p<0.01, ***p<0.001, as compared with control group (ANOVA followed by Tukey Kramer multiple comparison test).

Learning and memory involve mechanisms like acquisition, storage, consolidation, and recall. Active avoidance learning is reasonably good tests for cognitive function. The ability of the animal to identify the conditioning stimuli (buzzer) as a precursor of the unconditioned stimulus (shock) involves recall of task and may implicate long-term memory. In the present study Scopolamine significantly (p<0.05) increased the time taken to avoid the shock when compared to control, while Piracetam significantly (p<0.001) decreased the time taken to avoid the unconditioned stimulus at day 1, day 2, day 5 and day 9. *Tridax procumbens* at 3 mg/kg (i.p.) significantly (p<0.01) decreased
the time taken to avoid the unconditioned stimulus at day 1, day 2, day 5 and day 9 respectively while 6 and 9 mg/kg (i.p.) showed significant (p<0.001) avoidance of a shock at day 5. *Tridax procumbens* 3 mg/kg (i.p.) showed significant (p<0.01) activities at day 1, while *Tridax procumbens* at 6 mg/kg (i.p.) showed significant (p<0.05) activity at day 2, 5 and 9 when administered in combination with Scopolamine (Table 2). Thus data of the present experiment suggest that the drug-induced changes could be interpreted as a modification in the retrieval or recall phenomenon. From the present study, it is shows that the aqueous extract of *Tridax procumbens* exhibited potential Nootropic activity.

4. CONCLUSION
The use of plants, both the wild and domesticated species has been recorded since ancient times in almost all major civilizations. It is also dispensed as ‘Bhringraj’ which is well known Ayurvedic medicine for liver disorders. Antioxidant, antimicrobial, anti-inflammatory and immune modulatory properties have also been demonstrated. In the present study we have evaluated the nootropic activity of aqueous extract of *Tridax procumbens linn*, hence the aqueous extract of *Tridax procumbens linn*, has potential therapeutic value in alleviating certain memory impairment observed in Alzheimer disease.

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CONFLICT OF INTEREST
Authors have no any conflict of interest.

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