Cognitive enhancement activity of turmeric oil in amyloid beta (25-35) induced Alzheimer's model

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Abstract

Introduction: Turmeric oil (TMO) is a secondary metabolite of *Curcuma longa* Linn obtained by steam distillation of its rhizomes. It contains 50% ar-Turmerone as a major constituent. The antioxidant, anti-bacterial and anti- inflammatory activities of the Turmeric oil have already been established. It has been reported that, Curcumin (CM) is having a protective role in neuro inflammation and beneficial in Alzheimer's disease (AD). But the effect of TMO (fraction without CM) is not explored till now.
Aim: To evaluate the cognitive enhancement activity of turmeric oil using Amyloid Beta induced Alzheimer's model and comparing it with that of Curcumin.
Methodology: An ICV injection was performed to administer Aβ (25-35) peptide to both the lateral ventricles of the Wistar albino rats. Turmeric oil (400mg/kg), Curcumin (400mg/kg) and Donepezil (DPL) 10mg/kg were suspended in sunflower oil and administered orally from the second day of ICV injection. After 16 days of recovery period, *in-vivo* screening for spatial working, spatial learning and recognition memory were performed by Radial arm maze, Morris water maze and Novel object recognition task respectively. *In-vitro* study of acetyl cholinesterase inhibitory activity was also performed by modified Ellman's method.
Results: TMO significantly reduced the number of wrong entries in Radial arm maze compared to the Aβ group. DPL showed maximum reduction in number of wrong entries followed by CM and TMO. In Morris water maze task, TMO significantly reduced the escape latency when compared to the Aβ group. The effect of DPL in escape latency was more significant compared to CM and TMO. In Novel object recognition task, TMO could not alter the percentage time spent on the novel object compared to the Aβ group. DPL and CM significantly increased the percentage time spent on the novel object. Acetyl cholinesterase inhibitory activity of TMO, CM and DPL were 53.37%, 58.26% and 66.35% respectively at 10mg/ml concentration which denotes the enhancement of acetyl choline in brain. TMO provides a significant protection against spatial working and learning memory impairment, while its effect was not much affected in recognition memory impairment.
Conclusion: It is concluded that Turmeric oil enhances the cognitive activity compared to Curcumin and Donepezil.
Keywords: Cognition, Turmeric Oil, Amyloid Beta, Alzheimer's.

1. Introduction

Herbal drugs are widely used for the treatment of many diseases including Neuro-Degenerative (ND) diseases like Alzheimer's Disease (AD) and other memory related disease[1]. In Indian system of medicine, the following plants have promising activity in neuro-psycho-pharmacology. "*Allium sativum, Bacopa monnierae, Centella asiatica, Celastrus paniculatus, Nicotiana tabaccum, Withania somnifera, Ricinus communis, Salvia officinalis, Ginkgo biloba, Huperiza serrata, Angelica sinensis, Uncaria tomentosa, Hypericum perforatum, Physostigma venosum, Acorus calmus, Curcuma longa, Terminalia chebula, Crocus sativus, Enhydra fluctuans, Valeriana wallichii, Glycyrrhiza glabra*"[2].

In the present study, an attempt was made to explore the neuro-protective and nootropic potential of

Turmeric oil (TMO) derived from Turmeric. Turmeric, known as *Curcuma longa* L., is a perennial flowering plant belongs to the Zingiberaceae or ginger family. It is a spice native to India. The rhizome of the plant has been in medical practice for quite a very long time for its health benefits which includes anti-inflammatory, anti-diabetic, analgesic, anti-oxidant, antibacterial, anti-fungal, anti-protozoal, anti-ulcer, hypocholesteremic and anti-cancer activity [3].

Curcumin (CM), a diarylheptanoid obtained from *Curcuma longa* L. has a proven nootropic and neuro-protective activity [4], the compound is poorly bio-available and once it crosses BBB, it disappears from the brain very fast. The poor oral bioavailability of CM has been attributed to its poor aqueous solubility due to its partition coefficient 3.2 and extensive first pass metabolism [5]. TMO obtained from the CM free oleoresin of turmeric is a compound less explored for its cognitive enhancing properties. TMO, a lipophilic fraction from turmeric, exhibits several therapeutic potentials. TMO chiefly comprises ar-Turmerone (AT) and β-Turmerone[6]. Several medicinal and pharmacological properties such as antifungal [7], insect repellent, anti-bacterial [8], anti-platelet and anti-mutagenic [8] activities of TMO have been reported.TMO contains up to 50% of ar-turmerone[6]. It has been suggested that AT inhibits microglia activation, a property that may be useful in treating neurodegenerative diseases [9]. Recently, it is suggested that AT possesses anti-inflammatory properties resulting from the blockade of key signalling pathways in microglia. Because microglia activation is a hallmark of neuro-inflammation and is associated with various neurologic disorders [10], including neurodegenerative diseases and stroke, AT constitutes a promising therapeutic agent for various neurologic disorders.

Amyloid Beta (*Aβ*)is the main constituent of the plagues found in the brain of AD patients[11]. Hence, the direct injection of Aβ peptide is preferred for inducing AD in experimental models. Potent amnesic properties are reported for the 11-amino acid fragment of Aβ, Aβ (25–35). Aβ (25–35) is a subset of Aβ (1–42) located at the C-terminal end in the hydrophobic domain. This short peptide has been proposed to be a functional domain of Aβ responsible for its neurotoxic properties [11].

In this study, we have used Aβ (25-35) peptide through Intra Cerebro Ventricular (ICV) injection to produce cognitive impairment in rats and the two compounds (TMO and CM) were independently evaluated for its possible protective and ameliorative effects in cognitive impairment.

2. Materials and Methods

2.1 Animals

30 young male Wistar albino rats were procured from animal house of Department of Pharmaceutical Sciences, RIMSR, M G University. They were housed five per cage at 12:12 h light/dark cycle and fed ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 Test drugs:

Curcumin (CM) 95% and Turmeric oil (TMO) with 50% ar-Turmerone (AT) were obtained as a gift sample from Akay Flavours and Aromatics Pvt. Ltd, Cochin, Kerala, India.

2.3 Preparation of aggregated Aβ (25-35) peptide:

Commercially available Aβ (25–35) was purchased from Sigma Aldrich, St. Louis, USA and the peptide was aggregated by dissolving in sterile bi-distilled water at a concentration of 1 mg/ml, and stored at deep freezing temperature . It was kept incubated at 37˚ C for 4 days before the use[11].

2.4 Surgical Procedure [11,12–14]:

The animals were kept on overnight fasting on the day prior to surgery. The rats were administered with Oxytetracyclin inj. intra-muscularly (IM) one hour prior to the surgery to prevent infection. The rats were anesthetized intra-peritoneally (ip) with Ketamine (100 mg/kg) and Xylazine (10 mg/kg). A midline sagittal incision was made in the scalp using a scalpel blade. Holes were drilled in the skull over the lateral ventricles on both the hemispheres using the following coordinates: **0.8mm posterior to bregma; 1.5mm lateral to the sagittal suture.** All injections were made using a 10-μl Hamilton syringe. The needle of the micro-syringe was put **3.8mm beneath the surface of the brain**. All the groups except the control received 5μl of aggregated Aβ (25-35) in each lateral ventricle. Control group received bi-distilled water (10μl) instead of Aβ (25-35) peptide.

2.5 Post-surgical care

Oxytetracyclin was given once daily IM, for the next three days and Pentazocine was given for the next two days once daily subcutaneous (SC). The hind and fore limbs of the animals were cleaned with alcohol (90%) every 12 h to prevent any faecal contamination of wound and the bedding was changed every day. Food and water was supplied after 24 h of surgery. Betadine ointment was applied every 12 h for the next two days to prevent any chances of infection and faster wound healing.

2.6 *In-vivo* Screening

2.6.1 Radial Arm Maze (RAM)[15-17]:

Study design: Number of animals in each group: 6

Control group: Received bi-distilled water as I.C.V injection and 0.5ml/100g SFO orally.

Aβ group: Received Aβ (25-35) peptide as I.C.V injection

Standard group: Received Aβ (25-35) peptide as I.C.V injection and DPL 10mg/kg orally

Test 1: Received Aβ (25-35) peptide as I.C.V injection and CM 400mg/kg orally

Test 2: Received Aβ (25-35) peptide as I.C.V injection and TMO 400mg/kg orally

Procedure and Principle: The RAM was designed by Olton and Samuleson in 1976 to assess the spatial memory in rats. A standard RAM contains an octagonal open central platform of 34 cm in diameter and there were eight arms radiating from the platform. Each arm was 45 cm long and 10 cm wide. A recessed food well was located 2 cm from the end of each arm. The maze was elevated 70 cm above the floor level. The animals were fasted overnight before conducting the experiment. During this period the animals had access to water without limitations. Animals were subjected to 5 days of habituation trials pre-operatively. The rat was trained once a day: all arms were baited (one pellet per arm) at the beginning of each trial and the rat was allowed to move in the maze until either the 8 arm had been visited, 16 arm visits were made, or 10 min had elapsed. A re-entry into previously visited arms during a trial was recorded as an exploration error. The number of such errors was used as the main index of spatial memory impairment. After the last training session animals were returned to their normal diet and subjected to surgery and the animals were re-tested to perform the RAM 16 days after the surgery for 5 consecutive days.

2.6.2 Morris Water Maze (MWM)[18,19]:

Study design: Number of animals in each group: 6

Control group: Received bi-distilled water as I.C.V injection and 0.5ml/100g SFO orally.

Aβ group: Received Aβ (25-35) peptide as I.C.V injection

Standard group: Received Aβ (25-35) peptide as I.C.V injection and DPL 10mg/kg orally

Test 1: Received Aβ (25-35) peptide as I.C.V injection and CM 400mg/kg orally

Test 2: Received Aβ (25-35) peptide as I.C.V injection and TMO 400mg/kg orally

Procedure and Principle: MWM was designed by Richard G Morris in 1981 to assess the spatial learning and memory. It is widely used by researchers to evaluate the effect of neuro-cognitive disorders on spatial learning and their possible treatments.

Testing was conducted in the Morris water maze (diameter 1 m) with a "platform" (diameter 12.7 cm) which could be raised or lowered during a trial. The platform was located in the same quadrant throughout testing. The testing room contained a number of constant, salient visual cues.

During the training period, each rat received one training session each day for 7 days. During each daily session, the rat was placed in the water facing the pool wall at one of 3 start points (Except the platform quadrant). Upon release into the water, the rat was allowed to swim for 60 s to locate the platform which was raised to within 1.5 cm of the water (transparent) surface and escape from the water. Last 3 days of training and during the test days, the platform remained under the water and the water was rendered opaque. After escaping, the rat remained on the platform for 30 s before being removed. If the rat failed to escape within 60 s, it was guided to the platform (during training) and remained there for 30 s. The time taken by each rat to find the platform was measured. The test was started on 22nd day of surgery with an invisible platform for 3 consecutive days.

2.6.3 Novel Object Recognition Task (NORT)[18]:

Study Design: Number of animals in each group: 6

Control group: Received bi-distilled water as I.C.V injection and 0.5ml/100g SFO orally.

Aβ group: Received Aβ (25-35) peptide as I.C.V injection

Standard group: Received Aβ (25-35) peptide as I.C.V injection and DPL 10mg/kg orally

Test 1: Received Aβ (25-35) peptide as I.C.V injection and CM 400mg/kg orally

Test 2: Received Aβ (25-35) peptide as I.C.V injection and TMO 400mg/kg orally

The NORT takes advantage of a rodent's spontaneous preference to explore novel objects relative to familiar objects. This test is the benchmark test of recognition memory in the rodent and is dependent on the integrity of the hippocampus. Apparatus used for NORT was an opaque plastic chamber.

A single NORT consisted of a familiarization phase (encoding) followed by a prescribed delay interval and then a test phase (retrieval). During the familiarization phase, the rat was allowed to explore and become familiar with two identical objects placed in the chamber for 15 min. Following the familiarization phase, delay interval of 1 min was given before the test phase. During the test phase, the rat was allowed to explore two objects (one novel object and one familiar object). A total of 30 s time was given for the animal for the exploration. Object exploration was considered when the animal comes close to the object (nose within 2 cm of object). The dependent measure was the percentage of time that a rat spent exploring the novel object during the 30 s of object exploration. The objects were cleaned after every test to remove the odour of the animal.

2.6.3 Acetyl cholinesterase inhibitory activity (Modified Ellman's method) [20,21]

Principle: The principle of this method is the measurement of rate of production of thiocholine as Acetyl thiocholine iodide (ATCI) is hydrolysed in the presence of Acetyl cholinesterase enzyme to form thiocholine and acetate. Thiocholine then reacts with 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) to form a yellow coloured anion of 5-thio-2-nitro-benzoic acid. The rate of formation of coloured solution is measured under 412nm using a colorimeter.

Method: In a test tube, 0.4ml of acetyl cholinesterase, 0.5ml of 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) and 0.4ml of sample (DPL, CM and TMO) were added sequentially and mixed. It was then incubated for 15 minutes at 25^0C. 0.1ml Acetyl thiocholine iodide (ATCI) was then added to the above solution. The rate of absorbance was monitored at 412nm. The percentage inhibition was calculated by the following equation,

Percentage inhibition = A - (B – C) x 100/A

A = Control= [DTNB + ATCI + Water+ Enzyme]

B = Sample= [DTNB + ATCI + Sample + Enzyme]

C = Blank of sample= [DTNB + ATCI + Sample + Water]

3. Results

3.1 Radial Arm Maze
Table 1: Effect of Donepezil, Curcumin and Turmeric oil on the performance of Aβ (25-35) peptide injected rats in a Radial Arm maze.

Group	No. of Wrong Entries on day 17	No. of Wrong Entries on day 18	No. of Wrong Entries on day 19	No. of Wrong Entries on day 20	No. of Wrong Entries on day 21
Control	2.67±0.52	2.17±0.41	1.33±0.52	0.5±0.55	0.5±0.55
β	7.33±0.82	7.12±0.55	7.25±0.52	6.85±0.52	6.8±0.63
PL	3.33±0.52**	2.67±0.52**	2.17±0.41**	1.33±0.52**	0.67±0.52**
Test-1	5.17±0.98**	4.17±1.17**	3.33±1.21**	2.33±1.03**	1.5±1.05**
Test-2	5.67±1.03**	4.83±0.75**	4±0.89**	3±0.89**	2±0.89**

Values are presented as mean ± SD by one way Analysis of Variance (ANOVA) followed by Dunnett-compare all vs. toxic, n=6, **p<0.01
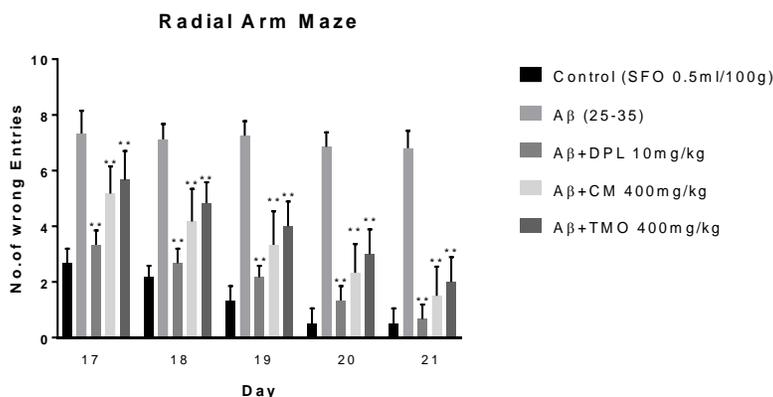

Fig. 1: Comparison of reduction in No. of wrong entries in Radial Arm Maze by different treatment groups. (Control, Aβ, DPL, CM and TMO)

In RAM task, TMO treated group showed 64.73% reduction in number of wrong entries over a period of 5 days. DPL and CM treated groups showed 79.88 and 70.98% reduction in number of wrong entries respectively over the same period. Aβ group and the control group showed 7.23 and 81.27% reduction in number of wrong entries respectively over the same period.

3.2 Morris Water Maze
Table 2: Effect of Donepezil, Curcumin and Turmeric oil in escape latencies of Aβ (25-35) peptide injected rats.

Group	Escape Latency on Day 22nd	Escape Latency on Day 23rd	Escape Latency on Day 24th
Control	31.33±3.44	26.83±2.31	22.33±2.25
Aβ	76.5±4.68	76.3±3.89	76.2±3.07
DPL	39±2.82**	33.83±2.63**	29.5±2.07**
Test-1	46.17±2.40**	41.33±1.86**	36.2±1.47**
Test-2	48.33±1.96**	44±1.67**	38.83±2.13**

Values are presented as mean ± SD by one way Analysis of Variance (ANOVA) followed by Dunnett-compare all vs. toxic, n=6, **p<0.01.
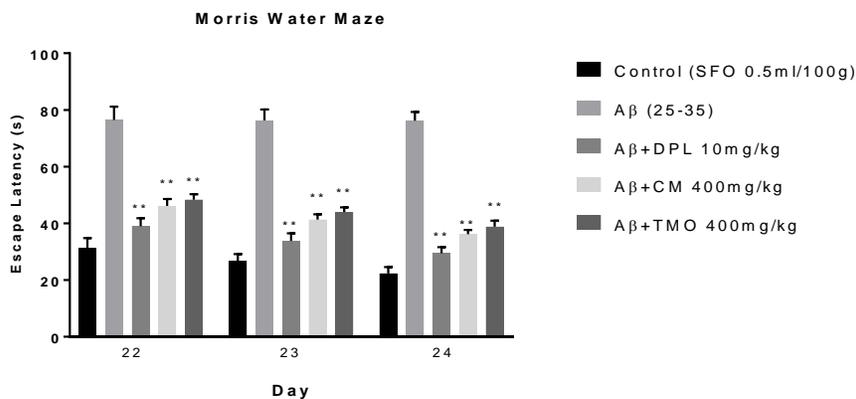
Fig. 2: Comparison of reduction in escape latencies in Morris Water Maze by different treatment groups. (Control, Aβ, DPL, CM and TMO)

In MWM task, the reduction in escape latency of CM and TMO treated group was 21.59% and 19.65% respectively over a period of 3 days. DPL treated group showed 24.36% reduction in escape latency over a period of 3 days. The Aβ group produced only 0.39% reduction in escape latency, while the control group made 28.72% reduction over the same period.

3.3 Novel Object Recognition Task

Table 3: Effect of Donepezil, Curcumin and Turmeric oil in recognition of novel object by Aβ (25-35) peptide injected rats (NORT).

Group	% time spent on the Novel object (%)
Control	75.53±7.21
Aβ	53.33±7.60
Standard	72.77±8.27**
Test-1	67.49±6.98*
Test-2	57.21±6.47

Values are presented as mean ± SD by one way Analysis of Variance (ANOVA) followed by Dunnett-compare all vs. toxic, n=6, *$p<0.05$, **$p<0.01$.
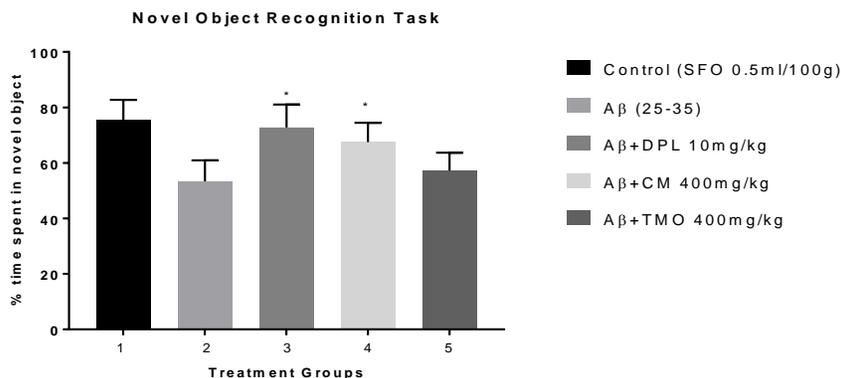

Fig. 3: % Time spent in the novel object by different treatment groups.(Control, Aβ, DPL, CM and TMO)

In NORT, the percentage time spent exploring the novel object by DPL, CM and TMO treated groups was 72.77, 67.49 and 57.21% respectively. The Aβ group spent only 53.33% time exploring the novel object. The control group spent 75.53% time in the novel object.

3.4 Acetyl cholinesterase inhibitory activity (Modified Ellman's method) [20,21]

Table 4: Acetyl cholinesterase inhibitory activity (% inhibition) of Donepezil, Curcumin and Turmeric oil

	DPL	CM	TMO
Percentage Inhibition at 25μg/ml	84.3± 0.25	14.63± 0.07	8.59± 0.25
Percentage Inhibition at 50μg/ml	89.5± 0.16	42.48± 0.33	28.63± 0.25
Percentage Inhibition at 100μg/ml	94.2± 0.15	58.26± 0.03	53.37± 0.18

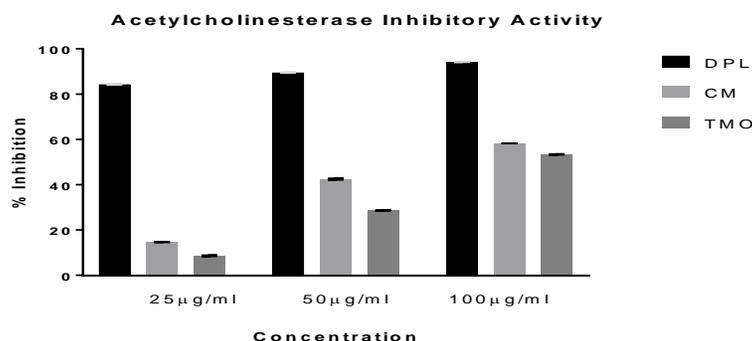Values are expressed as mean ± SD by one way ANOVA

Fig. 4: *In-vitro* **acetyl cholinesterase inhibitory activity of DPL, CM and TMO**

In the present study, DPL showed 94.2% inhibition at 100µg/ml concentration. The acetyl cholinesterase inhibitory activity of CM and TMO at the same dose was 58.26 and 53.37% respectively.

4. Discussion

AD is a progressive neuro-degenerative disorder and it is the most common cause of dementia [22]. An estimated 5.4 million Americans of all ages have AD in 2015. This number includes an estimated 5.2 million above the age of 65[22]. Though there has been extensive research going on in the field of AD, a promising cure is yet to be established [22,23].

This study was an attempt to explore the potential nootropic activity of TMO in comparison with CM. CM, the major bio-active compound in turmeric has a proven nootropic activity in many animal models of AD[24]. The neuro- protective and nootropic activity of the plant *Curcuma longa* Linn was focused on CM for many decades. Recently a less explored constituent (ar-Turmerone) of the plant has come to the limelight of researchers. AT has now proven to be effective in the regeneration of the neural stem cells [25]. Its anti-oxidant property is also established [9]. Turmeric oil, a secondary metabolite of turmeric, contains 50% of AT [6]. The anti-oxidant activity of TMO was proven to be due to the presence of AT [8]. TMO and CM were obtained as a gift samples from Akay Flavours and Aromatics Pvt. Ltd.

The study was conducted using **an Alzheimer's rat model induced by Aβ (25-35) peptide**. It was already proven that single I.C.V injection of Aβ (25-35) has potent amnesic properties in Wistar rats especially in short term memories like spatial working memory, spatial learning and recognition memory [11]. In the present study it was found that, **the I.C.V injection of Aβ (25-35) had resulted in an impairment of short term memory** compared to control group which received bi-distilled water instead of Aβ as I.C.V injection. This result was in accordance with the reports of Stepanichev *et al.*, (2003). The possible mechanisms of Aβ toxicity are

1) Neuro-degeneration in specific brain regions related to learning and memory processing.
2) Modulation of neurotransmission.
3) Direct or indirect interaction with molecular cascades linked with learning and memory.

In-vivo screening methods like, Morris Water Maze, Radial Arm Maze and Novel Object Recognition task were used for the evaluation of spatial learning, spatial working and recognition memory respectively[18].

Spatial memory is highly crucial and important parameter for assessing the cognitive capacity of an individual. The search for food, water and shelter are made possible by the spatial memory. So the assessment of these parameters is very critical in the study of drugs acting against cognitive impairment [26]. The present study finding revealed that test drugs were effective against the spatial memory impairment.

In day-to-day living **working memory** is a very important aspect of cognition. Working memory is a short term mental storage of pieces of critical information and processes them on and when required for performing complex cognitive tasks [27]. RAM is a true memory task in which rat is performing the task by checking off the arms using landmarks on a cognitive map. A landmark could be a chair, desk or the experimenter in the testing environment [27]. Thus RAM is an effective way to assess the spatial working memory of rodents. In the present study, an improvement in spatial working memory was evident in all the drug treated groups when compared to Aβ group (Table 1 & Fig. 1). TMO produced a significant reduction in number of wrong entries which was comparable to that of CM.

Spatial learning is a short term memory and a cognitive process allowing a person to remember different locations as well as spatial relation between the objects [27]. Spatial learning allows a person to navigate through a familiar location. MWM is an effective method to assess the spatial learning in rodents. Present study revealed that the maximum reduction in escape latency was exhibited by DPL compared to TMO and plane CM (Table 2 & Fig. 2).

All the drugs (DPL, TMO and CM) produced significant improvement in spatial learning compared to Aβ group. In RAM and MWM, CM treated group produced significant improvement in spatial working memory and spatial learning respectively. These results were in agreement with the reports of Oz *et al*[28] and Yanagisawa *et al*[33].

Recognition memory is the ability of an individual to recognize the previous events or objects. The novel environment is matched with the previously stored memory for the identification [29]. In the present study, NORT was performed to assess the recognition memory and it was found that, DPL and CM produced a significant increase in percentage time spent on the novel object compared to the Aβ group (Table 3 & Fig. 3). The recognition memory was improved significantly by DPL and CM. TMO could not alter the recognition memory when compared to the Aβ group.

Acetyl choline is the key Neurotransmitter in AD [30]. Damage to the cholinergic neurones (Acetyl choline producing) in the brain has been shown to be associated with memory defects in AD [29]. In brain, acetyl cholinesterase enzyme depletes the acetyl choline level by enzymatic cleavage. The drugs inhibiting the acetyl cholinesterase enzyme activity has been in the market as one of the main treatment options available for AD like Tacrin and DPL. In the present study, all the drugs possessed acetyl cholinesterase inhibitory activity. Maximum inhibitory activity was shown by DPL followed by CM and TMO (Table 4 & Fig. 4).

DPL was found more effective compared to CM and TMO in all the *in-vivo* and *in-vitro* screening. The effect of DPL in spatial memory and acetyl cholinesterase inhibition was in agreement with the reports published by Xing Yu *et al*[31] and Biswas *et al*[32] respectively. The high activity of DPL may be due to its potent acetyl cholinesterase inhibitory activity. Though TMO produced an acetyl cholinesterase inhibitory activity much lesser than that of DPL, its effect on spatial working memory, spatial learning was significant and comparable to that of CM. This could be an indication of mechanism of action of TMO apart from sheer acetyl cholinesterase inhibition for its nootropic activity. The major component of TMO which is AT has an established NSC proliferative action along with suggested inhibition of microglial activation [25], which may contribute to the protective effects of TMO. CM, an established nootropic and neuro-protective agent exerts its action through different mechanisms. CM inhibits Aβ aggregation inside the brain and produces lower molecular size Aβ that have weaker cell toxicity [33]. CM also possesses moderate acetyl cholinesterase inhibitory activity along with anti-oxidant, inhibition of microglial activation and anti-neuro inflammatory activity [34,35]. In the present

study, TMO produced a significant protective effect against the spatial memory impairment but the recognition memory was not much altered.

Further confirmatory studies on the cognitive enhancement activity of TMO could be relevant using different AD models to establish its significant role in AD and its mechanism of action.

5. Conclusion

It has been concluded that Turmeric oil has a significant cognitive enhancing capability in Aβ induced spatial and recognition memory impairment.

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