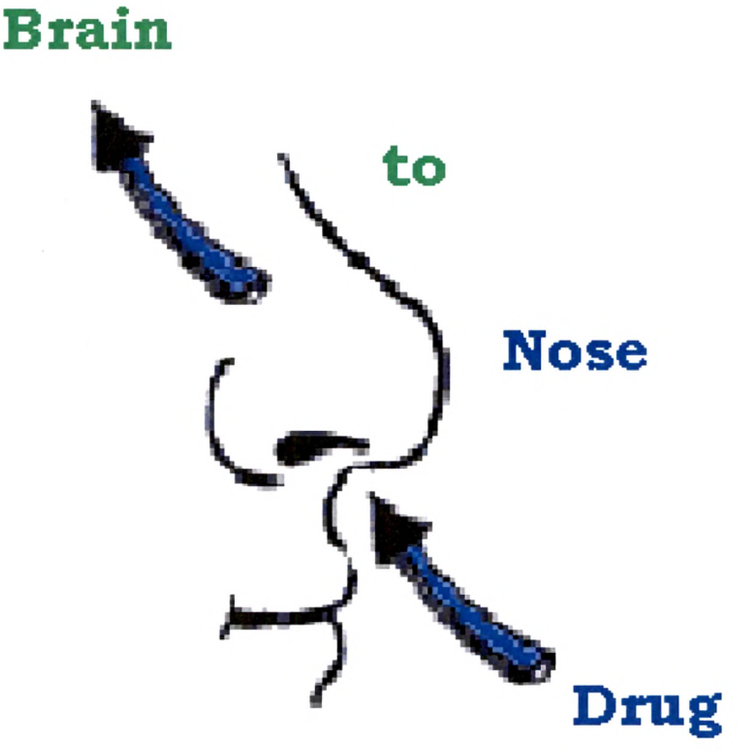


CHAPTER 8

PHARMACODYNAMIC STUDIES

8.1 Tacrine

8.1.1 Morris Water Maze Test:

All experiments conducted on animals were approved by the Committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. Balb/c mice (aged 4 to 5 months), weighing between 30 to 40 g were selected for the study on the basis of randomization technique.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by irreversible, progressive loss of memory followed by complete dementia (Scarpini et al. 2003). Cognitive impairment in AD is caused mainly by death of cholinergic neurons in basal forebrain area, though other neurotransmitter systems could well be involved. A deficit of acetylcholine in an AD brain is well known. Cholinesterase inhibitors have been reported to be effective in the treatment of AD. Scopolamine, a nonselective muscarinic receptor antagonist, has been used as a pharmacological tool to evaluate the effects nootropic drugs on memory deficits in experimental animals (Kim et al. 1999; Kim et al. 2003). Numerous studies show positive effects of acetylcholinesterase (AChE) inhibitors and muscarinic agonists on behavioral deficits in animals induced by the muscarinic receptor antagonist scopolamine (Bartus 1978; Murray et al. 1991; Rupniak et al. 1997; Ebert et al. 1998). For example, the AChE inhibitor tacrine (Cognex) and the muscarinic agonist milameline (CI-979/RU 35 926) reverse a scopolamine- induced impairment of radial arm maze performance in rats (M'Harzi et al. 1995; Callahan 1999).

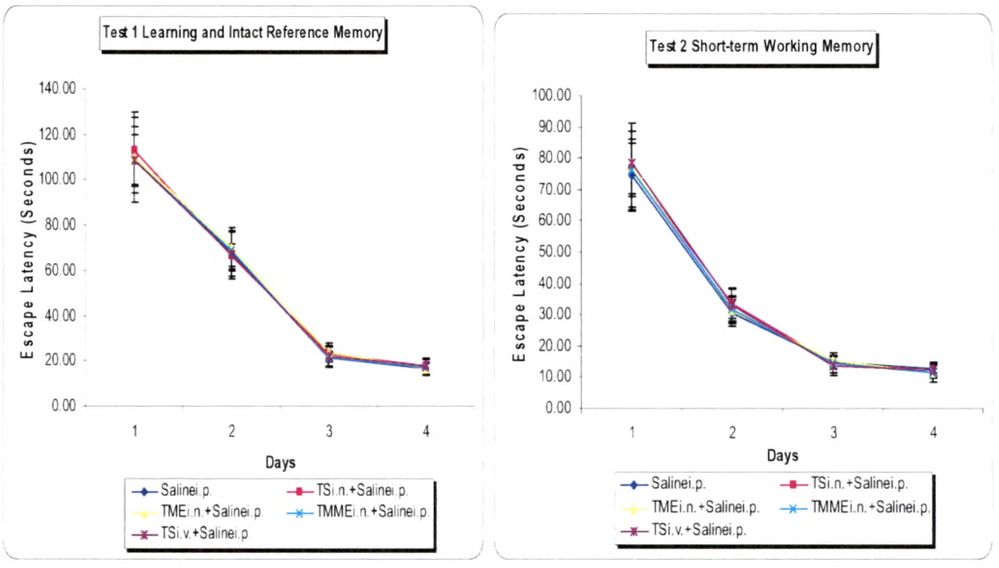
To evaluate the influence of developed tacrine formulations on learning and memory capacities, Morris water maze test was performed in scopolamine induced amnesia model in mice (Morris 1984; Lee et al. 2006). Morris water maze is a circular pool (90 cm in diameter and 45 cm in height) with a featureless inner surface. The pool was filled to a height of 25 cm with water. The pool was divided into four quadrants of equal area and a clear plexiglass column platform (6 cm diameter) was centered in one of the four quadrants of the pool and submerged just below the water surface. The mice could escape from water onto the platform and the time (in seconds) taken by the mouse was measured as escape latency. Mice were evaluated once daily for 2 water maze tests for 4 consecutive days. In the 1st test, mice were placed on the platform for 15 seconds and then

placed in the water. Escape latency (indicative of Learning and Intact Reference Memory) was measured. After 15 seconds on the platform the animals were placed back in the water (in previous position) and allowed to search for the platform (retained in previous position). Escape latency (indicative of Short-term Working Memory i.e. 2^{nd} test) was recorded. The platform location and the animal's starting point were held constant within two daily tests, but the location of the platform and the animal's starting point were changed every day. Amnesia was induced by intraperitoneal (i.p.) injection of scopolamine hydrochloride (0.4mg/kg of body weight (B.W.)) in 0.9% saline 30 min prior to testing.

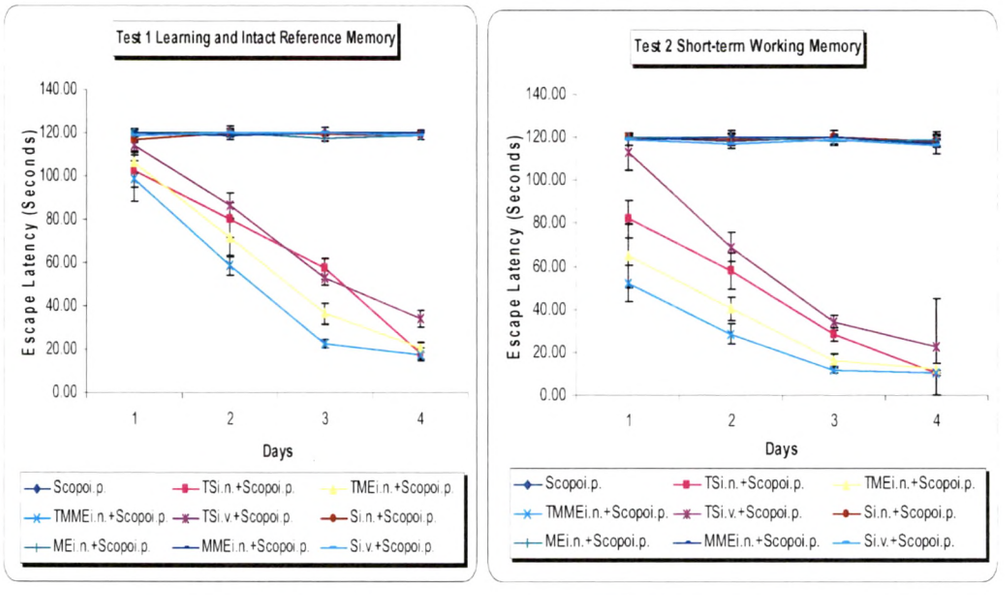
Mice were divided in 2 groups: Saline-treated (i.p.) and Scopolamine treated (i.p. 0.4 mg/kg). In both groups, animals (n=4) were treated with different i.n. and i.v. formulations of tacrine (i.e. $TS_{i.n.}$, $TME_{i.n.}$, $TMME_{i.n.}$, and $TS_{i.v.}$ – 1.3 mg/kg) 1 h prior to testing (i.e. 30 min prior to Scopolamine treatment) and evaluated once daily for 2 water maze tests for 4 consecutive days as described above. The TS (50 µL) containing 0.039–0.052 mg tacrine (equivalent to 1.3 mg/ kg B.W.) was injected intravenously (i.v.) through tail vein of mice. Similarly, TS/TME/TMME (10 µL) containing 0.039–0.052 mg tacrine (equivalent to 1.3 mg/kg B.W.) was administered (5 µL) in each nostril. Prior to nasal administration of the formulations, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of micropipette (10 µL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site. The mice were held from the back in slanted position during nasal administration of the formulations. Scopolamine-treated mice were also administered i.v. and i.n., the placebo formulations to check the influence of formulation components on scopolamine induced amnesia. The results obtained are recorded in Figure 8.1.

8.1.2 Statistical Analysis:

All data are reported as mean ± SD and the difference between the groups were tested using Student's t test at the level of $P< 0.05$. More than two groups were compared using ANOVA and differences greater at $P< 0.05$ were considered significant.



(A) Saline-treated Mice



(B) Scopolamine-treated Mice

Figure 8.1 Effects of different tacrine formulations after i.v. and i.n. administration on the escape latency achieved during the Morris water maze test in mice (n=4). (A) Saline-treated Mice and (B) Scopolamine-treated Mice. Error bars represent SD (n=4).

8.2 Donepezil

8.2.1 Morris Water Maze Test:

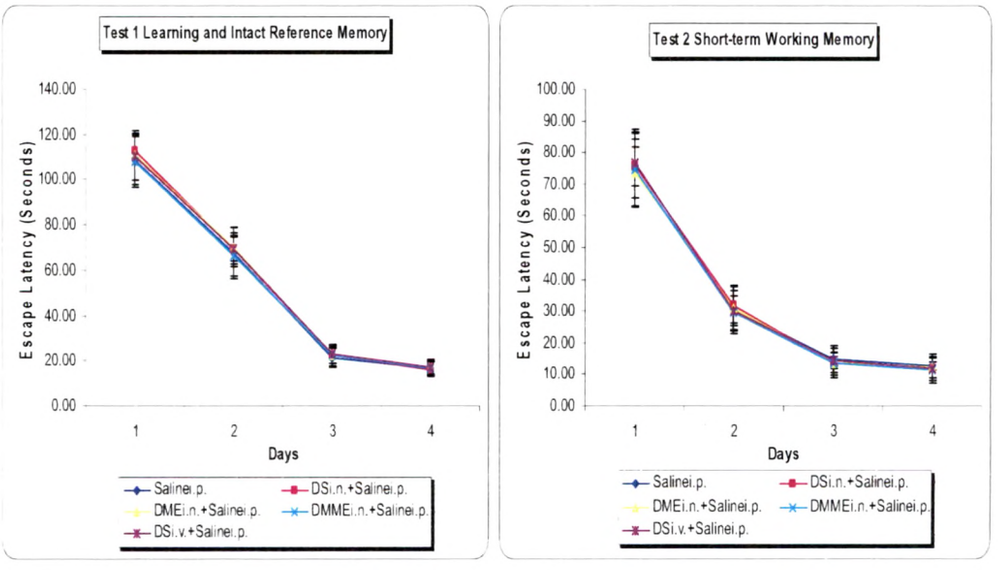
All experiments conducted on animals were approved by the Committee for the purpose of control and supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India, New Delhi, India. Balb/c mice (aged 4 to 5 months), weighing between 30 to 40 g were selected for the study on the basis of randomization technique.

To evaluate the influence of developed donepezil formulation on learning and memory capacities, Morris water maze test was performed in scopolamine induced amnesia model in mice (Morris 1984; Lee et al. 2006) as described in section 8.1.1.

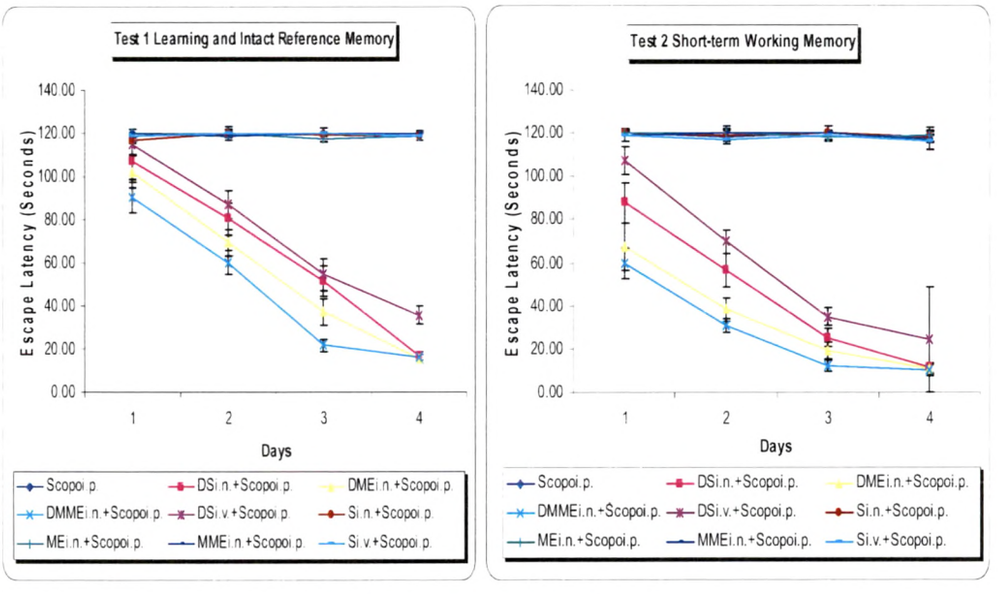
Mice were divided in 2 groups: Saline-treated (i.p.) and Scopolamine treated (i.p. 0.4 mg/kg). In both groups, animals (n=4) were treated with different i.v. and i.n. formulations of donepezil (i.e. $DS_{i.v.}$, $DS_{i.n.}$, $DME_{i.n.}$, and $DMME_{i.n.}$ – 0.65 mg/kg) 1 h prior to testing (i.e. 30 min prior to Scopolamine treatment) and evaluated once daily for 2 water maze tests for 4 consecutive days as described above. The DS (50 µL) containing 0.0233–0.0467 mg donepezil (equivalent to 0.65 mg/ kg B.W.) was injected intravenously (i.v.) through tail vein of mice. Similarly, DS/DME/DMME (10 µL) containing 0.0233–0.0467 mg donepezil (equivalent to 0.65 mg/ kg B.W.) was administered (5 µL) in each nostril. Prior to nasal administration of the formulations, the mice were partially anaesthetized by diethyl ether and the formulations were instilled into the nostrils with the help of micropipette (10 µL) attached with low density polyethylene tube having 0.1 mm internal diameter at the delivery site. The mice were held from the back in slanted position during nasal administration of the formulations. Scopolamine-treated mice were also administered i.v. and i.n., the placebo formulations to check the influence of formulation components on scopolamine induced amnesia. The results obtained are recorded in Figure 8.2.

8.2.2 Statistical Analysis:

All data are reported as mean ± SD and the difference between the groups were tested using Student's 't' test at the level of $P< 0.05$. More than two groups were compared using ANOVA and differences greater at $P< 0.05$ were considered significant.



(A) Saline-treated Mice



(B) Scopolamine-treated Mice

Figure 8.2 Effects of different donepezil formulations after i.v. and i.n. administration on the escape latency achieved during the Morris water maze test in mice (n=4). (A) Saline-treated Mice and (B) Scopolamine-treated Mice. Error bars represent SD (n=4).

8.3 Results and Discussion

8.3.1 Tacrine:

The effects of tacrine formulations after i.n. and i.v. administration on the escape latency achieved in the Morris water maze test in saline- and scopolamine-treated mice were shown in Figure 8.1. Saline-treated mice rapidly learned the location of the platform as indicated by a gradual decrease in escape latency. Minimum escape latency was achieved on day 3 in both of the tests and thereafter there was no significant decrease in escape latency observed (Figure 8.1 (A)). While in scopolamine-treated mice, a characteristic swimming behavior consisting of circling around the pool was observed and the latency period in learning and intact reference memory (test 1) and short-term working memory (test 2) remained unchanged throughout 4 days of testing period (Figure 8.1 (B)). Intranasal and i.v. administration of different tacrine formulations in saline-treated mice (Figure 8.1 (A)) and i.n./ i.v. administration of placebo formulations in scopolamine-treated mice did not result into any noticeable improvement in learning and memory capacities (Figure 8.1 (B)). While, i.n. and i.v. administration of tacrine formulations in scopolamine-treated mice antagonize scopolamine induced amnesia as evidenced by significant decrease in escape latency in both of the tests (Figure 8.1 (B)). These results indicated an increase in learning and memory capacities associated with tacrine. Following i.n. administration of TMME, mice learned to reach the platform within 3 days and exhibited behavioral pattern identical to saline-treated control mice. While, following $TS_{i.n.}$ and $TME_{i.n.}$, similar behavior was observed at the end of 4 days. In case of $TS_{i.v.}$, a noticeable decrease in the escape latency and improvement in learning and memory capacities were observed. But, it was slow compared to i.n. administrations and mice did not learn to reach the platform by end of 4 days. Thus, the results suggest fastest memory regain in scopolamine induced amnesic mice following intranasal administration of TMME and it further supports the findings of biodistribution studies.

8.3.2 Donepezil:

The effects of donepezil formulations after i.n. and i.v. administration on the escape latency achieved in the Morris water maze test in saline- and scopolamine-treated mice were shown in Figure 8.2. Saline-treated mice rapidly learned the location of the platform as indicated by a gradual decrease in escape latency. Minimum escape latency was

achieved on day 3 in both of the tests and thereafter there was no significant decrease in escape latency observed (Figure 8.2 (A)). While in scopolamine-treated mice, a characteristic swimming behavior consisting of circling around the pool was observed and the latency period in learning and intact reference memory (test 1) and short-term working memory (test 2) remained unchanged throughout 4 days of testing period (Figure 8.2 (B)). Intranasal and i.v. administration of different donepezil formulations in saline-treated mice (Figure 8.2 (A)) and i.n./ i.v. administration of placebo formulations in scopolamine-treated mice did not result into any noticeable improvement in learning and memory capacities (Figure 8.2 (B)). While, i.n. and i.v. administration of donepezil formulations in scopolamine-treated mice antagonize scopolamine induced amnesia as evidenced by significant decrease in escape latency in both of the tests (Figure 8.2 (B)). These results indicated an increase in learning and memory capacities associated with donepezil. Following i.n. administration of DMME, mice learned to reach the platform within 3 days and exhibited behavioral pattern identical to saline-treated control mice. While, following $DS_{i.n.}$ and $DME_{i.n.}$, similar behavior was observed at the end of 4 days. In case of $DS_{i.v.}$, a noticeable decrease in the escape latency and improvement in learning and memory capacities were observed. But, it was slow compared to i.n. administrations and mice did not learn to reach the platform by end of 4 days. Thus, the results suggest fastest memory regain in scopolamine induced amnesic mice following intranasal administration of DMME and it further supports the findings of biodistribution studies.

8.4 References

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