ผลของสารสกัดขมิ้นชันต่อความบกพร่องในการเรียนรู้และความจำที่ถูกเหนี่ยวนำโดยภาวะสมองขาดเลือดหรือสารสะโคโพลามีนในหนูถีบจักร

นางสาวรวิกานต์ นักขัตร

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EFFECTS OF TURMERIC EXTRACT (CURCUMA LONGA) ON IMPAIRMENT OF LEARNING AND MEMORY INDUCED BY EITHER CEREBRAL ISCHEMIA OR SCOPOLAMINE IN MICE

Miss Ravigarn Nagkhat

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Field of study	Pharmacology
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งานวิจัยนี้เป็นการศึกษาผลของสารสกัดขมิ้นชันต่อความบกพร่องในการเรียนรู้และความจำในหนูถีบจักรที่ถูกเหนี่ยวนำจากการทำให้สมองอยู่ในภาวะขาดเลือดโดยการผูกกั้นหลอดเลือดคอมมอนคาโรติดทั้งสองข้าง หรือการได้รับสารสะโคโพลามีน พบว่าหนูที่อยู่ในภาวะสมองขาดเลือดใช้เวลาในการหาแท่นพักนานขึ้นเมื่อทดสอบด้วยวิธีมอริสวอเตอร์เมส นอกจากนี้เมื่อทดสอบด้วยวิธีสะเต็บดาวพบว่า หนูใช้เวลาอยู่บนแท่นพักลดลงและจำนวนครั้งที่ก้าวลงจากแท่นพักเพิ่มขึ้น แสดงว่าหนูในกลุ่มนี้เกิดความบกพร่องในการเรียนรู้และความจำ แต่เมื่อให้สารสกัดขมิ้นชันทางปากในขนาด 100, 300 และ 1000 มิลลิกรัมต่อกิโลกรัมต่อวัน มีผลทำให้หนูใช้เวลาในการหาแท่นพักลดลงเมื่อทดสอบด้วยวิธีมอริสวอเตอร์เมส และจากการทดสอบด้วยวิธีสะเต็บดาวพบว่า หนูที่ได้รับสารสกัดขมิ้นชันในขนาด 300 และ 1000 มิลลิกรัมต่อกิโลกรัมต่อวัน ใช้เวลาอยู่บนแท่นพักนานขึ้น และจำนวนครั้งที่ก้าวลงจากแท่นพักลดลงเมื่อเทียบกับหนูในกลุ่มควบคุม แสดงว่าสารสกัดขมิ้นชันสามารถแก้ไขความบกพร่องในการเรียนรู้และความจำที่เกิดจากภาวะสมองขาดเลือดได้ และพบว่าสารสกัดขมิ้นชันทุกขนาดไม่มีผลต่ออัตราการเคลื่อนไหวของหนูที่อยู่ในภาวะสมองขาดเลือดแต่อย่างใด นอกจากนี้ยังพบว่าสารทดสอบสามารถลดระดับเอ็มดีเอที่เพิ่มขึ้นในสมองหนูที่อยู่ในภาวะสมองขาดเลือดได้

นอกจากนั้นสารทดสอบสามารถแก้ไขความบกพร่องในการเรียนรู้และความจำจากการได้รับสารสะโคโพลามีนได้เช่นเดียวกับการทำให้สมองอยู่ในภาวะขาดเลือดโดยการผูกกั้นหลอดเลือดคอมมอนคาโรติดทั้งสองข้างเมื่อทดสอบในวิธีมอริสวอเตอร์เมสและวิธีสะเต็บดาว และพบว่าอัตราการเคลื่อนไหวของหนูลดลงเมื่อได้รับสารสะโคโพลามีน แต่ไม่พบการเปลี่ยนแปลงใดๆ เมื่อได้รับสารสกัดขมิ้นชันทุกขนาด จากผลการทดสอบต่างๆ แสดงให้เห็นว่าสารทดสอบมีผลต่อระบบโคลิเนอร์จิก ในระบบประสาทส่วนกลาง โดยที่สารสะโคโพลามีนไม่มีผลต่อระดับเอ็มดีเอ

อาจกล่าวได้ว่าสารสกัดขมิ้นชัน สามารถแก้ไขความบกพร่องในการเรียนรู้และความจำจากคุณสมบัติต้านออกซิเดชั่น และน่าจะมีการออกฤทธิ์ต่อระบบโคลิเนอร์จิกในระบบประสาทส่วนกลางด้วย

สาขาวิชาเภสัชวิทยา (สหสาขาวิชา) ลายมือชื่อนิสิต รวิกานต์ นักขัตร

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ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

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KEY WORD: CURCUMA LONGA (Linn.)/ LEARNING/ MEMORY/ CEREBRAL ISCHEMIA/ MORRIS WATER MAZE/ STEP DOWN TEST/ LOCOMOTOR ACTIVITY TEST/ OXIDATIVE STRESS

RAVIGARN NAGKHAT: EFFECTS OF TURMERIC EXTRACT (CURCUMA LONGA) ON IMPAIRMENT OF LEARNING AND MEMORY INDUCED BY EITHER CEREBRAL ISCHEMIA OR SCOPOLAMINE IN MICE. THESIS ADVISOR: ASSOC. PROF. MAYUREE TANTISIRA, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. BOONYONG TANTISIRA, Ph.D., 73 pages. ISBN: 974-14-2286-5

The effects of turmeric extract (*Curcuma longa*) on learning and memory impairment induced by transient cerebral ischemia (bilateral occlusion of common carotid arteries, 2VO) was investigated in mice. The 2VO caused an impairment of learning and memory seen as an increase in the latency to find the platform in Morris Water Maze (MWM) test as well as a reduction of step-down latency and an increment of number of errors in step-down test. Administration of turmeric extract (p.o.) at doses of 100, 300 and 1000 mg/kg/day significantly reduced the latency to find the platform of 2VO mice in MWM test. Furthermore, in passive avoidance task, turmeric extract at doses of 300 and 1000 mg/kg/day were found to increase the step-down latency and decrease the number of error of 2VO mice. Similar results were observed in learning and memory deficit induced by scopolamine. In addition, it was found that injection of scopolamine but not 2VO significantly depressed locomotor activity and none of the observed effects was affected by the administration of turmeric extract. Based on the results that turmeric extract could antagonize amnesic effect of scopolamine, it is suggestive that turmeric extract might exert its memory enhancing effect through a correction of cholinergic deficit. Moreover, turmeric extract could suppress 2VO-induced increasing of brain malondialdehyde. Thus it is likely that memory enhancing effect of turmeric extract might be accounted by its antioxidative effect.

In conclusion, the present study has demonstrated the beneficial effects of turmeric extract for learning and memory deficit induced by cerebral ischemia and scopolamine. It is possible that antioxidant property of turmeric extract and its effect on cholinergic system could, at least partly, contribute to its positive effect on memory deficit in 2VO and scopolamine model. Thus, turmeric extract might be beneficial for memory impairment in Alzheimer's disease which oxidative stress and cholinergic system are underlying cause.

Field of Study Pharmacology (Inter-Department) Student's Signature Ravigarn Nagkhat

Academic Year 2005 Advisor's Signature Mayuree Tantisira

Co-Advisor's Signature Boonyong Tantisira

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LIST OF ABBREVIATIONS

AchE	Acetylcholinesterase
AD	Alzheimer's disease
anti-ChE	anticholinesterase
B.W.	body weight
°C	degree celsius
CBF	cerebral blood flow
CNS	Central Nervous System
EAE	experimental allergic encephalomyelitis
et al.	et alii, and others
g	gram (s)
MAO	monoamine oxidase
MDA	malondialdehyde
mg/kg	milligram (s) per kilogram
ml	milliliter (s)
mm	millimeter (s)
MS	multiple sclerosis
MWM	Morris Water Maze
NO	Nitric oxide
TBARS	thiobarbituric acid-reacting substances
TCM	traditional Chinese medicine
2VO	two vessel occlusion

CHAPTER I

INTRODUCTION

1.1 Background and rationale

Herbs are natural resources that are overlooked for a long time when sciences have an important role. Many people think that herbs are out-of-date and they hardly know about their advantages.

At present, herbs are widely accepted. People are more concerned about their health and they believe that herbs are safe therefore they are interested in using natural products. Many researchers try to develop more effective medicines with decreasing side effects from herbs.

In traditional practices of Ayurvedic and Chinese medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as Alzheimer's disease (AD). Many drugs currently available in Western medicine were originally isolated from plants, or are derived from templates of compounds isolated from plants. Some anticholinesterase (anti-ChE) alkaloids isolated from plants have been investigated for their potential in the treatment of AD, and are now in clinical use. Galantamine, isolated from several plants including *Lycoris radiata* Herb., which was used in traditional Chinese medicine (TCM), is licensed in the United Kingdom for the treatment of mild to moderate AD. Various other plant species have shown pharmacological activities relevant to the treatment of cognitive disorders, indicating potential for therapeutic use in disorders such as AD (Howes and Houghton, 2003).

One ancient Ayurvedic remedy is *Centella asiatica* (Umbelliferae), which is reputed to restore youth, memory and longevity. The essential oil (0.1% of the plant) extracted from *C.*

asiatica leaf contains monoterpenes, including bornyl acetate, α-pinene, β-pinene and γ-terpinene which are reported to inhibit AchE. The pharmacological basis to explain the reputed antiamnesic effects of *C. asiatica* has been explored experimentally. Studies have shown an alcoholic extract to be tranquillising in rats, an activity that was attributed to a triterpene, brahmoside. Further studies showed the extract of *C. asiatica* leaf to be sedative, antidepressant and potentially cholinomimetic *in vivo*. These findings suggest that *C. asiatica* may be appropriate to treat symptoms of depression and anxiety in AD, and that it may also influence cholinergic activity, and thus cognitive function. Cognitive-enhancing effects have been observed in rats following oral administration of an aqueous extract of *C. asiatica*; this effect was associated with an antioxidant mechanism in the CNS. An aqueous extract of *C. asiatica* leaf modulated dopamine, 5-HT and noradrenaline systems in rat brain and improved learning and memory processes *in vivo*. The triterpene asiatic acid (found in *C. asiatica*) and its derivatives have been shown to protect cortical neurons from glutamate-induced excitotoxicity *in vitro* (Howes and Houghton, 2003).

Ginkgo biloba L. has also been used traditionally in Iran to improve memory loss associated with blood circulation abnormalities. Numerous investigations have been conducted regarding the potential of *G. biloba* in cognitive disorders. The *G. biloba* extract Egb 761 has shown biological activities relevant to the treatment of cognitive dysfunction. There is some evidence (electroencephalographic data) to suggest that *G. biloba* extract Egb 761 has a local effect in the CNS; in addition, this extract has shown various biological activities relevant to the treatment of cognitive dysfunction. Favourable effects have been observed on cerebral circulation and neuronal cell metabolism, on the muscarinic cholinergic system, and the extract showed antioxidant activity. Egb 761 was also neuroprotective against β-amyloid and nitric oxide (NO)-induced toxicity *in vitro*, and could reduce apoptosis both *in vitro* and *in vivo*. *G. biloba* extracts have also been evaluated for their influence on cognitive function. Treatment with *G. biloba* extracts attenuated scopolamine-induced amnesia in rats, enhanced memory retention in both young and old rats and improved short-term memory in mice. With reference to the numerous studies

conducted, it is apparent that *G. biloba* may be useful in the treatment of AD symptoms, but further research is necessary to identify appropriate dosing regimens, potential effects of long-term use, interactions with other medicines, and standardization of extracts must also be a consideration (Howes and Houghton, 2003).

Regarded as a “rasayana” herb in Ayurveda (to counteract aging processes), *Curcuma longa*, known in English as “turmeric”, has also been used for culinary purposes. Turmeric is widely consumed in the countries of its origin for a variety of uses, including as a dietary spice, a dietary pigment, and an Indian folk medicine for the treatment of various illnesses. Much research has focused on curcumin, a curcuminoid from *C. longa* rhizomes. In particular, studies have shown that some curcuminoids are associated with antioxidant and anti-inflammatory activities. These two activities may be useful to improve memory impairment but studies with particular attention to cognitive disorders are lacking. Therefore, this study attends to study the effects of *Curcuma longa* on learning and memory impairment.

1.2 Research question

1. Can turmeric extract (*Curcuma longa*) improve learning and memory impairment induced by either cerebral ischemia or scopolamine in mice?
2. Can turmeric extract (*Curcuma longa*) attenuate the oxidative stress induced by cerebral ischemia in mice?

1.3 Hypothesis

1. Turmeric extract (*Curcuma longa*) may attenuate learning and memory impairment induced by either cerebral ischemia or scopolamine in mice.
2. Turmeric extract (*Curcuma longa*) may attenuate the oxidative stress induced by cerebral ischemia in mice.

1.4 Objective

1. To study the effect of turmeric extract (*Curcuma longa*) on learning and memory impairment induced by either cerebral ischemia or scopolamine in mice.

2. To study the effect of turmeric extract (*Curcuma longa*) on the level of oxidative stress induced by cerebral ischemia in mice.

CHAPTER II

LITERATURE REVIEWS

2.1 *Curcuma longa* Linn.

Curcuma longa Linn. is a member of ginger family, Zingiberaceae. Apart from this name, which is usually employed in scientific work, the synonym *Curcuma domestica* Val. is the designation most commonly found. The common names of this plant are Turmeric, Khamin (general), Khamin kaeng, Khamin yok, Khamin hua (Chiangmai), Khamin chan (Central, peninsular), Kheemin, Min (Peninsular), Taa-yo (Karen-Kamphaeng Phet), Sa-yo (Karen-Mae Hong Son) (Farnsworth and Bunyapraphatsara, 1992). It is a tropical plant native to South and Southeast Asia. It is a perennial herb; with a height of 3 to 5 feet. It has oblong, pointed leaves and bears funnel-shaped yellow flowers (Figure 2.1). The rhizome, which is thick, is the portion of the plant used medicinally (Thorne Research, 2001). Turmeric is used extensively in foods for both its flavor and color. In Ayurveda (Indian traditional medicine), turmeric has been used for its medicinal properties for various indications and through different routes of administration (Chainani-Wu, 2003).

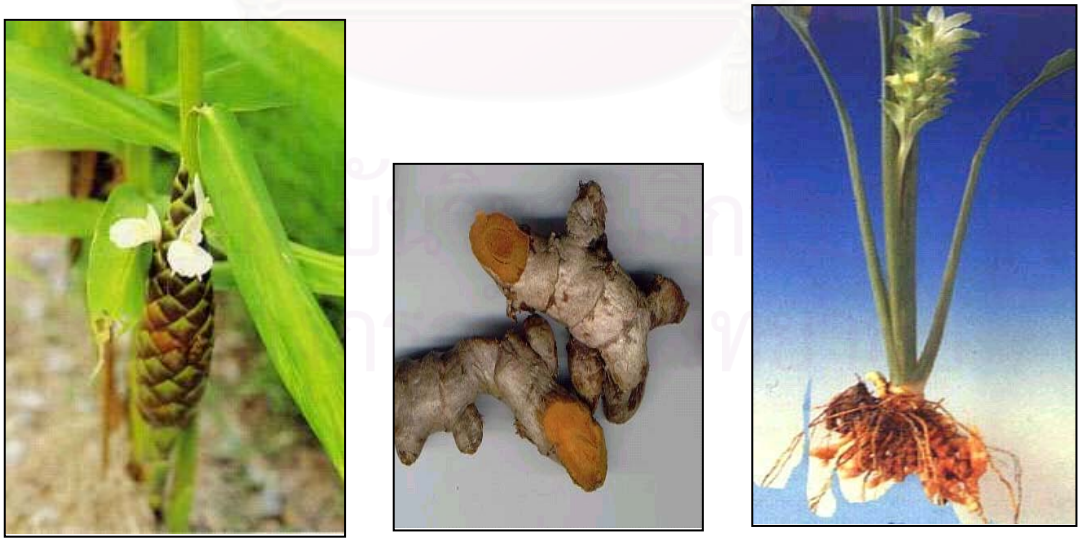


Figure 2.1 *Curcuma longa* Linn. (picture from www-ang.kfunigraz.ac.at)

2.1.1 Botany

Curcuma longa is a stemless rhizomatous herb; having rhizome fleshy, much branched, bright orange or yellow within and scented. Leaves emerge directly from the underground stem with overlapping petioles 8-15 cm long or more. They are light green, 30-40 by 8-10 cm, having thin ellipse-shaped or elongate lance-shaped blades. A cylindrical inflorescene about 10-15 by 5-7 cm, appears with the leaves and develops in their centre. It consists of large pale green, pouchlike, curved bracts, each with two or more pale yellow flowers except in the upper part, where the bracts are white and green or pink and without flowers. The tube-shaped calyx is splitted in one side to unequally teeth. The corolla-tube is more or less funnel-shaped, not exserted beyond the bract, with 3-lobed limb and white. The lateral staminode petaloid is rather long and folded under the dorsal petal. There is a central yellow band at the labellum. A fertile stamen with short filament, broad and constricted at the apex is found in the floret. The anther is versatile and usually spurred at the base; sometimes with a small crest at the connective. The ovary consists of 3-locules, each locule contains 2 ovules. The capsules are ellipsoid. Seeds are rare.

Curcuma longa is cultivated throughout the tropics. It grows very well in rather hot climates with high humidity at night. It grows well on well-drain loam; claywish or sandy soil are unsuitable (Farnsworth and Bunyapraphatsara, 1992).

2.1.2 Chemical constituents

The active constituents of turmeric are the curcuminoids and volatile oils including tumerone, atlantone, and zingiberone. The curcuminoids give turmeric its bright yellow color. The major curcuminoid is called curcumin (diferuloyl methane), which makes up approximately 90% of the curcuminoid content in turmeric, followed by demethoxycurcumin and bis-demethoxycurcumin (Figure 2.2) (Leung, 1980; Ruby *et al.*, 1995). Curcuminoids are derived from turmeric by ethanol-extraction. The pure orange-

yellow, crystalline powder is insoluble in water. The structure of curcumin ($C_{21}H_{20}O_6$) was first described in 1815 by Vogel and Pellatier and in 1910 shown to be diferuloylmethane by Lampe *et al*. Chemical synthesis in 1913 confirmed its identity (Lampe and Milobedeska, 1913).

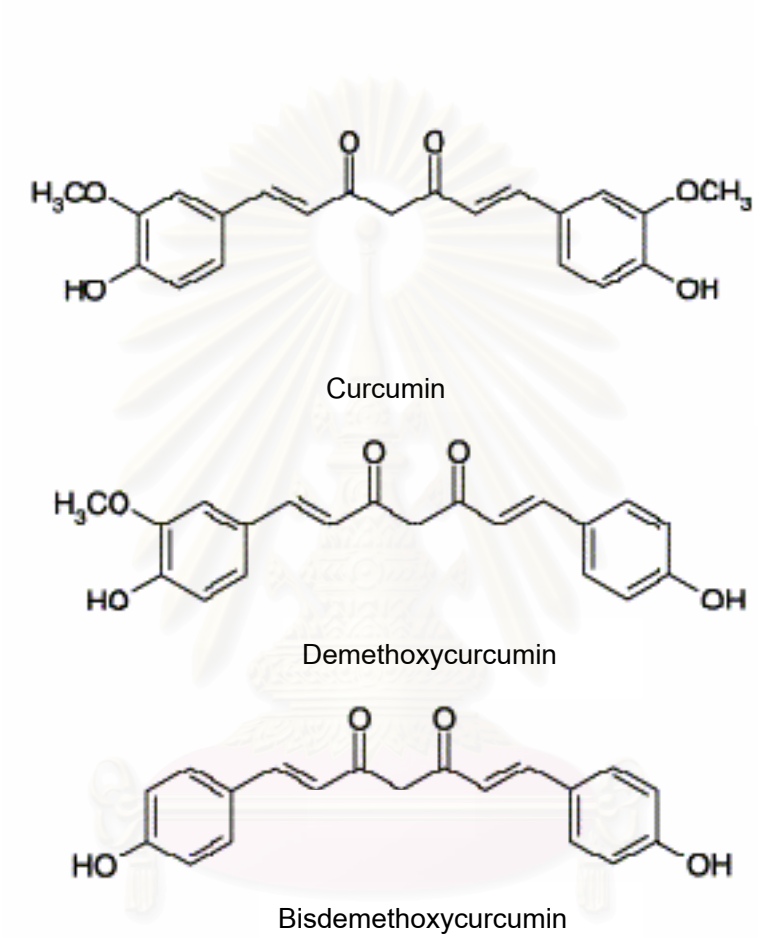


Figure 2.2 Structure of curcumin, demethoxycurcumin and bis-demethoxycurcumin (picture from Aggarwal *et al*., 2005).

2.1.3 Traditional applications

Turmeric is widely consumed in the countries of its origin for a variety of uses, including as a dietary spice, a dietary pigment, and an Indian folk medicine for the treatment of various illnesses. It is used in the textile and pharmaceutical industries (Srimal

and Dhawan, 1973) and in Hindu religious ceremonies in one form or another. Current traditional Indian medicine uses it for biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Jain and DeFilipps, 1991). The old Hindu texts have described it as an aromatic stimulant, and carminative (Nadkarni, 1954). Powder of turmeric mixed with slaked lime is a household remedy for the treatment of sprains and swelling caused by injury, applied locally over the affected area. In some parts of India, the powder is taken orally for the treatment of sore throat (Ammon and Wahl., 1991).

2.1.4 Pharmacological activities

There are several data in the literature indicating a great variety of pharmacological activities of *Curcuma longa* L., which exhibits activities against cancer, cardiovascular diseases, diabetes, Alzheimer's disease, multiple sclerosis (MS), cataract formation, HIV, and drug-induced nonspecific toxicity in the heart, lung, and kidney (Aggarwal *et al*., 2005).

These are the effects of *Curcuma longa* on the central nervous system. Curcumin was shown to be neuroprotective against ethanol-induced brain injury *in vivo* following oral administration; an effect that was related to a reduction in lipid peroxide levels and enhancement of glutathione in rat brain (Rajakrishnan *et al*., 1999). Some compounds from *Curcuma longa*, including curcumin, demethoxycurcumin, bisdemethoxycurcumin and calebin-A (and some of its synthetic analogues), were shown to protect PC12 cells from β-amyloid insult *in vitro* (Kim *et al*., 2001; Park and Kim, 2002); this activity was also suggested to be due to an antioxidant effect (Kim *et al*., 2001). An aqueous extract of *Curcuma longa* demonstrated antidepressant activity in mice following oral administration, which was associated with inhibition of brain monoamine oxidase A (MAO-A) (Yu *et al*., 2002). Curcumin inhibits experimental allergic encephalomyelitis (EAE) by blocking IL-12 signaling in T cells and suggest its use in the treatment of multiple sclerosis (MS) and other Th1 cell-mediated inflammatory diseases (Natarajan and Bright, 2002). Curcumin offered

significant neuroprotection in middle cerebral artery occlusion induced focal cerebral ischemia. The antioxidant property of curcumin may be the mechanism involved in neuronal protection in focal cerebral ischemia (Thiyagarajan and Sharma, 2004).

How curcumin produces its therapeutic effects is not fully understood, but they are probably mediated in part through the antioxidant and anti-inflammatory action of curcumin. Pharmacologically, curcumin is quite safe, and dose as high as 8 g/day have been administered orally to humans with no side effects. Numerous therapeutic activities as above, its pharmacological safety and its color qualifies curcumin as “Indian solid gold” (Aggarwal *et al*., 2005).

2.2 Memory

The brain is the organ that is responsible for what we call the mind. It is the basis for thinking, feeling, wanting, perceiving, learning and memory, curiosity, and behavior. Memory is a fundamental process, and without memory we are capable of nothing but simple reflexes and stereotyped behaviors. Thus, learning and memory is one of the most intensively studied subjects in the field of neuroscience.

Learning is defined as the process of acquiring knowledge about the world, and memory is defined as the retention or storage of that knowledge. According to these definitions, memory devides into 2 types, based on what kind of information is stored: declarative and procedural memory (Figure 2.3).

Declarative (Explicit) memory refers to the ability to remember names, faces, facts, events. Declarative memory depends on conscious reflection for its acquisition and recall, and it relies on cognitive process such as evaluation, comparison, and inference. Declarative memory is comprised of semantic and episodic components. Semantic memory includes verbal information as well as pictorial images. Episodic memory includes personal

and autobiographical memories, such as one's home address or activities yesterday. Semantic, visual and episodic memories are interrelated functionally and anatomically, but can demonstrate independence.

Procedural (Implicit or Non-declarative) memory refers to the ability to learn perceptual-motor tasks such as mirror reading. Procedural memory has an automatic or reflexive quality, and its information or readout is not independent on awareness, consciousness, or cognitive processes such as comparison and evaluation (Kupfermann, 1991).

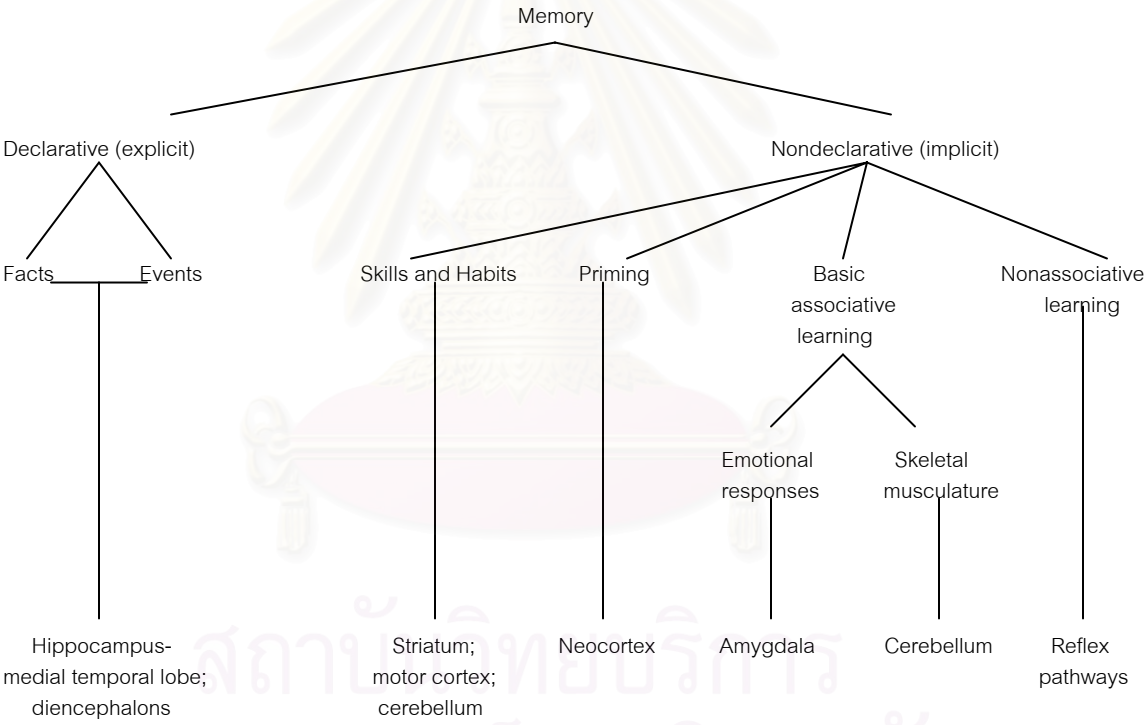


Figure 2.3 Types of memory (picture from Beggs *et al*., 1999).

2.2.1 Stages of memory

The process of memory formation is divided into 3 sequential steps: encoding, storage and retrieval. Encoding is the formation of mental memory traces; storage refers to the laying down of more permanent memory traces; and retrieval refers to the process of remembering.

Encoding can be further divided into the processes of holding and acquisition. Holding allows the temporary retention of information that exceeds the attention span. Acquisition is the process by which information is analyzed and organized. The encoding of memory often involves association with prior memories and cross-referencing among different sensory modalities and between language and emotional contexts.

Storing is the process by which transient memory traces are consolidated and reconstructed into stable or long-term memories. Memory traces are dynamic phenomena. They do not simply sit in a storage bin, but are linked with other memories and integrated with new experiences.

Retrieving allows selective scanning and activation of memories from short- or long-term memory stores. Failure to retrieve information leads to amnesia, in which the memories are intact but cannot be freely recalled. The simplest way to distinguish between amnesic disorders due to impaired retrieval and amnesic disorders due to impaired encoding and storage is to test both free recall and recognition. Patients with defective encoding or storage will fail both to recall or to recognize stimuli, while those with defective retrieval will fail to recall freely but will succeed in recognizing stimuli. An impairment of retrieval may underlie the memory disorders associated with basal ganglia and frontal lesions (Devinsky and Arnold, 1992).

2.2.2 The phase and timing of memory

A basic and generally accepted classification of memory is based on the duration of memory retention, and identifies three distinct types of memory: *sensory memory*, *short-term memory*, and *long-term memory* (Figure 2.4).

The *sensory memory* corresponds approximately to the initial moment that an item is perceived. Some of this information in the sensory area proceeds to the sensory store, which is referred to as *short-term memory*. Sensory memory is characterized by the duration of memory retention from milliseconds to seconds and short-term memory from seconds to minutes. These stores are generally characterised as of strictly limited capacity and duration, whereas in general stored information can be retrieved in a period of time which ranges from days to years; this type of memory is called *long-term memory*.

Additionally, the term *working memory* is used to refer to the short-term store needed for certain mental tasks - it is not a synonym for *short-term memory*, since it is defined not in terms of duration, but rather in terms of purpose. Some theories consider working memory to be the combination of short-term memory and some attentional control. For instance, when we are asked to mentally multiply 45 by 4, we have to perform a series of simple calculations (additions and multiplications) to arrive at the final answer. The ability to store the information regarding the instructions and intermediate results is what is referred to as working memory (Delis and Lucas, 1996).

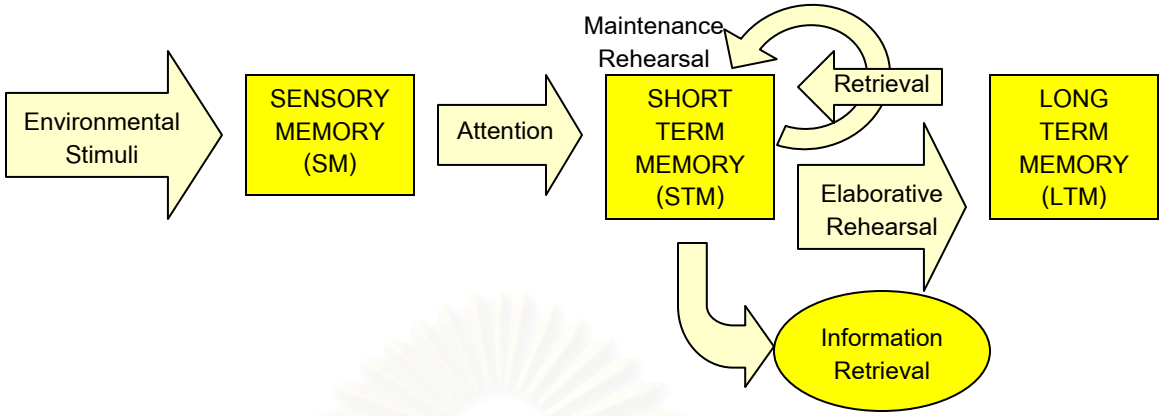


Figure 2.4 The memory processing (picture from www.dushkin.com).

2.2.3 Amnesia

Amnesia is a condition in which memory is disturbed. The causes of amnesia are organic or functional. Organic causes include damage to the brain, through trauma or disease, or use of certain (generally sedative) drugs. Functional causes are psychological factors, such as defense mechanisms. Hysterical post-traumatic amnesia is an example of this.

Amnesia can take 2 forms that are retrograde and anterograde. Retrograde amnesia refers to patients are unable to recall events occurring before the brain insult. Anterograde amnesia refers to new events are not transferred to long-term memory, so the sufferer will not be able to remember anything that occurs after the onset of this type of amnesia for more than a few moments. Both retrograde amnesia and anterograde amnesia can occur together in the same patient, and commonly result from damage to the brain regions most closely associated with episodic/declarative memory: the medial temporal lobes and especially the hippocampus (Devinsky and Arnold, 1992).

2.2.4 The brain mechanism of memory

Experiments on learning can be interpreted to say that explicit memory is first acquired through one or more of the three polymodal association areas of the cerebral cortex, namely prefrontal, limbic and parieto-occipital-temporal. Then, the information is transferred to parahippocampal and perirhinal cortices, entorhinal cortex, dentate gyrus, hippocampus, subiculum and back to entorhinal, parahippocampal and perirhinal cortex (Figure 2.5).

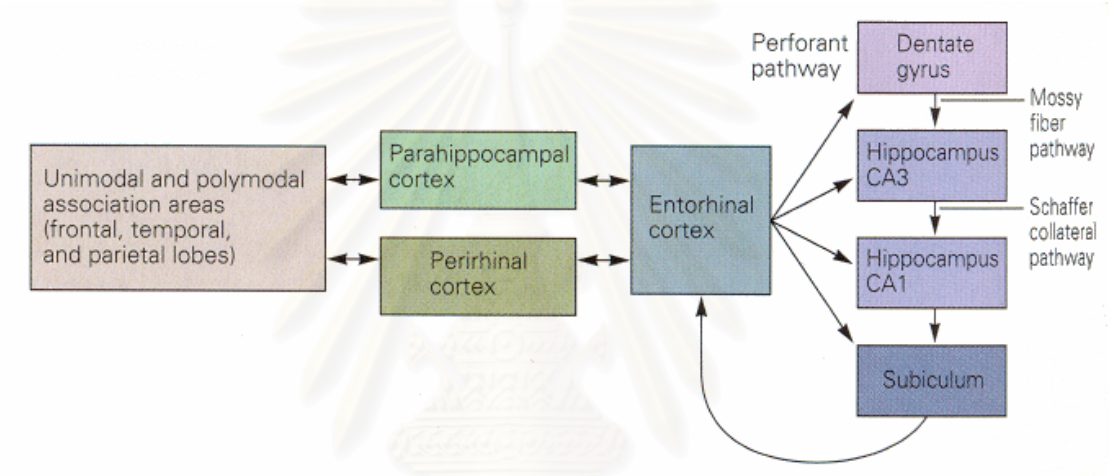


Figure 2.5 The input and output pathways of the hippocampal formation (picture from www.unmc.edu).

Different forms of learning are affected differentially by lesions in different locations. Damage to parahippocampal, perirhinal and entorhinal cortices produces greater deficits in memory storage for object recognition than does hippocampal damage. Right hippocampal damage produces greater deficits in memory for spatial representation, whereas left hippocampal damage produces greater deficits in memory for words, objects or people. In either case, the deficits are in formation of new, long-term memory; old memories are spared.

Implicit memories are stored differently depending upon how they are acquired. “Fear conditioning” (training that involves use of fearful stimuli) involves the amygdala. Operant conditioning involves the striatum and cerebellum. For example, eye blink conditioning is disrupted by lesions of the dentate and interpositus nuclei of the cerebellum. Classical conditioning, sensitization and habituation involve the sensory and motor systems involved in producing the motor responses being conditioned. Perhaps surprisingly, certain simple reflexes mediated by the spinal cord can be classically conditioned even after the cord has been surgically isolated from the brain. So, it appears that all regions of the nervous system may be capable of memory storage (Joseph, 1996).

2.3 Alzheimer’s disease

Alzheimer’s is the most common of a group of illnesses called dementias which affect the human brain. First described by Alois Alzheimer in 1907, it is a progressive disorder in which there is a slow but relentless destruction of nerve cells. This destruction does not occur uniformly, but affects certain areas such as the hippocampus and amygdale buried deep inside the brain, and parts of the outer (cortical) areas, thus leading to selective loss of mental function, especially memory (Roberts *et al*., 1993).

Alzheimer’s disease is estimated to affect approximately 15 million people worldwide, and the incidence increases from 0.5% per year at age 65 years to 8% per year at age 85 years. As more people live to old age, Alzheimer’s disease is becoming a greater medical and social problem. Alzheimer’s disease is characterized by progressive decline in memory, language, and other cognitive functions accompanied by concomitant behavioral, emotional, interpersonal, and social deterioration (Pratico and Delanty, 2000; Melanie and Peter, 2003). The neuropathological hallmarks of Alzheimer’s disease are the accumulation of extracellular amyloid plaque containing beta-amyloid and intracellular neurofibrillary tangles containing polymerized and hyperphosphorylated tau protein (Martin, 1999). The causes of these pathological changes are poorly understood. One widely held hypothesis is

that reduced choline acetyltransferase, which leads to reduced acetylcholine, causes pathology or death of many acetylcholine-producing neurons. Another theory is that an excess endogenous endotoxin plays an important role. Epidemiological studies demonstrate that relatives of Alzheimer's disease patients have an increased risk of developing the disease, indicating that genetics is a likely contributing factor in many cases. Finally, evidence suggests that in Alzheimer's disease, the brain is under increased oxidative stress and that this free radical attack may be an underlying source of neuronal damage (Markesbery, 1997).

Alzheimer's patients with dementia often have "disturbance in antioxidant balance which may predispose to increased oxidative stress" (Sinclair, 1998). Numerous experimental studies show that increased oxidative stress can impair memory function in laboratory rodents (Bruce-Keller et al., 1998; Rivas-Arancibia et al., 1998; Shufitt-Hale et al., 1998). Clinical trials on elderly human populations also show that antioxidants are associated with improved memory and learning performance (Perrig *et al*., 1997; Perkins *et al*., 1999; Sinclair *et al*., 1998).

Collectively, this evidence suggests that assessing and reducing oxidative damage may a beneficial clinical strategy to help, prevent or retard the development and progression of Alzheimer's disease.

2.4 Cerebral ischemia model

The goal of cerebral ischemia model is to reduce oxygen and glucose supply to brain tissue. This process produces brain injury via a variety of cellular and molecular mechanisms that impair the energetics required to maintain ionic gradients. The mechanisms involve a complex series of pathophysiological events that are dependent on the severity, duration, and location of the ischemia within the brain (Traystman, 2003). Disturbances of the cerebral circulation have been associated with the decline of cognitive

function in elderly subjects, as well as with the development of several types of dementia. The bulk of this evidence indicates that cerebral hypoperfusion may fail to satisfy the metabolic demands of neuronal tissue because of suboptimal delivery of vital nutrients to the brain. Owing to the inadequate energy supply, cognitive loss and memory deficits may develop (De Jong *et al*., 1999).

Chronically reduced cerebral blood flow (CBF) can trigger the degeneration of the capillary ultrastructure in the brain. Creating a reduction of CBF in laboratory animals can test such a presumed sequence of events best. The bilateral occlusion of the common carotid arteries (two vessel occlusion, 2VO) is a well characterized method in rodents. The 2VO paradigm is frequently discussed in the context of Alzheimer's disease because of the apparent prevalence of cerebral hypoperfusion in the disease. The 2VO model stands for the visualization of the cerebrovascular and behavioral consequences of the reduced CBF, whatever its trigger may be (Farkas and Luiten, 2001). The functional changes usually observed after 2VO consisted of visuo-spatial memory impairment, hippocampal gliosis, mean hippocampal CBF reduction of 32%, microtubule associated protein-2 loss in the CA1 apical dendrites (a marker of protein synthesis and pre-synaptic activity), cytochrome oxidase decline in CA1 and posterior parietal cortex (a marker of neuronal energy activity), increased hemeoxygenase-1 expression (a marker of oxidative stress), and extracellular deposits of amyloid precursor protein, normally localized to neuronal cell membranes (de la Torre, 2000).

2.5 Scopolamine model

On the basis of experimental as well as clinical evidences, central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions (Blockland, 1996; Vanderwolf, 1988). Blockade of central muscarinic acetylcholine receptor disrupts learning and memory functions in animals and human beings (Davis and Yamamura, 1978). Anticholinergic drug (muscarinic blocker)

such as scopolamine has been in use as potent dementic agent (Das *et al*., 2003). The memory deficits in Alzheimer's disease may be associated with the loss of cholinergic cortical innervation (Perry *et al*., 1977) and the available clinical treatments for Alzheimer's disease are acetylcholinesterase inhibitors (Sugimoto *et al*., 2000). Therefore, scopolamine-induced amnesia has been used as an experimental model for this illness.

2.6 The Morris Water Maze (MWM)

Morris water maze was devised by Prof. Richard Morris about 20 years ago. It has become a very popular paradigm for the study of spatial learning and memory in rodents. The test apparatus consists of a round pool of water in which a platform is submerged just below the water's surface. Typically, an animal learns to escape from the water by locating the hidden platform with the help of visual cues around the pool. The relative simplicity of the MWM task is undoubtedly one of the reasons for its continuing success. The MWM task has often been used in the validation of rodent models for neurocognitive disorders and the evaluation of possible neurocognitive treatments. Through its many applications, MWM testing gained a position at the very core of contemporary neuroscience research (D' Hooge and De Deyn, 2001).

2.7 Passive avoidance

Fear-motivated avoidance tests are usually based on electric current as source of punishment. In many tests, the floor of the apparatus is made up by a grid that can be electrified. In so-called consummator conflict tests, the animal receives an electric shock when touching food or water. Avoidance tests are divided into two categories: passive avoidance and active avoidance. In passive avoidance, the animal has to refrain from executing a previously response, e.g., touch food or water, step down from and elevated position (to a grid floor) or step into a narrow and apparently safer place (with a grid floor). Step-down or step-through tests are most frequently used to measure passive

avoidance behavior. The latency to refrain from performing the punished act expresses the ability to avoid (Myhrer, 2003).

2.8 Lipid peroxidation

Lipid peroxidation is the mechanism by which lipids are attacked by reactive oxygen species with sufficient energy to form a carbon radical that reacts with oxygen and results in a peroxyl radical, thus generating lipid peroxides. Lipid peroxides are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA). Therefore, measurement of malondialdehyde is widely used as an indicator of lipid peroxidation. Markers of brain lipid peroxidation have been the most studied indexes of oxidant stress in AD.

Lipid peroxidation has been quantitatively assessed by measuring malondialdehyde (MDA) levels by thiobarbituric acid-reacting substances (TBARS) assay; lipid peroxides; aldehydes; and isoprostanes. The majority of the published studies have used the TBARS test. It is easy to perform and inexpensive but also has significant shortcomings when used to assess lipid peroxidation in complex biological systems (Pratico and Delanty, 2000).

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental animals

All experiments were performed on male ICR mice, six-week old, and weighing 25-30 g. All animals were obtained from National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom, Thailand. Prior to testing, mice were housed in the animal house of the Faculty of Pharmaceutical Sciences, Chulalongkorn University and maintained on 12:12 light-dark cycle at controlled temperature (25±2°C) for at least one week. Food and water were given ad libitum. All behavioral experiments were carried out in a room adjacent to that in which the mice were housed under the same conditions of temperature and humidity. The experimental protocol was approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

3.2 Experimental instruments

1. pH meter (Beckman, U.K.)
2. Stopwatch (SEIKO)
3. Morris water maze set (Home made, Thailand)
4. Step down set (Home made, Thailand)
5. Locomotor activity set (UGO Basile, Comerico, Italy)
6. Automatic micropipette (Pipet-Lite^{TM}, U.S.A.)
7. Automatic mixer (Vertex, U.S.A.)
8. Homogenizer (Glas-Col, Terre Haute, U.S.A.)
9. Centrifugater (Sorvll, GLC-2B, U.S.A.)
10. Spectrophotometer (Shimadzu, UV1201, Japan)

3.3 Drugs and chemicals

1. Turmeric extract powder (Thai-China Flavors & Fragrances Industry Co., Ltd.) The compositions of turmeric extract are curcumin 72.40%, demethoxycurcumin 24.11% and bisdemethoxycurcumin 3.82%.
2. Tacrine (Sigma, U.S.A.)
3. Normal saline solution (Thai Nakron Patana Co., ltd., Thailand)
4. Scopolamine hydrobromide (Sigma, U.S.A.)
5. Tween 20 (The East Asiatic Co., ltd., U.S.A.)
6. Pentobarbital sodium (Sigma, U.S.A.)
7. Sodium hydrogen phosphate-2-hydrate (Sigma, U.S.A.)
8. Sodium dihydrogen phosphate-2-hydrate (Sigma, U.S.A.)
9. Acetic acid (Sigma, U.S.A.)
10. Sodium dodecyl sulfate (Sigma, U.S.A.)
11. Thiobarbituric acid (Sigma, U.S.A.)
12. N-butanol (Sigma, U.S.A.)
13. Pyridine (Sigma, U.S.A.)
14. 1, 1, 3, 3-Tetraethoxy-propane (Malondialdehyde) (Sigma, U.S.A.)

3.4 Preparation and administration of the test compound

In the present study, turmeric extract was suspended in vehicle (tween 20/water [1:5]) and given orally to mice once daily at doses of 100, 300 and 1000 mg/kg BW. Scopolamine and tacrine were dissolved in physiological saline. Scopolamine was intraperitoneally injected at a dose of 1 mg/kg BW. Tacrine was orally administered at doses of 0.5 and 2 mg/kg BW min 2VO and scopolamine model, respectively. The control animals in 2VO model were orally administered with vehicle (tween 20/water [1:5]). In scopolamine model, the control animals were intraperitoneally injected with saline.

3.5 Experimental methods

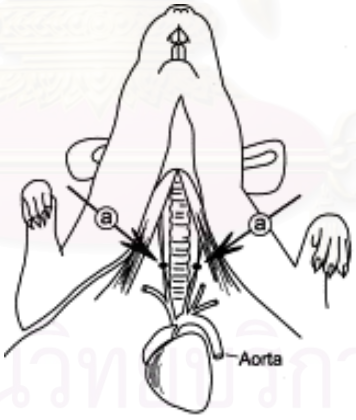
3.5.1 Experimental design

Animals were randomly divided into 2 groups. Cerebral ischemia to induce learning and memory impairment was performed on the first group whereas the second group was given scopolamine to induce learning and memory impairment. One hour before behavior testing (MWM, step-down test and locomotor activity test), vehicle (Tween 20/water 1:5) or the extracts of *C. longa* (100, 300 or 1000 mg/kg BW, dissolved in Tween 20/water) were administered orally through an intragastric feeding tube. Scopolamine was injected into the second group 30 min before behavior testing. Following the behavior test, the animals were sacrificed and whole brain was dissected for estimation the marker of oxidative stress (malondialdehyde).

3.5.2 Cerebral ischemia-induced learning and memory impairment

Mice were subjected to cerebral ischemia induced by 2VO plus hypotension (Figure 3.1). In brief, mice were anesthetized with sodium pentobarbital (Nembutal sodium solution, 60 mg/kg BW, intraperitoneal injection). Under deep anesthesia, the neck skin of mice was vertically incised. The common carotid arteries were exposed, carefully separated from the adjacent veins and sympathetic nerves, and then occluded by arterial clips. While the arteries were clamped, blood (0.3 ml) was withdrawn by cutting off the tip of the tail. Then, the artery clips were removed and cerebral blood flow was restored after 20 min. The skin incision was closed and the mice were kept in an air-condition room at 25°C. Sham-operated mice were subjected to the same procedure without carotid clamping and bleeding. After 24 h, the following MWM were carried out (Xu *et al*., 2000; Watanabe H. *et al*., 2003).

To study the effects of turmeric extract (*Curcuma longa*) on impairment of learning and memory induced by cerebral ischemia, six groups of animals were used. One group of sham-operated animals (n = 8) and one group of 2VO animals (n = 8) were administered with vehicle. Three groups of 2VO animals (n = 8 per group) were administered with the extracts of *C. longa* at the doses of 100,300 and 1000 mg/kg BW. The last group of 2VO animals (n = 8) were administered with tacrine at the dose of 0.5 mg/kg BW (positive control). All drugs were orally administered for 8 consecutive days. In each group of six animals three behavioral tests were performed, MWM test, step-down test and spontaneous locomotor activity test. MWM test was tested for 5 consecutive days after 2VO. Six days after 2VO the step-down test was performed and followed with spontaneous locomotor activity test for 2 days. Following three behavioral tests, the animals were sacrificed for an estimation of lipid peroxidation (Bejar *et al*.,1999).



2VO

Figure 3.1 Experimental mice model of cerebral ischemia (bilateral common carotid artery occlusion, two vessel occlusion: 2VO); (a) the common carotid arteries

3.5.3 Scopolamine-induced learning and memory impairment

To study the effects of turmeric extract (*Curcuma longa*) on impairment of learning and memory induced by scopolamine. Six groups of animals were used: groups of mice (n = 6 per group) were administered orally for 8 consecutive days, with vehicle, the extracts of *C. longa* (100, 300 or 1000 mg/kg BW) or tacrine (2 mg/kg BW) followed 30 min later by an intraperitoneal injection of scopolamine (1 mg/kg BW) or normal saline (0.1 ml). All mice were tested 20 min after the injection of scopolamine in the same manner as described for 2VO model.

3.5.4 Behavior tests

1. Morris water maze (MWM)

After 24 h of cerebral ischemia, the MWM was performed. The procedure used was a modification of that described by Morris (1984). The MWM consisted of a circular pool (Figure 3.2), painting with black color, which was 70 cm in diameter and depth of 13 cm of water with water maintained 25±1^{o}C. A platform (6 cm diameter) was situated 1 cm below the surface of the water. The pool was divided into four quadrants with platform in a fixed position in one quadrant. Daily swimming consisted of four trials in which the mice were placed in the water from four different starting points. A mouse was allowed to swim for a maximum of 60 sec to find the hidden platform where it was allowed to stay for 10 sec. This was conducted for 5 consecutive days. On the training day, if a mouse did not find the platform in 60 sec, it was placed on the platform by hand and remained there for 10 sec. The time to reach the platform (escape latency) was measured with a stopwatch (Watanabe H. *et al*, 2003).

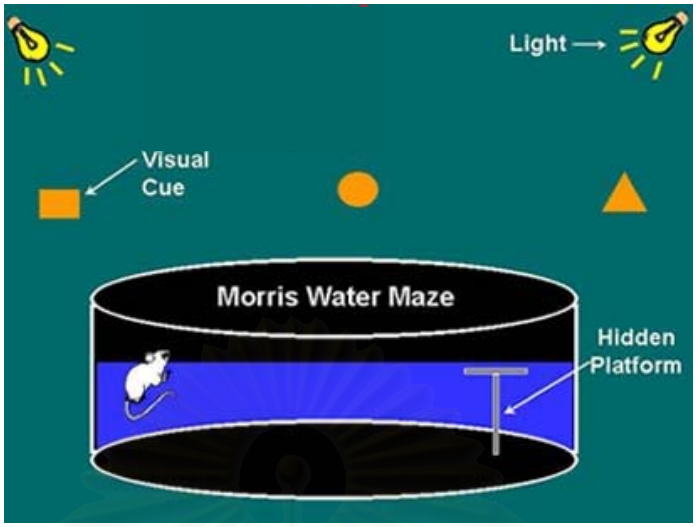


Figure 3.2 Equipment for Morris water maze test

2. Step-down test

A step-down passive avoidance was examined using apparatus consisted of plexiglass chamber (Figure 3.3). The inside dimensions of the activity cage are, length 35 cm; width 23 cm; and height 20 cm. The cage floor is made of evenly spaced stainless steel bars (3 mm diameter) that are spaced 11 mm apart, and a plastic platform (5 cm diameter, 4 cm height) set on the grid in one corner. Electric stimulation was given through the grid connected with a scrambled shock generator (1 Hz, 1 ms, 36 V dc). Step-down experiment was started after 24 h of the last MWM testing. Mouse was placed on the platform to get adapted to environment for 3 min without electric shock. Then electric shocks were delivered to the grid when the mouse stepped down from the platform. The cut-off time was 5 min. After 24 h of training, mouse was placed on the platform for retention test. The electric shocks were still delivered for 5 min. Step-down latency and number of errors were recorded. The time (step-down latency) that elapsed until the mouse stepped down from the platform was recorded. If the mouse did not step down from the platform within 300 sec, the retention test was terminated and the maximal step-down latency of 300

sec was recorded. An error was counted whenever the mouse stepped down from the platform and the number of errors made in 5 min was recorded (Luo *et al*., 2003).

Figure 3.3 Equipment for Step-down test

3. Spontaneous locomotor activity test

Each animal was placed in an activity cage consisting of plexiglass chamber and counting (Figure 3.4). The inside dimensions of the activity cage are, length 35 cm; width 23 cm; and height 20 cm. The cage floor is made of evenly spaced stainless steel bars (3 mm diameter) that are spaced 11 mm apart, connected to the circuit of counting unit. The registered numbers or counts of movements were recorded at 5 min intervals. The apparatus was placed in light and sound attenuated, and ventilated testing room with other behavioral testing apparatus (Jain *et al*., 2002; Gupta *et al*., 2003).

Figure 3.4 Activity meter

3.5.5 Lipid peroxidation assay

Following the behavioral testing, the animals were decapitated and the brains were quickly removed, cleaned with ice-cold saline and stored at -80°C.

1. Tissue preparation

Brain tissue samples were thawed and homogenized with 10 times (W/V) ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots of homogenates from mice brain were separated and used to determine the marker of oxidative stress (malondialdehyde).

2. Measurement of lipid peroxidation

Malondialdehyde (MDA), a measure of lipid peroxidation, was measured as described by Gupta *et al*. (2003). The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodiumdodecyl sulphate (8.1%) were added to 0.1 ml of processed tissue samples, then heated at 100°C for 60 min. The mixture was cooled with tap water and 5 ml of *n*-butanol/pyridine (15:1), 1 ml of distilled water was added. The mixture was vortexed vigorously. After centrifugation at 2500 rpm for 20 min, the organic layer was separated and absorbance was measured at 532 nm using a spectrophotometer. The concentration of MDA is expressed as nmol/g tissue (Gupta *et al*., 2003).

3.6 Data analysis

All data are expressed as the mean value for the group ± standard error of mean (SEM). Statistical analyses were performed by one-way ANOVA and Duncan post-hoc test for planned comparisons between control versus different treatment groups. A significance value of $P < 0.05$ was considered as statistically significant.

CHAPTER IV

RESULTS

4.1 Effects of turmeric extract on impairment of learning and memory induced by cerebral ischemia.

4.1.1 Effects of 2VO on spatial learning and memory performance.

The MWM performance in 2VO and sham-operated mice as measured by latency to reach the hidden platform during 5 consecutive days was summarized in Figure 4.1. The mean search time to find the platform on the training day between those of 2VO (47 ± 4.21sec) and sham-operated mice (44 ± 3.27 sec) did not differ. During days 1-5, mice subjected to 2VO required a longer time to locate the hidden platform than sham-operated mice. The escape latency was significantly delayed in 2VO mice as compared to the sham-operated mice. The escape latencies of 2VO and sham-operated mice on day 5 were 32 ± 4.50 and 8 ± 0.77 sec, respectively.

4.1.2. Effects of turmeric extract on spatial learning and memory performance in 2VO mice.

After 24 h of cerebral ischemia, the MWM test was performed. Turmeric extract was orally given to animals 1 h before testing. Administration of turmeric extract at doses 100, 300 and 1000 mg/kg BW and tacrine at dose 0.5 mg/kg BW attenuated the memory deficits in 2VO mice. The escape latency of turmeric extract-treated mice was shorter than that of the vehicle-treated mice. All doses of turmeric extract significantly improved learning and memory performance on day 4 and day 5. On day 5, the escape latencies of turmeric extract-treated mice at doses of 100, 300, 1000 mg/kg B.W. were 20 ± 4.89, 20 ± 2.78 and 14 ± 2.86 sec, respectively, whereas it was 16 ± 2.72 sec in tacrine-treated group (Figure 4.2).

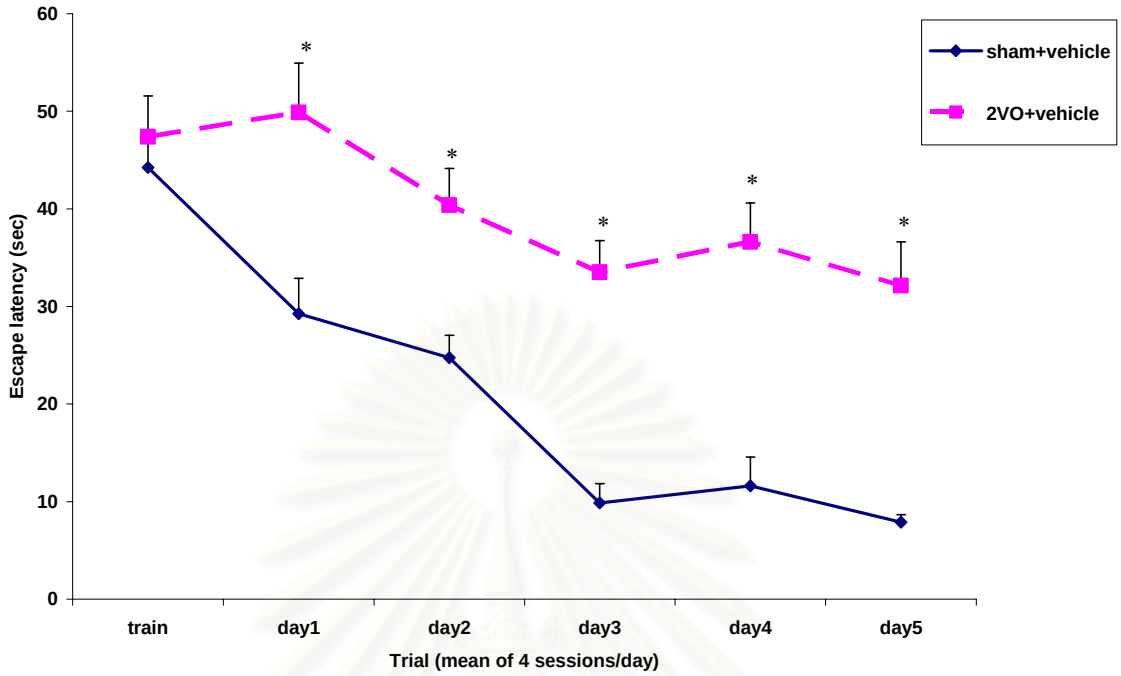


Figure 4.1 : The MWM performance in 2VO and sham-operated mice. The escaping latency onto the platform was measured during 5 consecutive days. Each data point represents the mean ± SEM (n=8) of four trials. A significant level of $P < 0.05$ was considered as a significant difference.

* Significantly different from values in sham-operated mice

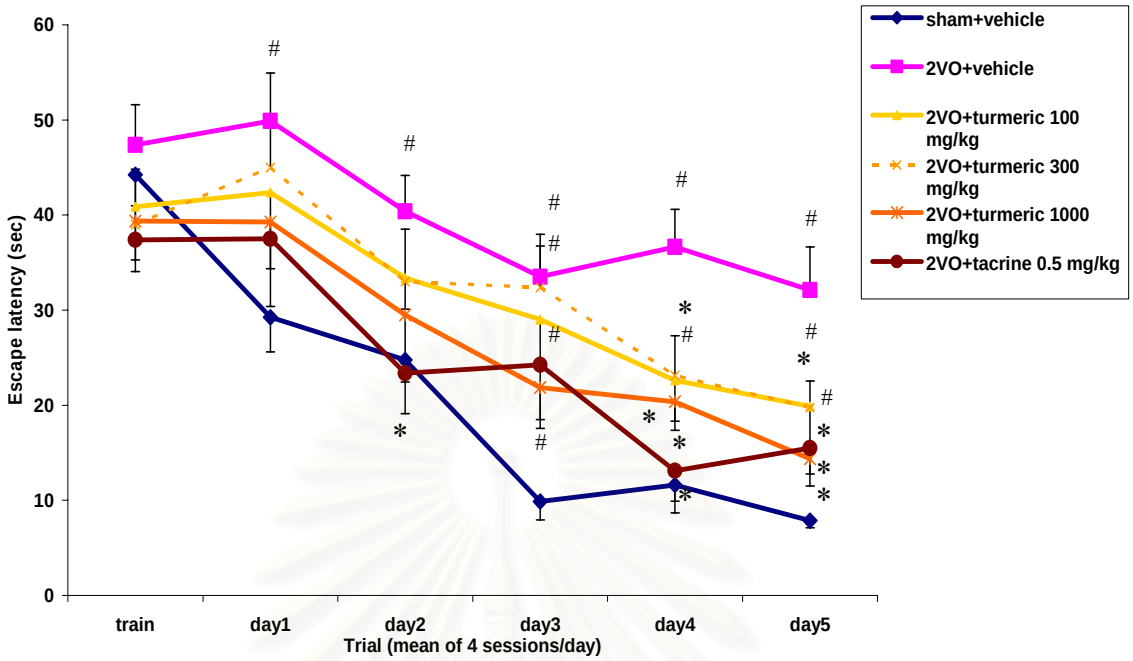


Figure 4.2 : Effects of turmeric extract on spatial learning and memory performance in 2VO mice. Mice were orally given with vehicle or turmeric extract at doses of 100, 300 and 1000 mg/kg BW, once daily. The escaping latency onto the platform was measured during 5 consecutive days. Each data point represents the mean ± SEM (n=8) of four trials. A significant level of $P < 0.05$ was considered as a significant difference.

Significantly different from values in sham-operated mice

* Significantly different from values in 2VO mice

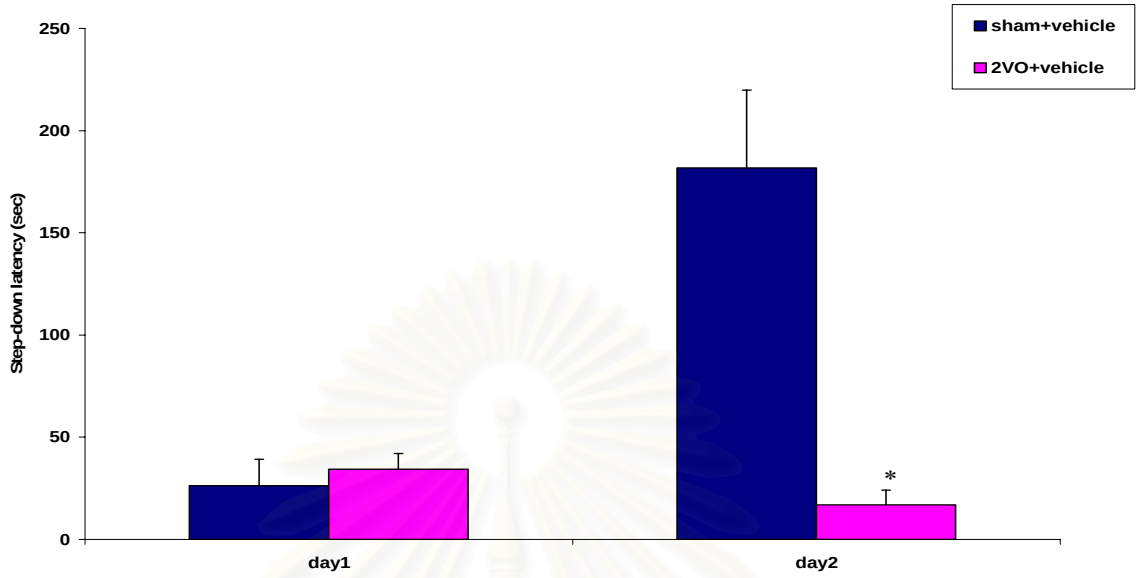
4.1.3. Effects of 2VO on step-down passive avoidance.

Impairment of learning and memory in step-down was observed after cerebral ischemia. As shown in Figure 4.3, 2VO caused a significant reduction in the step-down latency and increased the step-down errors on day 2. Step-down latencies were 182 ± 38.04 and 17 ± 7.29 sec in sham- and 2VO-operated mice, respectively. Numbers of errors were 2 ± 0.90 and 7 ± 2.45 in sham- and 2VO-operated mice, respectively.

4.1.4. Effects of turmeric extract on step-down passive avoidance in 2VO mice.

Similar to tacrine (0.5 mg/kg BW), treatment with turmeric extract at doses of 300 and 1000 mg/kg BW, p.o., significantly reversed the reduction in step-down latency and decreased the step-down errors of 2VO mice (Figure 4.4). Step-down latencies of turmeric-treated mice at doses of 100, 300 and 1000 mg/kg BW were 68 ± 32.61, 161 ± 45.99 and 257 ± 28.43 sec, respectively. Numbers of errors of turmeric-treated mice at doses of 100, 300 and 1000 mg/kg BW were 4 ± 1.17, 2 ± 1.00 and 0 ± 0.17, respectively. In tacrine-treated group, step-down latency and number of errors were 231 ± 38.08 sec and 1 ± 0.27, respectively.

(A)



(B)

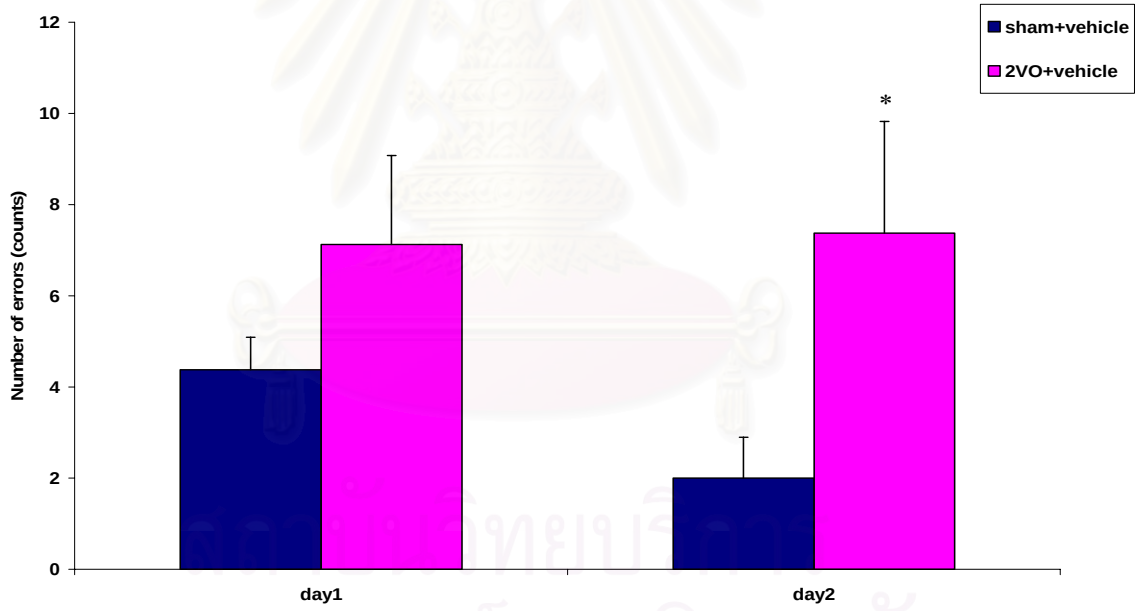
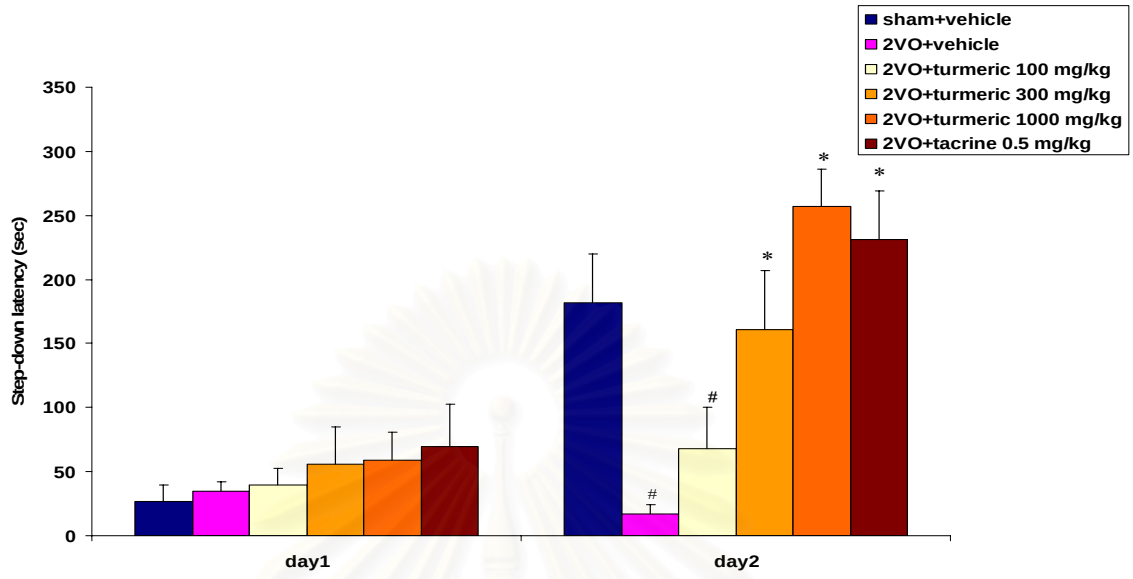


Figure 4.3 : Effects of 2VO and sham-operated mice on step-down test. Step-down latency (A) and number of errors (B) were expressed as the mean ± SEM (n=8). A significant level of $P < 0.05$ was considered as a significant difference.

* Significantly different from values in sham-operated mice

(A)



(B)

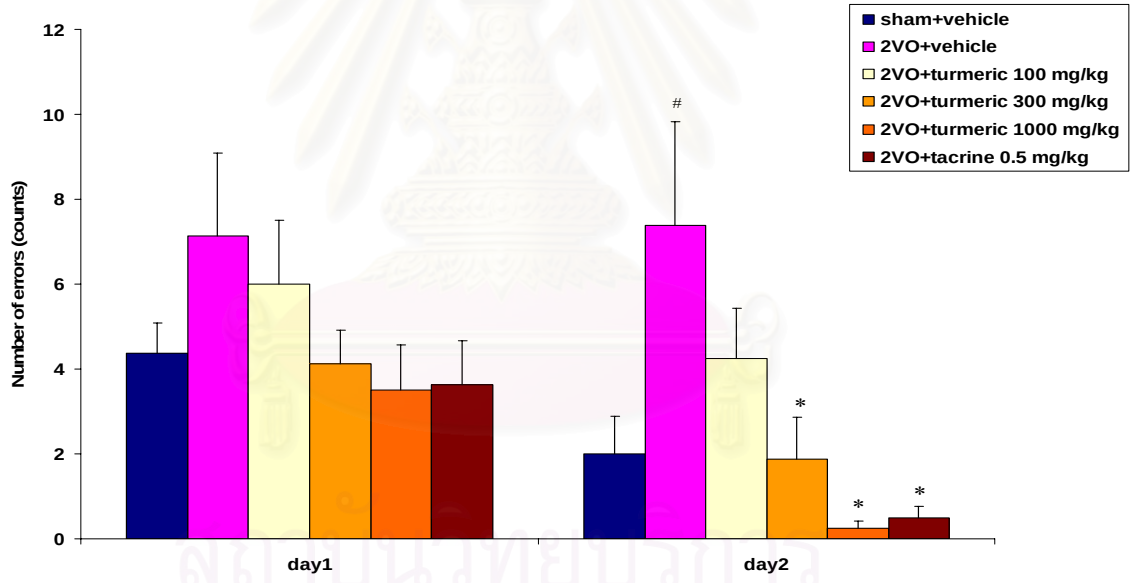


Figure 4.4 : Effects of turmeric extract on step-down passive avoidance in 2VO mice. Mice were orally given with vehicle and turmeric extract at doses of 100, 300 and 1000 mg/kg BW, once daily. Step-down latency (A) and number of errors (B) were expressed as the mean ± SEM (n=8). A significant level of $P < 0.05$ was considered as a significant difference.

Significantly different from values in sham-operated mice

* Significantly different from values in 2VO mice

4.1.5. Effects of 2VO on spontaneous locomotor activity.

The spontaneous locomotor activity, measured as movement counting during 5 minutes test period, in 2VO and sham-operated mice was shown in Figure 4.5. The spontaneous locomotor activity did not differ between 2VO and sham-operated mice.

4.1.6. Effects of turmeric extract on spontaneous locomotor activity in 2VO mice.

Oral administration of turmeric extract at doses of 100, 300 and 1000 mg/kg BW as well as administration of tacrine had no effect on spontaneous locomotor activity in 2VO mice. The results were shown in Figure 4.5.

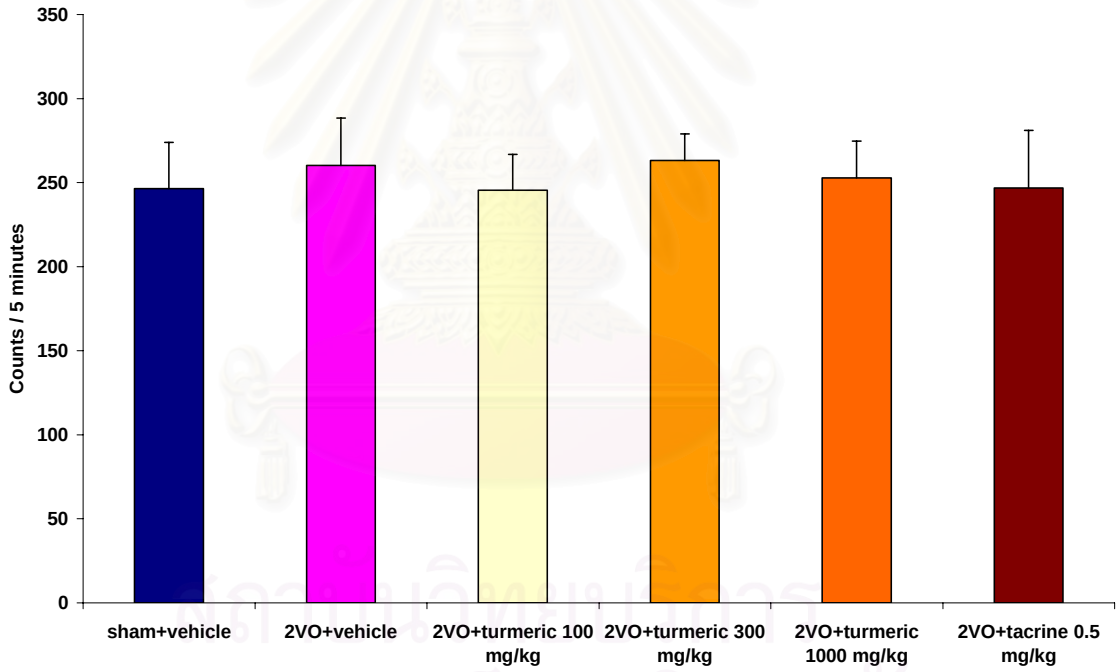


Figure 4.5 : Effects of turmeric extract on spontaneous locomotor activity in 2VO mice. Mice were orally administered with vehicle, turmeric extract at doses of 100, 300 and 1000 mg/kg B.W. or tacrine at dose of 0.5 mg/kg BW The registered numbers or counts of movements were recorded at 5 min intervals and expressed as the mean ± SEM (n=8). A significant level of $P < 0.05$ was considered as a significant difference.

4.1.7. Effects of 2VO on lipid peroxidation.

In the 2VO mice was observed significant increase in MDA levels as compared to the sham-operated mice. MDA levels of 2VO and sham-operated mice were 132.7 ± 12.88 and 36.2 ± 3.44 nmol/g tissue, respectively (Figure 4.6).

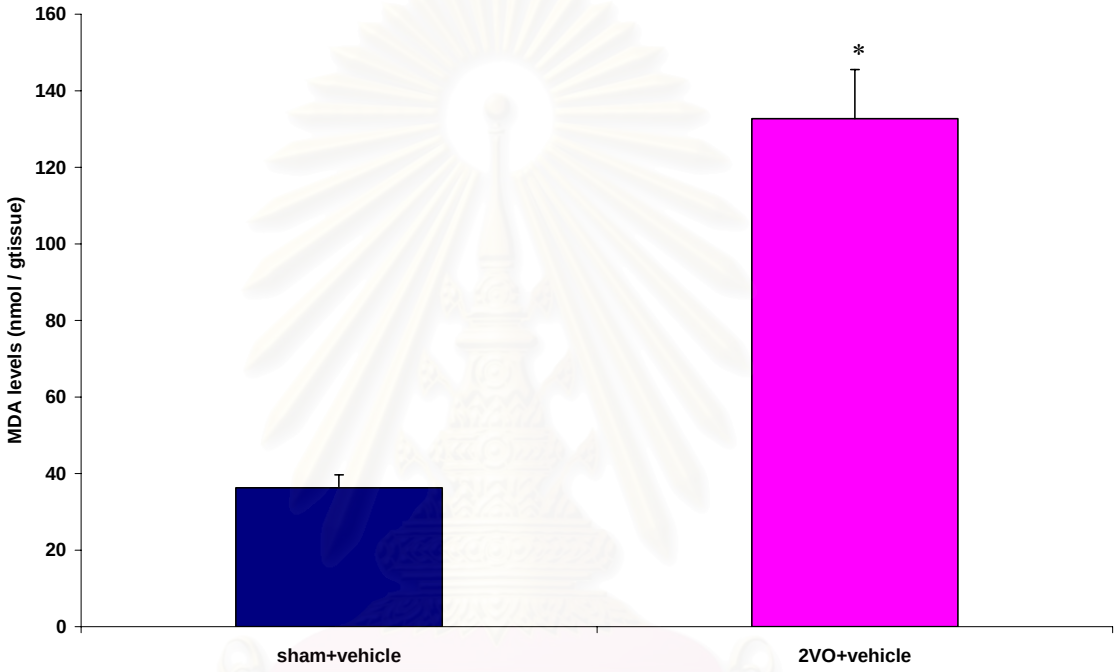


Figure 4.6 : Effects of 2VO on MDA levels in brains of mice. The concentration of MDA is expressed as nmol/g tissue (mean ± SEM; n=8). A significant level of $P < 0.05$ was considered as a significant difference.

* Significantly different from values in sham-operated mice

4.1.8. Effects of turmeric extract on lipid peroxidation in 2VO mice.

The effects of turmeric extract on lipid peroxidation in 2VO mice were shown in Figure 4.7. There was a significant decrease in 2VO induced-increment of MDA levels in both turmeric- and tacrine-treated group when compared to the 2VO mice. Administration of turmeric extract at doses of 100, 300 and 1000 mg/kg BW markedly reduced MDA levels to 44.5 ± 2.01, 53.0 ± 4.46 and 27.1 ± 3.43 nmol/g tissue, respectively. In tacrine-treated mice, MDA level was reduced to 33.4 ± 4.22 nmol/g tissue.

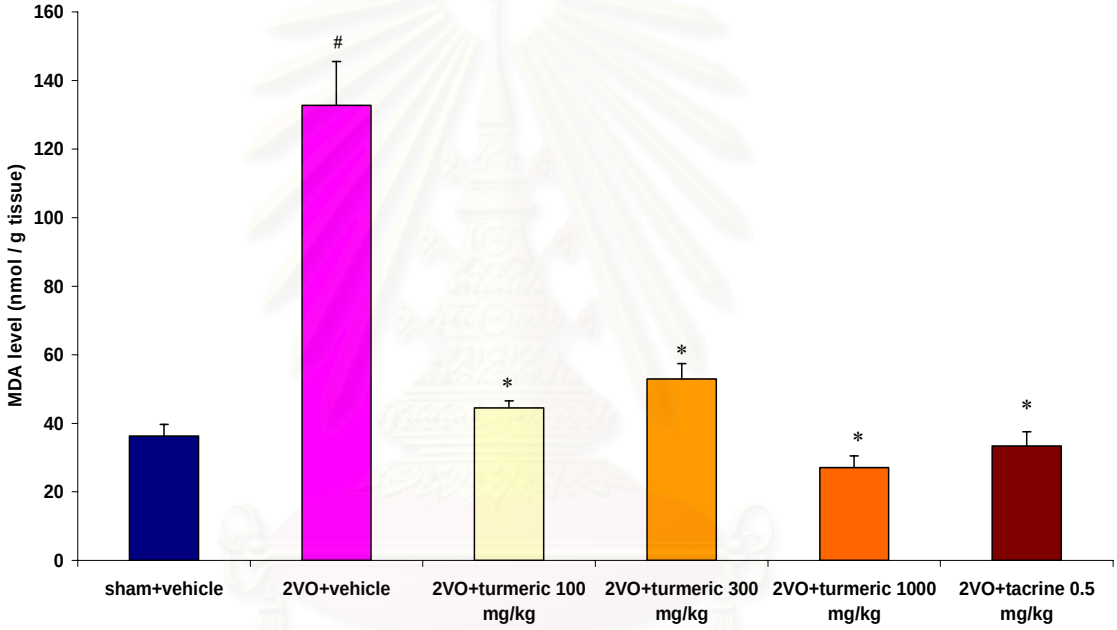


Figure 4.7 : Effects of turmeric extract on MDA levels in brains of 2VO mice. Mice were orally administered with vehicle, turmeric extract at doses of 100, 300 and 1000 mg/kg BW or tacrine at dose of 0.5 mg/kg BW The concentration of MDA is expressed as nmol/g tissue (mean ± SEM; n=8). A significant level of $P < 0.05$ was considered as a significant difference.

Significantly different from values in sham-operated mice

* Significantly different from values in 2VO mice

4.2 Effects of turmeric extract on impairment of learning and memory induced by scopolamine.

4.2.1. Effects of scopolamine on spatial learning and memory performances.

Effects of scopolamine (a muscarinic cholinergic receptor antagonist) administration (1 mg/kg BW, i.p., 30 min before the MWM test) on the MWM performance in mice were shown in Figure 4.8. In agreement with previous studies, scopolamine induced a state of amnesia by extending the escape latency to find the hidden platform in spatial memory task when compared to control (normal saline-treated). This effect was seen on the first day of trial and persisted throughout the whole trial schedule. The average escape latency times of the scopolamine-treated mice were significantly higher than those in normal saline-treated mice. The escape latencies of saline- and scopolamine-treated mice on day 5 were 12 ± 2.65 and 28 ± 3.60 sec, respectively.

4.2.2. Effects of turmeric extract on spatial learning and memory deficit in mice induced by scopolamine.

Effects of turmeric extract at doses of 100, 300 and 1000 mg/kg BW, p.o., on scopolamine-induced impairment of MWM performance in mice were shown in Figure 4.9. Tacrine-treated group was a positive control. All doses of turmeric extract significantly antagonized the effect of scopolamine on escape latency. On day 5, the escape latencies of turmeric extract-treated mice at doses of 100, 300, 1000 mg/kg BW and tacrine-treated mice were 17 ± 2.42, 18 ± 2.52, 11 ± 3.80 and 17 ± 5.05 sec, respectively.

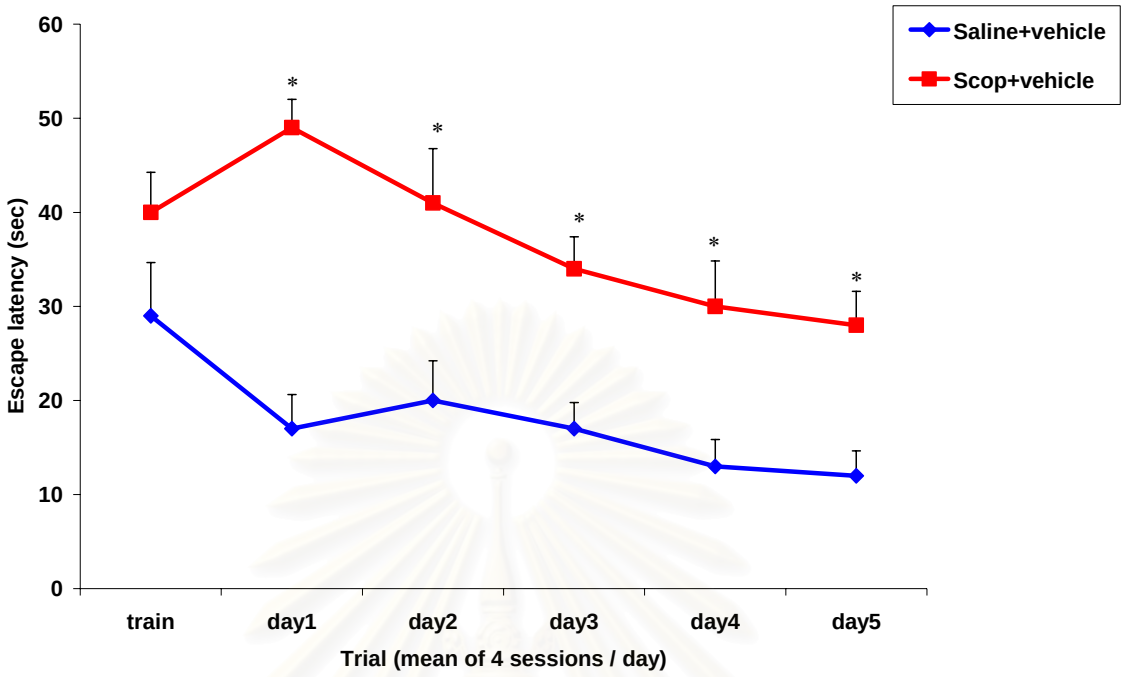


Figure 4.8 : Effect of scopolamine (1 mg/kg BW) on spatial learning and memory in the MWM performance. The escape latency onto the platform was measured on 5 consecutive days. Each data point represents the mean ± SEM (n=6) of four trials. A significant level of $P < 0.05$ was considered as a significant difference.

* Significant different from value in saline-treated mice

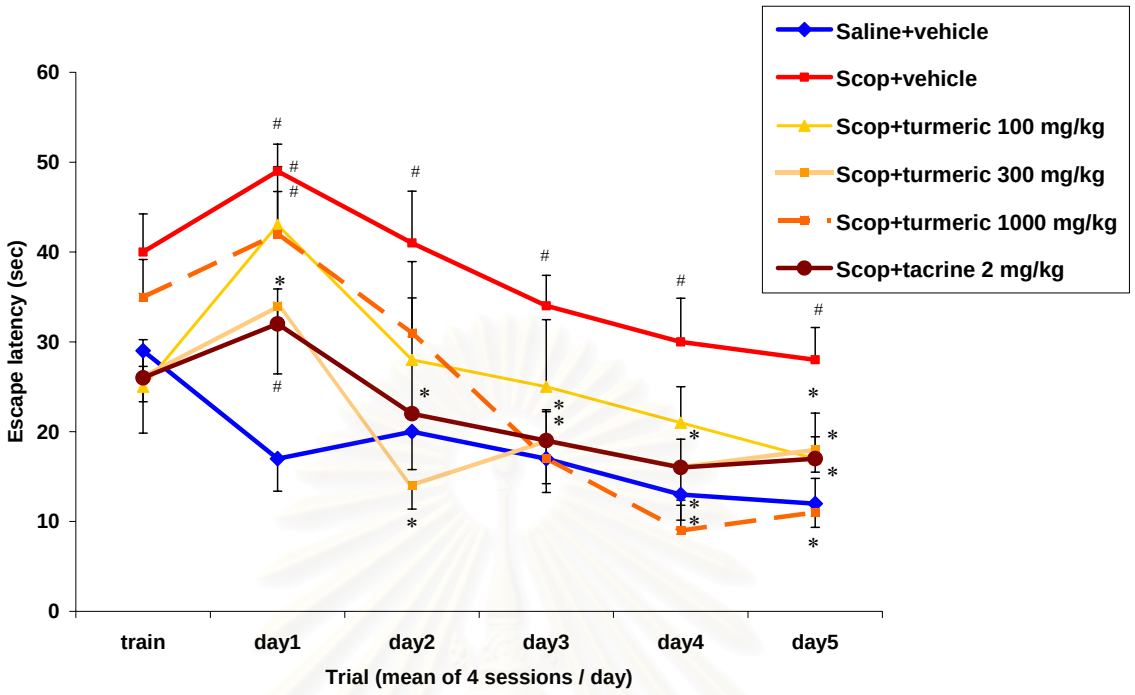


Figure 4.9 : Effect of turmeric extract on memory impairment induced by scopolamine (1 mg/kg BW) in the MWM performance. Mice were orally administered with vehicle, turmeric extract at doses of 100, 300 and 1000 mg/kg BW or tacrine at dose 2 mg/kg BW once daily. The escape latency onto the platform was measured on 5 consecutive days. Each data point represents the mean ± SEM (n=6) of four trials. A significant level of $P < 0.05$ was considered as a significant difference.

Significant different from value in saline-treated mice

* Significant different from value in scopolamine-treated mice

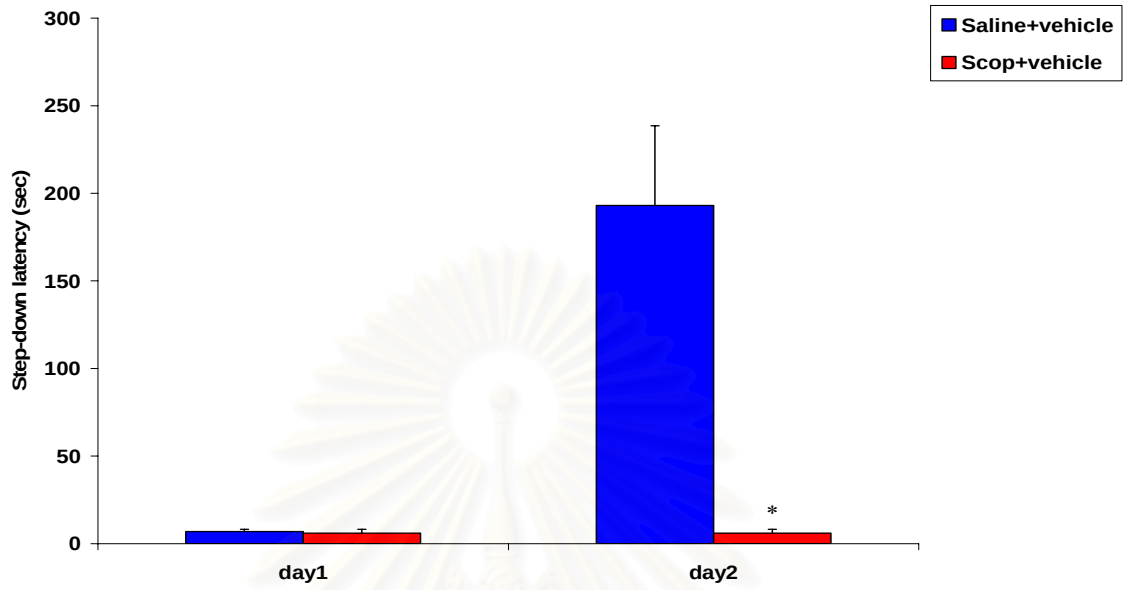
4.2.3. Effects of scopolamine on step-down passive avoidance.

As shown in Figure 4.10, scopolamine (1 mg/kg BW) significantly shortened the step-down latency and increased the number of errors on second day of step-down test when compared to saline-treated group. Step-down latencies were 193 ± 45.56 and 6 ± 2.15 sec in saline- and scopolamine-treated group, respectively on second day of step-down test. Numbers of errors were 1 ± 0.49 and 4 ± 0.31 in saline- and scopolamine-treated group, respectively on second day of step-down test.

4.2.4. Effects of turmeric extract on scopolamine-induced impairment of step-down passive avoidance.

Effects of turmeric extract at doses of 100, 300 and 1000 mg/kg BW, p.o., in step-down test were shown in Figure 4.11. Administration of turmeric extract at doses of 300 and 1000 mg/kg BW significantly increased the step-down latency as well as tacrine. The number of errors was significantly decreased in turmeric extract (100,300 and 1000 mg/kg BW)- and tacrine-treated group on second day of step-down test when compared to vehicle- treated group. Step-down latencies of turmeric-treated mice at doses of 100, 300, 1000 mg/kg BW and tacrine-treated mice (2 mg/kg BW) were 21 ± 1.86, 114 ± 13.68, 123 ± 8.41 and 116 ± 7.52 sec, respectively on second day of step-down test. Numbers of errors of turmeric-treated mice at doses of 100, 300, 1000 mg/kg BW and tacrine-treated mice (2 mg/kg BW) were 2 ± 0.81, 1 ± 0.17, 1 ± 0.00 and 2 ± 0.23, respectively on second day of step-down test.

(A)



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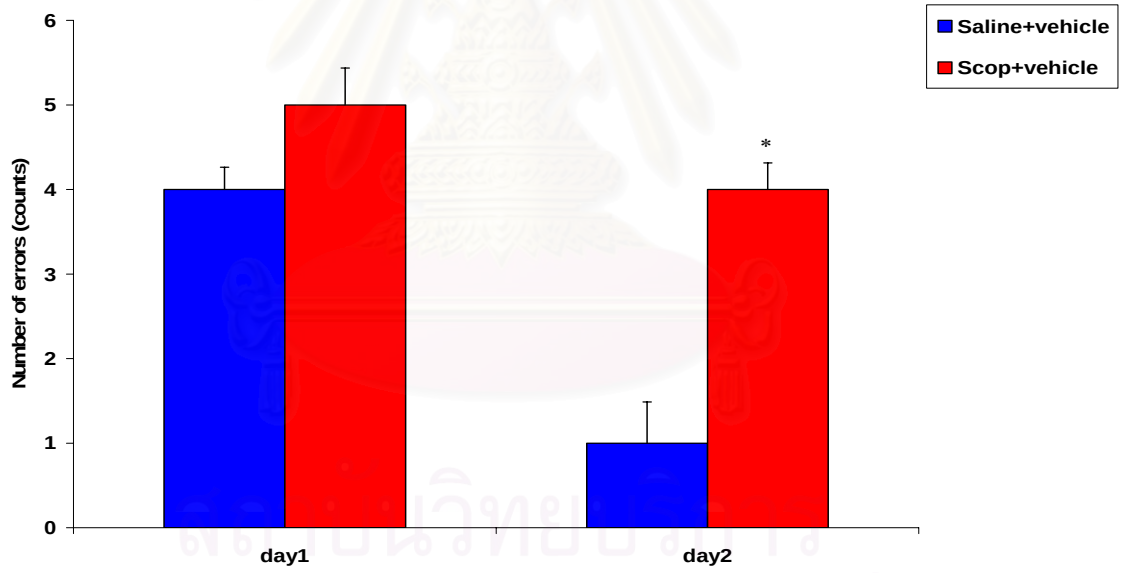
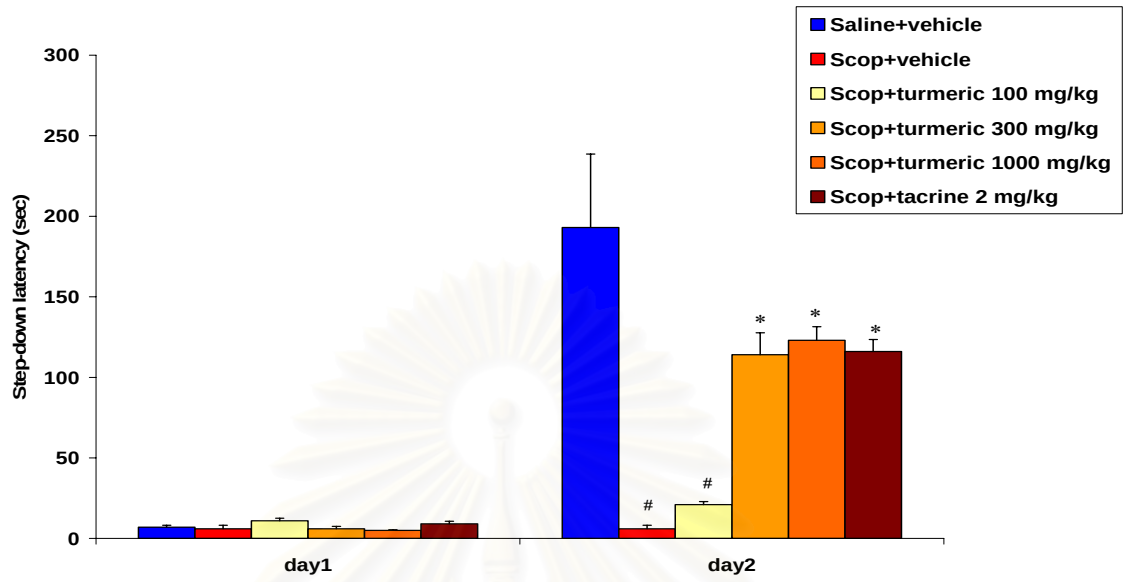


Figure 4.10 : Effects of scopolamine (1 mg/kg BW) on step-down passive avoidance test. Step-down latency (A) and number of errors (B) were expressed as the mean ± SEM (n=6). A significant level of $P < 0.05$ was considered as a significant difference.

* Significant different from value in saline-treated mice

(A)



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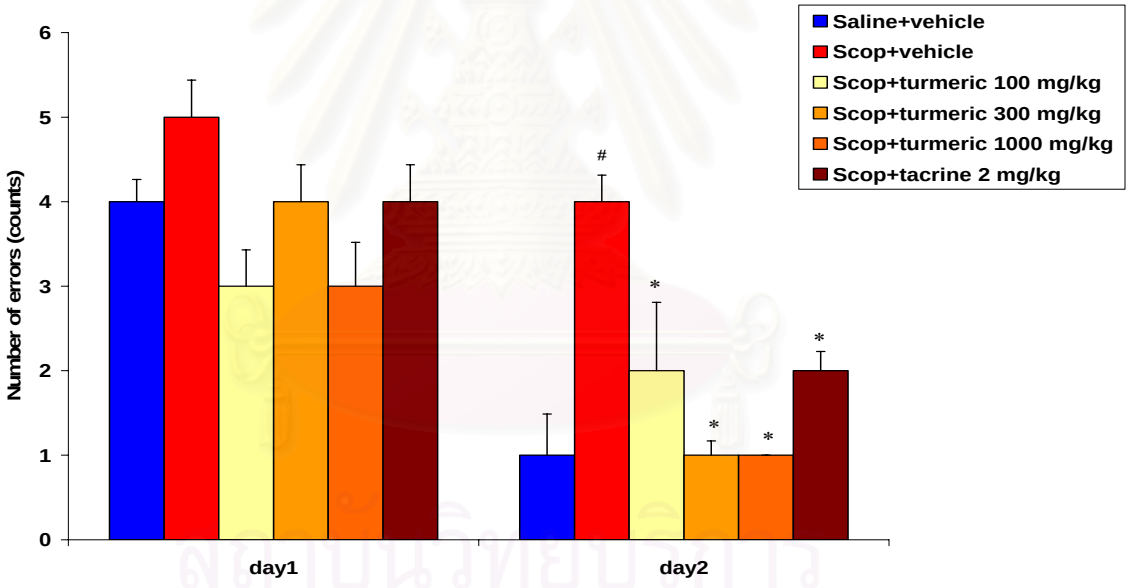

Figure 4.11 : Effects of turmeric extract on memory impairment induced by scopolamine (1 mg/kg BW) in step-down test. Mice were orally administered with vehicle, turmeric extract at doses of 100, 300 and 1000 mg/kg BW or tacrine at dose of 2 mg/kg BW once daily. Step-down latency (A) and number of errors (B) were expressed as the mean ± SEM (n=6). A significant level of $P < 0.05$ was considered as a significant difference.

Significant different from value in saline-treated mice

* Significant different from value in scopolamine-treated mice

4.2.5. Effects of scopolamine on spontaneous locomotor activity.

The spontaneous locomotor activity, measured as movement counting during 5 min test period, in scopolamine- and saline-treated mice was summarized in Figure 4.12. In scopolamine-treated mice, the spontaneous locomotor activity was significantly decreased when compared to that of saline-treated mice. The locomotor activities were 157.17 ± 11.02 and 0.50 ± 0.35 counts / 5 minutes in saline- and scopolamine-treated mice, respectively.

4.2.6. Effects of turmeric extract on spontaneous locomotor activity in scopolamine-treated mice.

All doses of turmeric extract and tacrine 2 mg/kg BW had no effect on spontaneous locomotor activity when compared to scopolamine-treated group. Administration with turmeric extract at doses of 100, 300 and 1000 mg/kg BW and tacrine (2 mg/kg BW) decreased the locomotor activities to 1.83 ± 1.07, 1.17 ± 0.67, 1.00 ± 0.56 and 1.83 ± 0.89 counts / 5 minutes, respectively when compared to saline-treated group. The results were shown in figure 4.12.

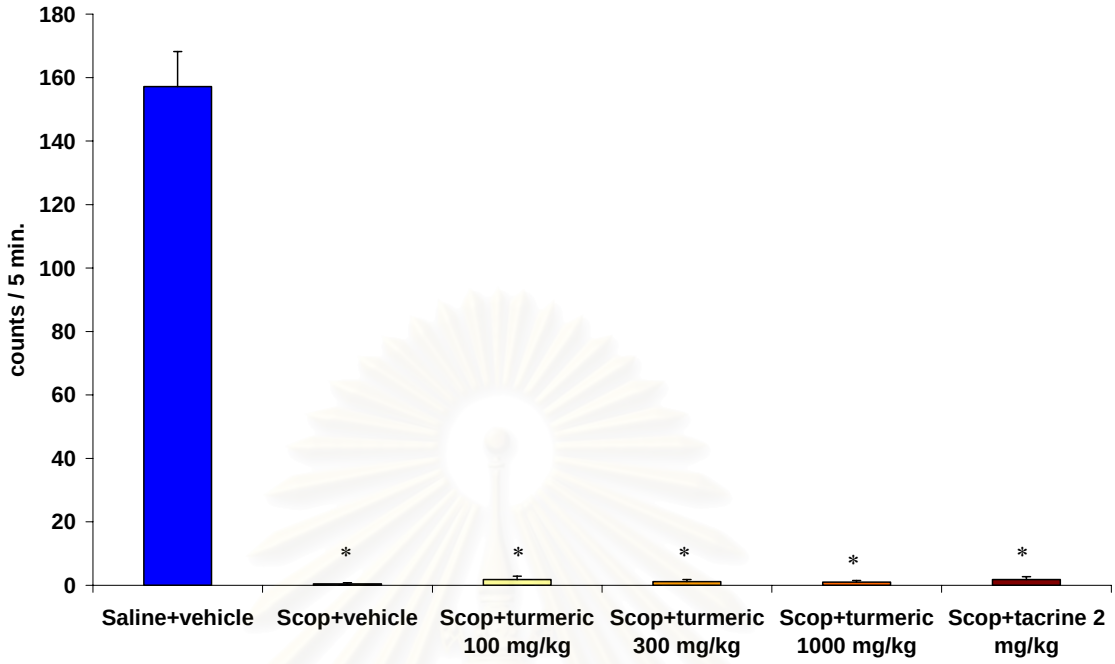

Figure 4.12 : Effects of scopolamine (1 mg/kg BW), turmeric extract and tacrine on spontaneous locomotor activity. Mice were orally administered with vehicle, turmeric extract at doses of 100, 300 and 1000 mg/kg BW or tacrine at dose of 2 mg/kg BW once daily. The registered numbers or counts of movements were recorded at 5 min intervals and expressed as the mean ± SEM (n=6). A significant level of $P < 0.05$ was considered as a significant difference.

* Significant different from value in saline-treated mice

4.2.7. Effects of scopolamine treated on brain lipid peroxidation.

Scopolamine had no effect on the MDA level. The brain MDA levels of scopolamine- and saline-treated mice after 8-day administration of vehicle were shown in Figure 4.13. There was no significant difference between MDA levels of scopolamine- and saline-treated mice.

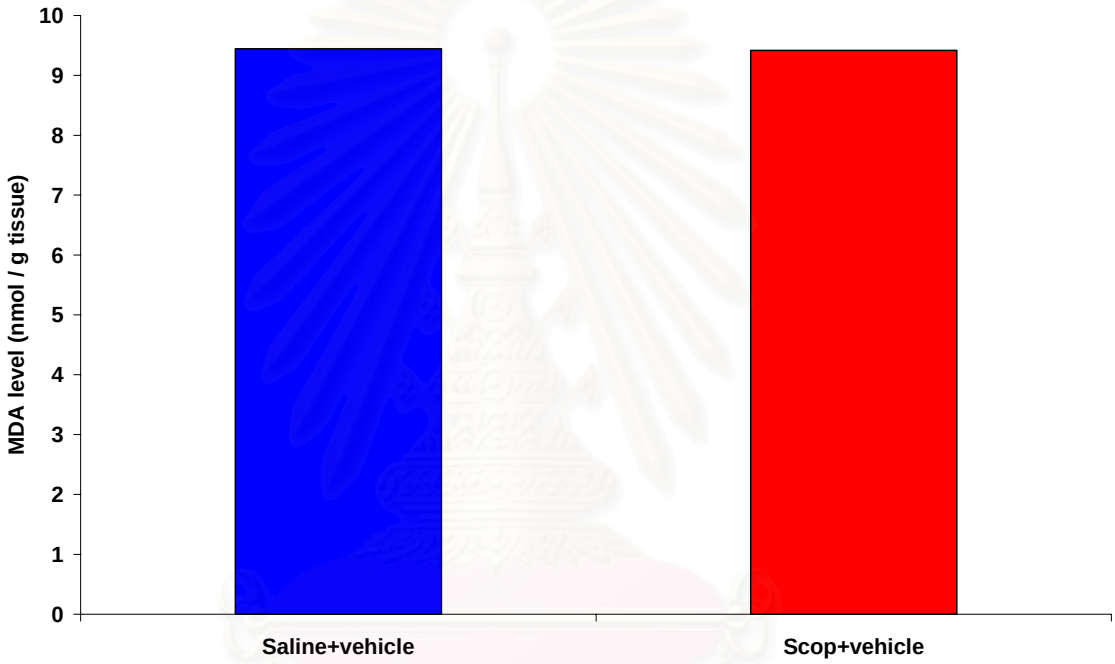

Figure 4.13 : Effects of scopolamine (1 mg/kg BW) on brain levels of MDA. The concentration of MDA is expressed as nmol/g tissue (mean ± SEM; n=6). A significant level of $P < 0.05$ was considered as a significant difference.

4.2.8. Effects of turmeric extract administration on brain lipid peroxidation in scopolamine-treated mice.

Effects of turmeric at doses of 100, 300 and 1000 mg/kg BW or tacrine at dose of 2 mg/kg BW, p.o., on brain lipid peroxidation were shown in Figures 4.14. At 8 days following testing; turmeric extract administration had no effect on MDA levels of the brain.

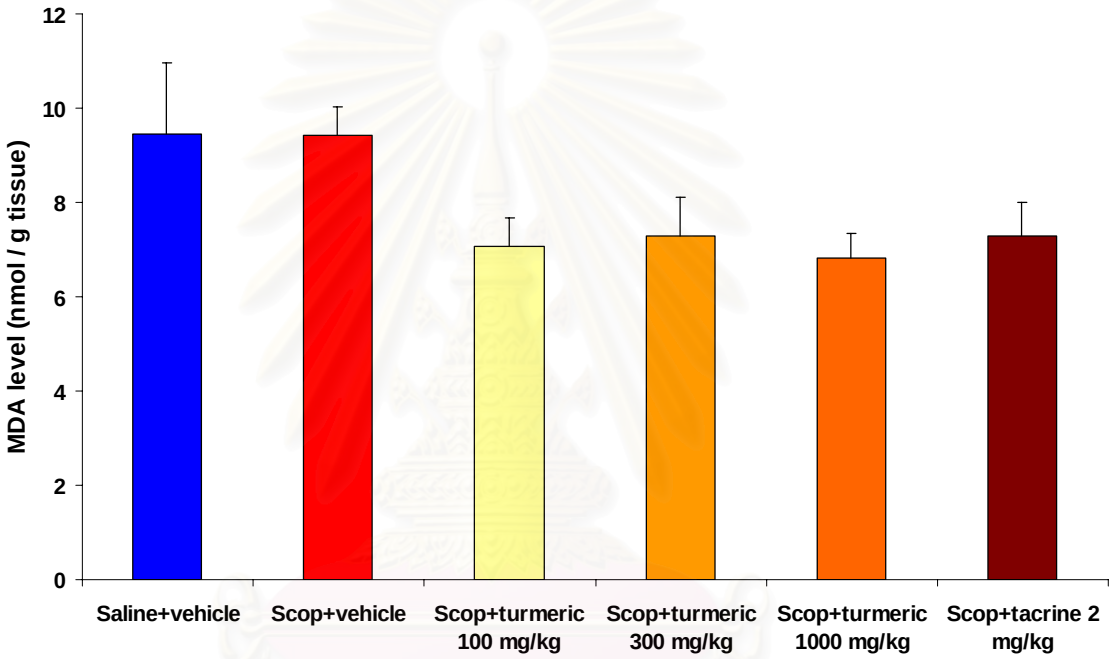

Figure 4.14 : Effects of turmeric extract on MDA levels in nmol/g tissue in scopolamine-induced disruption of memory (1 mg/kg BW). Mice were orally administered with vehicle, turmeric extract at doses of 100, 300 and 1000 mg/kg BW or tacrine at dose of 2 mg/kg BW once daily. The concentration of MDA is expressed as nmol/g tissue (mean ± SEM; n=6). A significant level of $P < 0.05$ was considered as a significant difference.

CHAPTER V

DISCUSSION AND CONCLUSION

There are various pharmacological activities of *Curcuma longa*. One of these is an antioxidant activity. This activity may associate with improving of learning and memory. In the present study, turmeric extract (*Curcuma longa*) was evaluated in mice for its effect on learning and memory impairment induced by either cerebral ischemia or scopolamine. In addition, the effect of *Curcuma longa* on lipid peroxidation was also performed.

The development of animal models of ischemia induced amnesia is vital to the analysis of the functional consequences of ischemia damage and to testing the behavioral efficacy of potentially therapeutic drugs (Xu *et al*., 2000). Transient cerebral ischemia provoked by bilateral common carotid artery occlusion or two-vessel occlusion (2VO) is a well known procedure to induce global and extensive brain injury and neuronal damage (Pulsinelli and Brierly, 1979). It is frequently discussed in the context of Alzheimer's disease because of the apparent prevalence of cerebral hypoperfusion in the disease (Farkas and Luiten, 2001).

In this study, mice were subjected to a 20-min period of cerebral ischemia produced by 2VO plus removal of 0.3 ml of blood from the tip of the tail. After 2VO procedure, we found that mice with transient cerebral ischemia showed an increasing in escape latencies in MWM but a decreasing in step-down latency while the number of errors was increased. These observations indicate that transient cerebral ischemia impaired spatial memory (MWM task) and passive avoidance task which concur with previous report (Itoh *et al*., 1993; Yamazaki *et al*., 1993; Hirakawa *et al*., 1994; Olsen *et al*., 1994; Li *et al*., 1999). In addition we found that 2VO had no effects on locomotor activity test indicating the memory deficit induced by 2VO did not involve motor system.

Previous report found that tacrine, a cholinesterase inhibitor, significantly improved the impaired learning performance of 2VO rats in the radial maze task, indicating the ameliorative effect of tacrine on the spatial working memory impairment induced by chronic cerebral hypoperfusion (Murakami *et al*., 2000). In the present study, we used tacrine as a positive control and it was shown to improve the memory deficit induced by transient cerebral ischemia in MWM and passive avoidance tasks.

Similar to tacrine, oral administration of turmeric extract significantly improved 2VO-induced deficit in learning and memory observed in MWM and step-down tests while no alteration in motor activity was observed. This result was similar for all doses of turmeric extract. This finding implied that beneficial effects of turmeric extract on impairment of learning and memory were unlikely to involve the improvement of motor function or activity.

Varying degrees of behavioral impairments are associated with aging and age associated neurodegenerative diseases. Among the prime candidates responsible for producing the neuronal changes mediating these behavioral deficits appear to be free radicals and the oxidative stress they generate. Oxidative stress refers to the cytotoxic consequences of oxygen radicals like superoxide anion, hydroxyl radical, and hydrogen peroxide, which are generated as byproducts of normal aberrant metabolic processes during aging and other neurodegenerative diseases and act on polyunsaturated fatty acids (PUFA) in brain, thereby propagating the lipid peroxidation. Potential antioxidant therapy should therefore, include either natural antioxidant enzymes or agents, which are capable of augmenting the functions of these enzymes (Kumar and Gupta, 2002). Therefore, the different doses of turmeric extract (100, 300 and 1000 mg/kg BW) were tested for their activity on the oxidative stress parameters (levels of MDA) in brain of mice.

In the present study, we found that the levels of MDA in 2VO mice were significantly increased when compared to those of sham-operated mice. This is supported by the previous report that bilateral common carotid artery occlusion followed by reperfusion

generated reactive oxygen species (ROS) (Sorrenti *et al*., 1994; Nakashima *et al*., 1999). Excessive generation of ROS results in the lipid peroxidation of the cell membrane and subsequent damage is reflected by accumulation of MDA, a byproduct of lipid peroxidation (Halliwell, 1991). Oral administration of turmeric extract at doses of 100, 300 and 1000 mg/kg BW showed a significant decrease in the MDA levels of the brain indicating attenuation of lipid peroxidation. These results agree with previous study that curcumin was also shown to inhibit lipid peroxidation in different animal models (Reddy and Lokesh, 1992; Sreejayan and Rao, 1994). In a focal cerebral ischemia model of rats, curcumin, a major component of curcuminoid extract, offered significant neuroprotection through inhibition of lipid peroxidation, increase in endogenous antioxidant defense enzymes and reduction in peroxynitrite formation (Thiyagarajan and Sharma, 2004). Accordingly in the present study, an increase in MDA was noted in 2VO mice which exhibited impairment of spatial learning and memory in MWM and passive avoidance tasks. Based on the finding that turmeric extract could ameliorate the lipid peroxidation in 2VO mice, it is suggestive that antioxidant property exerted by turmeric extract could contribute to its beneficial effect on impairment of learning and memory.

In addition to oxidative stress, it is well known that the cholinergic system in the central nervous system plays an important role in learning and memory function (Sarter and Bruno, 1994), and that brain cholinergic hypofunction causes dementia with symptoms such as memory loss and disorientation in cerebrovascular or AD (Coyle *et al*., 1983). Currently, the mainstay treatments for AD are acetylcholinesterase (AChE) inhibitors, which increase the availability of acetylcholine at cholinergic synapses. Until now, four AChE inhibitors have been approved by the FDA for the treatment of AD: tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®), and galantamine (Reminyl®) (Lahiri *et al*., 2002).

Evidences from animal and human studies indicate that learning and memory can be modified by drugs affecting central cholinergic function (Bartus *et al*., 1982; Collerton, 1986; Kopelman and Corn, 1988; Wu *et al*., 1996). For instance, such muscarinic

antagonists as scopolamine have been shown to impair memory, whereas such inhibitors of AChE as physostigmine, tacrine or velnacrine facilitate the cognitive process in animals and humans (Dawson *et al*., 1991; Braida *et al*., 1996; Rainer, 1997; Bejar *et al*., 1999). Scopolamine interferes with memory and cognitive function in humans and experimental animals by blocking muscarinic receptors in several brain regions (Beatty *et al*., 1986; Collerton, 1986; Kopelman and Corn, 1988). Therefore, the present study used scopolamine-induced learning and memory impairment model to evaluate the effect of turmeric extract on MWM and step-down test and used tacrine as a positive control. Unlike 2VO, impairment of learning and memory induced by scopolamine was not co concurrent with the increase of brain MDA, therefore antioxidative property of *Curcuma longa* was not demonstrable.

In MWM and step-down test we found that tacrine prevent memory deficit induced by scopolamine. This result was in agreement with previous report that tacrine antagonize the effects of scopolamine on spatial memory in the MWM and in passive avoidance tests (Dawson *et al*., 1991; Bejar *et al*., 1999). Oral administration of turmeric extract or tacrine attenuated learning and memory deficit in scopolamine model noted in MWM and step-down test. In locomotor activity test, scopolamine has been found to significantly reduce locomotor activity of mice. The same finding has been reported by Buxton *et al*., Kirkby *et al*., Pitkanen *et al*., and Ruotsalainen *et al*. Based on the results that all doses of turmeric extract and 2 mg/kg of tacrine given to scopolamine-treated animals had no effect on locomotor activity when compared to scopolamine-treated group. It can be concluded that the scopolamine-induced depression of motor activity had no significant effect on improved performances of turmeric- and scopolamine-treated mice in MWM and step-down tests. Cholinergic mechanism seemed to underlie beneficial effects of turmeric extract on impairment of learning and memory and the actual mechanism remains to be identified.

Neurodegenerative diseases such as AD are known to eventually be the result of neuronal cell death by many kinds of cause-induced excitotoxicity, oxidative stress,

inflammation and apoptosis (Kang *et al*., 2005). The initiating event or events leading to AD are unknown. Its pathophysiology is complex and likely involves multiple overlapping and perhaps redundant pathways of neuronal damage. One of the pathways of neuronal damage and death in AD is mediated by free radical injury (Practico and Delanty, 2000). If free radical injury is important in the initiation or progression of AD, then therapy with reduce oxidative injury and augment endogenous antioxidant defenses might prevent, delay, or ameliorate the disease process and diminish its human and social consequences. Evidence obtained in the present study suggests that turmeric extract could be useful in neurologic disorders, including AD (Delanty and Dichter, 2000).

Phytochemical investigations on turmeric extract have results in isolation of many active principles, like curcumin, demethoxycurcumin and bis-demethoxycurcumin. The best researched active constituent is curcumin (Leung, 1980). Curcumin exhibits strong antioxidant activity comparable to vitamins C and E (Toda *et al*., 1985). Our turmeric extract has curcumin as its major component. Therefore, it is likely that curcumin is responsible for an attenuation of oxidative damage elicited by the turmeric extract.

Several studies found that curcumin may be beneficial in neurodegenerative diseases such as AD. Lim *et al*. found that curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse model (Lim *et al*., 2001). In related studies, it has been shown that curcumin prevents Aβ-induced spatial memory deficits and Aβ deposits in Sprague-Dawley rats (Frautschy *et al*., 2001). Curcumin has also been shown to protect against Aβ-induced injury to neuronal cells (Park and Kim, 2002). These protective effects of curcumin are attributed mainly to its antioxidant properties and should be further exploited to develop novel drugs.

Several of the studies establishing curcumin's potential were carried out in animals. Further testing of curcumin in humans is required to confirm these observations. A clinical development plan for using curcumin to treat AD was recently described by the UCLA

Alzheimer Disease Center (ADC).

In conclusion the present study has demonstrated the beneficial effects of turmeric extract on learning and memory impairment in both of 2VO and scopolamine models. Based on the finding that 2VO but not scopolamine significantly increased the lipid peroxidation which could be ameliorated by turmeric extract. Therefore, it is possible that antioxidant property of turmeric extract could, at least partly, contribute to its positive effect on memory deficit in 2VO mice. Furthermore, the results that turmeric extract can attenuate learning and memory impairment induced by scopolamine did suggest the effect of turmeric extract on cholinergic system. Though further investigators are needed to clarify the mechanism of action of turmeric extract in details, the present study has clearly demonstrated the potential of turmeric extract to be further developed for dementia.

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APPENDICES

Effects of turmeric extract, vehicle and tacrine on performance of 2VO mice in MWM.

Group name	Escape latency in the MWM (sec)						
	n	train	Day1	Day2	Day3	Day4	Day5
sham+vehicle	8	44.25±3.27	29.25±3.63	24.75±2.30	9.88±1.95	11.63±2.94	7.88±0.77
2VO+vehicle	8	47.38±4.21	49.88±5.06$^{\#}$	40.38±3.77$^{\#}$	33.50±3.26$^{\#}$	36.63±3.97$^{\#}$	32.13±4.50$^{\#}$
2VO+turmeric 100 mg/kg	8	40.88±3.23	42.38±5.27	33.38±3.29	29.00±4.28$^{\#}$	22.63±4.31*	19.88±4.89*$^{\#}$
2VO+turmeric 300 mg/kg	8	39.00±5.83	45.00±4.12	33.00±5.51	32.38±5.60$^{\#}$	23.13±4.18*$^{\#}$	19.75±2.78*$^{\#}$
2VO+turmeric 1000 mg/kg	8	39.38±4.09	39.25±4.90	29.50±5.14	21.88±3.39	20.38±2.99*	14.38±2.86*
2VO+tacrine 0.5 mg/kg	8	37.38±3.33	37.50±7.12	23.38±4.28*	24.25±6.67$^{\#}$	13.13±3.22*	15.50±2.72*

Table 1 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of 2VO mice in MWM. The values are expressed as the mean ± S.E.M. of escape latency. Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

$^{\#}$Significantly different from value in sham operated control group. *Significantly different from value in 2VO mice group.

Effects of turmeric extract, vehicle and tacrine on performance of 2VO mice in step-down test.

	Group name	sham+vehicle	2VO+vehicle	2VO+turmeric 100 mg/kg	2VO+turmeric 300 mg/kg	2VO+turmeric 1000 mg/kg	2VO+tacrine 0.5 mg/kg
	n	8	8	8	8	8	8
Step-down latency	Day1	26.38 ± 12.85	34.38 ± 7.62	39.63 ± 13.15	55.75 ± 28.86	59.38 ± 21.12	69.75 ± 33.17
	Day2	181.75 ± 38.04	16.88 ± 7.29#	67.50 ± 32.61#	160.88 ± 45.99*	257.38 ± 28.43*	231.00 ± 38.08*
Number of errors	Day1	4.38 ± 0.71	7.13 ± 1.95	6.00 ± 1.50*#	4.13 ± 0.80	3.50 ± 1.08	3.63 ± 1.04
	Day2	2.00 ± 0.90	7.38 ± 2.45#	4.25 ± 1.17	1.88 ± 1.00*	0.25 ± 0.17*	0.50 ± 0.27*

Table 2 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of 2VO mice in step-down test. The values are expressed as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA and Duncan for comparisons. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

#Significantly different from value in sham operated control group. *Significantly different from value in 2VO mice group.

Effects of turmeric extract, vehicle and tacrine on performance of 2VO mice in spontaneous locomotor activity test (counts/5 minutes).

Group name	sham+vehicle	2VO+vehicle	2VO+turmeric 100 mg/kg	2VO+turmeric 300 mg/kg	2VO+turmeric 1000 mg/kg	2VO+tacrine 0.5 mg/kg
n	8	8	8	8	8	8
Locomotor activity (counts/5 minutes)	246.50 ± 27.43	260.13 ± 28.23	245.50 ± 21.35	263.25 ± 15.84	252.88 ± 21.81	246.75 ± 34.36

Table 3 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of 2VO mice in spontaneous locomotor activity test. The values are expressed as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA and Duncan for comparisons. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

Effects of turmeric extract, vehicle and tacrine on performance of 2VO mice in lipid peroxidation.

Group name	sham+vehicle	2VO+vehicle	2VO+turmeric 100 mg/kg	2VO+turmeric 300 mg/kg	2VO+turmeric 1000 mg/kg	2VO+tacrine 0.5 mg/kg
n	8	8	8	8	8	8
MDA levels (nmol/g tissue)	36.24 ± 3.44	132.70 ± 12.88$^{\#}$	44.54 ± 2.01*$^{\#}$	52.96 ± 4.46*$^{\#}$	27.12 ± 3.43*$^{\#}$	33.37 ± 4.22*$^{\#}$

Table 4 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of 2VO mice in MDA levels. The values are expressed as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA and Duncan for comparisons. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

$^{\#}$Significantly different from value in sham operated control group. *Significantly different from value in 2VO mice group.

Effects of turmeric extract, vehicle and tacrine on performance of mice induced by scopolamine in MWM.

Group name	Escape latency in the MWM (sec)						
	n	train	Day1	Day2	Day3	Day4	Day5
Saline+vehicle	6	29.33 ± 5.66	17.33 ± 3.63	20.17 ± 4.23	17.17 ± 2.78	12.50 ± 2.85	12.00 ± 2.65
Scop+vehicle	6	39.67 ± 4.25	49.17 ± 3.00#	40.67 ± 5.77#	33.83 ± 3.39#	30.33 ± 4.84#	28.00 ± 3.60#
Scop+turmeric 100 mg/kg	6	24.50 ± 2.28	42.67 ± 6.49#	28.33 ± 6.88	24.83 ± 7.45	20.50 ± 4.01	17.00 ± 2.42*
Scop+turmeric 300 mg/kg	6	26.17 ± 6.17	34.33 ± 7.57#	14.17 ± 2.61*	19.33 ± 5.78	16.33 ± 4.21*	18.17 ± 2.52*
Scop+turmeric 1000 mg/kg	5	35.00 ± 4.15	42.00 ± 4.73#	31.40 ± 7.92	17.40 ± 5.23*	9.20 ± 3.36*	10.60 ± 3.80*
Scop+tacrine 2 mg/kg	6	26.17 ± 4.24	31.67 ± 3.89*	21.83 ± 5.51*	18.50 ± 3.43*	15.50 ± 3.16*	16.50 ± 5.05*

Table 5 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of mice induced by scopolamine (Scop 1 mg/kg) in MWM. The values are expressed as the mean ± S.E.M. of escape latency. Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

#Significantly different from value in saline-treated group. *Significantly different from value in scopolamine-treated group.

Effects of turmeric extract, vehicle and tacrine on performance of mice induced by scopolamine in step-down test.

	Group name	saline+vehicle	scop+vehicle	scop+turmeric 100 mg/kg	scop+turmeric 300 mg/kg	scop+turmeric 1000 mg/kg	scop+tacrine 2 mg/kg
	n	6	6	6	6	5	6
Step-down latency	Day1	6.83 ± 1.16	6.33 ± 2.15	10.67 ± 1.55	6.33 ± 1.46	5.20 ± 0.38	9.00 ± 1.73
	Day2	192.83 ± 45.56	6.17 ± 2.15#	21.00 ± 1.86#	113.50 ± 13.68*	122.60 ± 8.41*	115.83 ± 7.52*
Number of errors	Day1	4.00 ± 0.26	4.50 ± 0.44	3.33 ± 0.43	3.50 ± 0.44	3.40 ± 0.52	3.50 ± 0.44
	Day2	1.17 ± 0.49	4.17 ± 0.31#	2.17 ± 0.81*	1.17 ± 0.17*	1.00 ± 0.00*	1.50 ± 0.23*

Table 6 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of mice induced by scopolamine (Scop 1 mg/kg) in step-down test. The values are expressed as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

#Significantly different from value in saline-treated group. *Significantly different from value in scopolamine-treated group.

Effects of turmeric extract, vehicle and tacrine on performance of mice induced by scopolamine in spontaneous locomotor activity test (counts/5 minutes).

Group name	saline+vehicle	scop+vehicle	scop+turmeric 100 mg/kg	scop+turmeric 300 mg/kg	scop+turmeric 1000 mg/kg	scop+tacrine 2 mg/kg
n	6	6	6	6	5	6
Locomotor activity (counts/5 minutes)	151.17 ± 11.02	0.50 ± 0.35*	1.83 ± 1.07*	1.17 ± 0.67*	1.00 ± 0.56*	1.83 ± 0.89*

Table 7 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of mice induced by scopolamine (Scop 1 mg/kg) in spontaneous locomotor activity test. The values are expressed as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

*Significantly different from value in saline-treated group.

Effects of turmeric extract, vehicle and tacrine on performance of mice induced by scopolamine in lipid peroxidation.

Group name	saline+vehicle	scop+vehicle	scop+turmeric 100 mg/kg	scop+turmeric 300 mg/kg	scop+turmeric 1000 mg/kg	scop+tacrine 2 mg/kg
n	6	6	6	6	5	6
MDA levels (nmol/g tissue)	9.45 ± 1.50	9.42 ± 0.60	7.07 ± 0.60	7.30 ± 0.82	6.82 ± 0.53	7.29 ± 0.72

Table 8 : The effect of different dose of turmeric extract (100, 300 and 1000 mg/kg) on performance of mice induced by scopolamine (Scop 1 mg/kg) in lipid peroxidation. The values are expressed as the mean ± S.E.M. Statistical analyses were performed by one-way ANOVA and Duncan for comparison. A significant value of P less than 0.05 ($P < 0.05$) was considered as statistically significant.

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